BOOK OF ABSTRACTS

Co-Editors: Luciano Adorini, Massimo Locati

ANNOTATIONS

In the following we are publishing the abstracts as submitted by the authors.

Missing session numbers represent sessions with no abstracts associated.
Missing presentation numbers represent talks with no abstracts received as per date of production. Bold presentation numbers indicate the presenting author.

The sessions are in numerical order.

Keys and Abbreviations:

IL1.01.01	**Invited Lecture**
LB.1	**Late Breaking Session 01**
LL.1	**Lunchtime Lecture**
P1.01	**Poster Presentation**
PL.1	**Plenary Lecture**
PS.1	**Lecture: Perspectives in Immunology**
S.1	**Symposium**
SS.1	**Sponsored Session**
W1.01	**Workshop**

The Editors

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ORAL PRESENTATIONS

Plenary Lectures

PL1.01
Mitochondrial transport by microtubule acetylation is essential to NLRP3-inflammasome activation

***S. Akira**, T. Misawa, T. Saitoh;*
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Detection of pathogen components and subsequent induction of innate immune responses, such as the production of inflammatory cytokines and type I interferon (IFN), is mediated by pattern-recognition receptor (PRR) that include Toll-like receptor (TLR), RIG-I-like receptors (RLR), C-type lectin receptor, NOD-like receptor and intracellular sensor for DNA (AIM2, DAI, IFI16 and DDX41). In addition to infectious insults, endogenous damage-associated molecular pattern molecules (DAMPs) also activate these receptors, resulting in sterile inflammation. In response to Nigericin, monosodium urate, or silica particles, NLRP3 forms the inflammasome with its adaptor protein ASC and mediates innate immune responses. Chemical compound screening revealed that tubulin polymerization inhibitors including colchicine specifically suppress NLRP3-inflammasome activation. NLRP3-inflammasome inducers cause aberrant mitochondrial homeostasis to reduce the NAD^+ level, which in turn inactivates the NAD^+-dependent α-tubulin deacetylase Sirtuin 2 (SIRT2), resulting in accumulation of acetylated α-tubulin. Accumulated acetylated α-tubulin mediates ASC-NLRP3 contact to promote NLRP3-inflammasome activation. These findings indicate that, in addition to direct activation of NLRP3, the creation of optimal sites for signal transduction by microtubules is required for entire activation of the NLRP3-inflammasome.

PL.3.01
On Immunity in, Biomarkers for, and Vaccines against Tuberculosis

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Tuberculosis (TB) is a health threat of global dimension. TB is also a highly interesting target for immunologic research since it reflects the outcome of a longstanding co-evolutionary process between the pathogen and the human host. Worldwide 2 billion individuals are infected with *Mycobacterium tuberculosis*, of whom 90% remain latently infected under control of a balanced immune response. In 10% of infected individuals TB emerges due to dysbalanced immunity. T lymphocytes are central regulators of protection and pathology and mononuclear phagocytes serve both as effectors against and as habitat for the pathogen. Interactions focus on granulomas which as solid granulomas contain the pathogen, and as necrotic/caseous granulomas promote disease and dissemination. (I) Immune mechanisms which control inflammation in TB will be discussed including sensing of pathogen patterns and the role of regulatory cytokines, chemokines and micro-RNA. (II) The current vaccine BCG prevents disseminated childhood TB but fails to protect against pulmonary TB. We have genetically modified BCG to improve its protective efficacy. This vaccine is currently undergoing chemical testing with promising results. (III) Global gene expression profiling has provided deep insights into the host response in TB. Biosignatures have been defined which distinguish latent infection without disease from active disease. Such biomarkers not only provide the basis for novel intervention measures but also shed light on mechanisms underlying protection/pathology. Reciprocity between basic and translational research will provide new avenues in TB research.

PL.4.01
Interactions between the cellular and the humoral arm of innate immunity

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Innate immunity consists of a cellular and a humoral arm. The role of negative regulators, including TIR8 (a member of the IL-1 receptor family) and D6 (a decoy and scavenger chemokine receptor) will be discussed. We have used the long pentraxin PTX3 as a paradigm for the humoral arm of innate immunity and its interplay with cells. PTX3 is a multifunctional soluble pattern recognition receptor characterized by a C-terminal domain highly homologous to C-reactive protein and serum amyloid P component, associated to a N-terminal domain unrelated to other known proteins. PTX3 is produced upon stimulation with proinflammatory cytokines and Toll-like receptor engagement by different cell types, including endothelial cells, monocytes/macrophages, dendritic cells, fibroblasts and epithelial cells. The molecule binds with high affinity complement component, microbial moieties, growth factors and apoptotic cells. PTX3 plays non-redundant functions including innate immunity against selected microorganisms to regulation of inflammation. In addition PTX3 has a regulatory function. PTX3 serves as a paradigm for the interplay between the cellular and the humoral arm of innate immunity.

Bottazzi B, et al. An Integrated View of Humoral Innate Immunity: Pentraxins as a Paradigm. Annu Rev Immunol. 2010, 28:157-83.
Deban L, et al. Regulation of leukocyte recruitment by the long pentraxin PTX3. Nat Immunol. 2010, 11:328-34.
Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. J Clin Invest. 2012, 122:787-95.

PL.6.01
Dissecting the antibody response to pathogens and self antigens

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Memory B lymphocytes and long lived plasma cells represent a repository of the antigenic experience of an individual. By analyzing the specificity and function of these cells we can gain insights into the human immune response to pathogens and vaccines, identify correlates of protection, and isolate neutralizing antibodies and protective T cells. To interrogate human memory B cell and plasma cell repertoires we developed two culture-based high-throughput methods that are used to isolate, with high efficiency, human monoclonal antibodies of distinctive specificities. Unusually potent neutralizing antibodies against human cytomegalovirus were isolated from infected donors and used to identify the viral ligands and to design an experimental vaccine. We also isolated antibodies of exceptional breadth, such as a pan-influenza A neutralizing antibody and an antibody that neutralizes both respiratory syncytial virus and metapneumovirus. By targeting conserved structures, these broadly neutralizing antibodies are less prone to select escape mutants and are therefore promising candidates for prophylaxis and therapy of infections as well as tools for the design of improved subunit vaccines. We also isolated and characterized autoantibodies from patients with pemphigus, pulmonary alveolar proteinosis and other autoimmune diseases. By reverting the antibodies to the germline we investigate the role of somatic mutations in the generation of broadly neutralizing antibodies and autoantibodies.

PL.8.01
The Inflammasome in Health and Disease

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Dysregulation of the immune system and host-microbiota interaction has been associated with the development of metabolic diseases such as obesity and diabetes. Recent studies in our lab have

elucidated the important function of inflammasomes as steady-state sensors and regulators of the gut microbiota. Mice with disrupted inflammasome pathway have been shown to develop a colitogenic microbial community, which resulted in exacerbation of chemical-induced colitis and diet-induced steatohepatitis. As increasing evidence has demonstrated the profound effect of intestinal microbiome on host metabolism, the goal of this study was to examine the role of inflammasome-regulated microbiota in the pathogenesis of obesity and associated metabolic disorders. Notably, we found that mice lacking the inflammasome adaptor ASC developed increased adiposity and impaired glucose tolerance on regular chow. When fed with a high fat diet (HFD), mice lacking ASC, NLRP6, caspase-1 or IL-18 all manifested exacerbated obesity, adiposity and insulin resistance. 16S-rRNA based metagenomic analysis demonstrated an altered fecal microbial composition in HFD-fed ASC KO mice. Cross-fostering to WT mothers and antibiotic treatment diminished the aberrant microbiota in ASC KO mice and abrogated their metabolic phenotype. Together, these data suggest that the inflammasomes play a critical role in regulating energy metabolism via modulation of the gut microbiome.

PL.9.01
Cancer Immunoediting: From basic mechanisms to novel immunotherapies

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Cancer Immunoediting is the process by which the immune system controls and shapes cancer. We originally envisaged and subsequently showed that, in its most complex form, cancer immunoediting occurs in three phases: Elimination (i.e., cancer immunosurveillance—the host-protective phase of the process); Equilibrium (i.e., immune mediated tumor dormancy—the phase in which tumor cells that survive elimination are subjected to immunologic growth control and undergo editing); and Escape (i.e., the phase where immunologically sculpted or immunoselected tumors with either reduced immunogenicity or enhanced immunosuppressive capacity emerge to become clinically apparent, progressively growing tumors). Strong experimental data supporting each phase of the process have been obtained using mouse models of cancer and compelling clinical data have revealed that a similar process also occurs in humans. Our efforts now focus on elucidating the molecular and cellular mechanisms that underlie each phase of cancer immunoediting and in identifying the critical checkpoints that regulate progression from one phase of the process to the next. This focus led to development of a novel method of cancer exome sequencing and its use in defining mutational tumor antigens as key targets for adaptive immunity in nascent developing unedited cancers. Furthermore, we showed that immunoselection is a major immunoediting mechanism. We are now applying this approach to identifying mutational antigens in clinically apparent, edited tumors that can be used as targets for different forms of cancer immunotherapy including checkpoint blockade therapy. Our long-term goal is to develop rapid, safe, specific and effective personalized cancer immunotherapies.

Perspectives in immunology

PS1.01
On Understanding Immunity or what Immunology is all about

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1) Definition of specificity; by phenolic haptens or by protective antigenic sites against infections? 2) Importance of antigen as the (major, only?) regulator of immunity. 3) Protective immunity by vaccines against the classical acute childhood infections (e.g. measles), against chronic persistent infections (e.g. TB, leprosy, HIV or malaria)? 4) How is there no reactivity against extralymphatic intact organs and self-antigens, except when these are brought into secondary lymphatic organs in sufficient amounts and for sufficient time periods? 5) Are there important effector T cell class differences induced by phagocytosed antigens? And is crosspresentation > 10^6 fold less efficient than direct presentation? 6) Affinity maturation of antibodies against poorly or noncytolytic infections is slow, and against infectious agents that cause cell death too slow, and therefore irrelevant 'for memory'. 7) So called immunological memory is an experimental artefact. It is the pre-existent level of protective (neutralizing) antibodies (or the number of pre-activated T cells) that determine protection. Re-stimulation of so called memory B- or T cells takes 2 - 5 days and therefore is generally too slow for efficient protection.
The discussed parameters and limitations of the immune system, and of its function, show that we cannot do better immunologically than co-evolution if we use the same tools as evolution has been using. But we certainly can do better if we use new tools not used by evolution such as antibiotics, antivirals or pharmacologically active small molecules.

PS.2.01
Primary T cell immunodeficiency and immunopathology

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Modern tools in genomics have made easier the identification of monogenic inherited diseases that include a number of T cell immunodeficiencies. Their study continues to provide a flurry of informations on T cell biology and mechanisms underlying immunopathological findings frequently observed in T cell ID such as inflammation and auto immunity. Therefore, T cell PIDs may serve as models to capture pathophysiology of more common immunopathological disorders. Examples will be presented and discussed.

PS.3.01
Beyond anti-TNF Therapy for Rheumatoid Arthritis: Can we get close to a cure?

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The treatment of rheumatoid arthritis has progressed in recent years, with the incorporation of low dose methotrexate and 'biologicals' into routine treatment of disease. Of the biologicals, the TNF inhibitors, both monoclonal antibody and monoclonal antibody-like TNF receptor Ig fusion protein have been the most successful. While treatment is beneficial in the majority of patients, the exact percentage depending on the stage of disease, this therapy does not yield a cure; but approximately a 50% benefit. Our challenge is thus to learn how to get closer to a cure. We and others have been working on a number of approaches. Our approaches include analysing the cytokine abnormalities in non-responder patients, analysing the inhibitory pathways which are not functioning effectively in RA patients, and selectively interfering with the source of the antigen, the citrullination pathway. In case these approaches fail to develop the necessary solutions, we are also working on an empirical approach, using CyToF (mass spectrometry linked to flow cytometry) to evaluate which signalling pathways are upregulated in active disease patients compared to inactive RA, or non-responders compared to responders.

PS.4.01
T cell receptors, their specificity and role in selection

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More than thirty years ago others discovered the phenomenon of positive selection in the thymus. During this event, thymocytes are selected to mature only if their receptors react with low affinity with the MHC + peptide combinations on thymus cortical epithelial cells. One consequence of positive selection is the fact that mature T cells are much more likely to react with any given foreign peptide bound to an allele of MHC that they encountered in the thymus (self restriction) than with peptides bound to foreign MHC. In the studies to be presented here we examined the structural basis for the bias for self restriction. Our results show that the phenomenon is governed by the variable regions and also by the CDR3, somatically created, regions of the T cell receptors on T cells.
The latter finding is unexpected because T cell receptor CDR3 regions are usually thought to react with the peptide bound to MHC, rather than the MHC itself. If the peptide is the basis for the MHC allele specificity of positive selection it is a mystery how the self peptides that drive positive selection can reproducibly resemble, in their amino acids that contact the T cell receptor, foreign peptides. The mysteryand its possible resolutions will be discussed.

PS.5.01
Macrophage Receptors and Immune Activation

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Monocyte/macrophages represent a family of specialised phagocytes present in lympho-haemopoietic and other tissues.I shall present an account of their origin, distribution and activation, emphasising tissue macrophage microheterogeneity in situ and plasticity. Recent microarray and proteomic analysis has established gene expression signatures which underlie their known properties, and point to novel functions. This will be illustrated by studies in human and mouse, with special reference to alternative activation of macrophages by Th2 cytokines. The talk will emphasise the role of non-opsonic plasma membrane receptors implicated in innate and adaptive immunity.
The Th2 cytokines IL-4, IL-13 and their receptors initiate a distinct alternative activation phenotype in macrophages. We have identified biomarkers such as transglutaminase 2 as conserved indicators of alternative activation in human as well as mouse macrophages in vitro. The expression of such markers has been validated in human and mouse asthma models and suggests heterogeneity in monocyte as well as tissue macrophage populations.

PS.7.01
IL-6: New era comes for the treatment of inflammatory autoimmune diseases

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IL-6 was originally identified as a T cell-derived cytokine, which induces antibody production in B cells. A series of subsequent studies have revealed that IL-6 has a pleiotropic activity in various tissues and cells and its deregulated expression is responsible for several chronic inflammations and hemopoietic malignancies. Humanized antibody against 80kd IL-6R (Tocilizumab) has shown significant therapeutic effect in RA, JIA, Castleman's diseases, several other autoimmune inflammatory diseases, such as progressive sclerosis, reactive arthritis, polymyalgia rheumatica, adult still's desease and uveoretinitis. Cytokine storm induced by hyperactivation of T cells has been shown to be controlled by Tocilizumab. Recently, TH17 is shown to be responsible for the pathogenesis of autoimmune diseases and IL-6 together with TGF-β are essential for the induction of TH17. We identified a new transcription factor required for Th-17 cell induction, which is induced by IL-6 and TGF-β. This molecule, aryl hydrocarbon receptor (Ahr) interacts with Stat1 and Stat5 and abrogates their negative activity in the induction of Th-17 cell differentiation. Experimental arthritis is completely abrogated in T cell-specific Ahr-deficient mice. Therapeutic effect of Tocilizumab indicates that overproduction of IL-6 is responsible for the pathogenesis of autoimmune diseases. We identified a novel molecule, Arid5a, which specifically stabilizes mRNA of IL-6, sustains its overproduction and plays an important role in the promotion of inflammatory processes and autoimmune diseases.

PS.8.01
Evolution of Adaptive Immunity

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An alternative adaptive immune system has been defined in the surviving jawless vertebrates, lampreys and hagfish. Surprisingly, in this ancient immune system, leucine-rich-repeat gene segments are used instead of immunoglobulin V(D)J gene segments to generate very large repertoires of antigen receptors, called variable lymphocyte receptors (VLR), for T-like and B-like lymphocytes. Functional interactions between these lamprey lymphocyte populations are implied by their reciprocal expression of interleukins (IL-8 and IL-17) and their cognate receptors. Immunological memory is suggested in lampreys by their heightened secondary VLR antibody responses and increased antibody affinity. The unique structural characteristics and specificities of the lamprey VLR antibodies indicate their potential value as diagnostic reagents for infections and malignancies in humans. The similarities between the alternative adaptive immune systems in jawless and jawed vertebrates suggest they evolved as parallel solutions that balance the advantages and disadvantages of a highly diverse repertoire of lymphocyte antigen receptors.
Supported by NIH grants R01AI072435 and R01GM100151

Lunchtime lectures

LL.1.01
Immunological tolerance: mechanisms and therapeutic applications

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The immune system exists in a balance between the generation of effector and memory lymphocytes to protect against pathogens and the generation of Foxp3+ regulatory T cells (Tregs) to prevent or limit inflammatory reactions. Failure of control mechanisms is the fundamental cause of many inflammatory disorders. We have exploited transgenic mouse models to examine T cell responses to systemic and tissue-restricted self antigens and how these responses are controlled. Exposure of naive CD4 T cells to systemic or tissue antigens under various conditions leads to the development of pathogenic effector cells, and inflammatory disease. The disease resolves spontaneously, associated with the generation and activation of Tregs. Tregs that encounter tissue antigen acquire increased suppressive activity, and a fraction of these Tregs persists as a memory population that continues to control subsequent inflammatory reactions in the tissue. Thus, Tregs go through a sequence of development in the thymus, activation in the periphery to perform their function, and survival as memory cells, which is fundamentally similar to the life history of all lymphocyte populations. The generation and maintenance of pathogenic effector T cells vs protective Tregs are determined by: i) the duration of antigen exposure, with persistent antigen reducing effectors and preserving Tregs, and ii) cytokines, especially IL-2, which, at low concentrations, preferentially expands and maintains Tregs.
Elucidating the stimuli that generate and maintain functional Tregs in tissues will likely be valuable for manipulating immune responses in inflammatory diseases and for optimal vaccination and cancer immunotherapy.

LL.3.01
T Cell Memory and Exhaustion

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Acute viral infections result in the generation of a long-lived and self-renewing pool of highly functional memory CD8 T cells. These memory CD8T cells play an important role in faster control of infection upon re-exposure to the same pathogen. In contrast, chronic antigen stimulation during persistent viral infections or during cancer can lead to functional exhaustion of CD8 T cells. A characteristic feature of these exhausted CD8 T cells is sustained expression of inhibitory receptors such as PD-1. It is now well established that PD-1 plays a major role in T cell exhaustion and that blockade of the PD-1 inhibitory pathway can restore function in exhausted T cells. The role of PD-1 in T cell exhaustion was first described in mice during chronic LCMV infection and these observations have been extended to other chronic infections in mice, non-human primates and humans. Most recently, PD-1 directed immunotherapy has shown promising results in a phase I clinical trial in cancer patients. In this talk I will describe a new role for PD-1 in regulating T cell differentiation and also discuss strategies for enhancing PD-1 directed immunotherapy.

LL.4.01
Calcineurin/NFAT control of innate immunity

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Dendritic cells (DC) are well known as initiators and regulators of immunity. Microbe's uptake, antigen processing/presentation and cytokine production are well known DC functions. In contrast, the network of signaling pathways leading to regulation of transcription and gene reprogramming still need to be determined to better understand fine-tuning of DC functions.
In this regard, the finding that Calcineurin/NFAT signaling pathway is activated in DC upon fungal and microbial encounter raise the question about the role of NFAT-regulated genes in DC biology. Interleukin2 (IL2) is one of the genes that we found to be regulated by Calcineurin/NFAT signaling in DC.
Herein, we describe recent insights in understanding the production of IL2 against microbial particulates. In particular, 1,3-β glucan triggers IL2 production and interestingly, the concentration, the size as well as the solubility of these microbial particulates determine different dose-responses. The different levels of IL2 are found to be dependent on Dectin-1 and Syk/PLCγ2 signaling which leads to the activation of the calcineurin/NFAT signaling pathway. While there is compelling evidence that transcriptional regulation of IL-2 in DC is tightly controlled, the mechanisms and the biological relevance in vivo are not yet fully understood. Therefore, targeted deletion of the IL2 gene in DC has been achieved by the generation of conditional IL2 flox-flox x CD11c-cre mice. Notably, the associated phenotype, as revealed by the CyTOF technology, clearly indicates a regulatory mechanism of DC-produced IL2 in vivo.

LL.5.01
Memory

***J. Weill**;*
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Most vaccines are antibody mediated but one does not have a clear picture on the heterogeneity among memory B cells and plasma cells generated by these vaccines and on how each subset will behave during a recall response. Recent studies in mice have underlined the role of the nature of the antigen in the generation of persistent germinal centers and how the latter may influence the response of the different memory B cell subsets: return to germinal centers to further mutate their Ig receptors, thus increasing the affinity and the diversity of the response and/or giving rise to antibody secreting plasma cells. Moreover most studies are done in mice but there are clear differences between the murine and human B cell compartment, notably concerning the controversial nature of the IgM "memory" sub-population. Finally some auto-immune diseases are clear examples of long-lasting B cell responses thus offering a panel of all the possibilities of the system.

LL.10.01
Non-canonical inflammasome activation targets caspase-11

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Caspase-1 activation by inflammasome scaffolds comprised of intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) and the adaptor ASC is believed essential for production of the pro-inflammatory cytokines interleukin (IL)-ß and IL-18 during the innate immune response. We found that C57BL/6 gene-targeted mice that caspase-11 (also known as caspase-4) is critical for caspase-1 activation and IL-1ß production in macrophages infected with *Escherichia coli, Citrobacter rodentium*, or *Vibrio cholerae*. Mouse strain 129 exhibited similar defects in IL-1ß production and harboured a mutation in the *Casp11* locus that attenuated caspase-11 expression. This finding is important because published targeting of the *Casp1* gene was done using 129 ES cells. *Casp1* and *Casp11* are too close in the genome to be segregated by recombination so the published *Casp1*$^{-/-}$ mice lack both caspase-11 and caspase-1. Interestingly, *Casp11*$^{-/-}$ macrophages secreted IL-1ß normally in response to ATP and monosodium urate, suggesting that caspase-11 is engaged by a non-canonical inflammasome. *Casp1*$^{-/-}$ *Casp11*$^{129mt/129mt}$ macrophages expressing caspase-11 from a C57BL/6 BAC transgene failed to secrete IL-1β regardless of stimulus, confirming an essential role for caspase-1 in IL-1ß production. Caspase-11 rather than caspase-1, however, was required for non-canonical inflammasome-triggered macrophage cell death, indicating that caspase-11 orchestrates both caspase-1-dependent and -independent outputs. Finally, loss of caspase-11 rather than caspase-1 protected mice from a lethal dose of lipopolysaccharide (LPS). These data highlight a unique proinflammatory role for caspase-11 in the innate immune response to clinically significant disease states.

LL.11.01
Molecular mechanism for antibody memory generation

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Recent studies have revealed that the mechanisms for generating immune and genetic diversity share several critical features. Meiotic recombination, V(D)J recombination, CSR and SHM all require H3K4 trimethyl histone modification on the chromatin of the target DNA. Screening of histone chaperones revealed the FACT complex, Spt5 and Spt6 are involved in epigenetic marking of AID targets. Genetic instability related to dinucleotide or triplet repeats depends on DNA cleavage at the aberrant DNA structure by topoisomerase 1 (Top1). We also showed that Top1 initiates DNA cleavage in both SHM and CSR. These similarities suggest that AID hijacked the basic mechanism for genome instability when AID evolved in jawless fish. We showed that AID reduces Top1 protein by translation suppression. AID augments the frequency of mutations approximately 1,000 folds by reducing the Top1 protein, enhancing the formation of aberrant (non-B) DNA structure in Ig as well as other loci. Although AID has the risk of introducing genome instability, evolution selected AID probably because of the advantage conferred on the host of being protected against pathogens by the enormous immunoglobulin diversification. We studied the mechanism for reduction of the Top1 protein by AID and found that the miRNA-Ago2 complex binds the 3' UTR of Top1 mRNA by AID activation. The results support the hypothesis that AID edits miRNA to down-modulate Top1 mRNA translation. Furthermore, we found UNG suppresses SHM by its scaffold function for correct repair. Another non-canonical function of UNG is involved in synapse formation of cleaved ends during CSR.

LL.12.01
Three Horsemen of the Anti-Apocalypse

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Immune response activation is necessary for anti-pathogen defense but can result in inflammation and/or life-threatening septic shock due mainly to TNFα production. An emerging concept is that several novel parameters cooperate to balance pathogen control against host damage. One such parameter is tissue-specific regulation of TNFα secretion by iRhom2. Another parameter is the pre-priming by commensal bacteria of innate immune responses, a process controlled in part by the newly identified IgM Fc receptor TOSO. Finally, regulation of TNFα production to prevent inflammatory damage is also mediated by the vagus nerve circuit, which involves the synaptic transmitter acetylcholine expressed by leukocytes. Thus, a future approach to both steering towards a desired immune response and mitigating inflammation and septic shock may be to manipulate iRhom2 activation, vagal nerve feedback circuitry and/or microbiotal influence where they impinge on the immune system. We will describe four intertwining projects whose results support this new thinking.

LL.14.01
Toward an integrative biology of T cells and dendritic cells

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T cells probe the surface of dendritic cells (DCs) in search of cues reflecting the antigenic and inflammatory status of the body tissues. It remains a daunting task to understand how T cell activation is regulated through the summation of a multitude of positive and negative inputs and how their integration contributes to the unfolding of appropriate T cell responses. One of our major objectives is to understand how mutations that reduce TCR signaling output paradoxically lead to severe immune pathologies in both the mouse and human species. More specifically, we would like to elucidate the mechanisms through which during physiological, antigen-driven T cell responses some signaling "hub" used by the TCR leads first to activation of intracellular signaling pathways and then exerts with a temporal delay a feedback inhibition that leads to rapid attenuation of the whole TCR signaling pathway. In the absence of such negative feedback, T cell responses evolves into chronic pro-inflammatory T cell responses that perpetuate themselves in a TCR-independent manner and induce the production of massive amounts of autoantibodies. After giving an overview of the major actors of T cell activation, we will present some recent genetic and proteomic approaches that we have developed to tackle the complexity of T cell activation under physiological conditions and at the systemic levels.
Key words: T cells, dendritic cells, functional genomics

LL.15.01
Immune tolerance mediated by T regulatory type 1 cells

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T regulatory type 1 (Tr1) cells are a population of Tregs induced in the periphery by IL-10. Tr1 cells control immune responses through IL-10 and TGF-β, and Granzyme B-mediated killing of myeloid APC.
Tr1 cells are identified by their unique cytokine profile (IL-10+IL-4-IL-17-). Tr1 cells express transiently FOXP3 upon activation; but FOXP3 expression never reaches the high levels characteristic of FOXP3+ Tregs. By gene expression profile of human Tr1 cell clones we identified two surface markers (CD49b and LAG-3) that are stably and selectively co-expressed on murine and human Tr1 cells. The co-expression of CD49b and LAG-3 allows tracking Tr1 cells in peripheral blood of patients who developed tolerance after allogeneic hematopoietic stem cells transplantation (allo-HSCT).
Proof-of-principle clinical trials in allo-HSCT demonstrated the safety of cell therapy with Tr1 cells polarized in vitro with DC-10. A phase I/II trial using this polarized Tr1 cells is planned in patients after kidney transplantation.
Results obtained in patients treated with in vitro generated Tr1 cells demonstrated the feasibility of this approach. However, the polarized Tr1 population includes contaminating non-Tr1 cells, which may represent a drawback for Tr1 clinical applications. The co-expression of CD49b and LAG-3 enables the isolation of highly suppressive Tr1 cells from in vitro polarized Tr1 cultures.
In conclusion, Tr1 cells are inducible Tregs that play a key role in maintaining immunological homeostasis. The identification of CD49b and LAG-3 as Tr1-specific biomarkers will facilitate the study of Tr1 cells in vivo and the use of Tr1 cells for forthcoming therapeutic interventions.

LL.16.01
The emerging diversity of immunological memory

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Memory plasma cells, memory B and memory T cells provide protective immunity and reactive memory to recurrent pathogens. Recent results challenge the current paradigm that all memory cells are circulating throughout the body in quest of antigen, and are maintained by homeostatic proliferation. This may be true for memory B and T cells of the peripheral blood, which decay in numbers over time, and thus provide a kind of systemic shortterm memory for´recently encountered pathogens. We have recently shown that the bone marrow, which is not drained by the lymphatic vessels, is home of professional T memory lymphocytes providing systemic longterm memory. These cells rest in terms of pro-liferation and gene expression, and express distinct markers of differentiation, e.g. CD69 and Ly6C, discriminating them from cells of the periphery. They are individually maintained in survival niches organized by mesenchymal stroma cells, which express interleukin-7. Some time ago, we had demonstrated a similar principle of organisation for memory plasma cells. Their precursors, plasmablasts of secondary immune reactions immigrate into the bone marrow, guided by CXCL12, and then dock onto CXCL12 expressing mesenchymal stroma cells, which organize their survival as resting memory plasma cells. To this end, they recruit accessory cells like eosinophilic granulocytes. Survival of the memory cells is conditional on being in the niche. A third type of memory, local memory, is provided by memory cells located in distinct tissues, like skin and lung. These cells provide efficient local immunosurveillance at the site of entry of a recurrent pathogen.

LL.17.01
Life and Death Decisions: Annexin AI expressed on apoptotic cells downregulates the immune response

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Removal of excess cells by apoptosis maintains tissue homeostasis. To preclude the development of autoimmunity, dying cells are rapidly cleared by neighbouring phagocytes, such as dendritic cells (DC). The uptake of apoptotic cells usually does not lead to an autoimmune response, even though many self-antigens are presented by professional antigen presenting cells. We reasoned that apoptotic cells might play an active part in preventing autoimmunity by modulating DC activity, possibly leading to the development of "tolerogenic" DC. To define signals on the surface of apoptotic cells involved in this process, we raised monoclonal antibodies against apoptotic cells and screened for their ability to differentiate between live and apoptotic cells. Using this approach, the lipid binding protein annexin A1 (Anx 1) was identified on the surface of early apoptotic cells. Recombinant Anx 1and Anx1-transfected insect cells confirmed a role for this protein in suppression of pro-inflammatory cytokine secretion, upregulation of costimulatory surface molecules and T cell stimulatory capacity of DC. In mice, exposure of Anx 1 on apoptotic

cells leads to non-activation of antigen-specific T cells. Our results indicate that Anx 1 acts as an endogenous anti-inflammatory signal on apoptotic cells, suppressing the immune response against self antigens. Thus, Anx 1 may contribute to the induction of peripheral tolerance.

LL.18.01
Tyrosine kinases and phosphatases in inflammation and autoimmunity

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The balance between activating signals, initiated by tyrosine kinases, and inhibitory signals, mediated by tyrosine phosphatases, is critical for determining the overall outcome of the immune response. Shp1 is one of the major tyrosine phosphatases responsible for inhibitory signaling in innate immune cells. Mice carrying mutations in *Ptpn6*, the gene encoding Shp1, are referred to as *motheaten* mice. These animals develop a complex autoimmune/inflammatory disease that results in early mortality. Shp-1 regulates multiple cell signaling pathways in different hematopoietic cell types; however, the cellular and molecular mechanisms of autoimmunity and inflammation in *motheaten* mouse strain remains unclear. Using floxed *Ptpn6* mice, we dissected the contribution of innate immune cells to the *motheaten* phenotype. *Ptpn6* deletion in neutrophils, achieved using MRP8-cre (*s100a8-cre*) resulted in cutaneous inflammation but not autoimmunity. By contrast, dendritic cell deletion, achieved using CD11c-cre (*Itgax-cre*) caused severe autoimmunity without inflammation. Genetic and biochemical analysis showed that loss of Shp1 in neutrophils resulted in Src-family and Syk kinase hyperactivation, which in turn lead to dysregulated integrin-mediated neutrophil activation. By contrast, in dendritic cells, lack of Shp1 primarily affected MyD88 dependent pathways, leading to exaggerated TLR and IL-1β signaling responses that drove autoimmunity. Deletion of the inhibitory Lyn kinase, which is upstream of Shp1, in dendritic cells, produced a similar TLR hyperresponsiveness, leading to autoimmunity, probably as a consequence of exaggerated responses to commensal flora. Our results demonstrate how distinct Lyn/Shp1-regulated pathways in different cells contribute to complex disease phenotypes.

LL.20.01
Toxoplasma recognition by TLR11/TLR12 heterodimmers: a tale of mice (cats) and men

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Mouse is the natural intermediate host for the protozoan Toxoplasma gondii. Given all the tools available to study mouse immunology, experimental murine toxoplasmosis became very attractive to study the immunological mechanisms of host resistance to protozoan infections. Intriguingly, recent studies reveal that mice developed two host specific (absent in men and most vertebrates) TLRs, i.e., TLR11 and TLR12 that work as heterodimers for detecting T. gondii. While these are the primary TLRs involved in rodent resistance to this parasite, in the absence of such TLRs, the nucleic acid sensing TLRs (NAS-TLRs) participate in detecting and mediating host resistance to acute infection with T. gondii.The absence of functional genes encoding TLR11 and TLR12 in human genome, lead us to investigate the importance of the NAS-TLRs, as alternative receptors for detecting T. gondii in men. Here, we will discuss the importance of TLRs on innate immunity of toxoplasmosis, giving emphasis to the evolutionary aspects of these findings, both on parasite life cycle and human disease.
Financial support: National Institutes of Health

LL.21.01
The defensin and HMGN1 alarmins have potent adjuvant effects

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High-mobility group nucleosome-binding protein 1 (HMGN1) is a potent alarmin that induces antigen-specific Th1 immune response by interacting with TLR4. Inoculation of EG7, a mouse thymoma transfected to overexpress OVA, into Hmgn1-/- and littermate-matched Hmgn1+/+ mice revealed that the tumor grew much faster in Hmgn1-/- than in Hmgn1+/+ mice. EG7-bearing Hmgn1-/- mice had fewer splenic OVA-specific CD8 cells, suggesting that endogenous HMGN1 contributed to the development of antitumor responses. To determine whether exogenous HMGN1 could also enhance antitumor defense, we inoculated EG7-N1, an HMGN1-expressing EG7 tumor cell line, or parental EG7 into C57BL/6 mice. EG7-N1 tumor grew slower than EG7 tumors in the mice, while both cell lines proliferated equally in vitro, suggesting that the murine immune system was more resistant to HMGN1 expressing tumors.We next constructed a series of eukaryotic expressing plasmids encoding the genes of HMGN1, gp100 (a murine melanoma-associated antigens), or HMGN1-gp100 fusion gene, and used these plasmids as DNA vaccines. C57BL/6 mice vaccinated by gene gun with various plasmids were subcutaneously implanted with B16F1 melanoma (2×104/mouse).Mice vaccinated with HMGN1-gp100 plasmid inhibited tumor growth, whereas control mice developed tumors. T cells from mice immunized with HMGN1-gp100 plasmid generated interferon-gamma and gp100-specific cytotoxic activity. Overall, the data illustrate that HMGN1 contributes to the generation of antitumor immunity and suggest that the alarmin HMGN1 may be used as an effective tumor vaccine adjuvant.

LL.26.01
Chemokine antagonism: an in vivo mechanism for regulating inflammation during viral infection and tumorigenesis

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We recently demonstrated the first in vivo evidence of dipeptidylpeptidase 4 (DPP4) mediated chemokine antagonism in human disease pathogenesis. DPP4 (also known as CD26) is a member of the family of X-prolyl dipeptidylpeptidases (DPPs), and is capable of enzymatically removing the N-terminal two amino acids of proteins. Specifically, we detected N-terminal truncation of the chemokine interferon-induced protein 10 (IP-10 or CXCL10) in chronic hepatitis C virus (HCV) patients, which correlated with higher DPP4 plasma activity, reduced trafficking of CXCR3-expressing lymphocytes and poor response to treatment. DPP4 truncation of IP-10 generates a dominant negative form of the chemokine, which binds its receptor, CXCR3, but does not induce signaling. As a result, we suggested that DPP4 perturbs lymphocyte trafficking in the context of HCV pathogenesis, thus accounting for DPP4 activity being a correlate of treatment failure. Our basic and translational research program is currently focused on the regulation of DPP4 and its ability to perturb lymphocyte trafficking in an in vivo setting, with potential impact on therapeutic vaccination or enhancement of naturally occurring immune responses. Additionally, we aim to establish new tools and unique insight into DPP4 regulation that may help integrate its role in different pathologic contexts and biological processes.

LL.28.01
Genetic determinants of B cell lymphoma transformation and immune escape

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Diffuse Large B cell Lymphoma (DLBCL) represents the most common form of B cell derived non-Hodgkin Lymphoma (B-NHL), accounting for ~30% of the de-novo diagnoses and also arising as a frequent clinical evolution of Follicular Lymphoma (FL), the second most common type of B-NHL. The molecular pathogenesis of DLBCL is associated with a number of genetic lesions that appear in part to

distinctly segregate with individual phenotypic subtypes of this malignancy, which appear to derive from germinal center B cell-like (GCB-DLBCL) or from post-GC B cells resembling in vitro activated B cells (ABC-DLBCL), suggesting that these two disease groups utilize distinct oncogenic pathways. However, the lesions that have been identified so far likely may represent only a fraction of those necessary for malignant transformation. Thus, the identification and functional characterization of the entire set of structural alterations present in the DLBCL genome is required for a complete understanding of its pathogenesis and for the development of rationally targeted therapeutic approaches. Toward this end, we have integrated whole-exome sequencing analysis and copy number variation analysis for a comprehensive definition of the DLBCL coding genome. The results have identified a novel set of recurrent genetic lesions, which, in turn, identify novel altered pathways In DLBCL, including those involving chromatin remodeling, germinal-center development and immune escape.

LL.34.01
A dance of death: HIV-1 and immunodominant T cells

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CD8+ T cell responses to viruses focus on small numbers of epitopes. Across a population immunoprevalent responses are determined by MHC type some of which become immunodominant. The immunodominance hierarchies reflect epitope quantity on infected cells and prevalence of HIV specific T cells in the naïve repertoire.
In acute HIV-1 infection, initial immunodominance hierarchies are influenced by sequence variability in the single infecting virus. The first T cells then rapidly select escape mutations, which quickly impair T cell control. New responses and escapes follow, shifting the patterns of immunodominance. The rate escape is determined by T cell response relative magnitude countered by fitness cost of each escape mutation. Escape slows as T cell response breadth increases, but all epitopes escape eventually and the virus persists, as ability to make new CD8 T cell responses is impaired.
CD4+ T cell responses to HIV-1 are less narrow and examination of the pre-immune CD4+ T cell repertoire shows that a considerable contribution to primary T cell response from cross reactive memory T cells as well as classical naïve T cells. Furthermore, epitopes are often presented by several different HLA class II allotypes. Thus rules for class II MHC epitope and CD4+T cell selection differ. The recent vaccine induction (Hansen et al Science 340, 940, 2013) of anti-SIV CD8+ T cells that respond to epitopes presented by class II MHC, with no clear immunodominance hierarchy, has major implications for future control of HIV-1 by vaccines.

Sponsored Sessions

SS.5 Infectious agents and autoimmunity - Part I (with the contribution of Italian Ministry of Health and Italian Multiple Sclerosis Foundation)

SS.5.02
Human genetics of infectious diseases

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The hypothesis that inborn errors of immunity underlie infectious diseases is gaining experimental support. However, the apparent modes of inheritance of predisposition or resistance differ considerably between diseases and between studies. A coherent genetic architecture of infectious diseases is lacking. We suggest here that life-threatening infectious diseases in childhood, occurring in the course of primary infection, result mostly from individually rare but collectively diverse single-gene variations of variable clinical penetrance, whereas the genetic component of predisposition to secondary or reactivation infections in adults is more complex. This model is consistent with (i) the high incidence of most infectious diseases in early childhood, followed by a steady decline, (ii) theoretical modeling of the impact of monogenic or polygenic predisposition on the incidence distribution of infectious diseases before reproductive age, (iii) available molecular evidence from both monogenic and complex genetics of infectious diseases in children and adults, (iv) current knowledge of immunity to primary and secondary or latent infections, (v) the state of the art in the clinical genetics of non-infectious pediatric and adult diseases, and (vi) evolutionary data for the genes underlying single-gene and complex disease risk. With the recent advent of new-generation deep resequencing, this model of single-gene variations underlying severe pediatric infectious diseases is experimentally testable.

SS.7 Innovative flow cytometry to decipher immune cell function (with the contribution of BD Bioscience)

SS.7.01
Innovative tools to incorporate low density antigens in multicolor panels

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Careful antibody panel design is a mandatory step for successful polychromatic flow cytometry. Factors to consider include instrument performance, level of antigen expression, fluorochrome brightness, and spillover. Until recently the limited availability of bright fluorochromes created a challenge when designing panels aimed at identifying several low expressed antigens simultaneously. The development of BD Horizon Brilliant polymer dyes opens a new era in flow cytometry due to their considerable brightness compared to conventional dyes, allowing for easier design and implementation of 10-12 color panels. Moreover, because of the brightness of these fluors, the resolution of dim markers can be greatly improved, allowing the development of high-sensitivity immunophenotyping. Finally, the availability of multiple dyes for different laser lines allows to build 4-5 color panels with minimal set-up requirements reducing the spillover concerns to their minimum.
In this tutorial we will present recommendations for multicolor panel design when incorporating these new reagents,as well as recommendations on instrument configuration and setup. Examples of surface and intracellular applicationsthat benefit from the use of these dyes will also be presented.

SS.7.02
Regulatory B cells maintain regulatory T cells whilst inhibiting Th17 and Th1 differentiation

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In the last few years, significant progress in understanding the biology of B cells and their function in health and disease has been attained. It is now well establish that in addition to produce antibody, B cells present antigens and produce a variety of cytokines. Amongst the different B cell subsets, those producing IL-10 have been ascribed with regulatory function. We have shown that regulatory B cells (Breg) restrain inflammation by controlling the expansion of TH1 and TH17 whilst contributing to the maintenance of regulatory T cells. Translation of our results to human has demonstrated the importance of Bregs in controlling inflammatory responses in patients with rheumatological disorders. In this presentation I will discuss the phenotype and function of Bregs in the context of healthy individuals and the impact that altered cellular function within the Breg

compartment has in patients with systemic lupus erythematous and rheumatoid arthritis. Of late there has been an exponential growth in the literature on regulatory B cells in the areas of infectious disease, autoimmunity and cancer. This reflects the interest in the therapeutic potential of this regulatory subset.

SS.8 Infectious agents and autoimmunity - Part II (with the contribution of Italian Ministry of Health and Italian Multiple Sclerosis Foundation)

SS.8.01
***Porphyromonas gingivalis* and autoimmunity in rheumatoid arthritis**

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Pathogenic autoantibodies in autoimmune disease are often disease-specific and antigen-specific. They tend to belong to "families" of biologically related proteins. In rheumatoid arthritis (RA), the family is that of citrullinated proteins. Although more than 20 potential citrullinated autoantigens have been described in RA, only four, citrullinated fibrinogen, vimentin, collagen and α-enolase, have be thoroughly characterised, epitope-mapped and animal models established.
Citrullinated α-enolase was discovered by my group over 8 years ago and having shown that it was a true autoantigen in approximately 50% of RA patients, we then went on to map its immunodominant epitope(s) to a peptide designated citrullinated enolase peptide-1 (CEP-1). Anti-CEP-1 is linked to the subset of RA in which smoking and shared epitope are major interacting gene/environment risk factors. Antibodies to the CEP-1 peptide have also been linked to *Porphyromonas gingivalis* infection, based on sequence homologies and cross reactivity between *P. gingivalis* enolase and the human antigen. More recently, we have shown that immunising DR4 transgenic mice with *P. gingivalis* enolase induces an erosive arthritis and autoantibodies to mammalian enolase. *P. gingivalis* produces a unique bacterial deiminase (termed PPAD) which can citrullinate bacterial antigens and human enolase. All of these developments show that defining an autoantibody to a specific antigen in RA supports the gene/environment/autoimmunity paradigm and provides indications of the upstream events in pathogenesis.

SS.8.02
Virus-host interactions in multiple sclerosis

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Genetic and environmental factors jointly determine the susceptibility to develop Multiple Sclerosis (MS). Collaborative efforts during the past years achieved substantial progress in defining the genetic architecture, underlying susceptibility to MS. Similar to other autoimmune diseases, HLA-DR and HLA-DQ alleles within the HLA class II region on chromosome 6p21 are the highest-risk-conferring genes. Less-robust susceptibility effects have been identified for MHC class I alleles and for non-MHC regions. The role of environmental risk factors and their interaction with genetic susceptibility alleles are much less well defined, despite the fact that infections have long been associated with MS development. Current data suggest that infectious triggers are most likely ubiquitous, i.e., highly prevalent in the general population, and that they require a permissive genetic trait which predisposes for MS development. Improving our understanding of the environmental factors involved in MS might lead to new and more effective approaches to prevent this disease.

SS.8.03
Epstein-Barr virus and lymphoid neogenesis in autoimmunity

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In recent years Epstein-Barr virus (EBV) has gained increasing credibility as the main culprit of some major B-cell related autoimmune diseases, like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and multiple sclerosis (MS). Evidence supporting a pathogenic role for EBV in these autoimmune diseases comes from epidemiological, serological, virological and histopathological studies. The presence of a higher viral load in the peripheral blood of SLE and RA patients and in the chronically inflamed target tissues in MS and RA, as well as quantitative and qualitative differences in the immune response toward EBV in SLE, RA and MS suggest poor control of viral infection. However, it is still unclear whether EBV dysregulation has a role in autoimmune disease development or acts as an activator of the underlying disease process. EBV offers numerous mechanisms to perturb the immune system, including immortalization of autoreactive B cells, molecular mimicry and stimulation of inappropriate immunopathological responses. In this presentation I shall summarize evidence linking EBV infection to formation of ectopic lymphoid tissue, a common feature of several organ-specific autoimmune diseases, and shall discuss the possibility that establishment of extra-lymphatic foci of EBV persistence and reactivation plays a role in inducing and sustaining B- and T-cell responses leading progressively to tissue destruction.

SS.11 Moving translational immunology forward through public-private partnership (with the contribution of IMI – Innovative Medicine Initiative)

SS.11.03
Translating inflammatory understanding towards patient benefit: Lessons from anti-TNF therapy

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Monclonal antibodies, first generated by Kohler and Milstein in the 70's were recognized as a wonderful invention. But there were significant hurdles in translating their potential into effective therapy and the monoclonal antibody era of therapeutics did not occur until 2000's. Hurdles which had to be overcome were the potential immunogenicity of monoclonal antibodies in patients, and the production of antibodies in sufficiently large quantities, Kgs to tons for treating humans. Reducing immunogenicity was achieved by molecular biological techniques, first 'chimerization', replacing the murine antibody backbone by human IgG, but leaving the mouse antigen combining site, to 'humanized' or fully human antibodies. Greg Winter contributed much in this latter field of generating human monoclonals.
Our contribution was to define a good target for antibody therapy, the cytokine TNF in rheumatoid arthritis, which if blocked yields sufficient clinical benefit to have become drugs of choice used in millions of patients. In 2011 this family of drugs became the best-selling class of pharmaceuticals, with sales of $25 billion. How we discovered this target and overcame the hurdles to getting this treatment adopted will be discussed.

Symposia

S.1 Pathways in inflammatory diseases

S.1.04
Tumor-induced myeloid-cell mediated immune suppression: Its all the RAGE

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Immunotherapies that activate innate and/or adaptive immunity have significant potential for the treatment and prevention of metastatic cancer. However, immunotherapies are frequently ineffective because cancer patients contain immune suppressive myeloid cells. “Myeloid-derived suppressor cells” (MDSC) and Tumor-Associated Macrophages (TAMs) are the dominant myeloid populations present in virtually all patients with solid tumors. Collectively, MDSC and TAMs facilitate tumor progression by suppressing T and NK cell activation, polarizing immunity towards a tumor-promoting type 2 phenotype, perturbing the trafficking of naïve T cells, promoting tumor vascularization, promoting tumor cell invasion and metastasis, and protecting tumor cells from chemotherapy. Multiple studies have confirmed that optimal immunotherapy requires the neutralization of MDSC and TAMs. Both MDSC and TAMs are driven by chronic inflammation which also enhances their synergism. Chronic inflammation is a complex phenomenon and there are many pro-inflammatory mediators. Many of these mediators are regulated by the pro-inflammatory alarmins High Mobility Group Box protein 1 (HMGB1) and S100A8/A9 which activate cells through the Receptor for Advanced Glycation Endproducts (RAGE) and Toll-like receptors (TLRs). HMGB1 is a major driver of MDSC accumulation and potency and mediates its effects by (i) increasing MDSC ability to suppress T cell activation; (ii) increasing the synergism between MDSC and macrophages by increasing MDSC production of IL-10 in MDSC-macrophage cross-talk; (iii) increasing the differentiation of MDSC from hematopoietic progenitor cells; and (iv) driving the ability of MDSC to prevent T cell trafficking by cleaving the T cell homing receptor L-selectin (CD62L).

S.1.05
Regulation of inflammation and mucosal innate immunity by HVEM, a TNF super family receptor

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The herpes virus entry mediator (HVEM) is a TNF super family receptor that binds multiple ligands, including TNF and Ig super family members. We have explored the role of HVEM in epithelial cells, a radiation resistant cell type critical for preventing chronic inflammation and enhancing host defense. During *Citrobacter rodentium* infection by oral gavage, *Hvem*$^{-/-}$ mice had impaired colonic epithelial responses, resulting in higher bacterial burdens, inflammation and increased mortality. HVEM stimulation induced epithelial responses by a novel signaling pathway, NIK-dependent Stat3 activation, resulting in the expression of genes important for mucosal immunity. HVEM signaling also induced IL-22R1 expression, which boosted IL-22 signaling, leading to further enhanced Stat3 activation in the epithelium. While HVEM or IL-22R signaling alone induced Stat3 activation in epithelial cells *in vitro*, during infection *in vivo*, both Stat3-activating pathways were required. Likewise, in *Streptococcus pneumoniae* infection, HVEM was required not only for survival and bacterial clearance, but also for Stat3 activation and induction of innate epithelial responses in the lung. In the *C. rodentium* model, HVEM controlled bacterial clearance by interacting with the Ig family molecule, CD160, which is prominently expressed by innate-like intraepithelial lymphocytes, while a different Ig family member, BTLA, is important in the lung. Our findings establish HVEM as a crucial regulator of innate defense at mucosal surfaces, including the gut and the lung, by acting as a signaling receptor enhancing epithelial Stat3 activation and inducing epithelial IL-22R1 expression.

S.2 Therapy of autoimmune and autoinflammatory diseases

S.2.04
Treg therapy in autoimmunity and transplantation: preclinical and clinical experiences

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Type I diabetic patients suffer from the lack of immune homeostasis and unregulated autoimmune destruction of islet β cells. The lack of regulation has been attributed, in part, to the loss of a highly specialized regulatory T cell. Accumulating evidence suggests that defective regulation is due to the instability and loss of Tregs in the inflamed pancreatic islets. Treg instability and loss results from reduced IL-2 sensitivity and IL-2 signaling via STAT5 as instability is reversed, and immune regulation restored, with IL-2 therapy. However, IL-2 therapy can also activate effector T cells and NK cell subsets as well as induce the expansion of unwanted cells such as eosinophils. Thus, the use of IL-2 immunotherapeutically will benefit from the generation of selective IL-2 muteins that can specifically target Tregs. This presentation will focus on several aspects of Treg and IL-2 biology including their function and stability in mouse and human autoimmune settings. The application of various immunotherapies in controlling Tregs in autoimmune lesions as well as clinical approaches to selectively enhance Treg function will be presented. This work is supported by NIH and JDRF.

S.3 Microbial triggers for inflammation

S.3.02
Commensal microbe-dependent induction of effector T cells in the lamina propria

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The vertebrate intestinal tract is colonized by hundreds of species of bacteria that outnumber the total cells in the host, yet must be compartmentalized and tolerated to prevent invasive growth and harmful inflammatory responses. Signaling initiated by commensal bacteria contributes to compartmentalization, preventing mononuclear phagocyte-mediated transport of the non-invasive bacteria to sites where adaptive immune responses can be induced, e.g. the mesenteric lymph nodes. Nevertheless, T cell responses against non-invasive commensals can occur, as exemplified by responses elicited by the segmented filamentous bacteria (SFB). SFB adhere to the epithelium in the terminal ileum of mice and induce differentiation of Th17 cells that contribute to autoimmune disease in susceptible mice. We found that intestinal Th17 cells from SFB-colonized mice have T cell antigen receptors (TCRs) largely specific for SFB proteins, suggesting that SFB antigen presentation occurs within a niche specialized for Th17 cell differentiation. Based on the mouse studies and evidence for Th17 cell involvement in rheumatoid arthritis (RA), we compared the fecal microbiomes of RA patients and healthy individuals, and found an over-representation of bacterial strains most closely related to *Prevotella copri* in newly-diagnosed untreated patients. The implications of how individual constituents of the microbiota contribute to the effector/memory T cell repertoire and to organ-specific autoimmune disease will be discussed.

S.3.03
Regulation of Pathogen Colonization by Virulence Factors and the Microbiota

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The mechanisms that allow pathogens to colonize the intestine and the indigenous microbiota to inhibit pathogen colonization remain

unclear. We found that that germ-free animals are unable to eradicate *Citrobacter rodentium*, a model for human infections with attaching/effacing (A/E) bacteria. These bacteria are food- and waterborne non-invasive pathogens which attach to and colonize the intestinal tract by inducing characteristic A/E lesions on the intestinal epithelium, leading to transient enteritis or colitis in humans. The genome of A/E pathogens including Enterohemorragic *Escherichia coli* (EHEC), enteropathogenic *E. coli* (EPEC) and *C. rodentium* harbor the locus of enterocyte effacement (LEE) that is critical for bacterial colonization and the ability to cause pathology. We found that early in infection, LEE virulence genes were expressed and required for pathogen growth in conventionally raised but not germ-free mice. LEE virulence gene expression was downregulated during the late phase of infection, which led to relocation of the pathogen to the intestinal lumen where it was out-competed by commensals. The ability of commensals to out-compete *C. rodentium* was determined, at least in part, by the capacity of the pathogen and commensals to grow on structurally similar carbohydrates. Moreover, dietary carbohydrates can influence the ability of members of the gut microbiota to out-compete the pathogen in the intestine. Our studies indicate the members of the microbiota use metabolic pathways to out-compete pathogens. Furthermore, intestinal pathogens have developed strategies to avoid competition with commensals based on the expression of virulence factors during the early phase of the infection.

S.3.04
Mechanisms and consequences of NFAT signaling pathway activation in innate immune cells in LPS-induced inflammatory conditions

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Innate immunity is the most ancient form of response to pathogens and it relies on evolutionary conserved signaling pathways, i.e. those involving the NF-kB pathway. Nevertheless, increasing evidence suggests that factors that have appeared more recently in evolution, such as the Nuclear Factor of Activated T cell transcription factor family (NFATc), also contribute to innate immune response regulation in vertebrates. We have observed that exposure to inflammatory stimuli, such as LPS, induces the activation of NFATc factors in innate immune cells, including conventional dendritic cells (DCs), with a mechanism TLR4-independent involving instead CD14. Therefore, from the one hand, the plasma membrane localized CD14 is required for the microbe-induced endocytosis of TLR4. This CD14-dependent endocytosis pathway is upregulated during DC maturation and is required for the efficient stimulation of antigen specific T lymphocytes. The CD14-dependent TLR4 endocytosis relies on the Syk-Plcγ2 pathway. On the other hand, CD14 promotes the activation of the NFAT pathway in DCs. In turn, NFATs contribute to the regulation of IL-2 and PGE2 production, antigen transport to the lymph nodes and DC life cycle. These three NFAT-controlled phenomena and the consequences of their activation will be discussed.

S.4 Leukocyte signaling

S.4.01
T cell metabolism and differentiation

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T lymphocytes use networks of serine/threonine kinases to interpret information from antigens, cytokines and nutrients to make appropriate responses that control T cell function. Two important nutrient controlled serine/threonine kinases for peripheral T differentiation are the mammalian target of rapamycin complex 1 (mTORC1) and the adenosine monophosphate (AMP)-activated protein kinase (AMPK). mTORC1 functions to control effector T cell differentiation whereas AMPK monitors energy stress to negatively regulate mTORC1 activity and to control the effector/memory transition of immune activated T cells. High-resolution mass spectrometry analysis of the T cell proteomes reveals that mTORC1 controls T cell differentiation by regulating the expression of key transcription factors that control the expression of effector molecules and the effector T cell migratory program. How is mTORC1 activity controlled during immune responses? A key insight is that antigen and cytokine receptors regulate mTORC1 by controlling the cellular uptake of glucose and the large neutral amino acid (LNAA) leucine. The intracellular supply of glucose and amino acids to T cells is thus regulated by pathogen exposure and the T cell antigen receptor (TCR). In the context of leucine uptake, a single System L transporter, slc7a5, mediates LNAA uptake in activated T cells. Strikingly, Slc7a5 null T cells cannot metabolically reprogram in response to antigen and fail to differentiate to effector cells. These findings reveal fundamental mechanisms linking pathogen control of nutrient uptake in T cells to serine/threonine kinase mediated signaling pathways that control T cell function.

S.4.03
Control of T cell signaling by membrane binding of TCR-CD3 ITAMs

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The CD3 epsilon and zeta cytoplasmic domains of the T cell receptor bind to the inner leaflet of the plasma membrane (PM), and a NMR structure showed that both tyrosines of the CD3 epsilon ITAM partition into the bilayer. Electrostatic interactions between acidic phospholipids and clusters of basic CD3 epsilon residues were previously shown to be essential for CD3 epsilon and zeta membrane binding. Phosphatidylserine is the most abundant negatively charged lipid on the inner leaflet of the PM and makes a major contribution to membrane binding by the CD3 epsilon cytoplasmic domain. We have found that TCR triggering by peptide-MHC complexes induces dissociation of the CD3 epsilon cytoplasmic domain from the plasma membrane. Release of the CD3 epsilon cytoplasmic domain from the membrane is accompanied by a substantial focal reduction in negative charge and available phosphatidylserine in TCR microclusters. These changes in the lipid composition of TCR microclusters even occur when TCR signaling is blocked with a Src kinase inhibitor. Local changes in the lipid composition of TCR microclusters thus render the CD3 epsilon cytoplasmic domain accessible during early stages of T cell activation. We propose that membrane binding sequesters tyrosine residues of an ITAM in the hydrophobic phase of the lipid bilayer and that receptor triggering induces release of an ITAM from the membrane through localized changes in the lipid microenvironment of TCR microclusters.

S.4.04
Tyrosine kinases in leukocyte activation and migration

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Myeloid leukocytes such as neutrophils or macrophages are critical components of innate immunity but their improper activation may also lead to tissue damage during autoimmune inflammation. We have previously shown that certain neutrophil responses require Src-family kinases, Syk and PLCγ2. Therefore, we tested the role of tyrosine phosphorylation pathways in in vivo inflammatory reactions. Src-family kinases, Syk and PLCγ2 were all found to be required for autoantibody-induced inflammatory reactions such as the K/BxN serum-transfer arthritis in experimental mice. Given the role of tyrosine kinases in β_2 integrin-mediated leukocyte activation, we hypothesized that Src-family kinases, Syk and PLCγ2 are also required for β_2 integrin-mediated leukocyte migration. Surprisingly, neutrophil migration in a conventional Transwell assay did not require Src-family kinases, Syk or PLCγ2 even though it was strongly reduced by the genetic deficiency of the β_2 integrin-chain CD18. In addition, the Src-family kinase inhibitor dasatinib did not affect in vitro neutrophil migration. In vivo competitive migration assays (in which wild type and knockout cells are allowed to migrate to the site of inflammation within the same animal) also revealed that Src-family kinases, Syk and PLCγ2 were not required for neutrophil or monocyte migration in sterile peritonitis or autoantibody-induced arthritis

models. On the other hand, tyrosine kinases were required for cytokine production by neutrophils and macrophages. Taken together, Src-family kinases, Syk and PLCγ2 are required for neutrophil activation and cytokine production but do not play any direct role in CD18-mediated migration of myeloid cells to the site of inflammation.

S.5 Myeloid cells

S.5.01
Colony stimulating factors and macrophage lineage phenotypes

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Macrophage-CSF (M-CSF or CSF-1) and granulocyte macrophage-CSF (GM-CSF) were originally defined as hemopoietic growth factors. Subsequent evidence indicates that they can also affect the function of mature myeloid cells, for example, macrophages/dendritic cells. There is heightened recent interest in their biology partly because clinical trials which target their action are underway in inflammation/autoimmunity.
The comparative effects of M-CSF and GM-CSF on murine and human monocyte/macrophage populations in vitro will be presented indicating marked differences and even opposite and competing responses. The relationships between the phenotypes of such CSF-1-treated populations to so-called M1 and M2 polarization states will be discussed. In vivo data arising out of their depletion/blockade, either in the steady state or during inflammation, indicate that M-CSF and GM-CSF both probably control macrophage lineage function and/or survival relatively late in lineage development, perhaps helping to explain the efficacy following their targeting. Unanswered questions will be raised and some future strategies recommended.

S.5.03
Fate mapping the origins of fetal and adult myeloid cells

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It has remained a challenge to measure in vivo the developmental flow from hematopoietic stem cells (HSC) to myeloid and lymphoid lineages. Therefore, information on key quantitative numbers that characterize the generation and maintenance of the immune lineages is scarce, including answers to the questions how many HSC are active at any time, and how many HSC or their progeny give rise to a particular cell type. We have devised a fate mapping strategy to induce a genetic and inheritable mark in long-term HSC in the bone marrow. In this system, we traced the hematopoietic output from HSC, and measured the developmental flow along hematopoietic branches. Frequencies of myeloid output from HSC are about ten-fold higher compared to lymphoid cells. Moreover, myeloid output is constant while lymphoid cell production appears sporadic during normal adult hematopoiesis. Bone marrow transplantation experiments into non-myeloablated recipients revealed a fast and complete conversion of monocytes from host to donor type. In contrast, the group of recently recognized tissue-resident myeloid cells, which include microglia in the brain, Kupffer cells in the liver, or Langerhans cells in the skin are not, or only incompletely replenished from adult bone marrow HSC. Using a lymphoid marker for fate mapping, we surprisingly traced the origin of many tissue-resident myeloid cells to a developmental window in the fetal liver. A lymphoid mark on fetal derived tissue-resident but not monocyte-derived macrophages suggests closer myeloid-lymphoid relationships in fetal development versus adult maintenance of the immune lineages.

S.5.04
Uncovering the regulation of gene expression in human neutrophils

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Historically, polymorphonuclear neutrophils have been considered as innate immune cells with an anti-infectious and pro-inflammatory functions, due to their ability to phagocytose and to produce powerful antimicrobial peptides, proteolytic enzymes and reactive oxygen intermediates. Recently, however, unsuspected biological features of neutrophils have been uncovered. Indeed, it has been shown that neutrophils also display a high degree of plasticity as well as contribute to the control of innate and adaptive immune responses. For instance, various experimental models have validated in vitro data proving that, after migration to an infection site, neutrophils are stimulated to synthesize and secrete many proinflammatory cytokines and chemokines. In such regard, it is well known that member of TLRs represent a class of receptors able to trigger neutrophil-derived cytokines and chemokines. However, our knowledge on the TLR-dependent signaling components and molecular mechanisms that, in neutrophils, regulate cytokine gene expression is still incomplete. Similarly, very little is known on the molecular bases underlying the differential capacity to express cytokines by neutrophils and autologous monocytes in response to the same TLR agonist. Data demonstrating that changes in chromatin organization and posttranslational modifications differentially control the transcriptional outputs also in human neutrophils will be presented.

S.5.05
Host vs. microbe in the human neutrophil phagosome

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In their capacity as the predominant circulating effector cell in human innate immune defense against infection, polymorphonuclear neutrophils (PMN) exert the majority of their antimicrobial action in phagosomes. Antimicrobial agents delivered from granules and reactive oxygen species generated *de novo* by the NADPH oxidase can reach high concentrations, including that of the potent antimicrobial agent HOCl generated by myeloperoxidase-mediated oxidation of chloride. Despite the toxic environment in PMN phagosomes, 10-20% of ingested *Staphylococcus aureus* (SA) survives and eventually escapes. To test the hypothesis that ingested SA repair host-induced damage as a strategy for survival in PMN, we examined the role methionine sulfoxide reductases (msr), enzymes that repair oxidized methionine residues, in the fate of ingested SA. A deletion mutant lacking two isoforms of msr (msrA1 and msrB) was more susceptible to killing by reagent H_2O_2 or HOCl and by PMN. In addition, transcription of *msrA1B* increased briskly after ingestion by normal PMN. Unexpectedly, *msrA1B* upregulation was even greater in SA ingested by PMN in which NADPH oxidase activity was blocked by diphenyleneiodonium or genetically absent (CGD PMN). Isolated PMN granules without exogenous oxidants stimulated increased expression of *msrA1B*, and screening of a library of two-component deletion mutants of SA suggested that vancomycin-responsive VraSR regulated *msr* expression, thereby providing a novel link between oxidative protein repair and cell wall homeostasis in SA. Identification of those transcriptional responses of ingested microbes that promote their survival in PMN will provide better insight into pathogenesis of infection and inspire novel strategies for antimicrobial therapy.

S.6 Lymphocyte development

S.6.01
Mechanotransduction processes involved in T cell development and cognate recognition

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During the processes of thymic development as well as immune surveillance, the surface molecules on T lineage cells are subject to piconewton scale mechanical forces. We have previously shown that the TCR is an anisotropic mechanosensor, converting mechanical energy into a biochemical signal upon specific but not irrelevant pMHC ligation. Resultant common TCR quaternary change rather than conformational alteration can best facilitate structural signal initiation because of the vast number of distinct TCR molecules. Moreover, in principle, sheer forces can form catch bonds at the TCR-pMHC interface to enhance binding. Given the key role of thymocyte trafficking between cortex and medulla during thymic selection, we further investigated mechanotransduction processes therein. We observed that on developing DP thymocytes, plexinD1 controls surface topology of nanometer-scaled β1 integrin adhesion domains. Furthermore, ligation of plexinD1 by sema3E in trans regulates individual β1 integrin catch bonds. Loss of plexinD1 expression reduces β1 integrin clustering thereby diminishing avidity, whereas sema3E ligation shortens individual integrin bond lifetimes under force to reduce their stability. Both decreased expression of plexinD1 during developmental progression and a thymic medulla-emanating sema3E gradient enhance thymocyte movement towards the medulla, thus enforcing the tightly-orchestrated lymphoid trafficking required for effective immune repertoire selection. In the absence of this regulated mechanobiology, autoreactivity develops. Our results demonstrate plexin-tunable molecular features of integrin adhesion relevant to many cellular processes.

S.6.02
Analysis of T cell fate and reactivity

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There is strong evidence that manipulation of T cell immunity can have clinically meaningful effects, not only in the field of infectious diseases but also in oncology. We have set out to develop and exploit technologies that can be utilized to dissect disease- and therapy-induced T cell responses in mice and men. First, to understand how the T cell based immune system can recognize human cancer, we have developed MHC-based technology to measure T cell reactivity against hundreds of (potential) T cell epitopes. Experiments that exploit this approach in conjunction with cancer exome sequencing to dissect T cell recognition of the consequences of the DNA damage in melanoma will be presented. Second, to understand more fundamental aspects of the generation of T cell responses, and in particular T cell diversity, we have developed technology for in vivo lineage tracing at the single cell level. Data obtained demonstrate that the fate of individual naïve T cells is highly discordant, with respect to initial clonal expansion and differentiation, and with respect to their ability to participate in recall responses. From these experiments we conclude that the reproducibility of T cell responses that we are used to see at the cell population level is due to the averaging of highly divergent cellular behaviors. Furthermore, the data demonstrate that asymmetric division of T cells is by itself insufficient to explain the generation of distinct cellular outcomes within the T cell lineage.

S.6.04
The role of self recognition in the development of various T cell subsets

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The healthy adaptive immune system is responsive to foreign antigens, and tolerant to self. However, several types of T cells have, and even require, substantial self-reactivity for their particular functions in immunity: nTreg cells, iNKT cells, and nIELs. We used a Nur77GFP reporter mouse to understand the self-reactivity of various T cell populations, and examine the consequences of NKT cell self-reactivity in particular. We identified a specific subset of iNKT cells that produces IL-4 in the steady state, particularly early in life. Through examination of lineage specific transcription factors, we define 3 subsets of iNKT cells_NKT1, NKT2, and NKT17, which exhibit substantial inter-strain variation in numbers. In several strains of mice, NKT2 cells are abundant and stimulated by self-ligands to produce IL-4. This steady state IL-4 conditioned CD8 T cells to become “memory-like”, increased serum IgE levels, and caused dendritic cells to produce chemokines. These data show that steady state IL-4 produced by self-stimulation alters immune properties under normal physiological conditions

S.6.05
Butyrophilin 3A1 binds phosphorylated antigens and stimulates human γδ T cells

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Human T cell receptor Vγ9Vδ2-expressing cells recognise phosphorylated prenyl metabolites (P-Ags) as antigens in the presence of antigen-presenting cells but independently of major-histocompatibility-complex, MR1 and CD1 molecules. We used genetic approaches to identify the molecule that binds and presents P-Ags. We used TCR Vγ9Vδ2 transgenic T cells as responders to stimulation with mouse-human hybrid somatic cell lines as antigen-presenting cells. Using a combination of somtic hybrids containing individual human chromosomes, we mapped the presence of the gene encoding the antigen-presenting molecule to human chromosome 6, in a position telomeric to MHC locus. By microarray we identified genes commonly expressed in antigen-presenting cells and found butyrophilin 3A1 (BTN3A1) as the molecule involved in activation of TCR Vγ9Vδ2 cells.
BTN3A1 bound P-Ags with low affinity, at 1:1 stoichiometry as detected by surface plasmon resonance and mass spectrometry. The structures of the BTN3A1 distal domain in complex with host- and microbe-derived P-Ags revealed an immunoglobulin-like fold, in which the antigens bound within a shallow pocket. Soluble Vγ9Vδ2 TCR interacted specifically with BTN3A1-antigen complex as shown by surface enhanced Raman scattering. Accordingly, BTN3A1 represents an antigen-presenting molecule for the activation of Vγ9Vδ2 T cells.

S.7 Cancer immunotherapy

S.7.03
TLR-ligand peptide conjugates for superior therapeutic vaccination against immunogenic cancers

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High-risk HPV type 16 is the most oncogenic virus type that cause cervical, vulvar and head and neck cancer. Therapeutic vaccination with a set of 13 overlapping synthetic long peptides (SLP) covering the entire sequences of the E6 and E7 oncoproteins of HPV16

resulted in complete or partial regression of premalignant lesions in >50% of women presenting with premalignant HPV16-positive vulvar intraepithelial neoplasia (VIN) (Kenter et al. NEJM 2009). In preclinical mouse models TLR ligand-peptide conjugates performed better as therapeutic vaccines than long peptides mixed with the same TLR ligands. Notably, covalent attachment of TLR ligands to synthetic long peptides allows superior Dendritic cell (DC) targeting with antigen and DC activation in vitro and in vivo, resulting in superior T cell priming and tumor control in these animal models. Subsequently D Filippov and colleagues developed a proprietary TLR1/2 ligand, based on knowledge of the crystal structure of its interaction with the TLR1/2 receptor.
In 2013 we will conduct a phase I/II toxicity/immunogenicity clinical trial in HPV16-positive head and neck cancer patients with a vaccine formulation consisting of synthetic long peptides conjugated to the improved TLR-ligand. Two peptides were selected from the set of 13 HPV16 SLP based on immunogenicity in vaccinated patients in previous trials. When these novel TLR ligand-HPV peptide conjugates were functionally tested *in vitro*,they both induced significant mouse and human DC maturation, as determined by IL-12 production and the upregulation of co-stimulatory molecules and other activation markers. Moreover, as determined by specific T-cell proliferation and cytokine production, the epitopes present in the peptides of both conjugates were much more efficiently recognized by HPV-specific human T-cell clones derived from VIN patients in comparison to mixtures of SLP and the same TLR ligand.Taken together, these results provide a solid basis for the further clinical development of proprietary TLR ligand -SLP conjugates.

S.8 Innate immune sensors

S.8.02
NLRs and inflammasomes in intestinal homeostasis, cancer and metastasis

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Sensing of microbial products and/or host-derived danger signals by pattern recognition receptors (PRRs) of the innate immune system is a key physiological response required to maintain homeostasis at the steady state and to induce defense mechanisms in pathological situations. However PRR responses need to be tightly regulated, as their aberrant activation is often associated with immunopathologies. The Nod-like receptors (NLRs), which constitute a subset of PRRs, are especially critical for ensuring host resistance, as evidenced by the high association of mutations and single nucleotide polymorphisms in NLR genes with human diseases. We have investigated NLR regulation, signaling mechanisms and effector functions in the gut. At the ICI, I will discuss how NLRs and associated inflammasomes are regulated, and will present our findings on the role of the inflammasome-IL-18 axis in intestinal homeostasis, colorectal cancer and metastasis to the liver. We show that in addition to maintaining intestinal homeostasis and preventing intestinal inflammatory pathologies, the inflammasome mediates tumor immune surveillance through NK cells and suppresses colorectal cancer growth in the liver. The mechanistic bases of these observations will be discussed.

S.8.03
Human NK cell receptors and pathogen infection

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It is well established that Natural killer (NK) cells play a crucial role in early immunity after hematopoietic stem cell transplantation because they are the first lymphocyte subset recovering after the allograft. Interestingly, in different patients undergoing umbilical CB transplantation discrete patterns of NK-cell development can be identified. In this context, we showed that , in a group of patients, a relevant fraction of NK cells already expressed a mature phenotype characterized by the KIR+NKG2A- signature 3-6 months after transplantation. In other patients, most NK cells maintained an immature phenotype even after 2 months. A possible role for cytomegalovirus in the promotion of NK-cell development was suggested by the observation that a more rapid NK-cell maturation together with expansion of NKG2C+ Siglec-7-NK cells was confined to patients experiencing cytomegalovirus reactivation. Our data support the concept that cytomegalovirus infection accelerates NK-cell development after HSCT leading to fully mature KIR+ NK cells displaying potent alloreactivity against KIR ligand mismatched targets.

S.8.04
Variable Natural Killer cell Receptors and Human Evolution

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Natural killer (NK) cells have distinctive roles in immunity and reproduction, which are controlled by variable receptors that recognize MHC class I molecules. The variable NK cell receptors present in human populations – the Killer-cell Immunoglobulin-like Receptors (KIR) - are specific to simian primates, species in which the KIR have progressively coevolved with MHC class I molecules. The emergence of the *MHC-A, B* and *C* genes in hominids drove the evolution of a system of KIR that recognises MHC-C molecules, which is currently most highly elaborated in chimpanzees. By contrast, the human system of KIR that recognise HLA-C appears to have been subject to different selection pressures that have acted in competition on the immunological and reproductive functions of MHC class I molecules. It is speculated that this compromise facilitated the development of the bigger brains that enabled archaic and modern humans to migrate out of Africa and populate other continents. Comparison of modern human populations reveals extraordinary diversity in their systems of KIR and HLA class I ligands.

S.9 Genetic and epigenetic control

S.9.01
A genomic framework for macrophage identity and functional specialization

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Cell identity is determined by a complex and highly dynamic interplay between cell intrinsic and lineage restricted developmental pathways on the one hand, and cell extrinsic and tissue specific micro environmental signals on the other. In this context, macrophages are a paradigmatic cell population in that their functional specialization in vivo reflects the impact of the local microenvironment onto the intrinsic macrophage differentiation program. This complex interaction leads to a variety of specialized macrophage types in different tissues as well as in physiological and pathological conditions. Genomic approaches developed in the last five years, and specifically the combination of RNA sequencing and Chromatin Immunoprecipitation (ChIP)-sequencing, have enabled an integrated and high resolution view of players and mechanisms that control macrophage plasticity in the context of different environmental conditions and at the same time enforce macrophage identity in spite of a changing environment. Principles of genomic regulation inferred from these experiments will be discussed.

S.9.02
Histone Mimicry and Antiviral Response

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In my presentation I will discuss histone mimicry and its potential physiological significance. Histone mimicry describes a phenomenon of structural and functional similarity between the N-terminal domain of histones and the non-histone proteins of distinct origin. I will

discuss how the presence of histone mimics in mammalian and viral proteins contributes to the regulation of gene expression. I will also discuss how synthetic compounds that mimic the histone function can regulate gene expression in model systems.

S.9.03
Metabolism, epigenetics and miRNAs - an unholy trinity controlling innate immunity

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In the field of inflammation research, the most important advances in the past 10 years has been in the uncovering of multiple pathways involved in innate immunity. Glucose metabolism has been shown to be required for induction of IL-1beta mRNA by TLR4. Succinate is a signal from the mitochondria elevated in response to TLR signalling, which is required for IL-1beta gene transcription via activation of HIF1alpha. The so-called Warburg effect of aerobic glycolysis is a feature of cells activated during inflammation (including Th17 cells) providing an added level of complexity to signalling pathways activated by TLRs during inflammation (1). One important output from these changes will be epigenetic modification, as histone and DNA modification depends on metabolic products such as acetylCoA and alpha ketoglutarate. MicroRNAs also participate in these processes, two examples being miR107, whose expression is decreased by LPS and which is involved in insulin resistance, and miR-210 which can target succinate dehyrdogenase. There is therefore likely to be important interactions between metabolism, epigenetics and miRNAs during innate immunity and inflammation.

1. O'Neill LAJ and Hardie DG (2013) Metabolism of inflammation limited by AMPK and pseudo-starvation. Nature 493, 346-355

S.9.04
Regulation of T lymphocyte effector functions by non-coding RNAs

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$CD4^+$ T-lymphocytes orchestrate adaptive immune responses by differentiating into various subsets of effector T-cells such as Th1, Th2, Th17, and Treg. These subsets have been described by master transcription factors that dictate the expression of cytokines and receptors, which ultimately define lymphocyte effector functions. However, the view of T lymphocyte subsets as stable and terminally differentiated lineages has been challenged by increasing evidence of functional plasticity within $CD4^+$ T-cell subsets, which implies flexible programming of effector functions depending on time and space of T cell activation. Among the mechanisms which define the plasticity of effector functions, regulatory non-coding RNAs appear to play a key role. Not only microRNAs have been proven to be important for $CD4^+$ T-cell differentiation, but it is also likely that the overall T cell functioning is the result of a multilayered network composed by coding RNAs and by short and long non-coding RNAs. Interestingly, activated lymphocytes release microRNAs that can be monitored in the bloodstream, and we have found that some serum miRNAs can be detected as biomarkers of vaccination and more in general of adaptive immune responses. Furthermore, the prompt reduction of intracellular level of some miRNAs linked to their release in the external milieu suggests that the selective extracellular disposal of miRNAs can be a rapid way to regulate gene expression during lymphocyte activation. In conclusion, the integrated study of all the nodes of this network will provide a comprehensive view of the molecular mechanisms underlying T cell functions in health and disease.

S.10 T and B cell repertoires

S.10.01
Thymic microenvironments that shape T cell repertoire

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During development in the thymus, a virgin repertoire of diverse TCR-alpha/beta recognition specificities in immature T cells is selected to form a functionally competent and self-tolerant repertoire of mature T cells. Positive selection supports the survival of potentially useful self-MHC-restricted thymocytes upon low-affinity TCR engagement, whereas negative selection deletes potentially harmful self-reactive thymocytes upon high-affinity TCR engagement. Recent advances in the biology of thymic stromal cells have indicated that a proximal interplay among developing T cells, dendritic cells, and thymic medullary epithelial cells that promiscuously express tissue-specific self-antigens is essential for the establishment of a self-tolerant TCR repertoire. It has also been pointed out that the formation of an immunocompetent TCR repertoire requires positive selection by thymic cortical epithelial cells that express unique protein degradation machineries, including the beta5t-containing thymoproteasome. These results suggest the vital role played by self-peptide repertoires specifically expressed by multiple thymic microenvironments in the development of an immunocompetent and self-tolerant T cell repertoire.

S.10.03
Control of early B cell development by the transcription factor Ikaros

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The zinc-finger transcription factors Ikaros (Ikzf1) is an essential regulator of B-cell development, which is arrested at an early lymphoid progenitor stage in *Ikzf1*$^{-/-}$ mice. Here, we have investigated the role of Ikaros in early B-cell development.
Conditional loss of Ikaros in *Cd79a*-Cre *Ikzf1*$^{fl/-}$ mice stringently arrested B-cell development at a transitional c-Kit^{low} pro-B cell stage. These pro-B cells did not proliferate although they expressed Igμ and surrogate light chains. ChIP- and RNA-sequencing identified 130 activated and 180 repressed Ikaros target genes, which code for cell surface receptors, signal transducers and transcription factors. Consequently, Ikaros-deficient pro-B cells did not undergo pre-B cell receptor signaling. Consistent with the identification of Ikaros target genes implicated in cell migration and adhesion, Ikaros-deficient pro-B cells failed to migrate in response to CXCL12 stimulation, but strongly adhered to VCAM1-coated surfaces in contrast to control pro-B cells.
At the genome-wide level, Ikaros bound to active promoters and enhancers. DNase I hypersensitive (DHS) site mapping and profiling of active histone marks revealed that Ikaros binding silenced the promoters of repressed Ikaros target genes. In contrast, Ikaros promoted transcriptional elongation and activated enhancers at activated target genes. Together, these data identified Ikaros as an important regulator of early B-cell development.

S.11 Viral infections

S.11.01
Viral regulation of inhibitory and activating Ly49 receptors

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Natural killer (NK) cells play an important role in the control of cytomegalovirus (CMV) infection. NK cells effector functions are mediated through a fine interplay between activating and inhibitory NK receptors. Ly49 is a family of mouse NK cell receptors which contains both inhibitory and activating members. Inhibitory Ly49 receptors screen the cells for the presence of MHC class I molecules

and play their part in the control of viral infection through the "missing-self" recognition mechanism. On the other hand, activating Ly49 receptors can recognize stand-alone viral proteins or viral proteins expressed with MHC class I molecules. We have previously shown that, in addition to Ly49H which recognizes virally encoded m157 protein, activating Ly49P, L and D2 receptors also play a role in the specific recognition of cells infected by mouse CMV (Pyzik M et al. *Journal of Experimental Medicine* 208:1105, 2011). MCMV encoded m04/gp34 was shown to be necessary, but not sufficient for a successful recognition of MCMV infected cells via these activating receptors. In this talk I will present our results on characterization of viral component needed for an efficient recognition of infected cells by several activating Ly49 receptors. The biological significance of viral regulation of various Ly49 receptors in virus control *in vivo* will be discussed.

S.11.03
Cytokine regulation of NK and CD8 T cell responses to acute viral infections

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There is limited genetic information available to deal with protection against an almost limitless range of infectious agents and against disease resulting from immune responses to infection. To deal with the challenge, the immune system has evolved remarkable flexibility in shaping endogenous responses. Work focusing on elements controlling cytokine effects on NK and CD8 T cells during viral infections will be reviewed. In particular, two mechanisms sensing infection conditions to induce intrinsic cellular changes altering cytokines responses will be discussed. The first is through modifying total protein levels of individual signal transducers and activators of transcriptions (STATs) to change functions of cytokines with preferred STATs but flexibility to use other STATs for activation. The second is the role for proliferation in changing responses to cytokines from immune enhancing with interferon (IFN) gamma production, to immunoregulating with IL-10 production. The mechanisms are being characterized in NK and CD8 T cell responses to type 1 IFNs and IL-12 during infections of mice with lymphocytic choriomeningitis virus (LCMV) and/or murine cytomegalovirus (MCMV). Taken together, the studies are making unexpected discoveries about the biological importance of experience for conditioning intrinsic cell responses to shape immunity and promote health during viral infections. Supported by the National Institutes of Health, USA.

S.12 Immunodeficiencies

S.12.02
Toward a genetic theory of childhood infectious diseases

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The hypothesis that inborn errors of immunity underlie infectious diseases is gaining experimental support. However, the apparent modes of inheritance of predisposition or resistance differ considerably between diseases and between studies. A coherent genetic architecture of infectious diseases is lacking. We suggest here that life-threatening infectious diseases in childhood, occurring in the course of primary infection, result mostly from individually rare but collectively diverse single-gene variations of variable clinical penetrance, whereas the genetic component of predisposition to secondary or reactivation infections in adults is more complex. This model is consistent with (i) the high incidence of most infectious diseases in early childhood, followed by a steady decline, (ii) theoretical modeling of the impact of monogenic or polygenic predisposition on the incidence distribution of infectious diseases before reproductive age, (iii) available molecular evidence from both monogenic and complex genetics of infectious diseases in children and adults, (iv) current knowledge of immunity to primary and secondary or latent infections, (v) the state of the art in the clinical genetics of non-infectious pediatric and adult diseases, and (vi) evolutionary data for the genes underlying single-gene and complex disease risk. With the recent advent of new-generation deep resequencing, this model of single-gene variations underlying severe pediatric infectious diseases is experimentally testable.

S.12.03
Hypomorphic, homozygous mutations in Phosphoglucomutase 3 impair immunity and increase serum IgE levels

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Recurrent bacterial and fungal infections, eczema and elevated serum IgE levels characterize patients with the hyper-IgE syndrome (HIES). Known genetic causes for HIES are mutations in proteins involved in signal transduction pathways, namely STAT3 and DOCK8. However, glycosylation defects have not been described in these patients. One crucial enzyme in a glycosylation pathway is Phosphoglucomutase 3 (PGM3), which catalyzes a key step in the synthesis of UDP-GlcNAc which is required for the biosynthesis of N-glycan and other essential glycopolymers. Aim of our study was to elucidate the genetic cause in patients who do not have mutations in STAT3 or DOCK8. Genetic linkage analysis revealed an 11.9 Mb linkage region on chromosome 6. Mutation detection in the positional candidate genes identified one homozygous mutation per family (L83S and E340del) in PGM3 (Phosphoglucomutase 3) that segregated perfectly with affection status and recessive inheritance. Sanger sequencing of PGM3 identified two additional HIES patients from Morocco with the homozygous L83S mutation and a Turkish patient with a third homozygous mutation (D502Y). Functional studies showed that these mutations are hypomorphic and leave intact the enzyme's phosphoglucomutase activity, but impact on the biosynthetic reactions involving UDP-GlcNAc. Glycomics analysis revealed an aberrant glycosylation pattern in leukocytes exemplified by a reduced branching of complex-type N-glycans. We concluded that the impairment of PGM3 function leads to a novel primary (inborn) error of immunity, as biallelic hypomorphic mutations are associated with impaired glycosylation and a hyper-IgE-like syndrome.

S.12.04
Immunoglobulin class switch recombination deficiencies

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Immunoglobulin class-switch recombination deficiencies (Ig-CSR-Ds) are rare primary immunodeficiencies characterized by defective switched isotypes' (IgG/IgA/IgE) production. Depending on the molecular defect in question, the Ig-CSR-D may be combined with an impairment in somatic hypermutation (SHM). Some of the mechanisms underlying Ig-CSR and SHM have been described by studying natural mutants in humans. This approach has revealed that T cell-B cell interaction (resulting in CD40-mediated signaling), intrinsic B-cell mechanisms (activation-induced cytidine deaminase-induced DNA damage), and complex DNA repair machineries (including uracil-N-glycosylase and mismatch repair pathways) are all involved in class-switch recombination and SHM. We herein describe INO80 deficiency as a new cause of mild CSR-D. INO80 is a key protein in an evolutionarily conserved chromatin remodelling complex that functions in DNA replication, repair and transcriptional regulation. We identified hypomorphic *INO80* mutations in two Ig-CSR-D patients. These mutations were associated with fibroblast hyper-radiosensitivity, which was corrected by wild-type INO80 expression. In CH12-F3 cells, down-regulation of INO80 and its partners Reptin and Pontin was associated with lower CSR. Finally, an abnormal separation of sister chromatids was observed upon INO80 downregulation in CH12-F3 cells, suggesting a defect in cohesin. Since INO80 and cohesin were shown to accumulate at Sα and Eμ regions of the IgH locus, we propose that the INO80 complex modulates cohesion function that could be required during switch region synapsis.

S.13 Autoimmune mechanisms

S.13.01
Primary Biliary Cirrhosis: The Environment and Autoimmunity

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Environmental stimulation is a major factor in the initiation and perpetuation of autoimmune diseases. We have addressed this issue and focused on primary biliary cirrhosis (PBC), an autoimmune disease of the liver. Immunologically, PBC is distinguished by immune mediated destruction of the intra hepatic bile ducts and the presence of high titer antimitochondrial autoantibodies (AMA) directed against a highly specific epitope within the lipoic acid binding domain of the pyruvate dehydrogenase E2 subunit (PDC-E2). Using quantitative structure activity relationship (QSAR) analysis on a peptide-xenobiotic conjugate microarray platform, we have demonstrated that when the lipoyl domain of PDC-E2 was modified with specific synthetic small molecule lipoyl mimics, the ensuing structures displayed highly specific reactivity to PBC sera, at levels often higher than the native PDC-E2 molecule. Furthermore, data on the immunological characterization of antigen and Ig isotype specificities against one such lipoic acid mimic; 6,8-bis(acetylthio)octanoic acid (SAc), when compared with rPDC-E2, strongly support a xenobiotic etiology in PBC. This observation is of particular significance in that approximately one third of patients who have taken excessive acetaminophen (APAP) developed AMA with same specificity as patients with PBC, suggesting that the lipoic domain are a target of APAP electrophilic metabolites such as NAPQI. We submit that in genetically susceptible hosts, electrophilic modification of lipoic acid in PDC-E2 by acetaminophen or similar drugs can facilitate loss of tolerance and lead to the development of PBC.

S.13.05
Genetic and Environmental Contributions to Human Autoimmune Disease

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GWAS identified genetic associations between common SNPs and risk of autoimmune diseases. We evaluate the extent of sharing for 107 disease-risk SNPs in seven diseases and developed a novel statistic for which detects association of a SNP to multiple phenotypes. We find evidence that 47/107 (44%) immune-mediated disease risk SNPs are associated to multiple immune-mediated diseases. Distinct groups of interacting proteins are encoded near SNPs that predispose to the same subsets of diseases; we propose these as the mechanistic basis of shared disease risk. We have begun efforts to identify the biologic effects of disease causing SNPs at non-coding regions of the genome where it has been difficult to assign function to DNA sequence and to compare these effects across different autoimmune diseases. We use fine-mapping genetic data to identify causal mutations and integrate these data with chromatin maps of ten defined human CD4+ T-cell populations and 56 additional cell-types. These investigations identified risk variants disrupting the enhancers of distinct cell types among the different autoimmune diseases. Finally, autoimmune disease results from untoward interactions between genetics and the environment. We recently showed that salt boost the induction of Th17 cells mediated by SGK1. The Th17 cells generated under high-salt display a pathogenic cytokine phenotype. Mice fed with a high-salt diet develop a severe form of EAE. Identifying specific sites where a single, non-coding nucleotide variant is responsible for disease risk may pinpoint specific disruptions of consensus transcription factor binding sites that ultimately define disease risk as related to environmental factors.

S.14 Gene and cell therapy

S.14.04
NKT cells as an ideal target for anti-tumor immunotherapy

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Human NKT cells bearing an invariant Vα24Jα18 antigen receptor, the counterpart of murine Vα14Jα18 NKT cells, mediate strong adjuvant activity by their production of IFN-γ upon stimulation with a specific ligand, α-GalCer. In the animal model, administration of α-GalCer-pulsed DCs into tumor bearing host completely eradicates metastatic tumors within a week. IFNγ produced by α-GalCer-activated NKT cells mediates strong adjuvant activity acting on NK cells and CD8 killer T cells at the same time to increase in their numbers and to enhance their cytotoxic function. Also, NKT cells induce maturation of immature DCs in the presence of α-GalCer, resulting in the recovery from immunodeficiency in the tumor-bearing host.
Based on the efficient anti-tumor effects of NKT cell-targeted immune cell therapy in the animal model, we conducted phase I/IIa clinical trials in patients with head & neck tumors (10 cases). No severe adverse event related to the treatment was observed. After the injection of α-GalCer-pulsed PBMC-DCs (10^8) into nasal submucosa together with $5x10^7$ in vitro activated autologous NKT cells, all patients responded well to be SD in 5 cases and PR in 5 cases. Tumor infiltrating lymphocytes (TIL) in PR and SR cases were significantly increased (10-100 times increase over untreated controls) 28 days after the NKT cell targeted therapy, which efficiently

recruits endogenous NKT cells. iPS-derived NKT cells are also effective to eradicate tumors. These results are encouraging and warrant further evaluation of the survival benefit of this NKT cell-targeted immunotherapy.

S.15 Cell trafficking

S.15.01
Chemokines: tails from the clinic

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Knowledge about the chemokine system has advanced from molecules to mice to man, with CCR5 and CXCR4 antagonists maraviroc and plerixafor now on the market for HIV/AIDS and hematopoietic stem cell mobilization, respectively. CXCR4 blockade is also a rational therapeutic strategy in WHIM syndrome, a rare congenital immunodeficiency disorder characterized by warts, hypogammaglobulinemia, infections, and myelokathexis (neutropenia due to impaired egress from the bone marrow) that is caused by gain-of-function mutations in CXCR4. We have developed a Phase I clinical trial to repurpose plerixafor in WHIM syndrome. In addition to defining parameters of safety and efficacy in this disease, the trial has identified new patient groups and a potential role for CXCR4 in G6PC3 deficiency, a newly discovered inherited disorder of glucose homeostasis that like WHIM syndrome is characterized by severe congenital neutropenia and myelokathexis. This trial is providing basic new insights about the role of CXCR4 in leukocyte trafficking in man, as well as information about the utility of plerixafor in WHIM syndrome and the feasibility of chronic blockade of this essential receptor in any disease where it may play a role in pathogenesis.

S.15.02
Chemokine Guidance in the Lymph Node Imprints Adaptive Immune Responses

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The differentiation of naïve CD4+ T cells into T helper cells is critical for generating adaptive immune responses. This process occurs in the lymph node (LN) where naïve CD4+ T cells are activated by cognate antigen loaded dendritic cells (DC) and differentiate into one of several lineages of helper T cell subsets, such as T helper type 1 (Th1), Th2, Th17, and T follicular helper (Tfh). While the cytokines and transcription factors that control this process are relatively well understood, far less is known about how this process is temporally and spatially orchestrated within LNs *in vivo*, where it initially takes shape. We have shown that the CXCR3 chemokine system is required for the optimal generation of interferon gamma secreting Th1 cells *in vivo*. Using a newly generated CXCR3 chemokine ligand CXCL9 and CXCL10 reporter transgenic mouse, called REX3 for Reporter Expression CXCR3 chemokine ligands, we have found that following immunization with LPS and poly I:C or infection with influenza, stromal cells in the interfollicular region of the LN predominately express CXCL9 while hematopoietic cells in the medullary and interfollicular regions express CXCL10. Dendritic cell-derived CXCL10 facilitates T cell-DC interactions in LNs during T cell priming and CXCL9 and CXCL10 guide the intranodal positioning of CD4+ T cells to interfollicular and medullary zones, respectively, in the LN following immunization and influenza infection. Thus, the CXCR3 chemokine system guides priming CD4+ T cells into specialized niches in the reactive LN that facilitate Th1 cell differentiation.

S.15.05
Dynamisms and regulation of tumor-associated myeloid cells

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Tumor growth is often associated with the aberrant systemic accumulation of mononuclear phagocytes, which have the potential to support tumor growth. The accumulation of tumor-associated macrophages (TAMs) is a dynamic process associated with medullary and extramedullary monopoiesis and subsequent chemokine-mediated cellular migration. Recent studies suggest that spleen-pool monocytes are a source of TAMs. However, the relative contribution of spleen- versus bone marrow (BM)-pool monocytes to the TAM population remains unclear.
Here, we track spleen- and BM-pool Ly-6Chi monocytes in tumor-bearing mice in which myeloid cells express KikGR, a fluorescent protein that is irreversibly converted from green to red upon exposure to violet light. Irradiation of either the BM (femur) or spleen allowed us to track the redistribution of Ly-6Chi monocytes from each of these pools over a 24 h period. Within 1 h, significant numbers of red cells appeared in the peripheral blood from each pool, and began to redistribute to the BM, spleen, and tumor. However, many more BM-pool cells than spleen-pool cells accumulated in the tumor due to intrinsic differences in the ability of spleen- and BM-pool monocytes to infiltrate and survive within tumors. Experiments using Fucci transgenic mice that express cell-cycle indicator proteins demonstrated that the BM remains the major site of monopoiesis even in the tumor-bearing state.
These findings provide evidence that the contribution of BM-pool monocytes to the TAM population is considerably greater than that of spleen-pool monocytes.

S.16 Antigen processing and presentation

S.16.01
Genome-wide analyses define new factors controlling MHC class I and MHC class II antigen presentation

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MHC class I present mainly peptides generated in cells by the proteasome to present a blueprint of the intracellular proteome to immune cells. MHC class II molecules perform an analogous job but present mainly peptides generated in endosomes to the immune system. The two systems are thus complementary and in assembly cover the entire proteome for presentation. Fully understanding these processes is important as it may allow manipulation and then skewing of immune responses. Various proteins have been identified that support peptide generation, transfer, folding and loading of MHC molecules. We will report technologies and results from genome-wide genetic screens for additional molecules controlling antigen presentation by MHC class I or MHC class II molecules. These define new biology but also define new and unique targets for manipulation of antigen presentation by MHC molecules, as will be illustrated.

S.16.02
Molecular Aspects of MHC class I-restricted Antigen Processing

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The recognition of virus-infected cells or tumor cells by CD8-positive T lymphocytes relies on the formation of complexes of MHC class I molecules with virus-derived or tumor specific peptides, respectively. The peptides are generated in the cytosol and are translocated into the endoplasmic reticulum (ER), where peptide binding occurs, by the dimeric ATP-dependent Transporter associated with Antigen Processing (TAP). TAP is a component of the multi-subunit Peptide Loading Complex (PLC). The PLC also incorporates the glycoprotein tapasin, a product of an MHC-linked gene, which physically links MHC class I-[[Unsupported Character - Symbol Font ]]$_2$-microglobulin dimers to the PLC. The preferential association of MHC

class I molecules with high affinity peptides is mediated by tapasin and is facilitated by an adaptation of the calnexin/calreticulin quality control cycle that normally facilitates the proper folding of a variety of glycoproteins in the ER prior to transport. The lectin chaperone calreticulin and the thiol oxidoreductase ERp57, two components of this folding pathway, are also associated with the PLC and play a critical role in the peptide binding process. The mechanism of action of the PLC and the nature of the quality control mechanisms underlying the formation of MHC class I-peptide complexes will be discussed.

S.16.04
Antigen crosspresentation in dendritic cells

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Dendritic cells represent a highly specialized hematopoietic lineage, whose main role is to sense infections in tissues and to activate specific T lymphocytes in lymphoid organs to mount immune responses adapted to the threat. The mode of dendritic cell activation defines a series of maturation programs that influence T cell activation deeply. To activate T lymphocytes, dendritic cells need to present peptides derived from infectious antigens on MHC molecules on their plasma membrane. The activation of CD8+ T cells to mount cytotoxic immune responses requires the loading of peptides from either endogenous (self or viral) or internalized (endocytosed or phagocytozed) antigens. The latter process, called cross presentation, is more efficient in dendritic cells than in other antigen presenting cells, at least in part due to specialization of dendritic cell's endocytic pathway. Indeed, antigen cross presentation involves several steps of intracellular membrane traffic, including the export of the internalized antigens to the cytosol, low levels of phagosome-lysosome fusion and the recruitment of ER-resident proteins to phagosomes. We have initiated the analysis of the effect of various inducers of dendritic cell maturation on these different steps of membrane traffic.

S.16.05
Regulatory T cells control Th1 priming by inhibiting CD70 expression: a role for intercellular transfer of CD27?

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Naturally occurring regulatory T cells (nTregs) ensure the integrity of the host by controlling reactivity to self as well as to foreign antigens. We sought to determine the mechanism by which nTreg restrict the development of MHC class I and class II-rectricted IFN-γ-producing cells *in vivo.* Here, we show that regulatory T cells selectively inhibit the CD70/CD27 pathway, while sparing interleukin 12 production. Consistent with a role for CD70, $CD27^{-/-}$ mice did not show enhanced Th1 Responses under conditions of regulatory T cell depletion.Our data reveal a novel mechanism of immune regulation in which intercellular transfer of intact CD27 results in CD70 downregulation on dendritic cells and impaired Th1-prone costimulation. The molecular mechanism of this transfer remains elusive and may include trogocytosis and/or tunneling nanotubes. In conclusion, our data indicate that nTreg control the expression of CD70 on dendritic cells, which has been shown to act as master regulator of tolerance versus immunity.

S.17 Complement and soluble mediators

S.17.01
Complement: more than a "guard" against invading pathogens

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Nearly a century after the significance of the human complement system was recognized, we have come to realize that its functions extend far beyond the elimination of microbes. It is increasingly perceived as an intricate network of effectors, regulators and regulators that not only drives core cascade functions in health and disease but also extensively communicates with associated physiological pathways ranging from immunity and inflammation to homeostasis and development. A steady stream of experimental data reveals new fascinating connections at a rapid pace; while opening unique opportunities for research discoveries, and therapeutic interventions against various diseases and clinical conditions. I will discuss our updated view of the function, structure and dynamics of the complement network, highlight its interconnection with immunity at large and with other endogenous pathways, and illustrate its multiple roles in homeostasis and disease. Finally, I will discuss the current status of complement therapeutics and various approaches for complement based therapies.

S.17.02
Functional aspect of the lectin complement pathway

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The complement system is an effector mechanism in both innate and adaptive immunity. It is activated through three pathways, the classical, alternative and lectin pathways. The lectin pathway plays a role in innate immunity. It is initiated by the binding of mannose-binding lectin (MBL) or ficolins to carbohydrates on the surfaces of pathogens. In the lectin pathway, mannose-binding lectin (MBL) and ficolins act as pattern recognition molecules for pathogens, resulting in the activation of MBL- associated serine proteases (MASPs; MASP-1, MASP-2 and MASP-3). Among these proteases, MASP-2 is a key enzyme that cleaves C4 and C2 to assemble a C3 convertase (C4b2a). To investigate the roles of MASP-1 and MASP-3, we generated a MASP-1- and MASP-3-deficient (M1/3 KO) mouse model, and found that the deficient mice lacked alternative pathway activation, because factor D (Df) remained as a proenzyme in the serum. MASP-1 and MASP-3 were able to convert the proenzyme of Df to an active form in vitro and in vivo. Last year, Degn et al. reported that in human serum neither MASP-1 nor MASP-3 is required for alternative pathway function using 3MC MASP-1/3 deficient sera (J Immunol 2012). We claimed this point and found that in human MASP-1/3 deficient sera human Df is still proDf by Western blotting. In human and mouse sera, thus, MASP-1 and MASP-3 seems to be involved in activation of both the lectin and alternative pathways.

S.17.03
Trick or treat - how do pathogens deal with the complement system?

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Despite the potent antimicrobial effects of the complement (C) system many pathogenic microbes are resistant to opsonophagocytosis or direct C-mediated killing. The pathogens escape C killing e.g. by expressing surface proteins that bind soluble C inhibitors, notably factor H or C4bp. More than ten different types of microbes (borrelia, pneumococci, group A and B streptococci, meningococci and many invasive Gram-negative enteropathogens) express factor H binding proteins (FHBP). Interestingly, FHBPs of microbes often have the same binding sites on factor H, either in domains 6-7 or 20. The importance of FHBPs is exemplified by the facts that 1) a polymorphic variant in domain 7 that predisposes to age-related macular degeneration protects individuals from group A streptococcal infections, 2) the site in domain 20 is used by a number of microbes extending from Gram-negative and positive bacteria to the yeast *Candida albicans* and 3) the FHBPs can be used as vaccines. As a novel C evasion mechanism we have found an ability of pathogenic bacteria to bind the key C components C3 and C4 in a form that prevents their activation. Microbial proteins that bind C3 and C4 noncovalently have been discovered e.g. from *Borrelia burgdorferi*, *Salmonella* and *Yersinia*. The ability to escape C is thus a key feature

in the pathogenicity and invasiveness of disease-causing bacteria. Complement resistance may also explain the species-specificity spectra of some of the pathogens. At an individual level, those who carry protective alleles of their C regulator genes may have survival benefit against microbial infections.

S.17.04
The complement system: friend or foe?

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Embryo implantation into the maternal decidua represents a real challenge for the maternal immune system during pregnancy. The system provides protection against pathogens and other noxious agents that may impair the progression of pregnancy and at the same time avoids attacking the developing fetus despite recognition of paternal antigens expressed on fetal trophoblast. These goals are achieving mainly through the contribution of the cellular component of the innate immune system, in particular NK cells and macrophages. However, recent data suggest that the complement system is also implicated in these processes. Most complement components and regulators are present in the decidual tissue and may originate both from the circulation and from local synthesis by various cell types including macrophages, fibroblasts, endothelial cells and extravillous trophoblasts. Their expression is regulated by cytokines that contribute to embryo implantation site inducing vascular remodeling. Recent findings have disclosed a novel role of C1q, the first complement component, which is synthesized by decidual endothelial cells (DECs) and invading extravillous trophoblast. C1q expressed on DECs promotes adhesion of endovascular trophoblast followed by partial replacement of endothelium, while C1q secreted by trophoblasts favors their migration through the decidua.
Local complement activation may impair fetal survival if it were uncontrolled by regulators expressed at critical sites in the feto-placental unit. However, unrestricted complement activation may overcome the protection of complement inhibitors resulting in tissue damage and poor pregnancy outcome. Anti-phospholipid syndrome and pre-eclampsia are two examples of pathologic pregnancies that are contributed to a large extent by complement activation.

S.18 Bacterial infections

S.18.01
Activation of inflammasomes by bacterial pathogens

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Inflammasomes are multiprotein complexes that assemble in the cytosol of cells in response to infection or other noxious stimuli. Once assembled, inflammasomes initiate downstream signaling by activation of downstream proteases, most notably Caspase-1 and Caspase-11. Once activated, these caspases initiate downstream signaling events that stimulate inflammation. For example, inflammasome activation results in processing of cytokines (interleukins-1beta and -18), production of eicosanoids, and a rapid lytic cell death called pyroptosis. There are several distinct inflammasomes, each of which responds to different stimuli. However, the mechanisms by which these stimuli actually lead to inflammasome assembly are poorly understood. In my talk I will discuss recent advances in our understanding of inflammasome activation in response to bacteria. Our data, and that of several other labs, support the idea that the mechanisms of inflammasome activation are surprisingly diverse. An important question that will be considered is whether there are any general principles underlying these diverse mechanisms.

S.18.02
Card9 Signaling Complexes in Innate Immunity

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Card9 is a non-redundant adapter protein that functions in the innate immune system in the assembly of multifunctional signaling complexes. These complexes are engaged upon bacterial, fungal, or viral recognition, and they are essential for host protection. Loss of function mutations in Card9 lead to immunodeficiencies in mice and man. Moreover, Card9 polymorphisms are commonly associated with human inflammatory diseases such as inflammatory bowel diseases. Card9 is required to link Syk-coupled C-type lectin receptors to NF-kappaB activation. Card9 signaling also responds to intracellular danger sensors, such as RIG-I-like receptors and Nod2. Mechanistically, Card9 cooperates with Bcl10 and the paracaspase, Malt1 for the activation of pro-inflammatory cascades. A better understanding of the mechanisms of Card9 activation will enable the development of agonists or antagonists, which might be useful in vaccination and therapies of immunopathologies, Here, we discuss recent insights into the molecular regulation of Card9 signaling and the function of Card9 in host defense and immune homeostasis.

S.18.03
Type I interferon suppresses Type II interferon-triggered human anti-mycobacterial responses

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The immune mechanisms that control resistance vs. susceptibility to mycobacterial infection in humans were investigated by studying leprosy skin lesions, the site where the battle between the host and the pathogen is joined. Using an integrative genomics approach, we found an inverse correlation between IFN-β and IFN-γ gene expression programs at the site of disease. The Type II IFN, IFN-γ and its downstream vitamin D-dependent antimicrobial genes were preferentially expressed in the lesions from patients with the self-healing tuberculoid form of the disease and mediated antimicrobial activity against the pathogen, Mycobacterium leprae, in vitro. In contrast, the Type I IFN, IFN-β, and its downstream genes, including IL-10, were induced in monocytes by M. leprae in vitro, and were preferentially expressed in the lesions of disseminated and progressive lepromatous form. The IFN-γ-induced macrophage antimicrobial response was inhibited by IFN-β and IL-10, by a mechanism involving blocking the generation of bioactive 1,25-dihyroxyvitamin D as well as inhibiting induction of antimicrobial peptides cathelicidin and DEFB4. The ability of IFN-β to inhibit the IFN-γ-induced vitamin D pathway including antimicrobial activity was reversed by neutralization of IL-10, suggesting a possible target for therapeutic intervention. Finally, a common IFN-β and IL-10-associated gene signature was identified in both the skin lesions of leprosy patients and in the peripheral blood of active tuberculosis patients. Together these data suggest that the ability of IFN-β to downregulate protective IFN-γ responses provides one general mechanism by which some bacterial pathogens of humans evade protective host responses and contribute to pathogenesis.

S.19 Immune surveillance and tumor immunity

S.19.02
Integrating immune and vascular signaling programs through lectin-glycan interactions

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In the postgenomic era, the study of the ‘glycome’- the whole repertoire of saccharides in cells and tissues- has enabled the association of unique glycan structures with specific physiological and pathological processes. The responsibility for deciphering this biological information is assigned, at least in part, to endogenous

glycan-binding proteins or lectins whose expression is regulated at sites of inflammation and tumor growth. With the overarching goal of generating more rational therapeutic strategies, our laboratory investigates the molecular interactions between endogenous lectins and N- and O-glycans leading to the control of immune tolerance, inflammation and angiogenesis in the tumor microenvironment. In the past years we have identified an essential role for galectin-1, an endogenous lectin with specificity for poly-N-acetyllactosamine-enriched glycans in promoting tumor-immune escape in several tumor models by selectively eliminating Th1 and Th17 responses, by instructing the differentiation of tolerogenic dendritic cells (DCs) and by promoting the expansion of FoxP3+ T regulatory cells. In the presence of galectin-1, DCs acquired an interleukin 27 (IL-27)-dependent regulatory function and promoted IL-10-mediated T cell tolerance. More recently, we found that interactions between galectin-1 and specific target N-glycans may couple tumor hypoxia to angiogenesis through mechanisms that are independent of HIF-1α. Targeted disruption of galectin-1-glycan interactions prevented hypoxia-driven angiogenesis and suppressed tumorigenesis in several models. Our results suggest a major role for lectin-glycan interactions in integrating immunoregulatory to vascular signaling programs during tumor growth and metastasis

S.20 T and B cell subsets

S.20.02
Transcriptional network controlling Th17 cell differentiation

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Upon activation, T cells undergo distinct developmental pathways, attaining specialized properties and effector functions. T-helper (TH) cells are traditionally thought to differentiate into TH1 and TH2 cell subsets. Recently, a subset of interleukin (IL)-17-producing T cells (TH17) distinct from TH1 or TH2 cells was described and shown to have a crucial role in the induction of autoimmune tissue injury. Accumulating data suggests that there are three distinct steps in Th17 differentiation: Induction, Amplification and Stabilization mediated by distinct cytokines and loss of any of the cytokines (TGF-b, IL-6, IL-21 or IL-23) in the pathway results in a defect in generation of Th17. However not all Th17 cells are pathogenic and induce autoimmunity, IL-23 is a key cytokine that induces pathogenicity in Th17 cells. Using expression profiling at very high temporal resolution, novel computational algorithms and innovative nano-wire based “knock-down” approaches, we have developed a regulatory network that governs the development of Th17 cells. The Th17 transcriptional network consists of two self-enforcing but mutually antagonistic modules, which are essential for maintaining a balance between Th17 and other CD4 T cell subsets. Using these approaches we have identified Serum Glucocorticoid Kinase-1 (SGK-1) a key kinase induced during IL-23R signaling. High concentration of NaCl also induces SGK-1 and promotes Th17 differentiation, therefore supporting the possibility that high salt diet promotes Th17 differentiation and provides a trigger for inducing autoimmune disease on a genetically susceptible background.

S.20.05
Development and function of dendritic cell subsets

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The BATF members of the AP-1 transcription factor family mediate lineage decisions and functions of several types of immune cells. Batf is required for TH17 development, for T follicular helper (TFH) function by controlling c-Maf and Bcl6 expression, and in B cells, for AID expression and activation of transcription of the I-region promoters. Batf3 controls terminal differentiation and viability of the Irf8-dependent $CD8\alpha^+/CD103^+$ subsets of conventional dendritic cells required for cross-priming of CD8 T cells and innate defense against Toxoplasma gondii. The unique lineage-specific functions of BATF family members depends on the interaction of their leucine zipper domains to interact with non-AP-1 factors, specifically Irf4 and Irf8, in forming complexes on compound recognition elements. Although this unique interaction clearly operates in many BATF-specific transcriptional circuits, additional non-IRF-dependent activities are apparent that are still unique and not mediated by other AP-1 factors, such as in the regulation of the immunosuppressive cytokine IL-10. While homeostatic development of $CD8\alpha^+$ DCs is Batf3-dependnet, we identified a novel physiologic pathway that operates during infections by intracellular pathogens and that employs Batf and Batf2 for molecular compensation of Batf3 function in DC progenitors. This compensatory pathway serves to expand this protective DC subset during infections by intracellular pathogens. The seminar will discuss this and other unique and recently identified aspects of dendritic cell physiology.

S.21 NK cells

S.21.01
Co-stimulation of natural killer cells overcomes anergy

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Natural killer (NK) cells are well known to integrate signals received from their activation and inhibitory receptors that recognize ligands on their cellular targets but our understanding of NK cell activation is still incomplete. These concepts are well illustrated by NK cell responses to murine cytomegalovirus (MCMV) which contains several open reading frames (ORFs) that down-regulate expression of MHC class I molecules on infected cells, presumably to evade virus-specific MHC class I-restricted T cells while releasing the MHC class I-specific inhibitory receptors on NK cells, making the infected cells more vulnerable to NK cells. However, NK cells do not directly attack MCMV-infected cells unless they express m157, a virus-encoded molecule that is a ligand for the Ly49H NK cell activation receptor. Yet, the transgenic expression of m157 does not lead to widespread NK cell activation and instead leads to “anergy” of Ly49H+ NK cells, a form of tolerance. Here I will describe our progress in understanding how interactions between Ly49H and m157 lead to NK cell activation during MCMV infection but anergy during non-inflammatory conditions. In essence, “co-stimulation” of NK cells by signals from other molecules and pathways overcomes “anergy” from activation receptor engagement alone.

S.21.02
Adaptive reconfiguration of the human NK-cell compartment in response to cytomegalovirus

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Human Cytomegalovirus (HCMV) infection promotes in healthy individuals a persistent expansion of a functionally competent NK-cell subset expressing high surface levels of the CD94/NKG2C activating receptor. $NKG2C^{bright}$ NK cells are NKG2A- CD16+, display inhibitory KIR and LILRB1 receptors for self HLA class I molecules, but bear low levels of NKp46 and NKp30 activating receptors. Only minor proportions of $NKG2C^{dim}$ NK cells with a distinct phenotypic profile are detected in HCMV seronegative as well as in a number of HCMV+ subjects.
The expansion of $NKG2C^+$ NK cells reported in other infectious diseases is systematically associated to HCMV co-infection. The reconfiguration of the NK cell compartment appears particularly marked in immunocompromised patients and in symptomatic congenital HCMV infection, suggesting that it is inversely related with the efficiency of the T cell response.
An *NKG2C* gene deletion has been reported in different populations (~4% $NKG2C^{del/del}$). *NKG2C* zygosis appears related with the

magnitude of the steady-state NK cell redistribution, and functional studies support that the genotype influences the response of $NKG2C^+$ cells to engagement of the receptor.
This feature of HCMV infection is reminiscent of the response of murine $Ly49H^+$ NK cells against MCMV, and it has been hypothesized that an interaction of the CD94/NKG2C receptor with HCMV-infected cells may drive the NK subset expansion. Yet, the role of $NKG2C^+$ cells in the control of HCMV infection, the molecular mechanisms underlying the reconfiguration of the NK cell compartment, and its putative influence on the immune response to other pathogens or tumours remain open issues.

S.21.04
Natural Killer cells, Innate Lymphoid cells and Immunity

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Innate Lymphoid cells represent an emerging group of cells that participates to innate and adaptive immune responses. Natural Killer (NK) cells belong to group 1 ILCs. NK cell distinguish normal cells that they spare, from target cells that they contribute to eliminate, through a variety of cell surface activating and inhibitory receptors, the engagement of which regulates NK cell activities. Among NK cell activating receptors, NK cell express the Natural Cytotoxicity Receptors (NCR), which have been shown since more than a decade to be involved in the activation of NK cells by tumor cells. The NCR family includes NKp46, NKp44 and NKp30. A subset of gut Innate Lymphoid Cells (NCR+ ILC3) also express NKp46 in human and mice. We generated a NKp46-iCre knock-in mice that allow us to selectively target genes in NK cells and NCR+ ILC3. Using this genetic model, we will present data on the mechanisms by which these cells adapt to their environment.

S.21.05
Molecular mechanisms of differentiation of pro-inflammatory gamma-delta T-cells

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Gamma-delta T-cells are innate-like lymphocytes that share many functional properties with NK cells. Among them, we have recently demonstrated that human gamma-delta cells employ NKG2D and natural cytotoxicity receptors (especially NKp30) to target hematological tumors. The anti-tumor function of gamma-delta cells is also tightly linked to their abundant secretion of interferon-gamma (IFN-g). On the other hand, we and others have shown that gamma-delta cells make a large contribution to the production of interleukin-17 (IL-17) in murine models of infection, autoimmunity and cancer. We further demonstrated that IL-17 versus IFN-g producing gamma-delta cells differentiate in the murine thymus and can be segregated on the basis of CD27 expression levels. However, the molecular determinants of their functional potential in the periphery remained unknown. We have now conducted a genome-wide characterization of histone H3 modifications, integrated with mRNA levels of transcription factors that control *Il17* or *Ifng* expression, in peripheral gamma-delta cell subsets. This revealed a large set of potential new players in type 1 (IFN-g) or type 17 (IL-17-producing) cell differentiation *in vivo*. Furthermore, it allowed us to show that CD27(+) gamma-delta cells are stably committed to express *Ifng* but not *Il17*, whereas CD27(-) gamma-delta cells display permissive chromatin configurations at both type 17 and type 1 gene loci and can differentiate into IL-17/ IFN-g double producers in IL-1b/ IL-23-rich microenvironments. We will discuss the implications of these findings for gamma-delta T-cell responses in infection, autoimmunity and cancer.

S.22 Costimulatory and inhibitory molecules

S.22.02
The role of CTLA-4 in regulating T cell responses

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The T cell immune system exists in a state of balance, poised to react to invading pathogens but at the same time constantly being restrained from attacking our own tissues. Several strategies are employed in order to minimise our own self-reactivity. First amongst these processes is the deletion of T cells in the thymus, however this process is incomplete and self-reactive T cells still populate our immune systems. A second layer of control is exerted by regulatory T cells (Treg) which act to restrain self-reactivity by dominantly suppressing T cell responses. How Treg function to prevent autoimmunity is therefore of considerable interest. The protein CTLA-4 is highly expressed on Treg and we have recently identified a novel molecular basis for CTLA-4 function where CTLA-4 acts as a molecular “hoover” removing stimulatory ligands from antigen presenting cells[1]. This talk will discuss the mechanisms of CTLA-4 function, the predictions generated by this mechanism of action and the implications for regulatory T cell function.
1. Qureshi, O.S., et al., Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science, 2011. 332: p. 600-3.

S.22.03
Neutrophil microparticles activate tissue protective signals. Mauro Perretti Barts and The London School of Medicine

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Altered plasma neutrophil microparticle levels have recently been implicated in a number of vascular and inflammatory diseases, yet our understanding of their actions is very limited. We have recently detailed the proteome of neutrophil microparticles observing presence of >300 proteins, which varied in large part in function of the mode of neutrophil stimulation (Dalli, Montero-Melendez et al., Mol Cell Prot 2013). Heterogeneity of vesicles released from the same cell type was also reflected in distinct modulation of target cells, like the endothelial cells. These data set the scene for further analyses in human samples, like septic plasma and rheumatoid arthritis synovial fluids. It was noted that microparticles generated in settings that mimic vascular inflammation seem to propagate and accentuate the inflammatory response whereas microparticles that mimic those produce by neutrophils in an exudate are able to active tissue protective circuits. The latter is being studies in the contact of joint inflammatory disease. Specific determinants for the bioactions afforded by these microparticles have been identified and are exploited for therapeutic opportunities.
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S.23 Vaccination

S.23.01
Vita-PAMPs: Signatures of microbial viability

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Can the innate immune system detect and respond to microbial viability? Using bacteria as a model, we found that indeed the very essence of microbial infectivity, viability itself, can be detected, and notably, in the absence of the activity of virulence factors. The microbial molecule that serves as the signature of viability is bacterial messenger RNA (mRNA), common to all bacteria, and without which bacteria cannot survive. Prokaryotic mRNAs also differ from eukaryotic mRNAs in several ways, and as such, these features all

fulfill the criteria for a pathogen-associated molecular pattern (PAMP) as originally proposed by Charles Janeway. Because these mRNAs are lost from dead bacteria, they belong to a special class of PAMPs, which we call vita-PAMPs. Here we discuss the possible receptors and pathways involved in the detection of bacterial mRNAs, and thus microbial viability. We also consider examples of vita-PAMPs other than bacterial mRNA.

S.23.05
Reprogramming the immune environment in cancer via dendritic cells

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T cells can reject established tumors when adoptively transferred into patients thereby demonstrating that the immune system can be harnessed for cancer therapy. Active immunotherapy with vaccines has the potential to induce tumor-specific effector and memory T cells that might control tumor outgrowth on the long term. Vaccines act through dendritic cells (DCs), which induce, regulate and maintain T cell immunity. The goal is to generate high-quality, high-sensitivity, poly-functional effector $CD8^+$ T cells that reject tumors and memory $CD8^+$ T cells that prevent relapse by targeting distinct DC subsets. Our pre-clinical studies demonstrate that Langerhans cells are superior to other DC subsets in their capacity to prime high-affinity $CD8^+$ T cells that can kill tumors. Our recent studies demonstrated the presence in breast cancer Th2 inflammation, which fosters breast cancer development. This is driven by the cytokine TSLP, which induces and maintains pro-tumor $CD4^+$ T cells via OX40L-expressing DCs. Thus, a better understanding of DC biology within the tumor environment will allow us to design novel immunotherapeutic strategies that reprogram DCs to induce tumor-rejecting Th1-mediated acute inflammation and strong tumor-specific $CD8^+$ cytotoxic T cells.

S.24 Protozoal, fungal and helminth infections

S.24.03
Immunity and tolerance to fungi: The contribution of gut microbiota via tryptophan catabolism

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Candida albicans is a commensal fungus of the oro-gastrointestinal tract, vagina and skin. In concomitance with defects in the innate and adaptive immune systems or microbial dysbiosis, the fungus may shift from a state of commensalism to parasitism and can cause severe chronic mucosal infections. IL-22, driven by intestinal microbiota, exerts a fine control over the initial fungal growth and tissue homeostasis at mucosal surfaces. This prompted us to decipher the signaling mechanism(s) linking the bacteria-fungal population dynamics with the mammalian host at mucosal surfaces. Endogenous tryptophan metabolites from resident microbiota have an important role in mammalian gut immune homeostasis, yet their potential contribution to fungal colonization and/or infection has never been addressed. We discovered a metabolic pathway whereby tryptophan metabolites from the microbiota balance antifungal mucosal reactivity in mice. Switching from sugar to tryptophan as an energy source−e.g., under conditions of unrestricted tryptophan availability−highly adaptive lactobacilli are expanded and produce an AhR ligand that contributes to AhR-dependent *Il22* transcription. The resulting balanced mucosal response provides colonization resistance to *C.albicans* and mucosal protection from inflammation. Thus, our study identifies a tryptophan metabolic pathway that is exploited by certain lactobacilli to optimize host immune homeostasis and fungal adaptation in vertebrate hosts and may offer a plausible explanation not only for susceptibility to infection in certain clinical settings but also for the strain-specific effects observed with probiotic lactobacilli administration, the empirical use of which as probiotics−to prevent *Candida* infection−has long been recommended but never mechanistically explained.

S.25 Innate lymphocytes and mucosal immunity

S.25.03
Treg induction by a rationally selected Clostridia cocktail from the human microbiota

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Manipulation of the gut microbiota holds great promise for treating inflammatory and allergic diseases. Although numerous probiotic microorganisms have been identified to date, there is a compelling need to discover organisms that elicit more robust therapeutic responses, are compatible with the host, and can affect a specific arm of the host immune system in a well-controlled, physiological manner. Here we used a rational approach to isolate CD4+Foxp3+ regulatory T cell (Treg)-inducing bacterial strains from the human indigenous microbiota. Starting with a healthy human faecal sample, a sequence of selection steps was applied to obtain mice colonised with human microbiota enriched in Treg-inducing species. From these mice, we isolated and selected 17 strains of bacteria based on their high potency in enhancing Treg cell abundance and inducing important anti-inflammatory molecules, including interleukin 10 (IL-10) and inducible T-cell co-stimulator (ICOS), in Treg cells upon inoculation into germ-free (GF) mice. Genome sequencing revealed that the 17 strains fall within clusters IV, XIVa and XVIII of Clostridia, which lack prominent toxins and virulence factors. The 17 strains act as a community to both provide bacterial antigens to T cells and to help polarize the T cells to become Treg cells by inducing the generation of a TGF-β-rich environment. Oral administration of the cocktail to adult mice attenuated disease in models of colitis and allergic diarrhoea. Use of the isolated strains may allow for tailored therapeutic manipulation of human immune disorders.

S.25.04
Mucosal T cells: Modelled to fit the challenges

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The T cells that reside as intraepithelial lymphocytes (IEL) within the epithelium of the gastrointestinal tract constantly face a plethora of foreign antigens. This challenge requires rapid and effective protection, however, with the least damage to the epithelium. Furthermore, since the majority of antigens are diet- or commensal-derived antigens, a strict requirement for maintaining a balance between protection and tolerance represents another challenge.
Most IEL are CD8αβ effector memory T cells (T_{EM}) with immediate protective functions. Unlike lymphoid memory cells, mucosal T_{EM} are controlled by an environmental-driven process that optimizes effective immunity by selectively preserving the highest affinity CD8αβ cytotoxic T lymphocytes (CTL). The selective accumulation of high

affinity CD8αβ T_{EM} provides a first line of defense with exceedingly responsive sentinels that can eliminate invading pathogens, before the pathogens or the systemic immune system can jeopardize the barrier integrity. In addition to the CD8αβ CTL, there are also CD4 T cells with a cytotoxic phenotype and recently we showed that these CD4 CTL are progeny of conventional CD4 Th cells that lost the Th transcription factor, ThPOK and post-thymically terminated the Th program and acquired the CTL phenotype instead. Both, the selective accumulation of high affinity CD8αβ T_{EM} and the functional switch of CD4 Th cells to CTL, are processes driven by the challenges imposed by the mucosal environment to keep a balance between optimal immune protection in the face of immune tolerance.

S.26 Microbiome and microbe adaptation

S.26.03
Trained immunity: innate immune imprinting of host-microbe interaction

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The inability of innate immunity to build an immunological memory, considered one of the main characteristics differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. Long-term reprogramming of innate immunity, that induces adaptive traits and has been termed trained immunity characterizes prototypical innate immune cells such as natural killer cells and monocytes, and provides protection against reinfection in a T/B-cell-independent manner. In contrast, trained immunity has been shown to be able to induce protection against reinfection in a monocyte-independent manner. Non-specific protective effects dependent on trained immunity have also been shown to be induced after BCG vaccination in humans. Specific signaling mechanisms including the dectin-1/Raf1 and NOD2-mediated pathways induce trained immunity, through induction of histone methylation and epigenetic reprogramming of monocyte function. Complex immunological and metabolic circuits link cell stimulation to a long-term epigenetic reprogramming of its function. The concept of trained immunity represents a paradigm change in immunity and its putative role in infection and inflammation may represent the next step in the design of future vaccines and immunotherapeutic approaches.

S.26.05
The endogenous microbiome controls development of spontaneous autoimmunity to immunologically privileged neuroretina

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Autoimmune uveitis that targets the neuroretina and is thought to be driven by a T cell response to retinal antigens is a significant cause of human blindness. Since retinal antigens are not expressed outside the eye, a major unanswered question is where do retina-specific T cells become activated to acquire the ability to enter the eye. Microbial triggers have been suspected, but never proven. R161H mice, which express a transgenic T cell receptor (TCR) specific for the retinal antigen IRBP, develop spontaneous uveitis by 2 months of age. If treated with a broad-spectrum antibiotic cocktail, or reared under germ-free conditions, these mice exhibit a marked delay in uveitis onset and intensity. Analysis of cells from the gut lamina propria of antibiotic-treated or untreated R161H mice, crossed to various reporter strains, demonstrated presence of IRBP-specific Th17 cells, which appear to become activated in the gut by signals that depend on the presence of microbiota. Activation is not dependent on IRBP, but nevertheless appears to involve the TCR. Upon adoptive transfer to RAG2-/- recipients, these lamina propria lymphocytes induced uveitis. We interpret these data to mean that commensal microflora or its metabolites activate autopathogenic retina-specific T cells, possibly through a process involving at least in part a TCR-mediated signal. We hypothesize that such effects might not be restricted to IRBP-specific T cells and uveitis. Our findings may help to explain the postulated connection in humans between microbial triggers and uveitis, and could have implications also other autoimmune diseases.

S.27 Immune memory

S.27.02
Class and specificity of the human T cell response to commensals, pathogens, and allergens

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To interrogate the naive and memory T cell repertoires we developed a high throughput cellular screening method based on T cell libraries of polyclonally activated and expanded T cells. These libraries are then interrogated, in an iterative fashion, to identify antigen-specific T cells and determine frequency, fine specificity, and cross-reactivity, and to isolate T cell clones. The T cell library method is not limited by the complexity of the antigen, neither by the HLA of the donor, and allows the detection of T cells specific for naturally processed antigens, even whole pathogens. Using this method we have been able to identify antigen-specific T cells in the naive repertoire and measure the distribution of memory T cells within distinct subsets of Th1, Th2, Th17 and Th22 cells. I will discuss how the new high-throughput methodological approaches that are becoming available may lead to a better understanding of the class and specificity of the human immune response, the lineage relationship between T cell subsets and their plasticity, and will be instrumental to increase our understanding of the immune response to commensal and pathogenic microbes as well as environmental allergens.

S.27.03
Origins of CD4+ memory T cells

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We studied the relationship between memory cell formation and protective immunity by tracking microbial peptide:MHCII-specific CD4+ T cells during a transient Listeria infection that is not controlled by CD4+ T cells and a persistent Salmonella infection that is. We found that CD4+ memory T cell population generated by transient Listeria infection declined with a half-life of 60 days after the bacteria were cleared. The memory cell population consisted of non-cytotoxic Th1 and Tfh subsets. In contrast, CD4+ memory T cells generated by low-level persistent Salmonella infection did not decline over a year unless the infection was eliminated with antibiotics. The memory population consisted exclusively of Th1 cells, some of which expressed Granzyme B and were cytotoxic. Thus, an infection that is controlled by CD4+ T cells generated cytotoxic Th1 cells that were stably maintained by peptide:MHCII presentation. These results suggest that vaccines that are cleared from the body will not produce protection from infections that are controlled by CD4+ T cells because these cells will not persist or acquire the necessary microbicidal functions.

S.27.04
TFH heterogeneity, memory and plasma cell survival niches

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The formation of optimal helper T cell memory during Salmonella infection is dependent on BCR-mediated recognition of the bacteria by B cells and their subsequent antigen presentation to T cells. The

formation of stable T follicular helper cell (TFH) populations has similar requirements of B cells. In addition, both seem to depend on expression of Bcl6 and ICOS ligation. However, the CXCR5+, ICOS+, bcl6+, PD1+ TFH formed early in Salmonella infection are not classical, in that they do not enter germinal centres (none form until much later) and nor do they support early, extra-follicular antibody production which is largely T-independent. We have characterised these Tfh-like cells to ask whether they resemble, classical TFH, short-lived effector cells or memory precursor effector cells. We have asked about the importance of follicular entry and interaction with B cells by constructing chimeric mice in which T cells cannot enter follicles. We will discuss this data in the context of the contrasting requirements for T cell follicular migration in the generation of effector T cell and memory responses after bacterial infection compared to immunization with non-replicating antigens.

S.28 Imaging and cell interactions

S.28.01
Getting to the center of the immunological synapse

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The recognition events that mediate adaptive cellular immunity depend on intercellular contacts between T cells and antigen presenting cells (APC). T cell signaling is initiated at these specialized junctions between T cells and APCs, known as the immunological synapse (IS), when surface-expressed antigen receptors (TCR) recognize peptide fragments of pathogens (pMHC) on APCs [1]. Using high resolution optical and electron microscopy, optical-EM correlation, and electron-tomography, we show that centrally accumulated TCR at the IS is located on the surface of extracellular microvesicles that bud at the IS center and are recognized by APC bearing cognate antigen. An early endosomal-sorting complex required for transport (ESCRT) sorts TCR for inclusion in microvesicles, while terminal ESCRT components mediate scission of microvesicles from the T cell plasma membrane. The HIV polyprotein GAG co-opts this process by displacing TCR from microvesicles, resulting in release of virus-like particles at the antigen-dependent IS. We conclude that post-signaling TCR accumulates at the IS center and is released in extracellular microvesicles by an ESCRT-dependent mechanism triggered by T cell activation. These microvesicles mediate intercellular communication in immune cell collaboration, and can be co-opted by HIV GAG for viral transmission across antigen-dependent synapses.
[1] Fooksman, D. R., et al. Annu Rev Immunol 28: 79 (2010)

S.28.03
What determines the directionality of leukocyte transendothelial migration?

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Directional guidance is a hallmark of immune cell trafficking. Although chemokines are critical for directional leukocyte crossing of endothelial barriers, it is still unclear how these cytokines drive this complex migration process. It is believed that transendothelial migration (diapedesis) is mediated by signals from endothelial-presented chemokines which trigger serial low affinity integrin-mediated adhesions coupled to highly dynamic actin-driven protrusions. Whether steep chemokine gradients are obligatory for diapedesis is still unclear. The blood vessel cells that promote leukocyte diapedesis produce their own chemokines or actively transport chemokines across endothelial barriers. One plausible mechanism for generation of chemokine gradients across these barriers is extensive washing of chemokines from the lumenal vessel aspects. In addition, we find a sharp enrichment of heparan sulfates (HS) at the basolateral aspects of lymphoid and non lymphoid post capillary venules. We propose that this basolateral pool of potential chemokine scaffolds can generate different chemokine gradients across variably inflamed endothelial barriers. While most leukocyte diapedesis depends on these HS scaffolds, subsets of effector T cells respond to endothelial produced inflammatory chemokines independently of endothelial HS. Strikingly, the ability of these lymphocytes to squeeze their nuclei through endothelial junctions does not depend on endothelial myosin-II mediated contractility. Thus, leukocyte transendothelial migration is guided by endothelial chemokines, integrin ligands and unique endothelial machineries that couple transient and confined endothelial barrier rupture events to directional leukocyte motility from the apical endothelial surface towards the endothelial basement membrane.

S.28.04
Visualizing and regulating the traffic of pathogenic CD8+ T cells within the liver

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Hepatitis B virus (HBV) causes a liver disease of variable duration and severity. HBV replicates noncytopathically in the hepatocyte and most of the liver injury associated with this infection reflects the immune response. Virus-specific effector CD8 T cells play a major role in the development of liver disease and the resolution of HBV infection during self-limited acute viral hepatitis. Viral persistence reflects the failure to induce CD8 T cells with full antiviral capacity, so that the infection is not cleared from the liver and a CD8 T cell-dependent chronic necroinflammatory process begins, often resulting in cirrhosis and hepatocellular carcinoma (HCC). The use of dedicated animal models coupled with advanced in vivo imaging helped us to show that the hepatic recruitment of pathogenic effector CD8 T cells is promoted by platelets. This effect appears to depend on platelets that - after having adhered to liver sinusoidal endothelial cells and become activated - provide a preferential surface onto which CD8 T cells arrest their passage within the hepatic microcirculation. CD8 T cells eventually detached from platelets start to crawl up and down the liver sinusoids in search of viral antigens. That platelets represent key players in the pathogenesis of viral hepatitis is also indicated by experiments we recently performed in a mouse model of chronic HBV infection. There, a continuous aspirin/clopidogrel therapy inhibiting platelet activation was shown to limit the hepatic accumulation of pathogenic effector CD8 T cells and the consequent liver disease, ultimately preventing the development of cirrhosis and HCC.

S.28.05
Using Advanced Dynamic and Multiplex Static Imaging to Probe Innate and Adaptive Immunity In Vivo

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Immune responses involve multiple cell-cell interactions within lymphoid tissues, trafficking of activated cells to sites of effector function, and the migration of such effector cells within peripheral tissues. To gain a more detailed appreciation of the relationship among cell movement, tissue architecture, and immune function, we have used intravital multiphoton microscopy and a novel immunohistochemical method to analyze immune cell dynamics and functional tissue micro-anatomy.
Our data show that T cells follow stromal pathways during migration in lymph nodes, which enhances interactions with DCs attached to the same FRC network. Additional chemokine guidance cues facilitate interactions among rare antigen-presenting and antigen-recognizing cells. Adhesive interactions regulating the duration of cell-cell association are also critical to for adaptive immune responses. In tissue sites, effector cells stop when they perceive adequate antigen and undergo transient local activation and polarized cytokine release, followed by tuning of their response to exisitng antigen levels. Innate

immune (neutrophil) responses have been dissected at the molecular level. The role of cell localization in both innate defense and adaptive immunity has been addressed using both dynamic imaging and a new method called histo-cytomtery that reveals at high resolution the spatial positioning of cells with complex phenotypes throughout lymphoid tissues. These observations show the power of in situ imaging in the acquisition of a more accurate picture of the molecular, cellular, spatial, and temporal aspects of cell function and signaling events in host immune responses.
This work was supported in part by the Intramural Research Program of the NIH, NIAID.

S.29 Allergy

S.29.01
Role of tissues in immune regulation in allergic inflammation

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In chronic allergic inflammation, dermis in the atopic dermatitis skin and submucosa in the asthmatic lung act like a peripheral lymphatic organ, where dendritic cells, T cells and B cells contact each other. This is followed by a second step of antigen-presentation and activation in the inflamed tissue. Immune system cells and their cytokines interact with resident tissue cells, which leads to a series of tissue events leading to proinflammatory cytokine and chemokine release from both sides. Some of these events seem to be part of the immune pathology, such as basement membrane thickening, epithelial death, desquamation and spongiosis, however, they also act as mechanisms that control the severity of tissue inflammation. These can be listed as: keep away effects; wash away effects and immune suppression. “Keep away“ effects play a role in allergen ignorance by decreasing the allergen burden. They are lamina reticularis thickening and allergen-specific secretory IgA, in addition to cough and mucociliary activity. “Wash away“ effects that decrease the intensity of inflammation are epithelial apoptosis, spongiosis, leakage and wash of proinflammatory cytokines and inflammatory cells away from the tissues towards the lumen. Direct “suppression” is taking place by allergen-specific B and T regulatory cells as well as “regulation” of B cells for the production of non-inflammatory antibody isotypes (more IgG4 and IgA, less complement activating antibodies and IgE).

S.30 Tolerance and transplantation

S.30.01
Immunoregulation in Transplantation

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Immune regulation is fundamental to any immune response to ensure that it is appropriate for the perceived threat to the host. Strategies for the induction of specific unresponsiveness to donor alloantigens currently under investigation in the clinic take advantage of two of the major mechanisms for the induction of tolerance to self antigens - deletion and immunoregulation/suppression.
We have demonstrated that human regulatory T cells expanded ex vivo can protect human allografts (skin and vessels) from rejection (2, 3). Together with other leukocyte populations, including regulatory T cells, B cells and macrophages as well as myeloid derived suppressor cells and dendritic cells, Treg contribute to the regulation of immune responses in vivo after cell or solid organ transplantation (1).
The identification and characterisation of Treg that can control immune responsiveness to alloantigens has opened up exciting opportunities for new therapies in transplantation. Phase1/2a clinical trials are in progress - www.onestudy.org.

1. Nadig et al. In vivo prevention of transplant arteriosclerosis by ex vivo-expanded human regulatory T cells. Nat Med. 2010;16(7):809-13.
2. Issa F et al . Ex vivo-expanded human regulatory T cells prevent the rejection of skin allografts in a humanised mouse model. Transplantation. 2010;90:1321-7.
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S.30.02
Mechanisms of Allograft Tolerance Achieved with Hematopoietic Cell Transplantation

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Immune tolerance would avoid the need for chronic immunosuppressive therapy, with all of its toxicities, that is currently required to prevent graft rejection. Following upon studies in rodents and large animals, we have performed combined HLA-mismatched (haploidentical) related donor kidney and bone marrow transplantation (CKBMT) to induce chimerism and tolerance in patients with renal failure in an ITN-sponsored trial. CKBMT recipients have tolerated their allografts for >3 to >10 years without immunosuppressive medication and developed donor-specific unresponsiveness in in vitro assays. Donor chimerism was present for less than 3 weeks in these patients. Tregs were enriched in PBMC CD4 cell populations in the first few months post-transplant and some of the patients’ PBMCs drawn within the first year revealed residual anti-donor reactivity in vitro when Tregs were depleted. However, MLR, CML and limiting dilution assays at later time points were suggestive of a deletional mechanism of tolerance. Assessing deletional tolerance directly has previously been impossible due to the unavailability of markers for the many thousands of T cell clones responding to HLA alloantigens. We have developed a new strategy for tracking the fate of donor-reactive T cell clones and preliminary data supporting a deletional mechanism of tolerance will be presented. This tolerance requires an active role for the kidney graft. Because other organs are less effective than kidneys at promoting tolerance in combination with transient mixed chimerism, our current studies in large animals aim to achieve durable mixed chimerism without toxicity. Encouraging preliminary data will be presented.

S.30.03
Clinical and Immunological Relevance of Antibodies in Transplantation

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The detection and characterization of anti HLA antibodies in organ transplantation and the clinical impact of their occurrence is an area of immense interest. In recent years, it has become clear that several HLA antibodies that develop post transplantation and are not necessarily donor specific, are also associated with poor graft survival. Indeed the presence of circulating anti-HLA antibodies along with positive C4d staining on allograft biopsies has been reported to have strong correlation with the development of chronic rejection in kidney transplant recipients. Our experience indicates that renal transplant patients who develop anti-HLA antibodies *de novo*, even though they are not donor specific (cross-match negative) have significantly poor graft survival as compared to the negative group. Further, the impact of non-HLA antibodies including MICA (MHC class I related chain A), angiotensin II type 1 receptor, vimentin, collagen V etc. have been documented in solid organ transplant outcome. It is now agreed that MICA antigens expressed on endothelial cells are an important antibody target towards graft destruction. Patients who develop both anti HLA and MICA antibodies reject their grafts more frequently than those having either of these antibodies. The current data also indicates that identification of *de novo* donor specific antibodies with the help of most accepted and sensitive luminex based single antigen assay is an important tool in addition to other cell based assays. Thus, the HLA and non-HLA

antibodies could not only be predictive of allograft failure, but also as an important prognostic marker in transplant subjects.

Late Breaking Sessions

LB.1 Late Breaking Session - Basic Research

LB1.01
Specification of innate type-2 lymphocytes by the transcriptional determinant Gfi1

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Innate type-2 lymphoid cells (ILC2s) play a pivotal role in protective immune responses against helminth parasites and are implicated in the pathophysiology of allergic inflammation. ILC2s are activated by the epithelial-derived cytokines IL-33 and IL-25 and are a prominent source of the type-2 effector cytokines IL-5 and IL-13. However, regulatory networks that orchestrate the generation and effector state of ILC2s are poorly understood. Here we report that the transcription factor Growth factor independence-1 (Gfi1) functions to control the development, activation, and proper specification of the ILC2 effector state. Accordingly, IL-33- or *Nippostrongylus brasiliensis*-instigated inflammation was severely impaired in the absence of Gfi1. Genetic and molecular analyses of ILC2s reveals a stringent requirement for Gfi1 in response to IL33, not IL-25, as Gfi1 directly targets and activates the IL-33 receptor. Strikingly, loss of Gfi1 in activated ILC2s results in a unique hybrid effector state, which is characterized by derepression of the IL-17 inflammatory program while retaining expression of genes associated with type-2 inflammation, including IL-13. Given that Gfi1 is implicated in reciprocally regulating Th2 and Th17 cell fates, our results reveal it to be a conserved regulatory component which functions in both innate and adaptive immune cells to sustain a type-2 cytokine response while repressing the IL-17 effector state. These findings may have important implications for the pathophysiology of severe asthma, which manifests deregulation of these two inflammatory states in a subset of patients.

LB1.02
Identification of a common monocyte progenitor

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Monocytes, macrophages and dendritic cells (DCs) are critical immune regulators and part of the mononuclear phagocyte system (MPS), a body-wide, cellular system of phagocytic cells. The MPS is multi-functional and involved in various biological processes, such as the maintenance of tissue homeostasis, pathogen defense and wound healing, as well as many pathological conditions including infectious diseases, cancer and atherosclerosis.
Monocytes and DCs are heterogeneous populations and sub-divided into distinct subsets. For monocytes, classical Ly6C-high and non-classical Ly6C-low monocytes, and for DCs, classical DCs (cDCs) and plasmacytoid DCs (pDCs) are the major subsets. From a developmental perspective, monocytes/macrophages and DCs share a common origin, having the macrophage/dendritic cell progenitor (MDP) as common precursor. Unlike for DCs, the distal differentiation pathways from the MDP towards monocytes/macrophages are currently not fully elucidated.
We now demonstrate in mice the existence of a clonogenic, monocyte/macrophage-restricted progenitor, termed the common monocyte progenitor (cMoP). Derived from the MDP, the cMoP is a proliferating cell found in the bone marrow, which generates both major monocyte subsets and macrophages, but not DCs. Using in-depth quantitative proteomics, we characterized the proteome changes during monocyte differentiation, giving insights into the molecular principles of developing monocytes such as their functional maturation. Thus, monocytes/macrophages can renew independently from DCs from a committed progenitor.

LB1.03
Distinct TLR4-expressing cell compartments control neutrophilic and eosinophilic airway inflammation

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Stimulation of TLR4 expressed by hematopoietic and airway epithelial cells can contribute to the airway immune response to lipopolysaccharide (LPS) and allergens such as house dust mite (HDM) or ovalbumin (OVA), however, the specific contribution of these cell compartments to the airway inflammatory responses remains under-defined. We used newly developed *Tlr4*$^{fl/fl}$ mice along with reciprocal bone marrow chimera techniques to define the relative contributions of airway epithelial and hematopoietic cell *Tlr4* expression to LPS- and allergen-induced airway inflammation. Our data indicate that airway epithelial cell *Tlr4* expression is required for the development of Th2-mediated eosinophilic airway inflammation following airway sensitization and challenge with HDM or OVA/LPS. Conversely, hematopoietic cell *Tlr4* expression is required for Th1- and Th17-mediated cytokine production and for neutrophilic responses to these same allergens, or in response to acute LPS challenge. Thus, *Tlr4* expression by hematopoietic and airway epithelial cells controls distinctive aspects of the airway immune response to inhaled allergens.

LB1.04
Seeing is believing: visualization of the thymic T-cell development in a quantitative and non-invasive manner

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The optical transparency of the thymus in medaka fish facilitates the in vivo characterization of molecular and cellular principles that underlie the development of the T-lymphocytes using high-resolution imaging. To investigate the dynamics of thymocyte movement within the thymus in real time, we use medaka fish transgenic reporter lines to visualize either thymocytes at different developmental stages or thymic stromal cells (e.g. thymic epithelial cells and dendritic cells). We have analyzed the migration of individual thymocytes into (and out of) defined areas within the thymus. Lymphocyte progenitors, which require a high level of chemokine receptor Ccr9a for thymus homing, begin to express the chemokine receptor ccr9b after interaction with cortical thymic epithelial cells through Notch1b/Dll4a signaling, after which they move to the center of thymus. Thymocytes in the central area do not proliferate and are less motile. Dendritic cells in the thymus can interact simultaneously with several thymocytes and are able to engulf one of the latter in less than thirty minutes. We have observed single dendritic cells containing up to five phagocytosed thymocytes. Taken together, we will illustrate how advanced genetic tools allow the visualization and tracking of cells to study the cellular processes associated with T-cell development in the thymus in a quantitative and non-invasive manner.

LB1.05
A global approach to study viral antigen presentation using immunoproteomics

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Current knowledge about the dynamics of antigen presentation to T cells during viral infection is very poor despite being of fundamental importance to our understanding of anti-viral immunity. We have used advanced mass spectrometry to simultaneously quantify the presentation of vaccinia virus peptide-MHC complexes (epitopes) on infected cells and the amounts of their source antigens at multiple times after infection. The results show a startling 10,000-fold range in abundance as well as strikingly different kinetics across the epitopes monitored. The tight correlation between onset of protein expression and epitope display for most antigens provides the strongest support to date that antigen presentation is largely linked to translation and not later degradation of antigens[1]. In depth interrogation of MHC bound peptides identified over 100 new vaccinia virus derived peptides, over 70% of which were immunogenic and some bearing unanimated post-translational modifications. This highlights the utility of a peptidomics strategy to map T cell epitopes, particularly for complex pathogens. Finally, using data independent acquisition approaches (SWATH-MS) we have monitored all viral and host cell proteins providing a global view of viral antigen expression and host cell responses. This study highlights the complexity of viral antigen presentation and demonstrates the weakness of simple models that assume total protein levels are directly linked to epitope presentation and immunogenicity.
[1] Croft, N. P., Smith, S. A., Wong, Y. C., Tan, C. T., *et al.*, Kinetics of Antigen Expression and Epitope Presentation during Virus Infection. *PLoS Pathog* 2013, *9*, e1003129.

LB1.06
Molecular characterization of a novel antigen specific, immunoglobulin expressing cell in immunized animals with phenotypic characteristics of both granulocytes and lymphoctes

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Scientists seek improved methods of generating antigen specific antibodies important for use in therapeutics, diagnostics and creating immune reagents. Here we used flow sorting to examine antigen specific cells from immunized mice. Following immunization with protein antigens, a novel immunoglobulin- bearing antigen-specific cell population was identified and named "V cells". This cell population lacks surface expression of B-cell lineage specific markers such as B220, CD19, as well as T-cell, natural killer (NK)-cell, NK T (NKT)-cell, basophil, and stem-cell specific markers as determined by flow cytometric analysis. The dual IgG/IgE expression in V cells was confirmed via confocal microscopy and Z-stack analysis. V cells have a polymorphonuclear giemsa staining pattern which resolves as a “donught” shape under confocal and electron microscopy. They cycle in the spleen, bone marrow, and blood of immunized mice, but not other tissues. Antigen-specific V cells appear following protein immunization as early as 7 to 14 days. Molecular analysis of mRNA from sorted V cells of individual animals has shown rearranged and mutated IgG/E and Kappa by RT-PCR. The dual IgG/IgE expression in V cells contradicts long standing theories molecular expression and cellular classification. V cells, bearing antigen specific IgG and IgE, have some central role in protective immunity, homeostasis and/ or allergy as they are engendered commonly in response to protein antigens.

LB.2 Late Breaking Session - Translational Research

LB2.01
Identification of specific markers for type 2 (pro-allergic) human dendritic cells: interest as follow-up markers for immunotherapy

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Dendritic cells (DCs) are involved in the initiation of regulatory and effector adaptive immune responses. Thus, markers of polarized DCs represent promising molecules to monitor immune responses following immunotherapy. Herein, we aimed to identify specific markers for type 2 DCs (DC2), ie. monocyte-derived dendritic cells (MoDCs) which promote Th2 allergic responses.
After screening more than one hundred biological and pharmaceutical agents, a cocktail of molecules capable of differentiating DC2 was selected. Such DC2 were confirmed to support the differentiation of IL-5 and IL-13 secreting $CD4^+$ T cells. While DC1 produced IL-1β, IL-6, IL-8, IL-10, IL-12p70 as well as TNF-α, DC2 secreted a distinct panel of effector cytokines ($IL\text{-}1\beta^-$, $IL\text{-}12p70^-$, $TNF\text{-}\alpha^-$, $IL\text{-}10^{low}$, $IL\text{-}6^+$ and $IL\text{-}8^+$). Both DC1 and DC2 over-expressed effector genes (e.g. MX1, NMES1, FSCN1 and IRF4) while down-regulating the expression of regulatory genes (e.g. C1QA, CATC, GILZ, STAB1 and RALDH1) specific for DCreg. Whole genome transcriptome comparison of DC1, DC2 and DCreg revealed extensive differences between these three DC subsets. When compared to non-treated MoDCs, DC1, DC2 and DCreg up-regulated 1617, 1493 and 186 genes and down-regulated 1917, 1882 and 197 genes, respectively. Interestingly, in DC2, 104 and 36 genes were specifically over-expressed and under-expressed, respectively, when compared with DC1 and DCreg.
Specific DC2 markers have been identified, allowing to distinguish such cells from effector DC1 or regulatory DC subsets. Those markers are being tested as follow-up read outs of efficacy for allergen immunotherapy.

LB2.02
Double-stranded RNA of intestinal commensal but not pathogenic bacteria triggers production of protective IFN-β

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The small intestine harbors a substantial number of commensal bacteria and is sporadically invaded by pathogens, but the response to these microorganisms is fundamentally different. We identified a discriminatory sensor using Toll-like receptor 3 (TLR3). Double-stranded RNA (dsRNA) of one major commensal species, lactic acid bacteria (LAB), triggered IFN-β production from dendritic cells (DCs) and protected mice from experimental colitis. Endosomal TLRs are required for this protective mechanism, because Tlr3-/- as well as Unc93b13d mice, in which endosomal TLR3, 7, and 9 are dysfunctional, were not affected by oral administration of LAB in colitis model. While digestion of dsRNA canceled in vivo effects, suggesting TLR3 initiates the anti-inflammatory process, we also found that cooperation of TLR3 and TLR9 maximized IFN-β production in vitro. Compared to LAB, pathogenic bacteria contained less dsRNA and induced much less IFN-β. Moreover, dsRNA was not involved in pathogen-induced IFN-β induction. These results identify TLR3 as a sensor to small intestinal commensal bacteria and contribute to the maintenance of immunological homeostasis.
Collaborators: Daisuke Kaneko, Ikuko Nishimura

LB2.03
Human antigen-specific CD4+CD25+CD134+CD39+ T cells are enriched for regulatory T cells and comprise a substantial proportion of CD4+ T cell antigen recall responses

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Human antigen-specific CD4+ T cells can be detected through the dual expression of CD134 (OX40) and CD25 after 44 hours stimulation with cognate antigen. We show that differential surface expression of CD39 on these cells enables isolation of a CD39+ Treg-enriched cell population. We have demonstrated that the majority of these CD4+CD25+CD134+CD39+ T cells originate from the peripheral memory CD4+CD45RO+CD25hiCD127loCD39+ Treg population. Dose response studies also revealed that the CD4+CD25+CD134+CD39+ Treg-enriched cell population responds to lower doses of antigen than the effector-like CD4+CD25+CD134+CD39− T cells. Across recall responses to a wide range of antigens, these Treg-enriched CD4+CD25+CD134+CD39+ T cells consistently formed a substantial proportion of the CD4+ T cell response. Viable, antigen-specific Treg enriched CD25+CD134+CD39+ T cells were isolated and expanded in vitro as T cell clones, that stably retained their antigen specificity and a Treg phenotype with high Foxp3, CD25, CTLA-4 and CD39 expression. Importantly, these clones could suppress autologous effector T cell proliferation and the magnitude of suppression positively correlated with CD39, Foxp3 and CTLA-4 expression. Collectively, our data show that antigen-specific CD4+CD25+CD134+CD39+ T cells are highly enriched for Tregs, form a surprisingly large component of CD4+ T cell recall responses and maintain a Treg-like phenotype upon in vitro expansion. Identification and isolation of these cells enables the role of Tregs in memory responses to be further defined, a greater understanding of disease pathogenesis and paves the way for developing this methodology as a novel immunotherapy.

LB2.04
Human memory Helios- FOXP3+ Treg encompass induced Treg that express Aiolos and respond to IL-1β by down-regulating their suppressor functions

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FOXP3$^+$ Treg are critical regulators of self-tolerance and immune homeostasis. In mice and humans, two subsets of FOXP3$^+$ Treg have been defined based on their differential expression of Helios, a transcription factor of the Ikaros family. Whereas the origin, specificity and differential function of the two subsets are yet controversial, their characterization has been thus far limited by the absence of surface markers that distinguish them. Here, we show that human memory Helios$^+$ and Helios$^-$ Treg are phenotypically distinct and can be separated *ex vivo* based on their differential expression of IL-1RI, that is restricted to Helios$^-$ Treg, in combination with CCR7. The two populations isolated using this strategy are distinct with respect to the expression of other Ikaros family members. Namely, whereas Eos, that has been reported to mediate FOXP3-dependent gene silencing, is expressed in Helios$^+$ Treg, Aiolos, that is involved in the differentiation of T_H17 and induced Treg, is instead expressed in Helios$^-$ Treg. In addition, whereas both subsets are suppressive *ex vivo*, Helios$^-$ Treg display increased suppressive capacity than Helios$^+$ Treg, but respond to IL-1β by down-regulating their suppressive activity. Together, these data support the concept that human Helios$^-$ memory Treg encompass induced Treg that can readily respond to changes in the environment by modulating their suppressive capacity.

LB2.05
B lymphocytes undergo TLR2-dependent apoptosis upon Shigella infection mediated by the virulence factor IpaD

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Shigella is a gram-negative enteroinvasive bacterium and the causative agent of bacillary dysentery, an acute recto-colitis. Antibody-mediated natural immunity to Shigella requires several episodes of infection to get primed and is short-lasting, suggesting that the B cell response is functionally impaired. Here we show that upon ex vivo infection of human colonic tissue, invasive S. flexneri interacts with and invades B lymphocytes. We observe the induction of a type three secretion apparatus (T3SA)-dependent B cell death in vitro, both in lamina propria B lymphocytes and the human CL-01 B cell line. This cell death and the parallel reduction of the B cell pool can also be observed in an in vivo mouse infection model. Intriguingly, Shigella-induced B cell death does not require bacterial invasion or injection of virulence effectors via the T3SA in vitro. Instead, the virulence factor IpaD triggers mitochondrial B cell apoptosis in the presence of bacterial co-signals that render B lymphocytes prone to die. We provide evidence that IpaD binds to and induces apoptosis via TLR2, a signaling pathway that has thus far only been considered as a mitogenic stimulus for B lymphocytes. Apoptotic B lymphocytes in close contact with Shigella displaying IpaD are also detected in isolated lymphoid follicles of rectal biopsies of naturally-infected individuals. These findings reveal a novel mechanism of T3SA action to induce B cell death by the binding of a virulence factor and reveal an efficient strategy by which entero-invasive pathogens could impair the priming of a protective immune response.

LB2.06
CD14+CD16-, CD14+CD16+ and CD14dimCD16++ monocyte subsets are changed in obesity

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Introduction: Obesity is associated with a low-grade inflammation in which monocytes play an important role. Three subpopulations of monocytes have been described: classical CD14+CD16- (CM), intermediary CD14+CD16+ (IM) and non classical CD14dimCD16++ (NCM) monocytes. We previously showed increased percentages and numbers of IM and NCM in obese subjects that decreased with weight loss. Here we characterize gene expression profile of the monocyte subpopulations in obesity.
Subjects and Methods: The CM, IM and NCM of 7 obese subjects (OB) before and after gastric bypass and 7 lean subjects (C) were sorted by flow cytometry. The expression of genes involved in monocyte functions including migration, adhesion, phagocytosis and cytokines production, was analyzed by Taq Man Low Density Array.
Results: The gene expression of CX3CR1 (fractalkine receptor) and TLR8 (Toll Like Receptor 8) was highly increased in the CM, IM and NCM of the OB group and decreased after surgery. The three subsets displayed different gene expression profiles in the OB group compared to the C group: the NCM expressed high levels of CSF1R (Colony Stimulating Factor 1 receptor), SELPLG (selectin P ligand) and IL1β, the IM were characterized by over-expression of CCR5 (C-C chemokine receptor type 5), TNFα and MCP1 and the CM expressed high levels of CCR2 and CD36.
Conclusion: In obese subjects, the three subpopulations display different gene expression pattern of molecules involved in migration, inflammation and antibody capture. CX3CR1 and TLR8 could be considered as a molecular signature reflecting modified functions of monocytes in obesity.
Support:CAPES,CNRS

Workshops

W1.01 Granulocytes

IL1.01.01
Role of NADPH oxidase-derived reactive oxygen species in human neutrophil IL-1 beta secretion

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Interleukin-1β (IL-1β) is a major pro-inflammatory cytokine synthesized in the cytoplasm as a precursor that has to be proteolytically processed to become biologically active. We determined that in response to LPS and LPS plus ATP, human neutrophil IL-1β processing is dependent on caspase-1 and on elastase and/or proteinase-3. The role of reactive oxygen species (ROS) in IL-1β processing remains still controversial and has not been studied in neutrophils. We found that upon stimulation, NADPH oxidase-deficient neutrophils activated caspase-1 and did not exhibit differences in NALP3 expression as compared to healthy neutrophils, indicating that ROS are neither required for inflammasome activation nor for its priming, as has been reported in macrophages. Strikingly, ROS exerted opposite effects on the processing and secretion of IL-1β; whereas ROS negatively controlled caspase-1 activity, as reported in mononuclear phagocytes, they were found necessary for IL-1β secretion, a role never previously described. The complex ROS-mediated regulation of neutrophil IL-1β secretion might constitute a physiological mechanism to control IL-1β-dependent inflammatory processes where neutrophils play a crucial role.

IL1.01.02
Characterization of cytosolic proliferating cell nuclear antigen (PCNA) in neutrophils: anti-apoptotic role of the monomer

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Neutrophils are deprived of proliferative capacity and have a tightly controlled lifespan to avoid their persistence at the injury site. We have previously described that the proliferating cell nuclear antigen (PCNA), a nuclear factor involved in DNA replication and repair of proliferating cells is a key regulator of neutrophil survival (J Exp Med 2010). The nuclear-to-cytoplasmic relocalization occurring during granulocytic differentiation was dependent on a nuclear export sequence that was exposed only in monomeric PCNA (J Biol Chem 2012). Nuclear PCNA functions are tightly linked to its ring-shaped structure, which allows PCNA to bind to numerous partner proteins to orchestrate DNA-related processes. We tested the hypothesis that monomeric PCNA could have a biological role in neutrophils. Using a combination of cross-linking and gel filtration experiments, both trimeric and monomeric PCNA were detected in neutrophil cytosol. The promyelocytic cell line PLB985 stably transfected to express the monomeric PCNAY114A mutant showed an enhancement of granulocytic differentiation as evidenced by an increased percentage of CD11b- and gp91phox-positive cells compared with the wild type trimeric PCNA. However, both trimeric and monomeric PCNA displayed a similar anti-apoptotic activity following treatment with gliotoxin or TRAIL compared to control PLB985. The molecular basis through which cytoplasmic PCNA exerts its anti-apoptotic activity in mature neutrophils may, at least in part, be independent of the trimeric conformation. Through an intimate comprehension of the functions of cytosolic PCNA, novel pathways regulating neutrophil survival can be unraveled and innovative agents can be developed to dampen inflammation where it proves detrimental.

W1.01.01
Chromatin configurations correlate with the differential capacity of human neutrophils and monocytes to express IL-6 in response to LPS

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IL-6 is a pleiotropic cytokine with a broad range of pro- and anti-inflammatory functions that is produced by appropriately stimulated monocytes. However, whether human neutrophils do so, remains still controversial in the literature. To clarify such issue, we explored with additional studies at epigenetic level, whether human neutrophils express IL-6 in response to lipopolysaccharide (LPS), which in autologous monocytes represents a major IL-6 inducer.
We found that highly purified (> 99,7 %) neutrophils stimulated at 5 million/ml with 100 ng/ml ultrapure LPS for 24 h released no or very negligible (< 20 pg/ml) amounts of IL-6, unlike autologous monocytes (50 ng/ml/2,5 million/ml). Neutrophils neither expressed IL-6 mRNA or IL-6 primary transcripts at time points (up to 6 h) in which they were maximally produced in autologous monocytes. Consistent with the lack of any IL-6 transcriptional activity, no increase of IL-6 promoter activity could be observed in neutrophils incubated with LPS for 4 h, as revealed by formaldehyde-assisted isolation of regulatory elements (FAIRE). In contrast, a 10-fold increase of nucleosome free DNA levels was detected at the IL-6 promoter of autologous monocytes treated as neutrophils. Furthermore, by chromatin immunprecipitation (ChIP) no H4Ac (an epigenetic marker of transcriptionally active chromatin) was detected at the IL-6 locus (spanning a 30 kb range) of neutrophils incubated with LPS for up to 5 h, in large contrast to monocytes.
Taken together, these results suggest that the differential capacity of human neutrophils and monocytes to express IL-6 upon LPS activation is likely controlled by epigenetic mechanisms.

W1.01.02
Influence of CD137L signalling in myelopoiesis during acute and chronic inflammation

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CD137 is a costimulatory molecule expressed on activated T cells. The signalling of CD137 into T cells upon ligation by its ligand, CD137L expressed on antigen presenting cells (APC), can potently enhance the activation of T cells. Reversibly CD137 can also induce signalling into APC via CD137L to promote activation and proliferation. The aim of this project is to investigate the role of CD137L on myelopoiesis under inflammatory condition. Our data show that in vivo CD137 represents a novel and potent growth and differentiating factor for murine myeloid cells during inflammation. In an acute peritonitis model there is a significantly larger increase of proliferating myeloid cells in the bone marrow of wild type (WT) than of CD137-/- mice. Further investigations revealed that the sources of CD137, which drives the enhanced myelopoiesis during inflammation, are CD4+ T cells. In vitro, WT activated CD4+ T cells induced higher expression of myeloid marker as well as enhanced proliferation of lineage negative progenitor cells than CD137-/- activated CD4 T cells. Interestingly, in an ageing model where mice experienced chronic inflammation, WT mice also have enhanced myelopoiesis and increased number of CD137+CD4+ T cells compared to CD137-/- mice, further strengthening the link between CD137 and myelopoiesis during inflammation. It is hypothesized that when inflammation is induced, T cells are activated and home to the bone marrow and interact with CD137L-expressing progenitor cells and myeloid cells. Future experiments will focus on exploiting the potential of CD137-CD137L interaction in monitoring myelopoiesis during different pathological conditions.

W1.01.03
Neutrophils have a negative impact on DC activation and disease progression following Leishmania major infection

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Neutrophils arrive rapidly and massively at the site of *Leishmania major* inoculation in the skin. They participate in shaping the immune response by the secretion of numerous factors including cytokines and chemokines and by interactions with antigen-presenting cells such as dendritic cells (DC). More specifically, neutrophils participate in the attraction of DCs to the site of infection and can either promote or inhibit their activation. In this study, we investigated the impact of neutrophils on the activation of DCs during the first days following *L. major* inoculation at the site of infection and the draining lymph node (dLN) in *Leishmania*-susceptible BALB/c mice and evaluated the consequences on disease progression. Transient depletion of neutrophils led to increased activation of DCs in the dLN three days post *L. major* infection whereas no detectable differences in DC activation were found at the site of infection. Moreover, mice depleted of neutrophils developed significantly smaller lesions and harbored decreased numbers of parasites in infected ears six weeks post *L. major* infection. Collectively, these data indicate that neutrophils have a negative impact on DC activation and disease progression in BALB/c mice during the onset of *L. major* infection.

W1.01.04
Novel neutrophil-T-cell interactions: Hormones and the regulation of T-cell responses

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Novel cellular cross-talk within the immune system is gaining new ground. Specifically, the interaction between cells of the innate and adaptive immune systems appear to have important implications in the maintenance of tolerance.
We have reported that the pre-menopausal estrogen, estradiol, induces a protective, anti-inflammatory phenotype in human neutrophils, modulating adhesion molecule expression and reducing their recruitment to sites of inflammation, *via* non-genomic modulation of the anti-inflammatory protein, Annexin-A1.
In the present study, we provide new evidence that treatment of neutrophils with the pregnancy hormones estriol (E3) and progesterone (P4) can induce a regulatory-like phenotype in T-cells. Briefly, neutrophils were treated with 100ng/ml of E3 and P4 for 30 min, washed and co-cultured with autologous T-cells (stimulated with anti-CD3/anti-CD28) for 5 days. We found that neutrophils treated with E3 and P4 did *not* suppress T-cell proliferation. Closer analyses revealed T-cells co-incubated with E3+P4-treated neutrophils (but not with neutrophils without hormone treatment), released high levels of IL-2 and IL-10, and expressed the Treg transcription factor, FOXP3. These IL-10-producing $FOXP3^{+ve}$ T-cells were also functionally *suppressive*.
Taken together, we provide evidence for a novel cellular cross-talk, where neutrophils, in the presence of pregnancy hormones E3 and P4, induce and expand a functionally suppressive regulatory T-cell population. These results could have important implications in our understanding of maternal-foetal tolerance during pregnancy.
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W1.01.05
Neutrophil Extracellular Trap (NET) formation in the pathogenesis of *Staphylococcus aureus* sepsis

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Sepsis remains a serious condition, associated with multiple organ dysfunction syndrome, often resulting in mortality. Neutrophils are one of the first cells which detect systemic infection as they continuously patrol the blood. During sepsis, neutrophils are often immobilized in the lungs and liver for yet unclear reasons. One possibility for this sequestration is to allow neutrophils to form neutrophil extracellular traps (NETs), composed of extracellular DNA, histones and granular proteins, including proteases. Although NETs are well characterized *in vitro*, their *in vivo* characteristics remain unclear. NETs function to trap and immobilize pathogens and subsequently contribute either directly, or indirectly to pathogen elimination. Persistent presence of NETs in vasculature, however, might also lead to bystander cell damage due to prolonged exposure to anti-microbial and proteolytically active NET components. We report here that systemic infection with *Staphylococcus aureus* led to profound neutrophil infiltration in the liver and strong NET formation. These processes occur within the first few hours and are observed, in real time, using spinning-disk confocal intravital microscopy. Interestingly, some NETs persisted in vasculature for several hours and this was associated with profound damage to the liver, a phenomenon that could be reduced by systemic DNase treatment. Importantly, the liver damage was not exclusively NET-dependent as the observed damage was also diminished in mice injected with transgenic *S. aureus* not encoding *a*-toxin. Thus we can conclude that NET formation during *S. aureus* infection partially contributes to liver damage. EK is supported by FP7-PEOPLE-2010-IOF (grant No.273340) from EU.

W1.01.06
Activated basophils form extracellular DNA traps able to kill bacteria

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Background: Basophils represent a small population of blood leukocytes constituting less than 1% of total leukocytes. They have been primarily associated with the pathophysiology of allergic diseases and parasitic infections. Basophils release granule proteins and some Th2 cytokines (IL-4/IL-13) following immunological and non-immunological activation; such secretion can be enhanced upon priming with IL-3. Moreover, basophils can bind various bacteria even in the absence of opsonizing antibodies.
Objective: To investigate whether basophils are able to form extracellular DNA traps able to kill bacteria.
Results: Basophils were able to form extracellular DNA traps containing mitochondrial DNA and the granule protein basogranulin following immunological and non-immunological activation in the absence of cell death. Although basophils have been described to express no functional NADPH oxidase, the generation of reactive oxygen species (ROS) was required for the formation of these extracellular DNA-containing structures. While basophils were unable to kill bacteria intracellularly owing to their inability to perform phagocytosis, basophil extracellular traps (BETs) exhibited bactericidal activity *in vitro*. BETs were present in inflammatory human and mouse skin.
Conclusions: Basophils mediate IgE-mediated and -independent antibacterial immune responses involving extracellular DNA traps.

W1.02 Mast cells

IL1.02.01
Mast cells and regulatory cells: a dialogue that can last a lifetime

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Mast cells (MC) have been considered for long time only for their role in the allergy, and for the patients the most hated cells in the body. In the past, some researchers have also proposed eradicating them to avoid suffering. However, MCs reputation has been turned around with progress in our understanding of their role in immune response. In fact, we have learned that the MCs play many roles as sentinels that regulate the immune response on invading bacteria. Moreover, they have a fundamental role in link the innate with adaptive immune system. Our recent studies and literature data on MC interactions with other cells of immune system point to their importance in the cross-talk with regulatory cells, mediating the maintenance of the regulatory T cell-dependent peripheral tolerance during skin allografts. Additionally, they have a continuous dialogue, influencing the development and activity of other regulatory cells too, as the recently discovered subset of B cells, the IL10-competent B cells. However, a membrane-studded of receptors do not limit the MCs to have interactions with only few cellular types, but allow those cells to enlist all the cell of the immune system and to be present in normal and pathological tissue with a different behavior. This capability added to the power of thousands of compounds that the MCs can release is useful to coordinate the activity of immune system but can be fickle allies too.

IL1.02.02
Basophils: new key players in lung and intestinal Th2 or Th17-associated chronic inflammatory disorders.

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Basophils, a rare type of cells, are typically implicated in Th2-associated diseases through IgE- dependent mediators/cytokines release and amplification of dendritic cell-induced naïve T cell development into Th2 effectors. Here, we report that basophils accumulated in the lungs of mice with allergic asthma. Passive transfer of highly purified pulmonary basophils to sensitized but non asthmatic mice induced airway inflammation, lung production of type 2 cytokines and unexpectedly, IL-17 release. Murine pulmonary as well as human circulating basophils directly acted on autologous memory CD4 T cells to enhance *in vitro* Th2, Th17 and Th17/Th1 memory T cell responses. Accordingly, IL-3 or IL-33-activated basophils amplified IL-4 or IL-17 release in effector memory (T_{EM}), central memory (T_{CM}) as well as in $CRTh2^+$ and $CCR6^+$ CD4 T cells, respectively. Mechanistic analysis revealed that enhancing effect of cytokine production by basophils did not require the presence of Ag, APC or TCR triggering and was partially mediated by histamine via H2 and H4 histamine receptors. We next demonstrate that basophils were not only detected in lungs of cystic fibrosis patients but also accumulated at inflamed sites in intestinal tissues of patients with the Crohn's disease and Ulcerative Colitis. Thus, we propose that basophils are key innate players in chronic inflammatory disorders beyond Th2 and IgE-associated allergic diseases.
This work was supported by CCFC, CIHR and Allergen funds.

W1.02.01
Seven color flow cytometry analyses of Allergin-1 expression and function on human primary bronchoalveolar and nasal mast cells

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We have recently reported a novel inhibitory immunoglobulin-like receptor, Allergin-1, expressed on mast cell (MC) and showed that mouse Allergin-1 inhibited FcεRI-mediated signal and degranulation from MC in vitro and in vivo (Hitomi, et al, *Nat Immunol.* 2010). Unlike mouse Allergin-1, human Allergin-1 consists of three splicing isoforms, including Allergin-1L, Allergin-1S1, and Allergin-1S2. However, the expression and function of each Allergin-1 on human primary MCs have not been determined due to a hard acquisition of primary human MC. To overcome this problem, we established an analytical method of very small number of human MCs in broncoalveolar lavage fluid (BAL-MCs) and nasal scratching sample (N-MCs) by using seven color flow cytometry. We defined MCs as $PI^-CD45^+Lin^-c\text{-}Kit^+Fc\varepsilon RI\alpha^+$ cells and characterized BAL-MCs and N-MCs by using two monoclonal antibodies (mAb) specific to Allergin-1S1 or Allergin-1S2. MCs accounted for 0.164 ± 0.049% (n=21) in the BAL fluid cells of patients with variable respiratory diseases and 0.535 ± 0.112% (n=9) in nasal scratching sample from allergic rhinitis patients. We also demonstrate that BAL-MCs and N-MCs preferentially expressed Allergin-1S1, but not Allergin-1L and Allergin-1S2. To examine the function of Allergin-1S1 on BAL-MCs, BAL-MCs were sensitized with TNP-specific IgE and then stimulated with either TNP-conjugated control mAb or TNP-conjugated anti-Allergin-1S1 mAb. The proportion of $CD107a^+$ cells in the BAL-MCs were analyzed by flow cytometry. Comparing to TNP-conjugated control mAb, TNP-conjugated anti-Allergin-1S1 mAb significantly decreased the $CD107a^+$ population. These results first demonstrate that human bronchial MCs express Allergin-1S1, which inhibits FcεRI-mediated signal and degranulation from MC.

W1.02.02
Soluble CD14 is necessary for lipopolysaccharide-mediated signalling in human intestinal mast cells

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Mast cells are known to be involved in pathogen recognition and host immune defence. At the gastrointestinal barrier, mast cells are confronted with a huge microbiota and the challenge to tolerate commensal microorganisms and to recognize and eliminate potential pathogens. Here, we examined the role of CD14 in lipopolysaccharide (LPS) mediated signalling in human intestinal mast cells (hiMC). HiMC were isolated from intestinal tissue and cultured with SCF and IL-4. Using cytometry, TLR-4, but not CD14, was detectable on hiMC. Consistently, triggering with LPS alone failed to stimulate degranulation or cytokine expression in hiMC. To investigate the relevance of CD14 in LPS-mediated signalling, hiMC were treated with LPS [1 µg/ml] alone or combined with 1 ng/ml - 1µg /ml soluble CD14 (sCD14). We found a dose dependent induction of the proinflammatory cytokines/chemokines IL-1β, IL-6, and IL-8 measured by real-time RT-PCR. Noteworthy, the chemokines MCP-1, MIP-1α, and -1β were not expressed in response to LPS/sCD14 stimulation. Moreover, an increased cytosolic content of IL-8 and IL-1β and the release of IL-8 was measured by ELISA upon combined activation of hiMC with LPS and sCD14. In summary, hiMC are not sensitive towards LPS alone due to the missing CD14 receptor. In contrast, LPS triggering in the presence of sCD14 results in a selective expression and release of proinflammatory cytokines. In vivo, sCD14 may be derived by other LPS provoked cells and consequently activate hiMC at the gastrointestinal barrier.

W1.02.03
Siglec-7 is an Inhbitory receptor on Human mast cells

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Allergic inflammation (AI) is a multiphase process which is involved in pathological disorders such as asthma and allergic rhinitis. AI is typically initiated when IgE-bearing tissue dwelling mast cells (MC) bind to an allergen throw the FcεRI -bound IgE. This leads to cross-linking of the FcεRI and activates the MC to release their mediators and to orchestrate the immediate, early phase of the allergic response. The subsequent recruitment and activation of inflammatory cells, particularly eosinophils (Eos) causes a late phase allergic response which often results in the chronic perpetuation of the

inflammatory reaction. Due to their key role in the initiation of AI a lot of research is focus on understanding the mechanism controlling MC and especially on the ones that inhibit them. Siglec-7 belongs to a family of lectins that recognize sialic acid. We have found that Siglec-7 is expressed in MC as a 75 kDa protein and can be found on MC grown from bone marrow derived precursors (CBMC) and on the MC-lines: HMC-1 and LAD-2. Siglec-7 activation, by cross-linking it with Abs, can only inhibit FcεRI-induced MC activation when Siglec-7 is coupled to FcεRI. Siglec-7 activation and coupling to FcεRI inhibited the release of mediators from all three major groups of soluble mediators released by MC: arachidonic acid metabolites, newly synthesized and preformed mediators. Activation of Siglec-7 leads to phosphorylation of tyrosine residues on Siglec-7 and associated molecules. In conclusion, the discovery of new inhibitory receptor on MC will enable the development of new treatments to allergy.

W1.02.04
The S1P/S1PR$_2$ axis controls T-cell infiltration in a mast cell-dependent mouse model of acute pulmonary inflammation

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Mast cells promote allergic inflammation by first secreting vasoactive mediators, including histamine and sphingosine-1-phosphate (S1P). However, the pathophysiology of acute allergic inflammation and its molecular mechanisms still remain unclear. We show that preventing signaling through the type 2 receptor for S1P (S1PR2) on mast cells by neutralizing its ligand S1P with a highly selective antibody Sphingomab^{TM} improved many inflammatory features associated with antigen (Ag) exposure, including circulating levels of histamine and chemokines and lung mast cell degranulation. Moreover, kinetic studies revealed that T cells and a few macrophages were recruited very early around pulmonary blood vessels within minutes after Ag challenge. This unexpected early T cell infiltration was accompanied with increased serum levels of RANTES/CCL-5 and suppressed in the absence of mast cells, S1PR2 or intact S1PR2 signaling. Optimal RANTES/CCL5 secretion by IgE/Ag-activated mast cells also required functional S1PR2. We propose that perturbing S1PR2 signaling in mast cells might constitute an effective therapeutic strategy to prevent early T cell infiltration associated with allergic inflammation and therefore its persistency. Supported by NIH grants R01 AI50094 to SS and U19 AI077435 to SS and JJR and K01AR053186 and R01 AI095494 to CAO.

W1.02.05
IgE-dependent mast cell hyperplasya in the intestine of cystic fibrosis mouse model needs KCNN4 channel activity

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Cystic fibrosis (CF) primary dysfunction in epithelial transport concurs with deregulation of the immune system in human patients and animal models. We observed that when the KCNN4 channel is genetically silenced in the CF mouse lethality is drastically reduced with no improvement of intestinal function. The KCNN4 channel is broadly expressed including the immune system. We aim to test if KCNN4 channel inhibition is involved in immune system dysfunctions associated with CF.
CF animals presented a 4-fold increase of intestinal mast cells, the value obtained in the KCNN4-null/CF mice was lower and comparable to controls. IgE-induced migration was 2-fold increased in control and CF mast cells compared with non-stimulated cells. Migration was blocked when the KCNN4 specific inhibitor TRAM-34 was used. Migration was completely impaired in the KCNN4-null and KCNN4-null/CF cells. Serum IgE level was increased in the CF as in the KCNN4-null/CF mice.
Inhibition of KCNN4 blocks migration in control and CF mast cells. Hyperplasia of mast cells in the CF mice is localized in the intestinal tissue and can be due to increased migration induced by the higher levels of IgE. Mast cell reduction on intestine of KCNN4-null/CF mice is due to the impairment in migration of mast cells and not to reduced IgE levels. The origin of IgE in the CF mice remains a matter of investigation. KCNN4 inhibtion can be a useful pharmacological target on inflammatory CF disease where mast cells can act as enhancers of inflammation.
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W1.02.06
Mast cells determine the adaptive immune response in contact hypersensitivity

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Contact allergens trigger a rapid inflammatory response of skin resident innate immune cells. By these inherent adjuvant-properties, sensitizing compounds elicit allergic-specific T cell responses. However, the mechanisms by which organic sensitizers trigger innate immunity are incompletely understood. Mast cells are considered important sentinel cells of innate immunity at body surfaces. Conflicting results were published on the role of mast cells in contact allergy based on mast cell-deficient kit mutant mice. We used our novel Cre/loxP-based mouse models of mast cell deficiency and mast cell-specific gene inactivation to study mast cell functions in contact allergy. We found that in the absence of mast cells, allergic responses to organic haptens were massively reduced. Mast cell-deficiency abrogated the early innate response to contact allergens as determined by quantification of vasodilatation, vessel permeability and neutrophil influx. Importantly, we demonstrated that DC migration from sensitized skin to lymph nodes and expansion of T lymphocytes was reduced in the absence of mast cells, and that the CD8$^+$ T cell response to secondary allergen encounter was impaired in mast cell-depleted mice. To address mechanisms of the mast cell effect on contact hypersensitivity, we generated mice lacking TNF selectively in mast cells. Also these animals featured reduced CD8$^+$ T cell-mediated inflammatory responses of allergen-challenged skin. However, we found that mast cell-derived TNF selectively supports migration and maturation of CD8$^+$ DCs responsible for CD8$^+$ T cell priming. In summary, we demonstrate that mast cell responses to contact allergens are essential for the induction of the pathogenic T cell response.

W1.03 NK cells and NK receptors

IL1.03.01
Cross-talk bethween human natural killer cells and macrophages

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We investigate the molecular pathways involved in the interaction between NK cells and unpolarized or polarized macrophages. M0 and M2 are unable to trigger NK cell function. Following exposure to microbial products (LPS or BCG), M1 polarizing M0 and M2 (but not endotoxin tolerant macrophages), induce strong activation of resting NK cells resulting in CD69, CD25 and CCR7 expression, IFN-γ production and acquisition of anti-tumor cytotoxicity. NK cells also enhance the “editing” capability becoming capable of killing iDC, M0 and M2, which express low, non protective amounts of HLA-I. NK cell activation mostly depends on the interaction of NKp46, DNAM-1 and 2B4 receptors (on NK) with their ligands on macrophages, and on the

IL-18R (on NK) that binds small amounts of IL-18 released by M0 and M2 upon TLR engagement (PNAS 2010). We also showed that M-CSF induces the expression of mIL-18 membrane-bound form of IL-18 (mIL-18) in a subset of M-CSF primed macrophages differentiating from both CD16- and CD16+ monocytes. Inhibition of caspase-1 reduces mIL-18 expression suggesting the requirement of an assembled inflammasome for IL-18 surface expression. While M2 polarization does not modify mIL-18 expression, M1 polarization induces the protease(s)-mediated shedding of mIL-18. Soluble IL-18 (sIL-18) acts in close cell-to-cell contact and is crucial for CCR7 expression and IFN-γ release by resting NK cells (EJI 2012). Recently we analyzed the functional outcome of the interaction between NK cells and Tumor-associated macrophages (TAM) from ascites of ovarian cancer patients.

IL1.03.02
Functionally distinct subsets of human NK cells

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Natural killer cells not only exert cytotoxic activity against tumor cells or infected cells, but also act to regulate the function of other immune cells by secretion of cytokines and chemokines. The major functional properties of NK cells are cytotoxicity and cytokine production. NK cells express a wide range of activating receptors, as well as inhibitory receptors that regulate NK cell activation and tolerance, and their functions are governed by a balance between activating messages transmitted by their activating receptors and inhibitory signals transmitted by their inhibitory receptors. Similar to the Th1 and Th2 subsets of $CD4^+$ and also $CD8^+$ T cells, NK cells are also divided into NK1 and NK2 subpopulations according to the profile of cytokine secretion. NK cells that are exposed to IL-12 *in vitro* (NK1) produce predominantly IFN-γ, whereas NK cells stimulated with IL-4 (NK2) produce IL-5 and IL-13. Similar to suppression of both cytokine production and antigen-specific proliferation of Th1 and Th2 cells by IL-10, IL-10-secreting NK cells suppress both allergen-stimulated T cells and PPD-stimulated T cell proliferation, whereas IFN-γ secreting NK cells did not show any suppression. Findings suggest the *in vivo* existence of a regulatory NK cell subset, which indeed may play an immune regulatory and suppressor role.

W1.03.01
NK cell subsets isolated from human thymus differ from peripheral blood NK cells in their phenotype and their cytotoxic and cytokine secreting capabilities

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Little is known about the functional role of human NK subpopulations in different lymphoid organs beyond blood. We therefore aim to characterize the phenotypical and functional profile of human NK cell subsets from different organs with special interest in thymic NK cells.
Human NK cells can be classified according to the expression of CD56 and CD16 into immunoregulatory and cytotoxic NK cells. In this study, we observed that especially the composition of NK subpopulations in the thymus differs from peripheral blood of both adult and children. Thymic NK cells have not only a lower CD56-expression but also a different ratio of $CD16^{high}$ / $CD16^{dim}$ NK cells. Extensive phenotypical analysis revealed significant alterations in expression patterns of KIRs, NCRs and other maturation or differentiation markers on thymic NK cells. Furthermore, functional assays revealed important differences of $CD16^{high}$ and $CD16^{dim}$ thymic NK cells. Whereas thymic $CD16^{high}$ NK cells showed high killing capacity of K562 tumor cells, $CD16^{dim}$ NK cells required an increased stimulation period with IL-2 to gain cytotoxic function. Dependent on IL-2 both subsets could reach the killing capability of unstimulated adult peripheral blood NK cells. Remarkably, only $CD16^{dim}$ NK cells were able to produce few amounts of IFN-γ upon stimulation with IL-2.

In summary, this comparative study of NK cell subsets provides important insight on the development and function of NK cells that will be of great value for the optimization of cellular therapy.

W1.03.02
Neuraminidase-mediated, NKp46-dependent, immune evasion mechanism of influenza viruses

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Natural killer (NK) cells play an essential role in the defense against influenza virus infections, one of the deadliest respiratory viruses known today. The natural killer receptor (NKp46), expressed by NK cells is critical in controlling influenza virus infections, as influenza-virus infected cells are eliminated through the recognition of the viral hemagglutinin (HA) protein by NKp46. Here we describe a novel immune evasion mechanism of influenza viruses that is mediated by the viral neuraminidase (NA) protein. We show, by using various NA blockers, that NA removes sialic acid residues from NKp46 and that this leads to reduced recognition of HA. Furthermore, we provide in vivo and in vitro evidence for the existence of this novel NA-mediated, NKp46-dependent immune evasion mechanism and demonstrate that NA inhibitors, which are commonly used for the treatment of influenza infections, are useful not only as blockers of virus budding but also as boosters of NKp46 recognition.

W1.03.03
NK cells from malignant pleural effusions are not anergic but produce cytokines and display strong anti-tumor activity on short-term IL-2 activation

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NK cells are a major component of the innate immunity and exert a potent anti-tumor effect both in vitro and in vivo. However, tumor infiltrating natural killer (NK) cells have been shown to display severely impaired functional properties. In this study, we analyzed NK cells isolated from pleural effusions (PE) in patients with primary or metastatic tumors of different origin (including mesotheliomas, lung carcinomas, breast, colon gastric and liver carcinomas). Freshly isolated PE-NK cells displayed a CD56bright phenotype and expressed normal levels of both activating receptors and HLA-Class I-specific inhibitory receptors. In addition, they rapidly released large amounts of IFN-γ and TNF-α upon stimulation. After short- or long-term culture in IL-2, they acquired a potent cytolytic activity against both allogenic and autologous tumor cells. Tumor cell lysis was primarily mediated by NKG2D and by NKp30 and, in part, by NKp46 and DNAM-1, in agreement with the expression of the corresponding ligands on tumor cells. The finding that PE-NK cells are not functionally impaired and can efficiently kill tumor cells upon IL-2 activation may offer an important clue for novel approaches in tumor immunotherapy.

W1.03.04
Unique CD56bright CD16+ NK cells infiltrate regional metastatic Lymph Nodes from stage III melanoma patients

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Over the past decades, the incidence of melanoma has been constantly increasing. Malignant melanoma has a high metastatic potential with limited treatment options. There are numerous arguments indicating that melanomas are immunogeneic and that the immune system can control the tumor growth in certain conditions.

We focus our interest on Natural Killer (NK) cells, potent cytotoxic effectors that control metastases in several murine tumor models.
We have investigated the ex vivo phenotype and function of NK cells infiltrating 21 metastatic lymph nodes (M-LN) from 16 stage III B/C melanoma patients. The tumor burden in the samples was assessed by the percentages of CD45- cells in the LN suspensions. Interestingly, the expression of NKp30 and NKG2D correlate with the proportions of CD45- cells (likely melanoma cells) that invaded the M-LN. Moreover, among M-LN NK cells, we have identified a subset of CD56brightCD16+ cells that express high level of activating NK receptors. Our results showed that M-LN NK cells required PMA/Iono to degranulate and produce IFNγ ex vivo, and the proportion of CD107a was inversely correlated to the percentage of CD45- cells. Immunoselected NK cells from M-LN and donor LN were activated for 6 days with IL-2 or IL-15. These cytokine activated NK cells efficiently and rapidly lysed metastatic melanoma cell lines.
The presence of mature CD56brightCD16+NCR+NKG2D+ NK cells subset in M-LN from melanoma patients and their high anti-tumor potential after cytokine activation are strong arguments for NK cell based therapy in patients with positive sentinel lymph node.

W1.03.05
NK cells targeting of human and mouse Cancer Initiating Cells

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Tumor cell populations have been recently proposed to be composed by two compartments: cancer-initiating cells (CICs) characterized by a slow and asymmetrical growth and the differentiated cancer cells with a fast and symmetrical growth. CICs play a crucial role in tumor recurrence. The resistance of CICs to drugs and irradiation often allows them to survive traditional therapy. Natural Killer (NK) cells are potent cytotoxic lymphocytes that can recognize tumor cells in human and mice.
Human: The NK cell recognition of tumor target cells derived from the two cancer cell compartments of human colon adenocarcinoma lesions. Our data demonstrate that freshly purified human allogeneic NK cells can recognize and kill Colorectal Carcinoma CICs while the tumors either autologous or allogeneic is less susceptible to NK cells. This difference in the NK cell susceptibility correlates with higher expression on CICs of ligands for NKp30 and NKp44 in the NCR group of activating NK receptors. In contrast CICs express lower levels of MHC class I, known to inhibit NK recognition, on their surface than the related tumor cells.
Mouse: The NK cell recognition of tumor target cells derived from the two cancer cell compartments of murine mammary carcinoma. Our data demonstrate so far in vitro that NK cells purified from C57/BL6 and Balb/c mice, selectively recognize the CICs derived from an epithelial murine ErbB-2+ positive tumor cell line, named TUBO.
This study strengthens the idea that biology based therapy harnessing NK cells could be an attractive opportunity in solid tumors.

W1.03.06
Novel mouse models reveal how expression of NKG2D and its ligands on various cell subsets affects immune responses

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NKG2D is an activating receptor expressed by all Natural Killer (NK) cells, a subset of T cells, and activated macrophages. NKG2D and its ligands, which are expressed on stressed or damaged cells, play important roles during viral infections, cancers, and autoimmune diseases, both in mouse and human. While NKG2D is well studied in NK cells, its function on other cells remains unclear, particularly in vivo. In this study, we examine the consequences of cell-specific ablation of NKG2D, or tissue-specific expression of its ligand Rae-1, on innate and adaptive immunity, using two novel mouse models. By crossing Cre expressing strains to our two newly generated mice bearing a floxed allele of NKG2D, or a Rosa26-LSL-Rae-1 transgene, we obtain conditional knockouts of NKG2D, or mice that express Rae-1 in a cell-specific manner. We are now able to dissect the contribution of each cell subset expressing NKG2D in models of viral infection, tumor rejection, and autoimmunity. In addition, we observed that NK cells chronically interact with myeloid cells in vivo, since expression of Rae-1 on these subsets, but not others, leads to down-modulation of NKG2D expression and impaired NKG2D-dependent functions. Data from these two new mouse models provide a better understanding of the role of NKG2D by defining its impact on different immune cell functions, and by mimicking physiopathological situations of patients in which a human counterpart of Rae-1 is expressed on various tissues.

W1.03.07
Regulation of the activating NKp30 ligand B7-H6 in tumor cells

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Natural Killer (NK) cells are innate immune cells sensing and eliminating stressed cells by an array of specialized cell surface receptors. Stressed, transformed or infected cells downregulate inhibitory ligands and upregulate ligands for activating NK cell receptors, resulting in target cell killing. The natural cytotoxicity receptor NKp30 plays an important role in the induction of NK cell responses against tumor cells. Recently, the ligand for NKp30 on the surface of tumor cells has been identified to be the novel B7-family member B7-H6. Due to the lack of B7-H6-specific monoclonal antibodies (mAbs), the expression pattern of B7-H6 has so far only poorly been explored. The regulation of B7-H6 surface expression on tumor cells also remains unknown.
Here, we describe two novel B7-H6-specific mAbs generated in our laboratory. These mAbs specifically bind recombinant B7-H6 in ELISA and B7-H6 expressed on the surface of B7-H6-positive cells in flow cytometry.
Using these mAbs, we demonstrate that B7-H6 is expressed on the surface of various tumor cell lines but not on healthy PBMCs and keratinocytes. B7-H6 regulation was studied by treating tumor cell lines with different reagents. The treatments with the strongest effect on B7-H6 surface expression were further investigated for the mechanisms involved. Finally, the consequences of B7-H6 modulation on NK cell effector functions were assessed.Our findings show that certain treatments have the capability to modulate B7-H6 expression on the surface of tumor cells which has important implications for the activation of NK cells

W1.03.08
Human M1 macrophages activate resting NK cell antitumoral activities through IL-15/IL-15RA transpresentation

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Natural killer (NK) cells are important effectors of innate immune responses, providing cellular immunity against tumor-transformed and virally-infected cells. Although NK cell-DC crosstalk is well established, information on NK cells interactions with macrophages is scanty.
Autologous human NK cells and monocyte-derived macrophages were obtained from buffy coats of healthy donors. Macrophages were polarized into M0, M1, M2 and Tumor Associated Macrophages (TAMs). The influence of polarized macrophages on NK cell anti-tumoral activities was analyzed treating NK cells with macrophage-conditioned media or performing direct NK cell-macrophage co-culture. NK cell activating receptors and degranulation levels were evaluated by FACS, IFNγ production was quantified by RT-PCR and ELISA.
Among the panel of activating receptors we evaluated, resting NK cells treated with M1-conditioned medium upregulated specifically

NKG2D, NKp44 and CD69. Additionally, they expressed enhanced levels of IFNγ transcript and released high amount of IFNγ. This effect was partly abolished by neutralizing IL-15/IL-15Rα. Moreover, M1-treated NK cells killed more efficiently the erythroleukemia cell line K562 in a NKG2D-dependent but IL-15/IL-15Rα-independent fashion. Direct co-culture experiments showed that M1-primed NK cells secreted high amounts of IFNγ. They further displayed higher cytotoxic activities towards K562, which was decreased by NKG2D neutralization and totally impaired by further blocking IL-15Rα.
In conclusion, we demonstrate in a human model an important role and a dual effect of IL-15/IL-15Rα complex in the crosstalk between NK cells and M1 macrophages: the soluble complex induces NK cell IFNγ secretion, whereas membrane trans-presentation enhances NK cell cytotoxicity towards tumor cells.

W1.03.09
Natural killer cell distribution and trafficking in normal and neoplastic human tissues

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Although natural killer (NK) cells have been detected in various lymphoid and non-lymphoid organs in mice, limited data are available regarding the distribution and trafficking of NK cells among human solid organs. To increase the current knowledge regarding the presence of NK cells in both healthy and pathological conditions, we analyzed a wide array of normal as well as neoplastic tissues derived from different human compartments. We found that NK cells were broadly distributed in most solid tissues, although, in general, the amount of NK cells significantly varied depending on the tissue/organ analyzed. Interestingly, NK cell distribution appeared to be subset-specific since some tissues were preferentially populated by CD56bright perforinlow NK cells while, others by the CD56dim perforinhigh cytotoxic counterpart. Accordingly, the gene expression level of chemotactic factors in the tissues analyzed matched the expression of chemokine receptors in the NK cell subsets infiltrating the tissues. Remarkably, the chemokine expression pattern of many human tissues resulted modified after neoplastic transformation. As a result, the relative proportion of the NK cell subsets infiltrating the tissues was different, in most cases toward a tumor- infiltrating NK cell population enriched in CD56bright perforinlow cells. Besides solid tissues, a CD3negCD56bright NKp46+ cell population was also detected in seroma fluids, which represents an accrual of human afferent lymph, suggesting that NK cells might leave peripheral solid tissues. Overall, our results support the notion that distinct subsets of NK cells populate, and recirculate through, the majority of human peripheral tissues driven by organ-specific chemokine expression patterns.

W1.03.10
The viral KSHV chemokine vMIP-II inhibits the migration of naive and activated human NK cells by antagonizing two distinct chemokine receptors

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NK cells are cells of the innate immune system able to rapidly kill virus-infected and tumor cells. Two NK cell populations are found in the blood; the majority (90%) expresses the CD56 receptor in intermediate levels (CD56Dim) while the remaining 10% express it in high levels (CD56Bright). NK cells are resident in some tissues and traffic to various infected organs through the usage of different chemokines and chemokine receptors. KSHV, like other viruses of the herpes family, is master of immune evasion having numerous sophisticated and versatile strategies to escape the attack of immune cells such as NK cells. Here, we investigate the role of the KSHV derived cytokine (vIL-6) and chemokines (vMIP-I, vMIP-II, vMIP-III), in immune modulation. Using transwell migration assay and fusion and recombinant proteins, we showed that out of the four cytokine/chemokines encoded by the KSHV, vMIP-II is the only one that binds to the majority of NK cells. We demonstrate that vMIP-II binds to two different receptors, CX3CR1 and CCR5, expressed on the naïve CD56Dim NK cells and on activated NK cells, respectively, and that the binding of vMIP-II to CX3CR1 and CCR5 blocks the binding of the natural ligands of these receptors, Fractalkine (Fck) and Rantes, respectively. Finally, we show that vMIP-II inhibits the migration of naïve and activated NK cells towards Fck and Rantes. Thus, the present study indicates a very dominant role for vMIP-II in preventing NK cell migration.

W1.03.11
Innate immunity driving tumor angiogenesis: the role of Natural Killer cells in non-small cell lung cancer

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Natural Killer (NK) cells are innate effector lymphocytes that potentially can control tumors by their cytotoxic activity. However, in cancer patients innate immune cells often show polarization to a proangiogenic protumor phenotype. The role of NK cells in tumor angiogenesis remains to be defined.
Here we investigated phenotype, function and subset distribution of NKs in tumor, adjacent normal tissue and peripheral blood and the correlation with proangiogenic factor production, i.e. VEGF, PlGF and IL-8/CXCL8 from patients with non-small cell lung cancer (NSCLC).
The CD56brightCD16- NK phenotype predominated in NSCLC samples while the CD56dimCD16+ cytotoxic NK phenotype prevailed in adjacent normal tissues and in non-oncologic lung tissues. The CD56+CD16- NK subset was associated with angiogenic cytokine production including VEGF, PlGF and IL-8. Patients with squamous cell carcinoma histotype (SQK) showed higher NK angiogenic factor production compared to those from adenocarcinoma (ADC) and control tissues.
Supernatants derived from NSCLC infiltrating CD56+CD16- NKs induced in vitro endothelial cell chemotaxis and formation of capillary-like structures, particularly evident in SQK specimens. As TGFβ1 expression has been associated with poorer survival in NSCLC SQK patients and polarization of peripheral NK cells towards an angiogenic decidual NK phenotype, we evaluated whether TGFβ1 could induce proangiogenic factor production. Exposure of NK cells of peripheral blood from healthy subjects to TGFβ1 induced up-regulation of VEGF and PlGF production.
Our data suggest that NK cells in NSCLC can be switched to a proangiogenic phenotype, particularly evident for SQK patients and partly mediated by TGFβ1.

W1.03.12
Defining the Lethal Hit: Perforin Forms Transient Pores on the Target Cell Plasma Membrane to Facilitate Rapid Access of Granzymes During Killer Cell Attack

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Cytotoxic lymphocytes serve a key role in immune homeostasis by eliminating virus-infected and transformed target cells through the perforin-dependent delivery of pro-apoptotic granzymes. However,

the mechanism of granzyme entry into cells remains unresolved. Using biochemical approaches combined with time-lapse microscopy of human primary cytotoxic lymphocytes engaging their respective targets, we defined the time course of perforin pore formation in the context of the physiological immune synapse. We show that upon recognition of targets, calcium influx into the lymphocyte led to perforin exocytosis and target cell permeabilisation in as little as 30-sec. Within the synaptic cleft, target cell permeabilisation by perforin resulted in the rapid diffusion of extracellular milieu derived granzymes. Repair of these pores was initiated within 20-sec and completed within 80-sec, thus limiting granzyme diffusion. Remarkably, even such a short timeframe was sufficient for the delivery of lethal amounts of granzymes into the target cell. Rapid initiation of apoptosis was evident from caspase-dependent target cell rounding within 2-min of perforin permeabilisation. The current study defines the final sequence of events controlling cytotoxic lymphocyte immune defence, in which perforin pores assemble on the target cell plasma membrane, sufficient to ensure efficient delivery of lethal granzymes.

W1.03.13
Gap Junction intercellular communication induces NK cell activation and modulates cytotoxic capacity

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Gap junctions (GJ) mediate intercellular communication between adjacent cells. Previously, we showed that connexin 43 (Cx43), the main GJ protein in the immune system, mediates antigen transfer between human DCs, being also recruited to the immunological synapse during T cell priming. This crosstalk contributed to T cell activation, intracellular Ca^{2+} responses, and cytokine release. However, the role of GJ in NK cell activation by DCs and NK cell-mediated cytotoxicity against tumor cells, remain unknown. Here, NK cells and monocyte-derived DCs were obtained from peripheral blood. A human melanoma cell line (Mel2) and K562 cells were used as target. Cx43 localization was quantified on NK-DCs and NK-tumor cell co-cultures by flowcytometry and immunofluorescence. GJIC was evaluated using Calcein transfer and GJ inhibitors. Cytotoxicity and Granzyme-B activity was evaluated and the induction of Ca^{2+} signaling detected with FLUO-4AM. We found that NK cells form functional Cx43-GJ with DC and tumor cells. Polarization of Cx43 at the NK/DCs and NK/tumor cells contact site was detected. Cx43-mediated communication between NK and DCs was bidirectional, although blocking Cx43-GJIC inhibited NK cell activation not affecting DCs phenotype or function. Blocking of Cx43, by an analogue peptide or RNA silencing, inhibited CD69, CD25 and IFN-γ-release by NK. Moreover, Cx43-GJIC blocking strongly inhibited tumor lysis by NK associated to Granzyme-B activity and Ca^{2+} influx inhibition. Thus, our data suggest that Cx43-GJIC may play an important role in the NK cell interactions with other immune cells as well as in the NK anti-tumor effector function.

W1.03.14
Analyzing and modeling the dynamics of differentiating and adoptively transferred NK cells in patients undergoing hematopoietic stem cell transplantation (HSCT)

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Mature NK cells are able to display graft versus leukemia, without inducing graft versus host disease. These unique properties make NK cells an ideal additional lymphocyte-graft, especially in haploidentical HSCT. Here, we combine multiparameter flow-cytometry and mathematical algorithms to monitor patients undergoing haploidentical HSCT followed by NK cell adoptive transfer in the frame of a phase I/II clinical trial. Acute myeloid leukemia patients were transplanted with $CD34^+$ cells followed by transfer of $CD3^-$ $CD56^+$ NK cells derived from the same haploidentical donor. NK cells were monitored between day 0 and 150 after transplantation by multiparameter flow-cytometry and data was analyzed applying principle component analysis (PCA). In the first week after transplantation, we could separately track NK cells transferred from the donor, those reconstituting from donor $CD34^+$ cells, as well as recipient ones. Transferred NK cells largely displayed a mature phenotype and their proliferative ability *in vivo* was restricted to the $CD62L^+$ subset. In the second week after transplantation, we detected a peak of highly proliferating immature NK cells derived from $CD34^+$ cells, which progressively differentiated into mature NK cells, gradually losing the expression of CD62L, CD56 and NKG2A, while acquiring KIR, CD16, and CD57. PCA defined a clear direction of maturation and supported the concept of NK cell
differentiation as a non synchronized continuum. Mathematical clustering led to a model in which 3 distinct phases can be distinguished during time. Hence, transferred NK cells are detectable, proliferate in vivo and represent the only circulating lymphocyte population early after haploidentical HSCT.

W1.04 NKT cells

IL1.04.01
Regulatory role of iNKT cells, from viral infection to type 1 diabetes

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Invariant natural killer T (iNKT) cells are regulatory T cells that can both inhibit autoimmune T cell responses and promote T cell responses to pathogens such as viruses. Since converging data in humans and mouse models suggest that viral infections influence the development of type 1 diabetes, we have investigated whether iNKT cells could prevent type 1 diabetes (T1D) during viral infections using LCMV, and Coxsackievirus B4 that has been implicated in the etiology of T1D. iNKT cells inhibit the development of diabetes upon both infections. After LCMV infection, iNKT cells promote CD8 antiviral response in the spleen required for the clearance of the virus. In the pancreas iNKT cells interact with plasmacytoid dendritic cells (pDC) to induce a strong type I IFN production to inhibit viral replication. In a second step, iNKT cells activate pDC to produce TGF-beta that induces anti-islet Treg cells thereby preventing local damage and diabetes development. Upon CVB4 infection, iNKT cells inhibit the development of diabetes by another mechanism. Locally in the pancreas, CVB4-induced diabetes is associated with a massive recruitment of inflammatory macrophages. However iNKT cells alter macrophage function and induce IDO expression that inhibits anti-islet T cell function and prevents diabetes onset. While these two studies highlight the regulatory role of iNKT cells, a subset of iNKT cells producing IL-17 is locally activated in the pancreas and can exacerbate the development of diabetes. Altogether these data reveal the complex role of iNKT in T1D and prompt further studies in patients.

IL1.04.02
Therapeutic potential of NKT cell adjuvant-based therapies for the treatment of B-cell lymphoma

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Natural killer T (NKT) cells are a unique subset of T lymphocytes that serve as a link between the innate and adaptive immune system through their ability to recognize lipid antigens presented in the context of the non-classical MHC class I molecule, CD1d and rapid release of cytokines following activation. NKT cells can mediate tumor immune-surveillance; however, NKT cells are numerically reduced

and functionally impaired in lymphoma patients. Unlike other cancers, most hematologic malignancies express CD1d molecules and co-stimulatory proteins needed to induce anti-tumor immunity by NKT cells, yet most tumors are poorly immunogenic. In this study, we assessed NKT cell function throughout lymphomagenesis in two spontaneous, *myc* oncogene-driven mouse models of B-cell lymphoma. At early stages, NKT cell responses were enhanced in lymphoma-bearing animals compared to disease-free animals. In lymphoma- bearing animals with splenomegaly, lymphadenopathy, and high cyclin D1 expression, NKT cells were physically and functionally reduced. Treatment of tumor bearing mice with a potent NKT cell agonist, α-galactosylceramide (α-GalCer), resulted in a significant decrease in tumor burden. *Ex vivo* studies demonstrated that NKT cells from α-GalCer treated mice remained responsive to α-GalCer stimulation unlike the anergic NKT cells from vehicle-control treated mice. These data demonstrate an important role for NKT cells in their capacity to directly mediate tumor regression. Therefore, vaccination strategies utilizing NKT cell agonists in combination with other targeted therapies may be an effective means to stimulate effective anti-tumor immunity in patients with hematologic malignancies.

W1.04.01
Development of MAIT cells in the human fetus

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Mucosal-associated invariant T (MAIT) cells are innate-like T cells that are abundant in mucosal tissues, liver and peripheral blood. Human MAIT cells express a semi-invariant TCR Vα7.2-Jα33 Vβ2/13, and recognise microbial vitamin B metabolites restricted by the evolutionarily-conserved MHC-Ib-related (MR1) protein. MAIT cells are identified as Vα7.2+ CD161hi T cells coexpressing IL-18Rα and the transcription factor PLZF. The majority of MAIT cells are CD8α+, coexisting as CD8αα or CD8αβ, with minor CD4+ or CD8/4 double-negative populations. Human MAIT cells are thought to expand and mature following the establishment of gastrointestinal microbiota after birth. However, there is no study on human MAIT cell development prior to birth and microbiota establishment.
Here we studied, for the first time, the development of MAIT cells in second trimester human fetal tissues. Fetal MAIT cells were present in the thymus predominantly as an immature CD8αβ single-positive population, with similar observations in the spleen and mesenteric lymph node. In contrast, acquisition of maturation markers and expansion of CD8αα population were found in fetal small intestine, liver and lung. Fetal MAIT cells readily proliferated and produced cytokines in response to Escherichia coli stimulation in vitro. Our results thus indicate that human fetal MAIT cells become functionally mature in peripheral/mucosal tissues, likely in the absence of established gut microbiota.

W1.04.02
miRNA regulation of iNKT cell development

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iNKT cells are a separate subset of T lymphocytes displaying innate effector functions. We have previously shown that microRNAs (miRNAs) control the unique iNKT cell developmental program. iNKT cells, and not T cells, are dramatically reduced and blocked at an immature stage in mice with a conditional deletion of Dicer, the RNAse III enzyme that produce mature miRNAs. Accordingly, thymic mature iNKT and T cells display distinct miRNA signatures. To unravel the miRNA-mRNA interaction network underlying iNKT cell development, we determined the gene expression profiles of miRNA-sufficient or -deficient thymic iNKT cells and T cells. Consistent with a tight dependence of iNKT cells on miRNA control of gene expression, we find that the absence of miRNA dramatically affect iNKT but not T cell transcriptome. We identify genes upregulated in iNKT cells lacking miRNAs compared to WT cells, which are involved in cell proliferation, DNA integrity at G1/S transition during cell cycle, thymic development and positive regulation of apoptosis. Amongst the transcripts modulated by the absence of miRNAs, we sought to enrich for genes targeted by single miRNAs expressed by iNKT cells. In this manner, we are now trying to characterize the genes that could be critically involved in the genetic program controlling iNKT cell development via miRNA regulation.

W1.04.03
The role of NKT cell TCR β-chain on antigen specificity

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The prototypic Natural Killer T (NKT) cell ligand, α-galactosylceramide (α-GalCer) has been shown to provide anti-tumour effects in various disease models, and is currently in clinical trials as an anti-cancer therapeutic. Despite maintaining a semi-invariant TCR repertoire, NKT cells have been demonstrated to respond to a range of different glycolipid antigens, with different antigens eliciting distinct responses, however the molecular basis for antigen discrimination by the NKT cell receptor is unclear. Utilizing a range of structurally distinct glycolipids we have demonstrated that modifications in the glycosyl headgroup directly impacted TCR interactions, which affected the NKT cell cytokine response. Although these glycolipid analogues were capable of activating both Vβ7 and Vβ8 NKT cells, those with an altered 4'-OH on the sugar biased the expansion of NKT cells expressing Vβ7. Modifications in the acyl chain did not appear to affect TCR interactions, however truncation of the phytosphingosine chain resulted in an induced fit mode of TCR binding, which reduced TCR affinity. Vβ7+ NKT cells have also been shown to preferentially respond to the endogenous glycolipid antigen isoglobotrihexosylceramide (iGb3), a potential tumour antigen. Data will be presented demonstrating that recognition of iGb3 is specific to a subset of the NKT cell population, which is biased towards those expressing Vβ7 and also appears to be favoured by particular TCR-Jβ regions. The specificity of iGb3 recognition supports the possibility that other antigen specific populations may be present within the NKT cell population despite the semi-invariant nature of the NKT cell TCR.

W1.04.04
Bacterial CD1d restricted glycolipids induce IL-10 production by human Tregs upon crosstalk with iNKT cells

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Invariant natural killer T cells (iNKT) and CD4+CD25+FOXP3+ regulatory T cells (Tregs) are important immune regulatory T cells with antigen reactivity towards glycolipids and peptides respectively. However, the functional interplay between these cells in humans is poorly understood. In this study we therefore investigated a reciprocal interaction between human Tregs and iNKT cells in function of pathogen related mechanisms of iNKT stimulation. Specifically, we explored iNKT activation by diacylglycerols derived from *Borrelia burgdorferi* (responsible for Lyme's disease) and *Streptococcus pneumonia* (causative agent of pneumonia). In addition, innate like activation of iNKT cells relying on IL-12/IL-18, was also evaluated. We show that Tregs suppress iNKT cell proliferation induced by CD1d restricted glycolipids and by innate like activation. Inhibition was related to the potency of iNKT agonists, making diacylglycerol iNKT responses very prone towards suppression. Cytokine production by iNKT cells was differentially modulated by Tregs as IL-4 production was more profoundly reduced as compared to IFN-γ. A compelling observation was the significant production of IL-10 by Tregs after cell-contact with iNKT cells, in particular in the presence of bacterial diacylglycerols. These iNKT-primed Tregs showed an

increased FOXP3 expression and superior suppressive function. Suppression of iNKT cell but not conventional T cell responses was IL-10 dependent, suggesting that there is a clear difference in mechanism between the Treg mediated inhibition of these cell types. Our data highlight a physiologically relevant interaction between human iNKT and Tregs upon pathogen derived glycolipid recognition with significant impact on the design of iNKT cell based therapeutics.

W1.04.05
CD1d-restricted phosphatidic acid-reactive T cells selectively inhibit glycolipid-reactive T cells and protect against inflammatory liver disease

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Lipid antigens bind CD1d and activate T-cells. Extensive work on T-cells reactive with glycolipid antigens such as αGalCer has identified their pathogenic or protective roles in inflammation, infection and cancer. Little is known about the biology and functions of T-cells reactive to phospholipid antigens such as phosphatidic acid (PA). Here, we used CD1d-phosphatidic acid (CD1d-PA) tetramers to identify T-cells in various lymphoid and other organs of mice. We found that 0.5-2% of lymphocytes in liver and 0.3-1% of lymphocytes in spleen stained for TCRβ and CD1d-PA tetramers. CD1d-PA tetramer+ cells did not stain with CD1d/αGalCer tetramers and did not respond to glycolipid antigen αGalCer. Upon in vivo priming with PA, TCRβ+CD1d-PA tetramer positive cells secreted IFN-γ. Intriguingly, activation of CD1d-PA tetramer+ T-cells markedly reduced the proliferation of αGalCer-reactive T-cells in vitro, ex vivo, and in vivo. We further found that in vivo activation of CD1d-PA T-cells reduced Concanavalin A-induced hepatitis that is known to be mediated by αGalCer-reactive T-cells. Thus, PA-reactive T-cells are a subset of CD1d-restricted T-cells that are phenotypically and functionally distinct from αGalCer-reactive T-cells. CD1d-PA T-cells also negatively regulate the function of αGalCer-reactive T-cells. Ongoing studies will investigate mechanisms whereby PA-reactive T-cells inhibit the function of glycolipid-reactive T-cells.

W1.04.06
Invariant NKT cells in Response to Dengue Virus Infection in Human

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Dengue infection is a global health problem without vaccine and specific treatment. Clinical severity varies from asymptomatic, dengue fever (DF) to a more severe dengue hemorrhagic fever (DHF). Adaptive immune responses were shown to be pathogenic in severe disease. Invariant NKT (iNKT) cells represent innate-like lymphocytes that play a critical role in several viral infections but their contribution in human dengue infection is not known.
In this study, PBMC from dengue infected patients (n=35) with varying severity and at various timepoints were analyzed by flow cytometry. iNKT cells were identified by PBS57loaded-CD1d tetramer and CD3. iNKT cell activation and subset were evaluated by the expression of CD69 and CD4/CD8 respectively. Data were also compared with other febrile illness (OFI) and healthy controls.
The percentage of $CD69^+$ iNKT cells was significantly higher during acute febrile illness than at 2 weeks after the infection in both DF and DHF but not in OFI group. Furthermore, DHF group showed significantly higher % $CD69^+$ iNKT cells when compared to DF, OFI or healthy control. The percentage and subset of iNKT cells neither change during the course of infection nor differ in various disease severity conditions.
Therefore, during the acute febrile stage of dengue infection, iNKT cells are activated. The level of activation correlates with the disease severity. Our finding suggests that iNKT cells play a role in immune response against dengue viral infection in human. Further study is being performed to delineate the detailed mechanisms of how iNKT cells contribute to the pathogenesis of this disease.

W1.05 Innate lymphocytes

IL1.05.01
Functional Education of iNKT Cells by Dendritic Cell Tuning of SHP-1

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iNKT cells play key roles in host defense by recognizing lipid antigens presented by CD1d. iNKT cells are activated by bacterial-derived lipids and are also strongly autoreactive towards self-lipids. iNKT cell responsiveness must be regulated to maintain effective host defense while preventing uncontrolled stimulation and potential autoimmunity. CD1d-expressing thymocytes support iNKT cell development, but thymocyte-restricted expression of CD1d gives rise to antigen hyper-responsive iNKT cells. We hypothesized that iNKT cells require functional education by CD1d+ cells other than thymocytes to set their correct responsiveness. In mice that expressed CD1d only on thymocytes, hyper-responsive iNKT cells in the periphery expressed significantly reduced levels of tyrosine phosphatase SHP-1, a negative regulator of TCR signaling. Accordingly, heterozygous SHP-1 mutant mice displaying reduced SHP-1 expression developed a comparable population of antigen hyper-responsive iNKT cells. Restoring non-thymocytes CD1d expression in transgenic mice normalized SHP-1 expression and iNKT cell reactivity. Radiation chimeras revealed that CD1d+ dendritic cells supported iNKT-cell up-regulation of SHP-1 and decreased responsiveness following thymic emigration. Hence, dendritic cells functionally educate iNKT-cells by tuning SHP-1 expression to limit reactivity.

W1.05.01
TSLP induces corticosteroid resistance of natural helper cell and evokes severe asthma

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Asthma is a common chronic lung disease from which 230 million people suffer. Although corticosteroid is an effective treatment for most patients, ~5% of patients respond poorly to a normal dose of corticosteroid. IL-33, an epithelial cell-derived cytokine, induces airway inflammation in the absence of acquired immune system during asthma. Natural Helper (NH) cell, a new member of innate immune system, produces a large amount of IL-5 and IL-13 in response to IL-33, and induces eosinophilia and goblet cell, both of which are prominent symptom of asthma. In this study, we examined the effect of corticosteroid on NH cells during asthmatic responses. NH cells induced to the lung by a classic antigen-induced asthma model or by intranasal injection of IL-33 were efficiently despaired by intraperitoneal injection of dexamethasone. On the other hand, airway inflammation induced by addition of IL-33 to a classic antigen-induced asthma model was resistant to dexamethasone. We found that induction of NH cells with eosinophilia and goblet cell hyperplasia triggered by IL-33 plus thymic stromal lymphopoietin (TSLP) was not blocked by dexamethasone treatment, suggesting that TSLP modifies the corticosteroid-sensitivity of NH cells. In fact, *in vitro* experiments showed that dexamethasone completely inhibits the proliferation and Th2 cytokine production of IL-33-stimulated NH cells, but addition of TSLP dampened the effect of dexamethasone. Our results collectively indicate that TSLP produced by epithelial cells causes corticosteroid resistance of NH cells and exacerbate the airway inflammation in asthma.

W1.05.02
Group 2 innate lymphocytes are critical for Th2 cell-mediated allergic lung inflammation

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Group 2 innate lymphoid cells (ILC2), also termed natural helper cells, are stimulated by epithelial cell-derived IL-33 and TSLP. Activated ILC2 produce large amounts of IL-5 and IL-13, but not IL-4. Intranasal administration of the protease allergen papain activates lung ILC2 and induces T cell-independent allergic lung inflammation. We generated ILC2-deficient mice by transplanting bone marrow cells from the RORα mutant *Staggerer* mice to lethally irradiated mice and tested the effects of ILC2-deficiency on Th2 cell responses. Repeated intranasal administration of the protease allergen papain into normal mice induced Th2 cell generation, high titre serum IgE and allergic lung inflammation. In contrast, Th2 cell generation by papain, which was IL-4-independent, was severely impaired in ILC2-deficient mice. The initial sensitization of control mice with papain activated ILC2 and induced an accumulation of activated DCs expressing CD40 in draining mediastinal lymph node (mLN) where naive T cells differentiated into Th2 cells, whereas the accumulation of $CD40^+$ DCs and Th2 cell differentiation in mLN was impaired in ILC2-deficient mice. The effects of IL-13 neutralization in control mice and IL-13 injection into ILC2-deficient mice showed that ILC2-derived IL-13 is critical for the accumulation of $CD40^+$ DC in mLN and Th2 cell differentiation. Thus, ILC2 not only produce Th2 type cytokines and mediate T cell-independent allergic inflammation but they also play a critical role in the initiation of Th2 cell-mediated allergic lung inflammation.

W1.05.03
Identification of IL-17+RORgt+CD34+ as lineage-committed progenitors of IL-22+NCR+RORgt+ Innate lymphoid cells

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The innate lymphoid cell (ILC) family includes Natural Killer (NK) cells, RORgammat+ ILC and ILC2. Due to their shared developmental requirements, such as dependence on Id2 and gamma-chain cytokines, a common ILC progenitor has been postulated. Distinct stages have been described for NK cell but not for RORgt+ ILC differentiation. We identified a new population of human CD34+ hematopoietic progenitor cells (HPC) characterized by the ex vivo expression of RORgt, CD117, alpha4beta7 integrin and ability to produce IL-17A and IL-17F. CD34+ RORgt+ HPC reside in tonsils and give rise to IL-22 producing RORgt+ ILC, but not to NK cells upon in vitro culture. Conversely, tonsil CD34+ RORgt- CD117- HPC can still differentiate either towards IL-22 producing RORgt+ ILC or towards NK cells. Our data indicate that CD34+ RORgt+ cells are lineage-specified progenitors of IL-22+ RORgt+ ILC and that tonsils may represent a preferential site not only for NK cell, but also for RORgt+ ILC differentiation.

W1.05.04
T-bet is required for the development of NKp46+ innate lymphoid cells (ILCs) via a notch dependent pathway

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Rorγt+ innate lymphoid cells (ILCs) are found enriched in the intestinal mucosa of adults and are crucial for maintaining the delicate balance of tolerance, immunity and inflammation in the gastrointestinal tract. Rorγt+ ILCs include CD4+ LTi cells, CD4- LTi cells and the NK cell receptor expressing NKp46+ ILCs. The developmental relationship between these subsets and the transcription factors regulating their differentiation is controversial. We have found that the transcription factor T-bet (encoded by Tbx21) is essential for the development of NKp46+ ILCs, but not for LTi cells. Furthermore, NKp46+ ILCs differentiated solely from the CD4- and not the CD4+ LTi population in a Notch and T-bet dependent manor. In line with this finding, Notch was able to rescue this defect in the absence of T-bet. Collectively, our data provides a developmental link between CD4- LTi and NKp46+ ILCs controlled by the actions of Notch and T-bet distinct from CD4+ LTi.

W1.05.05
Reduced MAIT cell frequency associated with enhanced cell death in systemic lupus erythematosus

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Background: Mucosal-associated invariant T (MAIT) cells are restricted by the MHC-related molecule-1 (MR1) and express an invariant TCRα chain: Vα7.2-Jα33 in humans and Vα19-Jα33 in mice. Like other innate-like lymphocytes, MAIT cells have been suggested to play both proinflammatory and regulatory roles in autoimmune models. In this study, we sought to investigate whether MAIT cells are relevant to systemic lupus erythematosus (SLE).
Methods: Peripheral blood MAIT cells were identified as $CD3^+\gamma\delta TCR^-V\alpha7.2^+TCR^+CD161^{high}$ cells by FACS. The usage of Vα7.2-Jα33 TCR of single-cell sorted cells was analyzed by PCR. Peripheral mononuclear cells (PBMC) were stimulated with anti-CD3mAb and anti-CD28mAb or various types of cytokines, and 6-7 days later, the cell proliferation was analyzed.
Results: The percentages of MAIT cells from SLE patients were about 10-fold lower compared with those from healthy subjects. Single-cell PCR analysis indicated that the decrease of MAIT cells in SLE patients was not a result of downmodulation of surface markers. Although MAIT cells from healthy subjects as well as lupus patients proliferated upon TCR or cytokine stimulation, repeated stimuli reduced MAIT cell frequency. Lupus MAIT cells had higher percentages of FAS^{high} cells and active caspase-3 or 7-AAD positive cells, suggesting that more MAIT cells are undergoing cell death.
Conclusions: This study demonstrates that increased cell death of activated MAIT cells may be responsible for reduced frequency of MAIT cells in SLE. As increased serum concentrations of various cytokines have been reported in SLE, the decrease of MAIT cells may reflect such pathological condition of the disease.

W1.05.06
The SLAM family receptor Ly9 (CD229) is a negative regulator of marginal zone B cells response

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Homophilic interactions between the SLAM family receptors are required for the development of innate-like T lymphocytes. Recently, we have shown that Ly9 differs from the other SLAMF members by acting as an inhibitory regulator of iNKT cell development and activation. Splenic marginal zone B cells are innate-like lymphocytes that are known to mount rapid and intense antibody responses to blood-borne pathogens. Here, we test the hypothesis whether Ly9 also plays a regulatory role in the function of these cells. Analysis of the B cell development in a *Ly9*-deficient ($Ly9^{-/-}$) mice (BALB/c genetic background) showed no major alterations in bone-marrow B cell subsets. In contrast, the absence of Ly9 lead to an expansion of splenic $B220^+$ B cells as compared to wild-type mice (62.12% ± 0.51 vs 50.20% ± 0.93). Interestingly, an increased number of marginal zone B cells was also observed in the $Ly9^{-/-}$ mice. *In vivo* humoral response was assessed by immunizing $Ly9^{-/-}$ mice with T-dependent (TNP_{31}-KLH), T-independent type I ($TNP_{0.3}$-LPS) and T-independent type II (TNP_{65}-Ficoll) antigens. Our data revealed that Ly9 deficiency resulted in a striking increase of hapten-specific antibody levels,

especially IgG2a, IgG2b and IgG3 following TNP-Ficoll immunization (days 7 and 14). This effect was concomitant with a greater marginal zone B cell expansion. In contrast, responses against T-independent type I and T-dependent antigens were not significantly affected in *Ly9*-deficient mice. In conclusion, Ly9 emerges as an inhibitory cell-surface receptor involved in marginal B cell development and effector function.

W1.06 Macrophages

W1.06.01
Stabilin-1/CLEVER-1, defines an immune suppressive monocyte population

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Stabilin-1/CLEVER-1 is a multidomain protein present in lymphatic and vascular endothelial cells and Type II immunosuppressive macrophages. It functions in scavenging, endocytosis and in leukocyte adhesion to and transmigration through the blood vascular and lymphatic endothelial cells. In this study, we have identified for the first time Stabilin-1/CLEVER-1 expression on blood monocytes in healthy individuals. We investigated the gene expression profile and functional difference between Stabilin-1/CLEVER-1 high and low/negative expressing monocytes. Microarray analysis from RNA of sorted CLEVER-1 high and low/negative revealed differences in the gene profile. However, SiRNA mediated suppression of CLEVER-1 on the monocytes did not result in any alteration in the selected genes, suggesting that CLEVER-1 does not directly regulate the synthesis of the differentially expressed genes. Tetanus toxoid stimulation assays showed that when co-cultured with T-cells in ELISPOT plate CLEVER-1 high monocytes produced less of IFN-gamma compared to CLEVER-1 low/negative monocytes. When monocytes were differentiated in vitro to type I and II macrophages by cytokine stimulation, CLEVER-1 expression was induced on type II macrophages whereas no expression in type I macrophages was detected**.** We also found that CLEVER-1 expression is reduced in blood monocytes, placental macrophages and macrophages in placental bed in pre-eclampsia (involving many proinflammatory changes) compared to normal pregnancy. Collectively, these findings suggest that CLEVER-1 high monocytes play an immune suppressive role in circulating blood.

W1.06.02
Mice depleted of neutrophils develop chronic inflammation and deregulated adaptive immunity

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Neutrophils are an important cell type in innate immune reactions. They are one of the first cells engaged in an inflammatory response and thus have the ability to orchestrate both adaptive and innate responses.There has been an intensive research focus to understand the regulation of inflammation by neutrophils, and one way to address this is to deplete them in vivo. In this study we depleted neutrophils by using the diphtheria toxin A (DTA) stopflox system. A selective depletion of neutrophils was noted but othermyeloid cells in different organs were unaffected. Mice lacking neutrophils developed a chronic systemic inflammation with inflamed lymph nodes and splenomegaly after immune triggering. Deregulation of both T and B cell functions was observed, with increased numbers of Th1 and Th17 cells, plasma cells and high titers of serum antibodies. These results indicate a hitherto unreported immunohomeostatic function of neutrophils.

W1.06.03
Glucocorticoid-induced leucine zipper (GILZ) inhibits inflammatory activation in macrophages via reduced MAPK signalling: role in endotoxin tolerance

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Induction of glucocorticoid-induced leucine zipper (GILZ) by glucocorticoids plays a key role in their anti-inflammatory action, whereas GILZ expression is reduced under inflammatory conditions. Thus, we aimed to investigate the role of GILZ in LPS-induced inflammation and LPS tolerance in human alveolar macrophages (AM) and murine bone marrow-derived macrophages (BMDM).
Treatment of AM and BMDM with LPS resulted in a rapid decrease of GILZ mRNA and protein levels. To assess the functional significance of GILZ downregulation we generated mice with a macrophage-specific GILZ knockout (GILZ KO) using the *Cre-loxP* system. GILZ KO BMDM displayed a higher responsiveness towards LPS, as shown by increased and prolonged IL-1β- and TNF-α-expression. LPS-mediated ERK activation was significantly enhanced in GILZ KO cells, whereas inhibition of ERK repressed the induction of proinflammatory mediators upon LPS challenge, suggesting that GILZ modulates inflammation *via* the ERK pathway.
Interestingly, GILZ downregulation was abrogated in LPS-tolerized AM, which was independent of endogenous glucocorticoid production and associated with increased GILZ mRNA-stability. In line with this finding, LPS-tolerant cells were not able to induce the mRNA-binding protein TTP, which was previously shown to be involved in GILZ mRNA-downregulation. LPS tolerance resulted in a diminished expression of proinflammatory mediators accompanied by an almost complete abrogation of ERK, p38 and SAPK/JNK activation. In contrast, MAPK signalling was preserved in LPS-tolerized GILZ KO BMDM. Accordingly, LPS-tolerant GILZ KO cells retained their ability to induce TNF-α, indicating that GILZ downregulation not only increases macrophage activation, but also critically influences their deactivation.

W1.06.04
CD300a is a new hypoxia-inducible gene and a regulator of proinflammatory cytokine production in human monocytes and macrophages

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Peripheral blood monocytes are recruited to inflammatory and tumor lesions where they undergo terminal differentiation into macrophages. Monocytes/macrophages integrate stimulatory and inhibitory signals present in the microenvironment through a defined repertoire of cell surface receptors, and deregulated expression of these molecules may result in amplification of inflammation or establishment of immune escape mechanisms. Characterization of the effects of pathologic stimuli on the expression and function of these receptors may, thus, contribute to our understanding of monocytic lineage cell functions in diseased tissues. A common feature at pathologic sites and an important regulator of monocyte/macrophage proinflammatory functions is represented by low partial oxygen pressure (hypoxia). We recently identified by gene expression profiling the leukocyte membrane antigen, CD300a, a member of the Ig-like CD300 superfamily of immunoreceptors, as a new hypoxia-inducible gene in primary human monocytes. In this study, we demonstrate that CD300a mRNA and surface protein are upregulated by hypoxia and the hypoxia-mimetic agent, desferrioxamine, in both monocytes and monocyte-derived macrophages. Stimulatory effects are reversible, being counteracted by cell reoxygenation or addition of iron sulfate. Interestingly, CD300a ligation by an agonist Ab delivers both inhibitory and activating signals in these cells, differentially affecting the production of the chemotactic and proangiogenic cytokines, CCL20 and VEGF, driven by hypoxia and desferrioxamine. These findings point to a role for this molecule in the regulation of monocyte/macrophage inflammatory and angiogenic activity at hypoxic sites, with potential implications for inflammatory diseases and cancer pathogenesis.

W1.06.05
Physical interaction between miR-155 and miR-146a gene loci: Co-regulation during macrophage activation and induction of endotoxin tolerance

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MiRNA expression profile of macrophages is altered during activation, contributing to the regulation of the inflammatory response. MiR-155 and miR-146a are induced upon activation of macrophages by LPS. Previous work from our group has shown that several miRNAs are regulated by Akt kinases and control the magnitude of the inflammatory response and their polarization (M1/M2) phenotype. Akt1 ablation results in hyper-responsiveness to LPS and abrogates development of endotoxin tolerance via upregulation of miR-155 (Androulidaki et al., Immunity 2009), while ablation of Akt2 gives rise to an anti-inflammatory phenotype, via miR-155 and miR-146a (Arranz et al., PNAS 2012 and unpublished results). Herein, we analyzed the mechanism that controls expression of miR-155 and miR-146a in response to LPS in naïve and tolerant primary macrophages and RAW264.7 cell line. Pri-miR-155 and pri-miR-146a were induced upon LPS stimulation. When macrophages became LPS tolerant, the expression of pri-miR-155 and pri-miR-146a was decreased, while upon a subsequent LPS stimulus, their expression is rapidly induced. ChIP analysis revealed that their genomic loci are regulated by the same transcription factors, namely C/EBPβ, NFkB and Bach2, while FISH experiments showed a monoallelic inter-chromosomal interaction between miR-155 and miR-146a at the stage of endotoxin tolerance. Genetic ablation of Akt1 or Akt2 differentially affected expression of miR155 and miR-146a and association of their gene loci. In conclusion, our data suggest that miR-155 and miR-146a are coordinately regulated at the transcriptional level and physical interaction of their genomic loci is observed in mesophasic nuclei at the stage of endotoxin tolerance.

W1.06.06
Single cell dynamics of macrophage activation and signalling

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Macrophages display a remarkable ability to decode inflammatory cues by adapting their physiology. In response, they propagate such cues by activating specific gene expression programs that involves secretion of pro- and anti-inflammatory cytokines. This signalling is coordinated by a set of molecular and cellular networks including the Nuclear Factor kappa B (NF-κB) and Signal Transducers and Activators of Transcription (STATs) systems downstream of Tumour Necrosis Factor α, Interleukins, and Interferon. Here, we apply an interdisciplinary systems biology approach to build a quantitative picture of macrophage-mediated inflammatory signalling at a single cell level. We investigate classical and alternative macrophage activation using Raw 264.7, J774 and bone marrow derived macrophages as a model system. Using live cell microscopy with cells expressing NF-κB and STAT fluorescent fusion proteins we characterise the dynamical interaction between the two systems. These are correlated with temporal patterns of cytokine expression. Finally, in a co-culture we investigate the signalling between activated macrophages and a reporter fibroblast cell line, demonstrating that classically activated macrophages have a reduced capacity to propagate NF-κB signalling across the reporter fibroblast cell line. Our analyses suggest a complex relationship between macrophage ability to decode and propagate inflammatory cues and dynamics of underlying molecular and cellular signalling networks. A more quantitative picture of these non-intuitive interactions could provide better understanding of inflammatory signalling.

W1.06.07
Revisiting the ontogeny of the mononuclear phagocyte system: embryonic and adult hematopoietic contributions

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The mononuclear phagocyte system (MPS) constitutes a network of heterogeneous cell populations that play a crucial role in tissue homeostasis and immunity and include monocytes, macrophages and dendritic cells (DCs). Macrophages and DCs are highly heterogeneous in phenotype and function and multiple subsets of these cells have been described. Our findings show that heterogeneity is also reflected in the origin of DCs and macrophages. Macrophages and DCs are thought to derive from a common adult bone marrow (BM) progenitor. However, certain subpopulations such as the Langerhans cells (LCs), the DCs of the epidermis, and microglia, the resident macrophages of the central nervous system (CNS) seem to arise from embryonic hematopoietic precursors that respectively seed the skin and the CNS prior to birth, before the onset of BM hematopoiesis. The origin of these embryonic precursors has remained unclear. We recently showed that post-natal hematopoietic progenitors do not significantly contribute to adult LCs and microglia and that both LCs and microglia develop from yolk sac (YS)-derived primitive macrophages. However, in contrast to microglia, which remain of YS origin throughout life, YS-derived LC precursors are largely replaced by fetal liver monocytes during late embryogenesis, establishing the dual origin of LCs. Similarly, fetal liver monocytes contributed to other tissue phagocytes during late embryogenesis and superseded YS contribution. Our results highlight the importance of embryonic myeloid development in the formation of tissue macrophages, key elements of the adult immune system.

W1.06.08
CX3CR1hi Ly6C- monocytes play an anti-inflammatory role during liver inflammation

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Two major subsets of blood monocytes can be distinguished, namely $Ly6C^{hi}CCR2^{+}CX_3CR1^{int}$ 'inflammatory' monocytes and $Ly6C^{-}CCR2^{-}CX_3CR1^{hi}$ 'patrolling' monocytes. Both $Ly6C^{hi}$ and $Ly6C^{-}$ monocytes are recruited to inflammation sites where they can differentiate into macrophages and/or DCs and exert protective or detrimental roles depending on the pathogenic trigger. We use experimental African trypanosomiasis as a model to study the role of these monocyte subsets in infection-associated liver inflammation. We have reported that $Ly6C^{hi}$ monocytes are recruited to the liver of *Trypanosoma congolense* infected mice and differentiate in classically activated monocytic cells (M1, including TIP-DCs and macrophages). By secreting TNF, these cells contribute to liver cell apoptosis/necrosis, resulting in organ failure and early death. IL-10, as suppressor of M1 pathogenic immune responses, plays a critical role in limiting liver injury and prolonging survival.We now document that $Ly6C^{-}$ monocytes were increasingly recruited to the liver of *T.congolense* infected mice. These IL-10 producing cells do not own a gene signature reflecting an alternative, M2 activation status and do not differentiate into macrophages/DCs. Using adoptive transfer, we revealed that $Ly6C^{-}$ monocytes could suppress production of TNF by $Ly6C^{hi}$ monocytes during infection, favor the differentiation of the latter cells towards M2-type macrophages and protect liver integrity.These data illustrate a regulatory role for Ly6C- monocytes in parasite-induced liver pathogenicity by limiting the pro-inflammatory capacities of $Ly6C^{hi}$ monocyte and skewing them to a M2 phenotype. Hereby, $Ly6C^{-}$ monocytes protect the liver from damage caused by a persistent pathogenic immune response.

W1.06.09
Targeting MIF/CD74 signaling pathway to dampen acute inflammation in autoimmune diabetes

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Autoimmunity leads to the activation of innate effector pathways, proinflammatory cytokine production, and end-organ injury. In type 1 diabetes (T1D), autoaggressive T cells and infiltrating monocytes/macrophages secrete cytotoxic parameters that trigger beta cell damage and promote further inflammation. Recently, macrophage migration inhibitory factor (MIF) has been identified as an upstream activator of the innate immune response that mediates the recruitment and retention of monocytes/macrophages via its receptor, CD74. However, up to date, a contribution of MIF/CD74 signaling pathways in T1D pathogenesis remains incompletely explored. Here we identified a population of monocytes/macrophages from non-obese diabetic (NOD) animals with a hyper-inflammatory status and elevated levels of CD74. Interestingly, the induction of this unique monocyte/macrophage phenotype, translated into their increased ability to activate islet-antigen reactive $CD4^+$ T cells. Moreover, a similar inflammatory signature and elevated CD74 expression was observed in $CD14^{high}CD16^{low}$ monocytes from T1D patients. Exposure of human T1D monocytes to danger signals revealed that the majority of monocytes producing high levels of intracellular TNFa also uniquely expressed CD74. The physiological importance of this cell subset is further supported by the abundance of $CD74^+$ cell subsets within inflamed pancreatic islets of diabetic prone animals and even more so in animals with established disease. Further studies are ongoing to investigate whether interference in the MIF/CD74 signaling cascade could prevent the onset of- or protect against established autoimmune diabetes.

W1.06.10
Role of M1 and M2 macrophages in phagocytosis and degradation of amyloid-beta in Alzheimer's disease transgenic mice

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Microglia and brain-infiltrating monocytes are functionally distinct cells with non-redundant roles in the CNS during injury and inflammation. In the brain of APP/PS1 transgenic mice, a model of Alzheimer's disease (AD), blood derived monocytes are of critical importance in restricting amyloid-beta (Aβ) plaque growth, whereas microglia can be completely depleted without any effect on amyloid deposition. There is also evidence that the microglial population is skewed from an alternatively activated (M2) phenotype to a proinflammatory (M1) phenotype as mice age. These studies highlight the importance of monocyte derived macrophages in AD mice, but the individual roles of M1 and M2 phenotypes in restricting amyloid deposition remains to be elucidated. Here we show that macrophages that are transplanted into the hippocampus of aged APP mice retain the ability to migrate toward amyloid plaques. Interestingly, we found M1 macrophages to be better at phagocytosis and degradation of Aβ. Surprisingly, although M2-stimulation increased scavenger receptor expression, which has been implicated in Aβ-binding, it did not correlate with increased Aβ phagocytosis in our assay. These data suggest that a certain degree of inflammation might be beneficial in reducing Aβ deposition in the brain of APP mice.

W1.06.11
Interleukin 17 receptor A regulates renal inflammation and aggravates fibrosis

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Background: The T cell cytokine IL-17 is an important pro-inflammatory mediator. We have previously shown IL-17 receptor expression on monocytes and a role of IL-17 in macrophage accumulation in peritonitis. We here investigated the role of the IL-17 receptor on myeloid cells in chronic renal inflammation and fibrosis.
Methods: Renal interstitial fibrosis was induced by unilateral urethral obstruction in mice reconstituted with *Il17ra*$^{-/-}$ or control bone marrow or a mixture of both. Renal leukocytes were assessed by flow cytometry, and kidney damage was scored by HE and sirius red staining.
Results: *Il17ra*$^{-/-}$ macrophages were significantly less abundant than wt cells in in the control, and much more markedly the obstructed kidney. Obstruction increased CD11c and F4/80 expression. This phenotype was significantly less pronounced in the cells deficient in IL-17 signaling. The proportion of fibrotic tissue area was significantly lower in mice reconstituted with *Il17ra*$^{-/-}$ bone marrow than mice with *wt* bone marrow.
Conclusion: Lack of IL-17 receptor A on the myeloid cells significantly alters inflammation and diminishes fibrosis in renal obstruction.

W1.06.12
IL-4 stimulates macrophage soluble Flt-1 and inhibits murine experimental choroidal neovascularisation

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Monocyte/macrophages contribute to pathological retinal and choroidal angiogenesis. The exact role myeloid derived cells play in neovascularisation remains poorly understood. We have found that IL-4-conditioned macrophages have therapeutic anti-angiogenic properties, notably soluble Flt-1 production. To interrogate the therapeutic potential of IL-4 to inhibit angiogenesis, laser-induced choroidal neovascularization (CNV) was performed with IL-4 intravitreal administration.
Bone marrow derived macrophages (BMDM) conditioned with IL-4 were assessed for sFlt-1 expression. The activity of HUVEC in IL-4-conditioned BMDM supernatant was assessed to determine the anti-angiogenic capacity of sFlt-1, whilst specificity of sFlt-1 function was confirmed following sFlt-1 siRNA knockdown. To determine the effects of IL-4 inhibition on angiogenesis in vivo, IL-4 was administrated intravitreally during CNV development. Resident macrophages and CD11b+ isolated retinal microglia were also examined for sFlt-1 production in response to IL-4. We found that IL-4 or IL-13-conditioned BMDM media significantly suppresses VEGF-mediated HUVEC activity, and the effect was reversed when sFlt-1 was knocked down in those conditioned BMDM. In vivo, giving IL-4 intravitreally during CNV decreased angiogenesis. In the retina, CD11b+, but not CD11b- cells, express sFlt-1, arginase-1 and YM-1 in the presence of IL-4.
We have shown that IL-4 induced macrophage sFlt-1 expression which in turn significantly decreased VEGF-mediated endothelial cell activity. IL-4 administration inhibited angiogenesis in laser-induced CNV. Retinal CD11b+ cells responded to IL-4, inferring that the macrophage is critical for the sFlt-1-mediated IL-4 inhibitory response. Our study supports the therapeutic potential of IL-4 to suppress pathological angiogenesis via induction of sFlt-1 from macrophages.

W1.06.13
CCR2 expression by macrophages promotes tumor progression through induction of IL-10 production

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Chemokines are well known to play a major role in tumor progression and metastasis. In particular CCL2 and its receptor CCR2 are over-expressed in several human cancers and their higher levels correlate with poor prognosis and shorter outcomes. Whereas CCR2 has been demonstrated to recruit monocytic cells such as tumor-associated macrophages (TAM), little is known about its role in macrophage polarization. In order to directly investigate this aspect wt and $CCR2^{-/-}$ macrophages were polarized with M1 and M2 stimuli and analyzed

for gene expression and cytokines production. While no difference was found in M2 polarized macrophages, $CCR2^{-/-}$ M1 or LPS activated macrophages showed higher expression of inflammatory genes and reduced production of the anti-inflammatory cytokine IL-10 and of the pro-angiogenic cytokine VEGF when compared to wt macrophages. After LPS and CCL2 co-stimulation, $CCR2^{-/-}$ macrophages showed reduced activation of NF-kB and of p38 MAPK when compared to wt macrophages indicating a cross talk between CCR2 and TLR4 signaling pathways. The contribution of CCR2 to cancer growth was evaluated with a trasplantable lung cancer model that grew slower when co-injected with $CCR2^{-/-}$ macrophages. Taken together these data indicate that CCR2 expression by macrophages not only induce their recruitment to tumor site but also affect their polarization and anti-tumoral potential.

W1.06.14
Matrix reprogramming of immune cells and its disturbance in tumor

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The rapid response of the immunity to new pathogens is due to the fact that immune mechanisms need not be formed de novo but are quickly reproduced from a ready-made immune matrix available for immune cell interactions in response to pathogens. Matrix points contain various immune cells. For interdependent reprogramming of different cells of the immune matrix, we suggest the term “matrix reprogramming”. The architecture of the immune matrix clearly demonstrates the homeostatic mechanism, including the sensor (macrophages), the regulator (APCs, Tregs and MDSCs) and the effector (lymphocytes), which eliminate pathogens and restore homeostasis. We describe matrix reprogramming as applied to an adequate immune response or its disorder in tumors. The tumor can affect any component of the matrix and transform the homeostatic mechanism into protumor programs. As a result, the immune matrix fails to restore homeostasis. Macrophages play a key role in the protumor transformation of matrix. Thus, macrophages are a very attractive target for pharmacology and biotechnology. We are developing a new cell biotechnology focused on the creation of macrophages with a stable antitumor phenotype. The first approach utilizes the blockade of macrophage receptors to reprogramming cytokines released by the tumor. The second approach is based on ramification of the intracellular signaling pathway and possibility to create a “smart” macrophage, which would not only prevent the protumor transformation of the immune matrix but would additionally enhance the antitumor response. The third approach also create a “smart” macrophage and based on incorporation of chimeric heteroreceptors into the macrophage membrane.

W1.07 Effector functions of phagocytes

W1.07.01
STIM2 is the essential STIM protein in non-SOCE calcium responses of macrophages in vitro and in vivo

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Macrophage physiology critically depends on Ca^{2+} signalling. STIM isoforms are mediators of the most common mode of calcium influx in non-excitable cells, Store-Operated Ca^{2+} Entry (SOCE). Here, the relative dependence of key aspects of macrophage physiology on the mobilization of Ca^{2+} from the internal stores and/or the extracellular compartment was evaluated. Among the functions studied, cytokine responses to TLR ligands and chemokine elicited motility were mainly dependent on the mobilization of intracellular Ca^{2+}. In contrast, FcγR-mediated phagocytosis, apart from internal stores, depends on Ca^{2+} influx and SOCE. STIM2, in addition to contributing in SOCE, is involved in the maintenance of the internal ER-store of Ca^{2+}. The consequences of STIM2 deficiency in these Ca^{2+} dependent functions were characterized and contrasted with the effect of STIM1 deficiency. The SOCE dependent FcγR-mediated phagocytosis was reduced in case of STIM2 deficiency. However, the resulting defect was smaller as compared to the consequences of STIM1 deficiency. Moreover, genetic deletion of STIM2 had no effect on the phagocytosis-promoting function of C5a in vitro and in a model of autoimmune hemolytic anemia in vivo. Effective cytokine production downstream of TLRs and elicited motility, however, strictly depends on the presence of STIM2. In vivo, *Stim2*$^{-/-}$ mice exhibited milder LPS-induced sepsis and thioglycollate-elicited peritonitis. Interestingly, none of these SOCE-independent functions were reduced in case of STIM1 deficiency, suggesting their being specifically mediated by STIM2.

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W1.07.02
Glomerular deposition of myeloperoxidase and NETs in human anti ANCA associated vasculitis correlates with DTH effector cell accumulation

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Myeloperoxidase (MPO) autoimmunity underlies most cases of anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis and glomerulonephritis. We sought evidence for the hypothesis that glomerular injury is initiated by ANCA induced neutrophil accumulation, degranulation and NET formation. This is substantially exacerbated by anti-MPO CD4 T cells recognising extracellular MPO then directing DTH effector macrophages. Forty eight renal biopsies from ANCA MPO positive patients with clinically active glomerulonephritis were examined by confocal microscopy for leukocytes and MPO. A significant infiltration of MPO+CD15+ neutrophils and MPO+CD68+ monocytes/macrophages was present in all biopsies. Significant correlation was found for simultaneous glomerular infiltration of CD4 T cells and MPO expressing cells ($P<0.05$), and simultaneous infiltration of CD15 and MPO positive cells, and CD68 and MPO positive cells ($P=0.004$, $P=0.035$ respectively). Interstitial CD15 positive cells and the total number of MPO positive cells correlated inversely with estimated glomerular infiltration rate at time of biopsy and at 1 year ($P<0.05$ and, $P<0.01$, respectively.) Extracellular MPO was found in 93% of all glomeruli and made up 23% of the total proportion of MPO observed within glomeruli and 31% of all the MPO in the interstitium. Neutrophil extracellular traps (NETs) were present in 61.3% of MPO-ANCA biopsies and there was a significantly more CD15 cells, total MPO expression and extracellular MPO in the glomeruli of patients with the presence of NETs ($P< 0.05$). This study provides evidence to support the hypothesis that neutrophil derived extracellular MPO acts as a planted autoantigen in glomeruli inducing T cell mediated DTH type injury.

W1.07.03
Leishmania inhibits antigen crosspresentation by cleaving VAMP8

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Soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) proteins mediate membrane fusion events involved in vesicular trafficking associated to phagocytosis and phagolysosome biogenesis. Targeting these proteins may therefore provide a potent immune evasion strategy to several pathogens, including Leishmania, as it may interfere with phagosome functions. Here we report that VAMP8 and other phagosomal SNAREs were cleaved upon infection with Leishmania promastigotes, and that this cleavage was mediated by the Leishmania surface zinc-metalloprotease GP63. In addition, we found that Leishmania promastigotes efficiently inhibited antigen crosspresentation in a GP63-dependent manner. Using cells from VAMP8 null mice, we found that this SNARE was required for antigen

crosspresentation. In the absence of VAMP8, phagosomal assembly of the NADPH oxidase complex was impaired, leading to a defective phagosomal proteolysis. Thus, we uncovered the existence of a novel mechanism used by Leishmania promastigotes to subvert an important phagosome immune function, whereby these parasites proteolytically degrade key regulators of vesicular trafficking.

W1.07.04
The inhibitory effect of secretory leukocyte protease inhibitor (SLPI) on formation of neutrophil extracellular traps

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Neutrophil extracellular traps (NETs), a web like structures composed of DNA and antibacterial proteins provide efficient means of eliminating invading microorganisms but can also present potential threat to host, as a likely source of autoantigens, for example. Therefore, it is important to understand not only how the NETs are generated but also to identify mechanism(s) that inhibit NET formation. Translocation of neutrophil elastase (NE) from primary granules into nucleus followed by NEdependent histone processing and chromatin decondensation is one of the key events implicated in NET release. Since neutrophils contain inhibitors of NE such as secretory leukocyte protease inhibitor (SLPI) and α_1-Proteinase inhibitor (α_1-PI), we hypothesized that these inhibitors would play a role in restricting NET generation. Here we demonstrate, that exogenous human recombinant SLPI but not α_1-PI dramatically inhibited NET formation in human and mouse neutrophils. Moreover, neutrophils isolated from SLPI-/ mice were much more efficient in generating NETs compared with neutrophils derived from wild-type (WT) mice. Finally, endogenous SLPI was found to co-localize with NE in the nucleus of human neutrophils *in vitro*, as well as *in vivo* in an inflamed skin of psoriasis patients. Since SLPI is considered a cytosolic and/or granule protein, its co-localization with NE in the nucleus might further support a role of SLPI in restricting NEmediated histone processing. Taken together, our findings identify controlling role of SLPI in NET generation.

W1.07.05
The role of iRhom2 (rhbdf2) during immune activation

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Innate immune responses are vital for pathogen defence but can result in septic shock when excessive. A key mediator of septic shock is TNF, which is shed into intercellular spaces after cleavage from the plasma membrane by the TNF convertase (TACE). Here we report that the rhomboid family member iRhom2 interacts with TACE and regulates TNF shedding in vitro and in vivo. In particular, iRhom2 is critical for TACE maturation and trafficking to the cell surface in hematopoietic cells. Compared to controls, gene-targeted iRhom2-deficient mice show reduced serum TNF after LPS challenge and can survive a lethal LPS dose. Furthermore, iRhom2-deficient mice fail to adequately control the replication of Listeria monocytogenes and thus succumb to even mild infections. Our study has identified iRhom2 as a novel regulator of innate immunity that may be an important target for modulating sepsis and pathogen defense. Moreover, iRhom2 may be a critical trigger for immune activation and disease pathogenesis.

W1.07.06
FAM19A4: a novel cytokine promotes phagocytosis of zymosan but negatively regulates TNF-α secretion associated with NF-κB pathway in macrophages

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Cytokines play a key role in the immune system. When pathogen infection occurs, macrophages undergo phagocytosis first, accompanied with inflammatory cytokines secretion. FAM19A4 is a novel cytokine identified by the strategy of immunogenomics. It is a classical secreted protein which can be inhibited by BFA. It is low expressed in most normal human tissues, but is up-regulated in inflammatory stimulated monocytes-macrophages both in THP-1 cells and human peripheral monocytes derived macrophages. FAM19A4 can enhance phagocytosis of murine macrophages against zymosan both in vitro and in vivo not only the percentage of cells that phagocytized zymosan, but also the number of zymosan particles phagocytized by one single cell. FAM19A4 leads to noticeably increasing the phosphorylation of Akt on basis of zymosan stimulation, but reduces TNF-α secretion in zymosan-challenged macrophages by impacting activation of p38 and ERK1/2 in NF-κB pathway, due to the degradation of p65 both in nucleus and cytoplasm. These findings provide a novel cytokine FAM19A4 expressed by stimulated macrophages, which can promote phagocytosis of macrophages by activating Akt, but impact TNF-α increasing with challenge by inhibiting NF-κB pathway.

W1.08 Dendritic cell subsets

IL1.08.01
Increased numbers of monocyte-derived DC in the lymph node during successful tumor treatment with immune activating agents

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Local treatments with selected TLR ligands or bacteria such as Bacillus Calmette-Guerin increase anti-tumor immune responses and delay tumor growth. It is thought that these treatments may act by activating tumor-associated DC, and supporting the induction of anti-tumor immune responses. However, common parameters of successful immune activation have not been identified.
We compared treatments with different immune activating agents for ability to delay tumor growth, improve priming of tumor-specific T cells, and induce early cytokine production and DC activation. Treatment with poly I:C or a combination of monosodium urate crystals (MSU) and Mycobacterium smegmatis (M. smeg) was effective at delaying the growth of subcutaneous tumors, and reducing lung metastases. In contrast, LPS, and MSU or M. smeg alone had no activity.
Effective treatments required both NK cells and CD8+ cells, and led to increased T cell priming and the infiltration of NK cells and CD8+ T cells in tumors. Unexpectedly, both effective and ineffective treatments increased the expression of costimulatory molecules on DC in the tumor-draining lymph node. In contrast, only effective treatments induced the rapid appearance of a monocyte-derived DC population in the lymph node, early release of IL-12 and IFN-gamma, and low IL-10 in the serum.
Our results suggest that the activation of existing DC subsets is not sufficient for the induction of anti-tumor immune responses, whereas early induction of Th1 cytokines and monocyte-derived DC are features of successful activation of anti-tumor immunity.

IL1.08.02
Innate sensing of bacterial and endogenous nucleic acids by plasmacytoid dendritic cells: a novel role for interleukin 26

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Plasmacytoid dendritic cells (pDC) are professional IFN-α producing cells with the ability to sense viral infections via endosomal TLR7 and TLR9. While efficiently responding to viral nucleic acids transported into the cell during the infectious process, extracellular microbial or self-nucleic acids are unable to activate pDC due to the intracellular seclusion of these receptors. We found that interleukin-26, a Th17-derived cytokine belonging to the IL-20 family, is a highly cationic amphiphatic antimicrobial protein capable of directly killing bacteria such as pseudomonas, enterococcus, and staphylococcus via the mechanism of membrane pore formation. Upon killing of bacteria, IL-26 was found to bind the microbial DNA released into the extracellular compartment and to promote the internalization of the DNA complexes into endosomal compartments, leading to activation of TLR9 in pDC. Similarly, Th17-derived IL-26 was found to form complexes with DNA released by dying host cells and trigger innate immune activation of pDCs with production of IFN-α. These results identify a role of IL-26 as an antimicrobial protein and a regulator of innate immune activation extracellular microbial and self-nucleic acids, providing new insights into the potent antimicrobial and pro-inflammatory function of Th17 cells.

W1.08.01
Characterization of human afferent lymph dendritic cells from seroma fluids

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Dendritic cells (DCs) migrate from peripheral tissues to secondary lymphoid organs (SLO) through afferent lymph. Due to limitations in investigating human lymph, DCs flowing in afferent lymph have not been properly characterized in humans until now. In this study, DCs contained in seroma, an accrual of human afferent lymph occurring after lymph node surgical dissection, were isolated and analyzed in detail. Two main DC subsets were identified in seroma corresponding to the migratory DC subsets described in lymph nodes, i.e. $CD14^+$ and $CD1a^+$. The latter also included $CD1a^{bright}$ Langherans cells. The two subsets appeared to share the same precursor and be developmentally related; both of them spontaneously released high levels of TGFβ and displayed similar T cell activating properties. On the other hand, they differed in membrane molecule expression, including TLRs, in their phagocytic activity and in the expression of proteins involved in antigen processing and presentation. It is worth noting that while both subsets were detected in seroma in the post-surgical inflammatory phase, only $CD1a^+$ DCs migrated via afferent lymph in steady state conditions. Therefore, the large amount of DCs contained in seroma fluids allowed us a proper characterization of human DCs migrating via afferent lymph.

W1.08.02
Plasmacytoid, conventional and monocyte-derived dendritic cells undergo a profound and convergent genetic reprogramming during their maturation

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Dendritic cells (DC) express receptors sensing microbial, danger or cytokine signals, which combination triggering drives DC maturation and functional polarization. Maturation was proposed to result from a discrete number of modifications in conventional DC (cDC) contrasting to a cell-fate conversion in plasmacytoid DC (pDC). cDC maturation is generally assessed by measuring cytokine production and membrane expression of MHC class II and co-stimulation molecules. pDC maturation complexity was demonstrated by functional genomics. Here, pDC and cDC were shown to undergo profound and convergent changes in their gene expression programs *in vivo* during viral infection. This observation was generalized to other stimulation conditions and DC subsets, by public microarray data analyses, PCR confirmation of selected gene expression profiles, and gene regulatory sequence bioinformatics analyses. Thus, maturation is a complex process similarly reshaping all DC subsets, including through the induction of a core set of NFKB- or interferon-stimulated genes irrespective of stimuli. This work received funding from CNRS, Inserm, Agence Nationale de la Recherche (ANR) (pDCphysiology, ANR-07-MIME-018-01; EMICIF, ANR-08-MIEN-008-02 and PhyloGenDC, ANR-09-BLAN-0073-02), and the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013 Grant Agreement no. 281225).

W1.08.03
The dendritic cells of the bone marrow

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Dendritic cells (DC) are antigen presenting cells that are vital for initiating immune responses. They are found throughout the body and within all secondary lymphoid organs however those that might be found in the bone marrow (BM) have not been characterised. Here, for the first time, we reveal the 5 distinct DC subsets found in murine BM. We show that all of these DC subsets display unique functions, including the pathogens they recognise, the type of T cell they activate, and the proinflammatory cytokines and chemokines they produce upon activation. We identify that certain DC subsets of the BM show homology to those found in the spleen through their ability to cross present antigen to CD8 T cells or promote CD4 T cell expansion. However, both phenotypical and functional differences are evident between the DC of both organs. Unlike those of the spleen, certain DC of the BM produce large amounts of the neutrophil chemoattractant CXCL1 suggesting their importance, not only as antigen presenting cells, but as promoters of cell migration to and within the BM microenvironment itself. The BM is known to harbour latent HIV and cytomegalovirus. Recently it has also been shown to be a hideout of *Mycobacterium tuberculosis*. Our elucidation of the DC subsets within the BM identifies novel cellular targets for clinical manipulation of immune responses within the BM to overcome persistent infections by pathogens such as these. The DC of the BM may also be important targets in the prevention of graft versus host disease.

W1.08.04
Unraveling the fingerprint of human tolerogenic DC-10, a dendritic cell subset involved in the establishment of peripheral tolerance

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Dendritic cells (DCs) are critically involved in limiting immunity and in promoting tolerance. We identified DC-10, a subset of human tolerogenic DCs characterized by the high IL-10/IL-12 ratio and the co-expression of high levels of the tolerogenic molecules HLA-G and immunoglobulin-like transcript (ILT)4. DC-10 are potent inducers of adaptive type 1 regulatory T (Tr1) cells, well known to promote and maintain peripheral tolerance.
To characterize DC-10 and to investigate their tolerogenic potential, we compared DNA microarray-based transcriptional profiling of in vitro generated tolerogenic DC-10 and immunogenic mature myeloid DC (mDC). Through the screening of gene differentially expressed by human DC-10 we identified a unique pattern of DC-10-specific markers which will be used to improve their identification *in vivo*, and thus to better define the biology of *in vivo*-occurring DC-10. Moreover, DC-10 showed a molecular signature of anti-inflammation, immuno-modulation, and pro-angiogenesis. DC-10 show up-regulated IL-10- and Tissue Growth Factor (TGF) beta-dependent pathways, whereas down-modulate the signaling of pro-inflammatory cytokines. Moreover, DC-10 have an interesting matrix remodeling signature, that places them as hypothetical determinants of the tolerogenic environment in peripheral tissues.
These results represent the first step forward to further dissect the biology of DC-10 and will be instrumental to design novel approach for improving their in vitro generation for clinical use.

W1.08.05
In vivo roles of XC chemokine receptor 1-expressing dendritic cells

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Dendritic cells (DCs) are professional Ag presenting cells linking innate and adaptive immunity. DCs consist of various subsets, which exert subset-specific functions. Murine splenic CD8α$^+$ DCs are characterized by high ability to produce proinflammatory cytokines, to ingest necrotic cells and to cross-present antigens for inducing CD8 T cell responses. But, it still remains largely unknown how this DC subset is involved in various immune responses or inflammatory processes in vivo. In order to address this issue, the system for specific ablation of this DC subset in mice is quite useful. A chemokine receptor, XC chemokine receptor 1 (XCR1), is highly and selectively expressed in CD8α$^+$ DCs. We have first generated the mutant mice in which Diphtheria toxin A subunit (DTA) was designed to be expressed only in XCR1 expressing cells (XCR1-DTA mice). In XCR1-DTA mice, CD8α$^+$ DCs subset was constitutively ablated, but not in any other cells. In other tissues, including lymph node (LN) and intestinal lamina propria, CD103$^+$ DCs, which are closely related to splenic CD8α$^+$ DCs, were also ablated. XCR1-DTA mice showed no sign of autoimmune diseases or myeloproliferative disorder, which can be seen in the mutant mice lacking all CD11c$^+$ cells. XCR1:DTA mice should be useful for elucidating in vivo functions of XCR1 expressing DCs including CD8α$^+$ and CD103$^+$ DCs. Analysis on XCR1-DTA mice will be presented.

W1.08.06
Resident CD11b+Ly6C- lung dendritic cells are responsible for allergic airway sensitization to house dust mite in mice

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Conventional dendritic cells (DCs) are considered as the prime initiators of airway allergy. Yet, it remains unclear whether specific DC subsets are preferentially involved in airway allergic sensitization. Here, we systematically assessed the respective pro-allergic potential of individually sorted lung DC subsets isolated from house dust mite antigen (HDM)-treated donor mice, following transfer to naïve recipients. Transfer of lung CD11c+CD11b+ DCs, but not CD11c+CD11b-CD103+ DCs, was sufficient to prime airway allergy. The CD11c+CD11b+ DC subpopulation was composed of CD11c+CD11b+Ly6C+ inflammatory monocyte-derived cells, whose numbers increase in the lungs following HDM exposure, and of CD11c+CD11b+Ly6C- DCs, which remain stable. Counterintuitively, only CD11c+CD11b+Ly6C- DCs, and not CD11c+CD11b+Ly6C+ DCs, were able to convey antigen to the lymph nodes and induce adaptive T cell responses and subsequent airway allergy. Our results thus support that lung resident non-inflammatory CD11c+CD11b+Ly6C- DCs are the essential inducers of allergic airway sensitization to the common aeroallergen HDM in mice.

W1.09 Dendritic cell differentiation and function

IL1.09.01
Dendritic cells identified in the human system

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Most of the knowledge available on dendritic cell (DC) ontogeny, differentiation and functions has so far been derived from either mouse models or DCs generated ex vivo from human precursors employing different combinations of cytokines. As a result, the depiction of DCs present in the human body, in several aspects, remained vague.
Lately, we gained access to substantial information on human DC compartments, starting to identify the different roles of migratory and lymphoid tissue resident DCs as well as their peculiar molecular patterns. Notably, even in the absence of inflammation, specific subsets of human DCs continuously migrate from peripheral tissues to secondary lymphoid organs via afferent lymph, possibly playing relevant roles in the maintenance of peripheral tolerance to self antigens.
These recent studies open the way to further work on the functional role of distinct human DC subsets migrating from peripheral tissues to secondary lymphoid organs to exert their protective or tolerogenic immune functions, most likely depending on signals received in peripheral tissues.
This field of investigation might strongly contribute to the improvement of modern vaccination strategies as well as other immune-based therapies for human diseases.

IL1.09.02
Molecular regulators of DC function

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In order to fully understand mechanisms of autoimmunity and immune surveillance, my lab has been exploring a variety of molecular mechanisms that regulate the ability of dendritic cells to induce CD8 immunity. To follow the induction of CD8 cells in vivo, we have generated a new way to induce a tissue specific response using our RIP-GP mouse model. RIP-GP transgenic mice express the

lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) on the [[Unsupported Character - Symbol Font ]]-islet cells of the pancreas directed by the rat insulin promoter. Our previous studies have shown that virus infection in RIP-GP mice, or P14/RIP-GP double transgenic mice treated with peptide and DC maturation signals (anti-CD40, TLR, HSP-70) leads to the induction of tissue specific immunity and diabetes. We have recently developed a model showing that TLR matured bone marrow derived DCs pulsed with GP peptides can induce diabetes in RIP-GP single transgenic mice within 10 days. This allows us to evaluate the importance of various molecules expressed by DCs, using bone marrow derived DCs from various gene deficient mice.
Although conventional pathogen derived signals are important for DC maturation and the subsequent activation of CD8 immunity, there are other ways to alter DC function and allow the activation of CD8 T cells in vivo. I will present data on our recent studies using a variety of knock out DCs. Data suggests that there are multiple factors that maintain steady state DCs, and the molecular programming of DCs is a critical step that regulates immunity.

W1.09.01
Defining Dendritic Cells by Ontogeny

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Mononuclear phagocytes sample the environment for signs of damage or infection. The classification of these cells as macrophages or dendritic cells (DC) has traditionally been done on the basis of differences in cell morphology, expression of specific markers or of select functional attributes. However, these attributes are not absolute and often overlap, leading to difficulties in cell type identification. To circumvent these issues, we have generated a model to define DC based on their ontogenetic descendence from a committed precursor. We show that in mice precursors of conventional DC but not other leukocytes are marked by expression of DNGR-1/CLEC9A. We generated a mouse model to genetically label Clec9a-expressing conventional DC precursors and their progeny with yellow fluorescent protein (YFP). Genetic labeling of these cells and their progeny specifically traces cells traditionally ascribed to the DC lineage and the restriction is maintained after infection. Notably, in some tissues cells previously thought monocytes/macrophages are in fact descendants from DC precursors. These studies provide the first in vivo model for lineage tracing of DC and allow the definition of DC based on ontogenetic rather than phenotypic, morphological or functional criteria. These studies establish DC as an independent immune lineage and distinguish them from other leukocytes, thus paving the way to unraveling the functional complexity of the mononuclear phagocyte system.

W1.09.02
Lymphatic endothelium forms Integrin-engaging 3D structures during DC transit across inflamed lymphatic vessels

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Dendritic cell (DC) transmigration across the lymphatic endothelium (LEC) is critical for the initiation and sustenance of immune responses. Under non-inflammatory conditions DC transit across LEC has been shown to be integrin-independent. In contrast, there are increasing evidences on the participation of integrins and their ligands in DC transit across lymphatic endothelium under inflammation. In this sense, we describe the formation of ICAM-1 (CD54) -enriched 3D structures on LEC/DC contacts, as these DC adhere to inflamed skin lymphatic vessels and transmigrate into them. In vitro imaging revealed that under inflammation, ICAM-1 accumulated on microvilli projections surrounding 60% of adhered DC. In contrast, these structures were scarcely formed in non-inflammatory conditions. Besides, the ICAM-1-enriched microvilli were important for promoting DC transendothelial migration (TEM) and DC crawling over the LEC surface. Microvilli formation was dependent on the presence of integrins and of their high affinity epitopes on the DC side. Finally, we observed that LEC microvilli structures appeared in close vicinity to CCL21 depots, being their assembly partially inhibited by CCL21-neutralizing antibodies. Therefore, under inflammatory conditions, integrin ligands form 3D membrane projections around DCs. These structures, offer docking sites for DC transit from the tissue towards the lymphatic vessel lumen.

W1.09.03
MyD88 signaling drives steady-state intestinal CD103+ DC migration to draining mesenteric lymph nodes

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Intestinal homeostasis and induction of systemic tolerance to fed antigens (i.e. oral tolerance) relies on steady-state migration of small intestinal lamina propria dendritic cells (DCs) into draining mesenteric lymph nodes. $CD103^+$ DCs are the major migratory antigen-presenting cell population in the small intestine and appear to play a central role in initiating immune responses to soluble luminal antigens and inducing expression of gut-homing receptors on T cells. Here we demonstrate that the steady-state mobilization of $CD103^+$ DCs into the MLN is governed by the Toll-like receptor (TLR) signaling adaptor molecule MyD88 and occurs independently of TNF-α. Likewise, TLR signaling through the adaptor molecule TRIF and downstream production of type I interferon are not required. Experiments on CD11c-Cre x $MyD88^{fl/fl}$ mice lacking MyD88 selectively in $CD11c^+$ cells resulted in impaired steady-state $CD103^+$ DC migration, indicating a potential role for DC intrinsic MyD88 signaling in driving intestinal DC migration in the steady state.

W1.09.04
IL-27 in human secondary lymphoid organs attracts myeloid DC and impairs their HLA class I-restricted antigen processing and presentation

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DC are the most potent professional APC that in the immature state are positioned throughout the peripheral tissues by acting as sentinels and sensing the presence of antigens. When activated, DC migrate to lymphatic vessels and into lymph nodes, where they can direct antigen-specific T cell responses. Different cytokines play crucial role in inflammation and in polarizing immune responses, including IL-27 that may exert pro-and anti-inflammatory functions. Although the activity of IL-27 is well characterized in T, NK and plasma cells, only limited information is available on the role of IL-27 on human DC. Since the latter cells act as a key players in both adaptive and innate immunity, we have investigated the effects of IL-27 on human DC. We provide the first evidence that i) in human secondary lymphoid organs macrophages are the major source for IL-27, ii) immature and mature human DC express functional IL-27R, ii) IL-27 exerts immunosuppressive activity by crippling the antigen processing machinery in immature DC under steady-state conditions and after pulsing with a viral antigen, and iii) IL-27 is chemotactic for human DC. Our results highlight novel immunoregulatory functions of IL-27 that may be relevant in immune responses during infection and cancer.

W1.09.05
The kindlin-3-beta2 integrin interaction controls dendritic cell activation and Th1/Th2 priming

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Beta2 integrins play fundamental roles in leukocyte trafficking and signaling, but integrin proximal events in these processes remain

poorly characterized. Here, we show that mutation of the kindlin-3 binding site in the beta2 integrin cytoplasmic domain caused a loss of dendritic cell firm adhesion to integrin ligands. Surprisingly, the loss of beta2 integrin-mediated adhesion and cytoskeletal organization resulted in increased Syk and downstream signaling, and an elevated dendritic cell activation phenotype in terms of cytokine production and MHC class II and co-stimulatory molecule expression. This elevated dendritic cell activation state resulted in increased priming of CD4 T cell responses both *in vitro,* in a co-culture with TCR transgenic T cells, and *in vivo*, using a dendritic cell adoptive transfer model. Specifically, we found that in the absence of kindlin-3-beta2 integrin interactions in dendritic cells, Th1 priming *in vivo* was enhanced whilst Th2 responses were severely impaired. Thus, beta2 integrin binding to kindlin-3 integrates the cell exterior to the actin cytoskeleton, which restricts signaling in dendritic cells, thereby dampening their activation status and modulating *in vivo* immune responses.

W1.09.06
Deletion of IL-4 receptor alpha on dendritic cells renders mice hypersusceptible to Leishmania major infection

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Susceptibility to Leishmania major infection in BALB/c mice is driven by T helper 2 (Th2) responses producing interleukin (IL)-4 and IL-13, which share a common receptor subunit, the IL-4 receptor alpha chain (IL-4Ra). However, global abrogation of IL-4Ra is not sufficient to confer complete resistance to L. major, specific abrogation of IL-4Ra on CD4+ T cells leads to resistance, indicating a protective role for IL-4Rα signalling on non-CD4+ T cells. To investigate this further, CD11ccreIL-4Ra-/lox BALB/c mice were generated by gene targeting and cre/loxP recombination under control of the CD11c promoter. DNA, protein and functional characterisation demonstrated abrogated IL-4Ra expression on dendritic cells and alveolar macrophages only. Following infection with L. major, CD11creIL-4Ra-/lox mice became hyper susceptible to disease, showing earlier and increased footpad swelling, necrosis and parasite burden, increased Th2 cytokine and i type 2 antibody responses, as well as impairment in classical activation of macrophages, measured by iNOS expression. Hyper susceptibility in CD11ccreIL-4Ra-/lox mice was accompanied by a striking increase in parasite burdens in peripheral organs such as the spleen, liver and even brain. CD11b+ inflammatory DCs were the major infected cell population and showed deficiencies in iNOS production and increased parasite loads. IL-4Ra-deficient DCs produced reduced IL-12 but increased IL-10 due to impaired DC instruction and had increased mRNA expression of IL-23p19 and activin A. Together, these data suggest that abrogation of IL-4Ra signaling on DCs is detrimental to the host, leading to rapid disease progression, dissemination, and increased survival of parasites in infected CD11b+ DCs.

W1.09.07
Chronic hypoxia reprograms human immature dendritic cells by inducing a proinflammatory phenotype and TREM-1 expression

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Dendritic cells (DCs) are powerful antigen-presenting cells central in the orchestration of innate and acquired immunity. DC development, migration, and activities are intrinsically linked to the microenvironment. DCs migrate through pathologic tissues before reaching their final destination in the lymph nodes. Hypoxia, a condition of low partial oxygen pressure, is a common feature of many pathologic situations, capable of modifying DC phenotype and functional behavior. We studied human monocyte-derived immature DCs generated under chronic hypoxic conditions (H-iDCs). We demonstrate by gene expression profiling the up-regulation of a cluster of genes coding for antigen-presentation, immunoregulatory, and pattern recognition receptors, suggesting a stimulatory role for hypoxia on iDC immunoregulatory functions. In particular, we show that H-iDCs express TREM-1, a member of the Ig superfamily of immunoreceptors and an amplifier of inflammation. This effect is reversible because H-iDC reoxygenation results in TREM-1 down-modulation. TREM-1 engagement promotes upregulation of T-cell costimulatory molecules and homing chemokine receptors, typical of mature DCs, and increases the production of proinflammatory, Th1/Th17-priming cytokines/chemokines, resulting in increased T-cell responses. These results suggest that TREM-1 induction by the hypoxic microenvironment represents a mechanism of regulation of Th1-cell trafficking and activation by iDCs differentiated at pathologic sites.

W1.09.08
The mitochondrial protein, TCAIM, abolishes T cell priming of dendritic cells by inhibiting TLR-induced IL-2 expression

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We previously showed that TCAIM, T cell activation inhibitor - mitochondrial, is highly expressed in CD11c+ dendritic cells (DCs) of tolerance developing recipients and that the encoded protein is exclusively localized within mitochondria. Recently the importance of mitochondria in the regulation of host innate immune signaling, e.g. importance of TRAF6 translocation to mitochondria upon TLR1/2/4 stimulation resulting in release of mROS, has been demonstrated. Here we show that LPS-stimulated Ad-TCAIM transduced DCs failed to induce proliferation of and cytokine expression by allogeneic CD4+ and CD8+ T cells. A transcriptional profiling revealed a dramatically reduced IL-2 mRNA expression in LPS-stimulated TCAIM overexpressing DCs as compared to control-transduced DCs. This was accompanied by reduced mROS formation. Addition of IL-2 to DC-T cell co-cultures nearly completely restored T cell priming capacity of TCAIM overexpressing DCs. Thus TCAIM interferes with mitochondria-dependent events of TLR signaling in DCs and subsequently impairs their T cell priming capacity.

W1.09.09
PAF induces regulatory DCs through two independent mechanisms: amplification of COX2/PGE2 loop and induction of IL10

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The platelet activating factor receptor (PAFR) is a G-protein coupled receptor present in plasma and nuclear membranes of leukocytes. In macrophages, activation of PAF-R is associated to a suppressor phenotype. In the present study we investigated whether it has a similar effect on dendritic cells (DCs) and the mechanisms involved. Murine (BALB/c) bone marrow-derived dendritic cells were obtained after 6 days culture with GM-CSF. Maturation was induced by LPS. The PAFR-antagonists WEB2170 and PCA4248 and the prostaglandin (PG) synthesis inhibitors, Indomethacin and NS-398 were added to DCs 30 min before LPS. The agonist, methylcarbamyl-PAF (cPAF), was added together with LPS. RT-PCR and ELISA were employed to measure mRNA and protein for COX2, IL10 and IL12. PAF-R mRNA and protein is expressed in both types of DCs. PAFR-antagonists significantly increased lymphocyte proliferation in an antigen-specific proliferation assay and reduced IL10 and PGE2 production, indicating that PAFR engagement in DCs promotes suppressor functions through these molecules. PAFR-antagonists did not affect the expression of DCs MHCII and co-stimulatory molecules. IL10 was potentiated by cPAF and unaffected by PG synthesis inhibitors. IL12 was not affected by PAFR agonist nor antagonists. PAFR-antagonists did not affect COX2 mRNA but reduced PGE2 while cPAF potentiated its production. We propose that PAF is involved in two independent suppressor mechanisms in DCs: amplification of the loop COX2/PGE2 through its production induced

by LPS and potentiation of IL10, shifting DCs towards a regulatory phenotype. By increasing the antigen-presenting function of DCs PAFR-antagonists may be valuable co-adjuvants in vaccines.

W1.09.10
Glucocorticoid-Induced Leucine Zipper expression in dendritic cells controls Treg induction in vivo

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We have previously demonstrated that human dendritic cells (DCs) treated in vitro with glucocorticoids, IL-10 or TGF-β up-regulate the glucocorticoid-induced leucine zipper (GILZ) protein. We further established that GILZ over-expression turns DCs into a regulatory phenotype, generating CD25hiFoxP3+CTLA-4+ IL-10-producing antigen-specific regulatory T cells (Tregs) that inhibit T cell responses in an IL-10 dependent manner. To gain insight into the in vivo importance of GILZ expression in DCs for Tregs induction, we generated CD11c-GILZ transgenic mice. DCs from these mice constitutively overexpress GILZ to the same extent as glucocorticoid treated DCs. They display normal DC subsets distribution in both spleen and lymph nodes. GILZhi DCs phenotype is not different from that observed in DCs from control littermates, and their antigen uptake and processing capacity is not significantly modified. Tregs characterization in young mice hosted in an EOPS animal facility does not show any accumulation nor activation of these cells. However, Tregs analysis in older mice reveals a significant increase of CD4+FoxP3+ Tregs in the thymus, spleen and in the skin draining and mesenteric lymph nodes. Furthermore, when OVA-loaded GILZhi BMDCs and OT-2 T-cells are adoptively transferred in mice, in vivo OVA-specific T cell proliferation and activation are reduced, and this is associated with an increase in the CD25+FoxP3+ T cell population. Thus, our work shows that GILZ overexpression in DCs is sufficient to impede their capacity to activate T cells in vivo, most probably through Treg induction by a mechanism that does not require DCs phenotypic alteration or reduced antigen capture/processing.

W1.09.11
The inhibitory NK receptor Ly49Q protects plasmacytoid dendritic cells from TLR9-triggering cell death by assuring lysosomal integrity

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Ly49Q is an ITIM-bearing lectin-type NK receptor highly expressed in plasmacytoid DCs (PDCs), and associates with classical MHC class I in cis-configuration. We have reported that Ly49Q-/- mice showed impaired production of TLR9-mediated type I IFN production and diminished protection to mouse CMV infection. We also have revealed that Ly49Q colocalized with TLR9/CpG-ODN at the endosomes/lysosomes and affects their trafficking, which is shown to be important for TLR9 signaling.
We here show that Ly49Q is crucial for protection of lysosome-dependent PDC death under TLR9 stimulation. We found that Ly49Q-deficient (Ly49Q-/-) and an ITIM-less Ly49Q-expressing transgenic mice showed failure of intrasplenic redistribution of PDCs after CpG-ODN stimulation. Such anomalous Ly49Q-/- PDCs behavior was partly due to their decreased migration ability, but largely due to cell death. CpG-ODN-induced Ly49Q-/- PDC death was accompanied by cathepsin B leakage into the cytoplasm, and inhibited by cathepsin B- or caspase-1-inhibitor. These results suggest that CpG-ODN-induced Ly49Q-/- PDC death is lysosome-dependent process, and that Ly49Q exerted an influence on lysosomal integrity. It has been demonstrated that such cathepsin B- and caspase 1-dependent inflammatory cell death, so-called “pyroptosis”, causes IL-1β production through inflammasome activation. We also found that Ly49Q-/- PDCs secreted increased amounts of IL-1β after CpG-ODN stimulation. β2m-/- PDCs stimulated with CpG-ODN also showed pyroptotic cell death and produce IL-1β. These results strongly suggest that Ly49Q-MHC class I interaction facilitates PDCs survival through regulation of lysosomal integrity.

W1.09.12
Membrane transfer from tumor cells generates plasmacytoid dendritic cells cross-dressed with exogenous antigens

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The potential contribution of plasmacytoid dendritic cells (pDCs) in the presentation of tumor cell antigens still remains to be elucidated and some controversies currently exist regarding the ability of pDCs to phagocyte particulate antigens and cross-present them to MHC class I-restricted T lymphocytes.
Here, we show that human pDCs are prone to acquire membrane patches and associated molecules from cancer cells of different histotypes. Conversely, pDCs were not able to internalize cell membrane fragments by phagocytosis. The transfer of membrane patches to pDCs occurs in a very short time and require cell-to-cell contact interactions. Remarkably, HLA complexes were also transferred from tumor cells and the acquired antigens could efficiently be recognized by antigen-specific T cells on pDCs.
The exchange of exogenous antigens between pDCs and tumor cells appears to occur also *in vivo*, as pDCs isolated from human colon cancer tissues displayed a strong surface expression of Epithelial Cell Adhesion Molecule.
These data demonstrate that pDCs are particularly prone to acquire membrane patches from contiguous tumor cells by a cell-to-cell contact-dependent mechanism that closely resembles “trogocytosis”. This phenomenon may allow pDCs to proficiently present tumor cell-derived antigens despite their limited properties of endo-phagocytosis.

W1.09.13
Myeloid cells in the islets of Langerhans are key players in presentation of islet antigens and trophic function: identification of their phenotype and ontogeny

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The entry of autoreactive T cells to their target organ is important in autoimmunity since this initiates the inflammatory process. Islets of Langerhans contain a monocyte/macrophage-derived cell with important features and properties. This islet myeloid cell plays a central role in the interaction with diabetogenic T cells. We have studied in-depth the phenotype of the islet myeloid cells in the NOD and non-diabetic mouse strains and find 2 myeloid subsets: one subset (~85%), expressing MHC-II, CD11c, CD11b, F4/80 and LysM; and a minor subset, (~15%) expressing MHC-II, CD11c, CD11b, CD103, BTLA and LysM. Islet myeloid cells are stable in numbers and decrease 30% 7 days after whole body irradiation. Studies testing eGFP bone marrow transplantation has shown that islet myeloid cells can be replaced as early as 2 weeks post-transplant. Finally, we consider that in addition to their role of antigen presentation, the islet myeloid cell has a different and perhaps a more important role in islet physiology, which is to maintain the health of the islet. When a genetic mutation affects the presence of myeloid cells in islets (CSF-1-deficient mice), islets are reduced in size and show an altered glucose homeostasis. Our findings highlight the importance of the islet myeloid cell as central in presentation of islet antigen and trophic function of the islet. Their phenotype, ontogeny and trophic identification, will bring insights into diagnostics and point of interventions in diabetes.

W1.09.14
Dendritic cells partake in regulation of ovarian physiology

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Ovulation and inflammation share common attributes including the invasion of immune cells. We hypothesized that these cells, specifically dendritic cells (DCs), play a role in ovulation and corpus luteum formation. To follow the ovarian DCs we used transgenic mice, in which the expression of CD11c is conjugated to YFP. To explore their origin we transplanted WT ovaries under the kidneys capsule of YFP-CD11c transgenic hosts. For their conditional ablation, we injected diphtheria toxin into transgenic females, in which the expression of simian diphtheria toxin receptor is under the control of the CD11c promoter. We found a small ovarian resident CD11c positive- F4/80 negative-cells, apparently DCs prior to ovulation. Following the ovulatory stimulus, DCs are recruitment from the circulation and massively accumulate in the newly formed corpus luteum. We further revealed that CD11c positive cells are essential for expansion of the cumulus oocytes complex, the release of the ovum from the ovarian follicle, as well as for the formation of a functional corpus luteum. These effects of CD11c positive cells are mediated by upregulation of ovulation-essential genes and stimulation of lymphangiogenesis. Unexpectedly, we detected a remarkable anti-inflammatory capacity of the CD11c positive cells, which seemingly serves to restrict the ovulatory-associated inflammation. Our results provide strong evidence for the involvement of DCs in the ovulatory response. Moreover, the novel reported functions of ovarian dendritic cells unveil the wide diversity in their activities, beyond their classical immunologic role, which is most likely relevant to many biological systems as well as pathological conditions.

IL1.10.02
Association of TIR domains underlying immunosuppression in bacterial infection and activation of MyD88 mediated cell signaling associated with B-cell lymphoma

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Cells participating in innate immune system respond to pathogen associated molecules by activating Toll-like receptor (TLR) signaling pathways leading the inflammatory response. Excessive and/or inappropriate TLR signaling pathway activation can on the other hand lead to different acute or chronic inflammatory disease development including sepsis, rheumatoid arthritis, systemic lupus erythematosus and cancer. TLR and TIR-domain containing adaptor interactions represent potential sites for specific TLR signaling pathway inhibition. Expression of proteins with TIR domains are used by bacteria and viruses to suppress activation of TLR signaling response to infection. On the other hand mutations within the TIR domain of MyD88 are associated with lymphoma. We demonstrate that coiled-coil mediated dimerization of TIR domain TcpB of Brucella potentiates inhibition and prevents constitutive activation of innate immune response. Genetic fusion of TIR domains of human TLRs and MyD88 adaptor inhibits activation, similar to the monomeric TIR domain but leads to the constitutive activation of TLR signaling pathway at overexpression. We were able to design a TIR domain containing protein that lacks the constitutive activation and is superior to any described TIR-domain-containing protein in inhibiting TLR activation. Additionally those properties of dimeric TIR domain proteins provide an additional insight into the molecular mechanism of intracellular TLR signaling pathway, which is also used by the constitutive activity of MyD88 mutations found in B-cell lymphoma.

W1.10 Innate sensors

IL1.10.01
The long pentraxin PTX3: a paradigm for humoral pattern recognition molecules

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Soluble pattern recognition molecules (PRMs) are components of the humoral arm of innate immunity; they recognize pathogen-associated molecular patterns (PAMP) and are functional ancestors of antibodies, promoting complement activation, opsonization, and agglutination. In addition, several PRMs have a regulatory function on inflammation.
Pentraxins are a family of evolutionarily conserved PRMs characterized by a cyclic multimeric structure, and on the basis of structure, they have been divided into short and long families. C-reactive protein (CRP) and serum amyloid P component are prototypes of the short pentraxin family, while pentraxin 3 (PTX3) is a prototype of the long pentraxins. PTX3 is produced by somatic and immune cells in response to proinflammatory stimuli and Toll-like receptor engagement, and it interacts with several ligands and exerts multifunctional properties. PTX3 gene organization and regulation have been conserved in evolution, thus allowing its pathophysiological roles to be evaluated in genetically modified animals. Gene targeting of PTX3 has unequivocally defined functional roles of this molecule in innate immunity and inflammation. The molecular mechanisms underlying these properties indicate that PTX3 plays a role similar to that of antibodies, including in complement activation, opsonization, and glycosylation-dependent regulation of inflammation. In addition, recent data show that PTX3 is involved in the orchestration of tissue repair and remodeling, thus suggest that matrix and microbial recognition are common, ancestral features of the humoral arm of innate immunity.

W1.10.01
Viral inhibition of DNA-PK-dependent DNA sensing

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The host response to DNA virus infection includes the direct detection of viral DNA by the innate immune system which then initiates an inflammatory response. The pattern recognition receptors responsible for DNA sensing are under intensive research and, although several putative sensors have been proposed, in most cases their biological relevance is currently unknown. Here we show that DNA-dependent protein kinase (DNA-PK) can act as a DNA sensor in fibroblasts and in vivo and is important for sensing DNA viruses such as vaccinia (VACV) and herpes simplex virus (HSV) 1. In addition we present the discovery that a VACV protein, C16, can inhibit DNA sensing by directly binding DNA-PK and disrupting its ability to interact with foreign DNA. Removal of the gene which encodes C16 from VACV attenuates the virus in vivo and results in a more potent innate immune response to the infection. This discovery not only validates the biological relevance of DNA-PK as a DNA sensor for DNA viruses but furthers our understanding of how viruses such as VACV evolve to combat the host immune response.

W1.10.02
Hepatitis C virus degrades Riplet ubiquitin ligase to escape host innate immune response

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The innate immune system is essential for controlling viral infections. RIG-I is a cytoplasmic viral RNA sensor and triggers the signal to induce type I interferon production through IPS-1 adaptor molecule. Riplet ubiquitin ligase is essential for RIG-I activation and mediates K63-linked polyubiquitination of RIG-I C-terminal region. Hepatitis C

virus (HCV) is a major cause of hepatocellular carcinoma. HCV NS3-4A protease has the ability to suppress host innate immune response. The NS3-4A protease cleaves a peptide bond just after Cys-805 of IPS-1 to abrogate RIG-I signaling, and an IPS-1 C508A mutant protein is resistant to NS3-4A cleavage. Interestingly, we found that NS3-4A could reduce RIG-I signaling in response to HCV RNA even in the presence of the IPS-1 C508A mutant protein. We found that the Riplet protein level was severely reduced in human hepatocyte cell lines that contains HCV full-length replicons. Moreover, ectopic expression of NS3-4A reduced the Riplet protein level and abrogated Riplet-mediated RIG-I polyubiquitination. Within Riplet RING-finger domain is a sequence similar to NS3-4A target consensus sequence. Mutations within Riplet RING-finger domain rendered the Riplet protein resistant to NS3-4 protease activity. Although Riplet was required for RIG-I foci formation in cytoplasm in response to viral infection, RIG-I foci were not detected in cells infected with HCV JFH1. Taken together, our data indicate that HCV degrades Riplet ubiquitin ligase to escape host innate immune response.

W1.10.03
Lysosomal Dnase2a is required for the autophagic clearance of self damaged nuclear DNA

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Deficiencies in nucleases that degrade DNA lead to accumulation of self DNA, activation of innate immune responses and development of autoimmune disorders in mice and humans. How undegraded DNA accumulates and leads to immune responses in these deficiencies is not well understood. Here, we investigate the cell type specificity, source and sub-cellular localization of accumulated DNA in the absence of the major lysosomal DNA degradation enzyme, Dnase2a. Deletion of this nuclease results in inflammation and chronic polyarthritis that resembles human. We find that high levels of undegraded DNA accumulate in both phagocytic and non-phagocytic cells of Dnase2a-deficient mice. This excess DNA is of nuclear origin and co-localizes outside the nucleus with γ-H2AX that marks damaged double-stranded DNA, and increases in abundance after induction of DNA damage. DNA appears to escape from the nucleus via nuclear buds and speckles and requires autophagosomes and lysosomes for degradation. A deficiency in autophagy leads to higher levels of extra-nuclear DNA. Furthermore, an innate immune response is induced in either Dnase2a-deficient or autophagy-deficient cells, but is abolished upon depletion of the Sting-mediated cytosolic nucleic acid sensing pathway. Our results reveal a process by which damaged nuclear DNA is transported via nuclear buds and autophagosomes from the nucleus to the lysosome where it is degraded by Dnase2a, thus preventing self DNA recognition and activation of innate immune responses. Such a process may help explain how inflammation is regulated in diverse conditions, including cancer, chemotherapy and aging when damaged DNA is generated at high levels.

W1.10.04
Commensal microbiota monitoring by RLRs regulates contact hypersensitivity response

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RIG-I like receptors (RLRs) play a major role in response to cytosolic viral RNAs by initiating an antiviral immune response through the recruitment of the mitochondrial adaptor protein MAVS. A novel regulatory role of the RLRs pathway in sterile inflammation has been recently identified in a murine model of experimental colitis. Using another sterile inflammation model, Contact Hypersensitivity (CHS) to the strong hapten 2,4-dinitrofluorobenzen (DNFB), we showed that MAVS-deficient mice developed an exacerbated response. We characterized this inflammatory response at the challenge site of MAVS-deficient mice and demonstrated a higher oedema due to an increase of immune cell infiltrate with higher proportion of cytotoxic CD8+ T cells and an enhanced production of inflammatory cytokines. As it has been suggested that the monitoring of commensal bacteria by RLRs may be involved in the regulation of intestinal inflammation, we evaluated the role of the microbiota in the enhanced CHS response in MAVS-deficient mice. We performed cohousing experiments and showed that the inflammatory phenotype of MAVS-deficient mice can be transferred to WT mice. Moreover, antibiotic treatment of MAVS-deficient mice strongly inhibited the increased CHS response, confirming the importance of commensal microbiota in the regulation of CHS. Altogether, our results highlight that monitoring of commensal microbiota by RLRs is involved in the regulation sterile inflammatory CHS response.

W1.10.05
Essential role for Bruton's tyrosine kinase outside the B cell compartment in bacterial infection

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Bruton's tyrosine kinase (Btk) is a key component in B-cell receptor and Toll-like receptor signaling. Lack of functional Btk renders humans and mice highly susceptible to infections. Aim of our study was to determine the role of Btk in the host defense response against the capsulated bacterium *Klebsiella pneumoniae*.
Btk-KO mice inoculated intranasally with *K.pneumoniae* were highly susceptible to infection as reflected by increased bacterial counts in the lung, increased dissemination of bacteria to other organs within 24-36 hour and increased mortality. Impaired bacterial clearance was associated with elevated inflammation. Natural IgM antibodies against *K.pneumoniae* were found in WT mice (but not in Btk-KO mice) and these antibodies facilitated phagocytosis of *K.pneumoniae* by alveolar macrophages. Strikingly, Btk-KO mice of which the B cell compartment was 'rescued' by reinforced expression of Btk (Btk-KO/CD19-Btk Tg mice, with normalised natural IgM levels), were not protected against *K.pneumoniae* infection and demonstrated similar susceptibilty as Btk-KO mice. Moreover, B-cell deficient (muMT) mice displayed similar bacterial clearance in lungs and other organs as WT mice. Finally, Btk-KO mice with reinforced expression of Btk in $MHCII^+$ cells (Btk-KO/MHCII-Btk Tg mice), i.e. B cells and macrophages, showed an almost complete reversal in susceptibility to *K.pneumoniae*.
These data demonstrate that B cells and natural IgM antibodies contribute little to nothing to the early host defense response against *K.pneumoniae.* Moreover, these data indicate that Btk is essential for receptor signaling outside the B-cell compartment during *K.pneumoniae* infection and suggest an important role for Btk in myeloid cells during bacterial infection.

W1.10.06
Genetic variants in donor *PTX3* impair antifungal immunity and predispose to invasive aspergillosis after stem cell transplantation

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Invasive aspergillosis is a major complication associated with hematopoietic stem cell transplantation, although individual risk varies considerably. Given the pivotal role of the long pentraxin 3 (PTX3) in antifungal immunity, we investigated the contribution of genetic variation in PTX3 to risk of invasive aspergillosis in a discovery cohort of 229 recipients of allogeneic hematopoietic stem cell transplants and their donors. The analysis was validated in a large multicenter study involving 107 cases of invasive aspergillosis and 223 matched

controls. A donor homozygous haplotype (h2/h2) in PTX3 increased risk for invasive aspergillosis in the discovery (adjusted hazard ratio, 3.15; P=0.002) and validation (adjusted odds ratio, 2.78; P=0.03) studies. Functionally, the h2/h2 haplotype led to decreased expression of PTX3 in neutrophils, presumably due to impaired mRNA stability, resulting in defective phagocytosis and clearance of the fungus. PTX3 deficiency was confirmed in bronchoalveolar lavage and lung specimens from transplant recipients with h2/h2 donors. In conclusion, our findings disclose a crucial role of PTX3 to human antifungal immunity and point to PTX3 deficiency as an important risk factor for invasive aspergillosis in stem cell transplant recipients.

W1.11 Toll-like and pattern recognition receptors

IL1.11.01
Regulation of the innate immune responses in wild mice

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Immune response leads to inflammation, which, if excessive, can often lead to the programmed death. How do genes control the balance between inflammation and cell death? To answer this question, we have elected to study the immune responses in so called wild-derived mice (WDM), which evolved from common ancestor with classical laboratory mice more than a million years ago and therefore share only 11% of their genome with laboratory mice. Such divergence of WDM results in phenotypic differences between WDM and classical laboratory mice, which we investigate by means of classical genetic analysis. In the last few years, we established several genetic screens in the WDM including hyper- and hypo-responsiveness to TLR-agonists, resistance to TLR-mediated necroptosis, in vitro responses to infectious pathogens, resistance to septic shock in vivo. In our published and ongoing research, we show that forward studies in WDM provide insights into human biology that would not be available without access to this largely untapped reservoir of genetic diversity. Because we have not exhausted the genetic diversity available in the WDM, we propose to further explore it in newly established genetic screens.

W1.11.01
Emerging roles of an innate immune regulator TAPE in the endosomal Toll-like receptor (TLR) and cytosolic RIG-I-like receptor (RLR) pathways

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Pattern-recognition receptors (PRRs) function to trigger innate immune defenses upon sensing pathogen-associated molecular patterns (PAMPs) from pathogen infection. Of these PRRs, endosomal TLRs, cytosolic RLRs, and DNA sensors are implicated in detecting microbial nucleic acids. Upon ligand stimulation, these PRRs activate major downstream pathways leading to the production of inflammatory cytokines and type I IFNs. Remarkable progress has been made in defining the importance of PRRs in innate immunity. Yet, signaling networks underlying these PRR pathways still remain complex. Our recent work has led to uncover an innate immune regulator termed TAPE (TBK1-Associated Protein in Endolysosomes), also known as CC2D1A/Freud-1/Aki-1. TAPE is located in the endolysosomal compartments. TAPE interacts and synergizes with TBK1 and TRIF to enhance IFN-β activation. TAPE knockdown abolishes the TLR3 and TLR4 pathways to IFN-β production. Furthermore, we extend the biological role of TAPE to the regulation of RLR signaling. TAPE functionally and physically interacts with RIG-I, MDA5 and IPS-1/MAVS to regulate IFN-β activation. TAPE impairment blocks IFN-β production upon RLR ligand stimulation or RNA virus infection. Our data demonstrate a critical role for TAPE in linking TLR3, TLR4, and RLRs to innate immune responses. Furthermore, we have generated immune cell-specific TAPE conditional knockout mice to explore in vivo roles of TAPE in regulating the endosomal TLR, RLR, or other innate sensing pathways.

W1.11.02
Smurf2 negatively regulates RIG-I-like receptor -mediated immune signaling

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The primary role of the innate immunityis to limit the spread of infectious pathogens, by recognition by Toll-like receptors (TLR) and RIG-like receptors (RLR).Innate immune response is under tight control to avoid unwanted tissue damage. Here, we identified smurf2, one of the Smad ubiquitin regulator factor proteins (the HECT family of E3 ligase members), as an important negative regulator of virus-triggered type I IFN signaling. Overexpression of Smurf2 inhibits Sendai virus-induced IFN and ISRE activation and the expression of downstream molecules. The E3 ligase defective mutant Smurf2 C716A loses the ability to suppress virus-induced type I IFN signaling, suggesting that this effect is dependent on the ubiquitin ligase activity of smurf2.Compared to WT cells, Smurf2 knockout MEF cells dramatically promote SeV induced IFN and ISRE activation and the production of Interferon-β. It promotes the K48 ubiquitination of target proteins. Further studies are ongoing to charaterize the signaling molecules which Smurf2 targets.

W1.11.03
PIAS1 negatively modulates virus triggered type I IFN signaling by blocking the DNA binding activity of IRF3

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During viral infection, production of proinflammatory cytokines including type I interferons (IFNs) is under stringent control to avoid detrimental overreaction. The protein inhibitor of activated STAT (PIAS) family proteins have been recognized as anti-inflammatory molecules by restraining type I IFN induced amplifying signaling. Here we identified PIAS1 as an important negative regulator of virus-triggered type I IFN signaling. Overexpression of PIAS1 repressed virus-or RIG-I like receptor stimulated type I IFN transcription, whereas knockdown of PIAS1 expression augmented virus-induced production of type I IFNs. PIAS1 with a mutation in the SAP domain retained the inhibitory function in virus-induced IFN transcription, but abolished the inhibition in IFN-stimulated signaling. SUMO E3 ligase activity dead mutant PIAS1/C350S still had the comparable inhibitory function with WT PIAS1.Further study indicated that PIAS1 interacted with IRF3 and inhibited the DNA binding activity of IRF3. Therefore,PIAS1 is important in maintaining proper amounts of type I IFNs and restrains its magnitude when the antiviral response intensifies.

W1.11.04
The evolutionary landscape of the NLR family is reflected in the species-specific response to infection

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Activation of the NLR family of cytoplasmic pattern recognition receptors initiates an innate immune response to a wide range of exogenous and endogenous ligands. This leads to a pro-inflammatory response via NFκB and stress kinase signalling pathways; and/or the secretion of interleukin-1β following caspase-1 processing by a multi-protein complex termed the inflammasome.
Humans possess 22 NLR proteins, but this number varies in other species. We have analysed the evolutionary patterns of the NLRs across a range of vertebrate species including mammals, birds, amphibians and fish. There are clear species specific expansions, and losses, of subsets of this receptor family including one pair of

receptors that have undergone linked genetic retention or loss across multiple species. The key inflammasome forming NLRs are almost entirely restricted to mammals; birds possess a very limited subset of receptors; fish show extensive receptor specific expansions; and the coelacanth is an evolutionary midpoint in NLR evolution. In addition a putative new NLR protein has been identified bioinformatically.
Functional analysis of the cellular response to ligand stimulation in different species supports the crucial role of these receptors in the innate immune response and provides a rationale for the different susceptibilities of species to infections such as Salmonella.

W1.11.05
CD14 as a key regulator of TLR-mediated responses of microglia

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Toll like receptor 4 (TLR4) is best-known for its recognition of lipopolysacharide (LPS), the cell wall component of Gram-negative bacteria. Binding of this ligand to TLR4 triggers the response via both MyD88- and TRIF-dependent signalling pathways, leading to production of cyto- and chemokines and thereby alarming and attracting the peripheral immunocompetent cells to invade into the site of insult. However, TLR4 is only fully functional in complex with several co/receptors, such as CD14. Our latest data show that CD14 increases the sensitivity towards LPS in a cell type-specific manner, making microglia far more sensitive to LPS than bone marrow and peritoneal macrophages. While application of low dose LPS into striatum of CD14ko brains reveals minor neutrophil recruitment compared to wildtypes, high LPS leads to augmented neutrophil influx. This phenomenon correlates well with *in vitro* data, revealing CD14 absence in microglia upon high LPS challenge to result in excessive but selective chemokine production, with highest impact on the neutrophil chemoatractant CXCL1. For such regulatory abilities, CD14 requires its membrane insertion and prolonged functionality, pointing to an involvement of signalling. Thus far we excluded the role of Syk and PLC that in comparison to dendritic cells do not have a contribution in CD14-dependent signalling in microglia. Importantly, we found various factors regulating the expression of CD14 receptor itself, which could thereby determine the extent and impact of a CD14-mediated control over TLR4 functions. Supported by the DFG (FOR1336).

W1.11.06
Bruton's tyrosine kinase plays an essential role in TLR-dependent human DC activation

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Bruton's tyrosine kinase (btk) plays an essential role in various biological functions of different cell types. Mutations in btk lead to the X-linked form of Agammaglobulienmia (XLA) in humans or in the xid phenotype in mice. However, in recent years the important physiological function of btk has been investigated in various cell types , such as macrophages, NK cells, neutrophils and others, mainly by using the animal models (xid mice). Furthermore, btk was recently linked to the innate immune system, in particular the TLR pathway. However, the TLR9 pathway in dendritic cells from XLA patients has not been investigated yet. We decided therefore to investigate whether Btk influences human DC responses upon TLR engagement. We show that btk plays an essential role in moDC responses to CpG: although responses to LPS/TLR4 induce normal DC activation in terms of up-regulation of activation markers, the CpG/TLR9 pathway is completely impaired in XLA patients. Furthermore, cytokine production upon TLR9 activation in XLA patients is radically impaired in terms of IL-6, IL-10, IL-12 and TNF-a production. Interestingly, btk resulted essential for STAT1 up-regulation in a TLR9-dependent manner. The important role of btk in human DC activation was confirmed after incubation of healthy DCs with the specific btk inhibitor resulting in impairment of TLR9 responses as seen in XLA patients. These findings may be of important significance for better understanding and managing clinical complications in different clinical conditions, such as agammaglobulinemia and lymphoid malignancies.

W1.11.07
Lysosomal oligopeptide transporter SLC15A4 regulates Toll-like receptor 7/9-mediated autoantibody production

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Lysosome is a key compartment that regulates the signaling from Toll-like receptors. We have reported that Solute Carrier family (SLC) 15A4, a lysosomal oligopeptide transporter expressed in the immune and nervous cells, played crucial roles in TLR7-, TLR9- and NOD1-mediated cytokine production in dendritic cells (DCs).
We here show that SLC15A4 is also expressed in B cells, and plays crucial roles in B cell functions. Lack of SLC15A4 reduced the serum IgG2c antibody level, which was caused by inability of SLC15A4-deficient B cells to produce IgG2c antibody in response to TLR stimulation. When SLC15A4-deficient B cells were stimulated by TLR7 or TLR9 ligand, cytokine production such as IFN-beta or IL-6 was impaired. We next examined TMPD-induced SLE model whether SLC15A4 was involved in autoantibody production since IgG2c antibody is thought to be one of the pathogenic factors in mouse autoimmune disease. SLC15A4-deficient mice failed to produce autoantibody such as anti-RNP and anti-DNA IgG antibodies. These results indicated that SLC15A4 contributes to pathogenesis of TLR7- or TLR9-dependent autoimmune diseases.
SLC15A4 gene is reportedly associated with the autoimmune disorders such as inflammatory bowel diseases and systemic lupus erythematosus in human. Although further investigation should be necessary, our findings suggest that SLC15A4 could be a novel therapeutic target for the autoimmune diseases.

W1.11.08
NLRC5-mediated regulation of MHC class I immune responses

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MHC class I and class II molecules are critical for the activation of human adaptive immune responses. Although a master regulator of MHC class II genes, CIITA (MHC class II transactivator) has been recognized for nearly two decades, the mechanism of MHC class I expression has been largely unknown until recently. We found one of NLR protein, NLRC5 is a MHC class I transactivator (CITA). Whereas CIITA can strongly induce MHC class II and related genes such as invariant chain, NLRC5 can specifically associate with and activate promoters of both classical (HLA-A, B, C) and non-classical MHC class I genes. Strikingly, NLRC5/CITA regulates not only MHC class I, but also regulates functionally related genes such as TAP1, LMP2 and b2microglobulin. In NLRC5 deficient mice, the expression of MHC class I and related genes is selectively downregulated and CD8 T cell responses are impaired. Therefore, NLRC5/CITA and CIITA regulate concerted expression of genes in MHC class I and class II pathways, respectively.

W1.11.09
Co-ligation of TLR3 and Dectin-1 agonists potentiates Th1 polarization capability on monocyte derived Langerhans cells in vitro

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Langerhans cells represent the first dendritic cell subtype that encounters foreign pathogens due to its localization in skin and stratified mucosa. Their potential to initiate T-cell responses has recently been challenged. Since viral and fungal infections are among the most common in these tissues, we have studied effects of Toll-like receptor 3 (TLR3) and Dectin-1 agonists, polyinosinic:polycytidylic acid (poly I:C) and curdlan, respectively, on maturation of their in vitro counterparts, monocyte derived Langerhans cells (MoLCs). Immature (im)MoLCs were generated in the presence of GM-CSF, IL-4 and TGF-β1 and then treated with polyI:C, curdlan or their combination for 2 days. Both single or combined treatments elicited phenotypic maturation of imMoLCs with increased allostimulatory capacity. Poly I:C-treated MoLCs up-regulated both the production of IFN-γ and IL-17 by alloreactive CD4+ T cells while curdlan-treated MoLCs stimulated only the production of IFN-γ, compared to imMoLCs. Poly I:C+curdlan-treated MoLCs produced higher concentrations of IL-12 and IL-10 and stimulated more strongly the production of IFN-γ by alloreactive CD4+ T cells then curdlan-treated MoLCs. At the same time, poly I:C+curdlan-treated MoLCs produced more IL-23 but less IL-27 and were weaker stimulators of IL-17 by alloreactive CD4+ T cells then poly I:C-treated cells. Poly I:C+curdlan-treated MoLCs showed higher expression of CD40 and CCR7 then both poly I:C- and curdlan-treated MoLCs. Ligation of both TLR3 and Dectin-1 agonists on imMoLCs upregulated their Th1 polarization capability compared to single agonists. This finding suggests that MoLCs could be considered for their potential use in immunotherapy.

W1.11.10
TLR-9 dependent dendritic cells activation by filamentous bacteriophage targeting DEC-205

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Based on a modification of the phage display technology, we developed and described an antigen delivery system based on the filamentous bacteriophage fd, able to elicit full-spectrum of antigen-specific immune responses. We improved this system by constructing phage particles displaying a single chain antibody fragment known to bind the mouse dendritic cells (DC) surface molecule DEC-205, and thus enabling direct delivery of phage particles to DC. We demonstrated that targeting DCs via DEC-205 delivers phage particles into late endosome/lysosome LAMP-1 positive compartments. Moreover we observed that DCs targeted by fd virions via DEC-205 upregulate accessory molecules, produce IFN-alpha and IL-6, and mediate an increased antigen presentation. We found that these activities of fd-antiDEC-205 phage particles were TLR-9 dependent. Since to become active TLR-9 translocate into LAMP-1 positive compartments, we suggest that phage particles, containing a single strand DNA genome reach in CpG motifs, are able to intercept and trigger the active TLR-9 innate immune receptor and thus to enhance the immunogenicity of the displayed antigenic determinants.

W1.11.11
Bacterial 23S rRNA is recognized by the endosomal TLR13 unless it is modified to constitute erythromycin resistance

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The innate immune system, being very sensitive, tends to over-amplify inflammatory signals. Accordingly, bacterial infections often cause septic shock. Major intiators of sepsis are bacterial infections with Escherichia coli and Staphylococcus aureus. Analyzing their interaction with the host, we observed recognition of a segment within bacterial 23S ribosomal (r) RNA through TLR13 in mice. The same segment is a target for antibiotics like macrolide, lincosamide and streptogramin group (MLS) antibiotics (including erythromycin). As a resistance mechanism bacteria prevent binding of MLS antibiotics to 23S rRNA by inducing N-methylation at a specific adenosine (A) upon acquisition of a distinct erythromycin resistance related methyltransferase (e.g. ErmB or ErmC) via horizontal gene transfer. Next to post transcriptional RNA modification, mutation of the specific A (e.g. A→G, found also in eukaryotic 28S rRNA) renders bacteria resistant. Of note, both RNA alterations abrogated activation of TLR13, thereby enabling immune evasion.
Utilizing Tlr23479-/- mice, we observed Gram negative bacterial total RNA to be non TLR13 activating in contrast to purified Gram negative bacterial 23S rRNA. We speculate that it is another RNA subspecies that inhibits the stimulative capacity of 23S rRNA.

W1.11.12
TLR triggered MAPK signalling is responsible for the regulatory phenotype of macrophages in response to Schistosoma mansoni cercarial secretions

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The interaction between antigen presenting cells in the skin with molecules secreted by cercariae of *Schistosoma mansoni* constitutes the first point of contact between the host's immune system and the pathogen. Macrophages (MΦ) may play a pivotal role in modulating the immune response in the skin as they readily take up the molecules secreted by cercariae upon invasion.
MΦ produce high levels of IL-10 when exposed to cercarial excreted/secreted (E/S) products, but it is unknown what signalling pathway(s) drive the production of IL-10 rather than IL-12 , or how they are linked to innate recognition of E/S antigens. Our findings demonstrate that TLRs, particularly TLR-4 and TLR-2, play a fundamental role in the induction of IL-10. Furthermore, MyD88 is essential for the activation of multiple MAPK pathways which in turn control E/S product induced IL-10. Selective chemical inhibition of specific pathways allowed us to determine the contribution of each signalling cascade. We determined that the activation of the MEK/Erk and p38 induced the production of IL-10, whilst it negatively affected IL-12. Furthermore, p38 activation leads to the phosphorylation of CREB, which is likely responsible for the observed effects on both IL-10 and IL-12. Finally, we show that TLR-4 and TLR-2 are directly implicated in the activation of these pathways.
From these findings we propose a mechanism by which *S. mansoni* E/S products induce a specific MAPK signalling cascade that triggers IL-10 production in MΦ by binding their TLRs, thus polarizing the immune response in the skin.

W1.11.13
Redox-based control of synergy and antagonism among TLRs modulates cytokine production by monocytes

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In human monocytes, single stimulation of different TLRs results in induction of IL-1β and of cytokines downstream of it. The process is tightly regulated by redox signaling. Here we show that the simultaneous administration of agonists of TLR 2, 4 and 7, at doses providing optimal monocyte activation when used individually, strongly enhances IL-1β but severely impairs IL-1Ra and IL-6 secretion and induces oxidative stress. Antioxidants restore IL-1Ra and IL-6 secretion, demonstrating the functional link between generation of oxidative stress and impairment of secretion of cytokines downstream of IL-1β. When TLRs are concomitantly triggered by lower doses of the three agonists, which are unable to induce monocyte activation if provided individually, oxidative stress is prevented and cytokine secretion is maintained. Thus, multiple triggering of TLRs and sustained doses of single agonists concur to the loss of redox control and the consequent block of secretion of cytokines downstream of IL-1β. In conclusion, we have identify a novel redox-mediated mechanism responsible for the correct development of TLR-triggered inflammatory response. This mechanism provides a molecular basis to the unbalance between pro- and anti-inflammatory circuits occurring in pathologic conditions such as sepsis and chronic diseases, and open the way to new strategies for controlling inflammation.

W1.11.14
A TNFα-CCL20-CCR6 axis drives Nod1-induced B cell recruitment

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Innate immune responses provoke the accumulation of leukocytes at sites of inflammation. In addition to monocytes and granulocytes, B cells also participate in anti-microbial innate immune responses, however the mechanisms for recruitment of B cells to sites of inflammation are not well understood. To study B cell accumulation following systemic inflammation, we used a model synthetic ligand that stimulates a specific pattern recognition molecule, Nod1. Upon exposure to Nod1 agonists, both B cells and neutrophils rapidly accumulate within the spleen and dendritic cells migrate into the periarterial lymphoid sheath. Nod1 stimulation led to a marked increase in several chemokines within the spleen, including CXCL13, CCL2 and CCL20. While the Lymphotoxin pathway was critical for the induction of the B cell chemoattractant CXCL13 in response to Nod1 agonists, B cell accumulation within the spleen following Nod1-induced systemic inflammation was independent of the Lymphotoxin pathway. In contrast, a CCR6/CCL20 chemokine loop instructed rapid B cell recruitment to the spleen in response to systemic administration of Nod1 agonists in a TNFα-dependent manner. These results reveal a novel mechanism for rapid mobilization of B cells during inflammation, and shed light on how B cells participate in innate immune responses to microbial stimulation.

W1.12 Lectins and glycoimmunology

IL1.12.01
Galectins in Inflammation and Immunity

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Galectins are a family of β-galactoside-binding proteins. They can bind to cell-surface glycans, thereby affecting a variety of cellular processes. They are also present in the cytosol and nucleus and can influence cellular functions through interacting with other regulators in a non-glycan-dependent fashion. Recombinant galectin-1 and -9 have been shown to induce apoptosis in T cells, by engaging cell surface glycoproteins, and suppress certain inflammatory responses when administered in vivo. Recombinant galectin-3 has been shown to promote the inflammatory responses by activating various leukocytes and inducing migration of monocytes/macrophages.
We demonstrated that endogenous galectin-3 is recruited to the cytoplasmic side of the immunological synapse in activated T cells and promotes HIV-1 viral budding in a fashion that is dependent on Alix. We also found galectin-3 suppresses the bacteriocidal effects of macrophages on Listeria monocytogenes and that the protein is accumulated around the bacteria that are escaping the phagosomes.
We demonstrated that galectin-3 negatively regulates the Th cell response in the Th1 and Th17 directions through dendritic cells. By using galectin-3-deficient mice, we established that galectin-3 promotes allergic airway inflammation and airway hyperresponsiveness, as well as allergic skin inflammation, through promoting the Th2 response.
Galectin-7 is down-regulated in human psoriatic lesions compared with non-lesional sites. We demonstrated that knocking down galectin-7 expression in the human keratinocyte cell line HaCaT resulted in increased production of the inflammatory cytokines IL-6 and IL-8, in response to various immune stimuli. Additional results suggest that galectin-7 has a suppressive effect on the inflammatory response in keratinocytes.

IL1.12.02
Molecular interplay between sialic acids and siglecs in subversion of host innate immune response by Pseudomonas aeruginosa

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Pseudomonas aeruginosa (PA) is an opportunistic pathogen and associated with immune-compromised host. We have demonstrated PA (PA+Sias) adsorbed both α2,3- and α2,6-linked Sias from the host serum. Sialic acid-binding immunoglobulins like lectins (siglecs) are usually present on different immune cells. They are important in cellular interactions with pathogens and functional consequences of the engagement of notorious pathogen like PA with siglecs are unclear. Innate immune cells mainly neutrophils and macrophages generally express receptor like siglec-1, siglec-5 and siglec-9 on their surface. PA+Sias interact maximally with NK-cells and monocytes through siglec-7 and siglec-9 via α2,6- and α2,3-linked sialic acid respectively. Adsorbed Sias on PA also show association with both human macrophages and neutrophils mainly via α2,3-linked sialic acid-siglec-9 dependent manners. In contrast PA shows negligible binding with both B- and T-cells. Both macrophages and neutrophils also exhibits enhance phagocytosis of PA+Sias compared to PA-Sias. The interaction of PA with neutrophils shows reduced oxidative burst, releases of elastase and enhances expression of IL-10 in transcriptional and translational levels. All these events result decreased NETs formation thus establishing the role of adsorbed Sias that can weaken neutrophil's protective activity. Enhanced survivality of PA demonstrated by significant increased in CFU counts supported strongly for their resistance towards NETs. It may consider as a general mechanism for pathogens that cannot synthesize Sias in order to subvert immunity. Such Sias-siglec interaction also may be accompanied by modulation of Th1 and Th2 cytokines which may likely be due to the function of TLR and its downstream signaling.

W1.12.01
C-type lectin MCL is a receptor for mycobacterial cord factor TDM and is critical for Mincle induction

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We have previously reported that Mincle is a receptor for mycobacterial tuberculosis. Mincle recognizes mycobacterial glycolipid, trehalose dimycolate (TDM) and triggers inflammatory

responses such as cytokine/chemokine production and lung granuloma formation. These TDM responses were totally abolished in Mincle-deficient mice, suggesting that Mincle is a dominant receptor for TDM. Although Mincle expression was barely detected under steady-state conditions, TDM stimulation strongly induced Mincle expression. These data prompted us to hypothesize that an alternative TDM receptor expressing under resting conditions may promote the initial induction of Mincle. We have searched for the novel TDM receptor and finally identified MCL (macrophage C-type lectin, also called clec4d, clecsf8) as a candidate receptor. MCL is constitutively expressed in myeloid cells. MCL directly bound to TDM and transduced activation signals via ITAM-bearing adaptor molecule FcRgamma. We generated MCL-deficient mice and analyzed the function of MCL in TDM responses. MCL-deficient mice failed to induce Mincle expression upon TDM stimulation. As a result, TDM-induced inflammatory responses including cytokine/chemokine production and lung granuloma formation were significantly impaired in MCL-deficient mice. Finally, we analyzed the function of MCL against mycobacterial infection. MCL-deficient cells showed the significant reduction of Mincle induction and compromised immune responses against mycobacterial infection.
In summary, MCL is a dominant TDM receptor at resting stage and triggers Mincle induction for efficient immune responses against mycobacteria.

W1.12.02
Targeting C-type Lectin receptors on dendritic cells for neonatal adjuvant setting

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Early life is characterized by a high susceptibility to infection, poor innate responses, and biased CD4 T cell response to many vaccines to TH2. New adjuvant settings should take into consideration these particularities of the neonatal innate immune system, such as its poor capacity to respond to TLR activation. We are thus investigating alternative innate pathways to TLRs for dendritic cell activation, such as C type lectin receptors (CLRs) that signal through the Syk/CARD9 pathway, to prime TH1 protective response in neonates.
Among CLRs, targeting Dectin-1 was evaluated using curdlan, a beta 1-3 glucan, as an adjuvant in neonatal vaccination inducing strong Th1 and Th17 responses. In a subunit vaccine including TB10.4 antigen and curdlan, protective response were induced against aerosol challenge with Mycobacterium tuberculosis H37Rv. The efficiency of Curdlan relies on the mobilization of dermal dendritic cells as well as RORgt-gamma-delta T cells in the dermis of neonatal mice.
The potency of beta-glucans was also evaluated on human neonatal dendritic cells for their capacity to induced appropriate inflammatory responses.
This work illustrates the potency of Dectin-1 as an emerging adjuvant candidate. We showed that Dectin-1 triggering can induce pro-inflammatory cytokines both in mice and in humans. Signal through Dectin-1 was able to induce protective TH1/TH17 response in newborn mice and protect them from MTB infection. These results show the potential of CLR related pathways as a relevant target for future adjuvant development.

W1.12.03
A novel role of beta-glucan in improving anti-tumorimmunity by regulating monocytic myeloid-derived suppressor cells

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Accumulating evidence has shown that a population of cells with suppressive activity called myeloid-derived suppressor cells (MDSCs) contributes to the negative regulation of immune responses and plays an essential role in tumor-induced immunosuppression. Promoting MDSCs differentiate into mature myeloid cells without suppressive capacity might be considered one of the most promising approaches in cancer immunotherapy. β-Glucans have been reported to function as potent immunomodulators to stimulate innate and adaptive immune responses, which contributes to their anti-tumor property. We firstly found the expression of dectin-1 (receptor of β-glucan) on MDSC. Therefore, we hypothesized that WGP (whole β-glucan particles) treatment through dectin-1 pathway may circumvent MDSC-mediated immune suppression.
Here we investigated the effect of particulate β-glucans on MDSCs and found that β-glucan treatment could promote the differentiation of M-MDSCs (monocytic MDSCs) into a more mature $CD11c^{+}$ $F4/80^{+}$ $Ly6C^{low}$ population via dectin-1 pathway in vitro, which is NF-κB dependent, and the suppressive function of M-MDSCs was significantly decreased. Treatment of orally administered yeast-derived particulate β-glucan drastically down-regulated MDSCs but increased the infiltrated DCs and macrophages in tumor-bearing mice, thus eliciting CTL and Th1 responses, inhibiting the suppressive activity of regulatory T cells, thereby leading to the delayed tumor progression.
We show here for the first time that β-glucans induce the differentiation of MDSCs and inhibit the regulatory function of MDSCs, therefore revealing a novel mechanism for β-glucans in immunotherapy and suggesting their potential clinical benefit.
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W1.12.04
Soluble CD52 is a negative regulator in the innate immune system

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CD52 is a small, heavily glycosylated molecule comprising a short peptide linked to the cell membrane via a GPI anchor. The physiological role of CD52 has been elusive, but we have recently shown that T cells with high expression of CD52 are immunosuppressive via the release of soluble CD52. In the current study, we investigated the effect of recombinant soluble CD52 (CD52-Fc) in the innate immune system.
In response to LPS, CD52-Fc in a nanomolar dose-response range inhibited cytokine production by human and mouse monocytes and dendritic cells. Similarly, CD52-Fc inhibited responses to other innate immune stimuli including Pam3CSK, CPG, Lysteria monocytogenes, Poly I:C, and the inflammasome activators monosodium urate, alum and nigericin.
Cleaving the sialylated N-linked oligosaccharides of CD52 reversed the inhibitory effect of CD52 on LPS-induced IL-1β secretion, demonstrating the requirement of the oligosaccharide moiety for the inhibitory effect of CD52. Moreover, the inhibition was dependent on sialic acid, because desialylation with A. ureafaciens neuraminidase also reversed the inhibitory effect of CD52-Fc. In accord with this finding, preliminary studies showed that CD52-Fc interacts with the lectin receptor, Siglec 10.
The inhibitory effect of CD52-Fc was applied to a model of sepsis in C57Bl/6 mice: CD52-Fc (10 μg/kg) markedly reduced plasma cytokine concentrations 2 h following i.p. injection with, or up to one hour, after LPS (15 mg/kg).
Our findings demonstrate that soluble CD52 suppresses innate immune responses and is a candidate immunotherapeutic agent.

W1.12.05
Galectin-3 promotes cell-to-cell transmission of HIV-1

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Galectin-3 (Gal3), a β-galactoside-binding lectin, has been reported to regulate various immune functions. Furthermore, reports indicate that Gal3 expression is greatly induced by HIV-1 infection in T cells. However, the role of Gal3 in HIV-1 infection remains unclear. Lipid raft integrity plays a critical role in virological synapse formation during HIV infection, and previously we have demonstrated that endogenous Gal3 is accumulated in lipid rafts of dendritic cell surface membrane. Therefore, we hypothesized that galectin-3 may play a role in viral cell-to-cell transmission during virological synapse formation. Here, we show that Gal-3 was co-localized with Gag and Env in lipid raft at cell-to-cell junction of HIV-1-infected Magi5 (HeLa cells expressing CD4, CXCR4 and CCR5) and Hut78 $CD4^+$ T cells. In addition, results from conventional cell-to-cell transmission assay indicate that HIV-1 transmission efficacy is significantly attenuated in Gal3 knockdown Magi5 and Hut78 T cells ($p<0.05$). On the other hand, HIV-1 transmission efficacy is significantly increased by overexpression of Gal3 in Jurkat T cells ($p<0.05$). Moreover, Gal3-promoted HIV-1 cell-to-cell transmission efficacy is positively correlated with the expression level of Gal3 in the effector cells ($p<0.05$). Time-lapse confocal microscope indicates colocalization of Gal3-EGFP and HIV Gag-iCherry at virological synapses between effector and target cells. In addition, both Gal3-EGFP and Gag-iCherry were cotransmitted from the effector cells to the target cells via virological synapses. These results were confirmed by using human primary $CD4^+$ T cells. Altogether, our studies demonstrated an active role of Gal3 in HIV-1 cell-to-cell transmission and revealed a potential target for HIV-1 therapy.

W1.12.06
Galectin 3 affects DCs:NKT cell interaction in the development of αGalCer-induced hepatitis

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We used α-galactosylceramide (αGalCer) induced liver injury, well established murine models of NKT cell mediated hepatitis, to study the role of Galectin 3 (Gal-3) in acute liver injury.
We tested susceptibility to αGalCer-induced hepatitis in galectin-3-deficient (Gal-3KO) mice and wild-type (WT) C57BL/6 mice.
One microgram/mouse of αGalCer i.v. significantly enhanced expression of Gal-3 on dendritic cells (DCs) and NKT cells of WT mice. Gal-3KO mice were less sensitive to αGalCer-induced hepatitis as evaluated by liver enzyme test, histology, cytokine production and intracellular staining of immune cells in the liver. The level of IL-10 in the sera and percentage of IL-10-producing NKT cells were significantly higher in αGalCer-treated Gal-3KO mice. Percentage of liver infiltrating CD11c (+) DCs, CXCR3(+) DCs, CD1d(+) DCs and TNFα-, IFNγ-, and IL-12-producing DCs was significantly lower in αGalCer-treated Gal-3KO mice. In vitro, αGalCer-loaded DCs, isolated from livers of untreated Gal-3KO mice, produced significantly higher amounts of IL-10 and significantly lower amounts of IFNγ compared to DCs from WT mice confirming that. αGalCer-induced production of IL-10 and IFNy by DCs is Gal-3 dependent. Liver DCs (5x105/mouse) of untreated WT mice, but not from Gal-3KO mice, transferred in Gal-3KO recipient significantly enhanced αGalCer-induced hepatitis. Pretreatment of WT mice with a selective inhibitor of Gal-3 led to the attenuation of αGalCer-induced liver injury and provided similar effect as Gal-3 deletion.
Gal-3 plays an important pro-inflammatory role in αGalCer-induced hepatitis by affecting DCs:NKT cell interaction in the liver.
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W1.13 Fc and Fc-like receptors

IL1.13.01
Inside-out and outside-in Fc receptors: impact on antibody therapy of cancer

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Fc receptors are an important bridge between the cellular and humoral branches of the immune system. Residing on monocytes, granulocytes, platelets, B lymphocytes, NK cells and dendritic cells, triggering of Fc receptors by complexed antibodies can lead to e.g. phagocytosis, respiratory burst, cytokine production and antigen presentation. Over the past decades, Fc receptors got more attention because of their important role in antibody therapy. Immunotherapy using monoclonal antibodies (mAb) has emerged as an excellent therapeutic strategy for cancer, immune deficiencies, immune regulation and autoimmunity. The first FDA-approved antibody for human neoplasia was Rituximab, targeting B-cell CD20 for treatment of non-Hodgkin's lymphoma and immune diseases.
An important mechanism of action of rituximab is antibody-dependent cellular cytotoxicity (ADCC), depending on Fc receptor bearing effector cells, such as NK cells and macrophages. In vitro also complement dependent cytotoxicity (CDC) and apoptosis induction are described as important mechanisms. The Immunotherapy group investigates the importance of these distinct effectormechanisms in vivo, including the possibility of apoptosis induction by crosslinking of Fc-receptors. For the latter purpose an transgenic mouse was developed, with normal expression of Fc receptors, but without the signaling of these receptors.(de Haij et al, Cancer Research 2010). Also the effectiveness of IgA as a novel isotype in antibody therapy is being explored in vivo (EMBO MM, in press). Knowledge of the biology of both IgG and IgA receptors is essential for these investigations.

IL1.13.02
Fc gamma Receptors: a 50 year anniversary

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Fcgamma R are an heterogeneous family of molecules of the Ig superfamily with effector and immunoregulatory functions. The Fcgamma RI bind IgG with high affinity whereas FcgRII, FcgRIII and FcgRIV (in the mouse) bind IgG immune complexes. They comprise several activating receptors characterized by an Immunoreceptor Tyrosine Activation Motif (ITAM) in their intracytoplasmic region or in their associated chains, and a unique single chain inhibitory receptor, FcgRIIB, with an Immunoreceptor Tyrosine Inhibition Motif (ITIM) in the intracytoplasmic domain.
Fcgamma R are expressed by most if not all cells of the immune system. The innate cell subpopulations co-express activating and inhibitory Fcgamma R, whereas B cells selectively express inhibitory FcgRIIB and NK cells mostly FcgRIII. Expression of FcgRII can be found on T lymphocytes as well. The ectopic expression of FcgRIIB can be found on some cancer cells such as in human metastatic melanoma.
I will present the FcgR family members and their functions to introduce the workshop.

W1.13.01
Role of IgG, IgG receptors and neutrophils in anaphylactic reactions

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Allergic reactions are generally considered to be triggered by the activation of mast cells or basophils, when IgE antibodies bound to their receptors (FcεRI) get crosslinked by specific antigens. Challenging this dogma, we could recently demonstrate that a murine model of allergic shock (anaphylaxis) depended primarily on IgG, IgG receptors and the activation of neutrophils (JCI 2011). Anaphylaxis was abolished in mice lacking all activating IgG and IgE receptors (FcRγ-/- mice). Interestingly, transfer of human neutrophils into

antigen-immunized FcRγ-/- mice restored a shock upon antigen challenge, suggesting a) that neutrophils alone can induce anaphylaxis, and b) that human neutrophils bare IgG receptors (FcγRs) responsible for this reaction.
Human neutrophils constitutively express FcγRIIA (CD32A) and can induce FcγRI (CD64) expression under inflammatory conditions. As both receptors are activating IgG receptors, we hypothesized that hFcγRIIA and/or hFcγRI could contribute to allergic reactions.
Using unique models of hFcγR-transgenic mice, we could show that both hFcγRIIA and hFcγRI were sufficient to induce anaphylactic reactions in mice. hFcγRI-induced anaphylaxis depended on the activation of neutrophils (Blood 2013), whereas both neutrophils and monocytes contributed to hFcγRIIA-triggered reactions. Supporting a role for these IgG receptors and neutrophils in human anaphylaxis, we found that human neutrophils released anaphylactogenic mediators upon stimulation with IgG immune complexes (Blood 2012).
Together our data suggest that IgG antibodies as well as their receptors, e.g. hFcγRIIA or hFcγRI, may contribute to these afflictions in humans and might therefore be novel targets for anti-allergic therapy.

W1.13.02
Role of the phagocytic synapse in the recognition of bacteria and the subsequent induction of Th17-responses

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Airway infections caused by extracellular bacterial pathogens such as *Staphyloccus aureus* and *Streptococcus pneumoniae* cause millions of deaths every year. In response to pneumococcal replication, bacterial lysis occurs which leads to the release of many pathogen-associated molecular patterns. Immune cells may recognize soluble ligands (released during lysis) or intact bacteria and induce a local Th17 response, ultimately leading to bacterial clearance. The presence of opsonizing antibodies may also be relevant, as cross-talk between Fc gamma receptors and pattern recognition receptors signaling pathways may affect the ensuing cellular response.
The aim of this study is therefore to examine the immune response to soluble and particulated bacterial components and examine the effects of IgG. In the presence or absence of purified human IgG, primary human immune cells (PBMCs) were stimulated with surface-coated or soluble TLR/CLR ligands, including whole bacterial lysate. We then examined the early innate response (IL-6, TNFa, IL-23 and IL-1b) as well as the late cytokine response (IL17a, IFNg). Our results suggest that stimulation of PBMCs with surface-coated, but not soluble IgG induces production of TNFa, IL-1b and IL-17A. Co-stimulation with surface-bound IgG and Pam-3-CSK (either soluble or surface-bound) induced a synergistic IL-1b response compared to co-stimulation with either Pam-3-CSK or IgG alone, suggesting molecular cross-talk between FcγR and TLR signaling pathways. Currently, experiments are ongoing to identify the exact innate signaling pathways that lead to this synergistic effect in the context of the 'phagocytic synapse'.

W1.13.03
Anti-inflammatory activity of IVIg mediated through the activating FcγRIII (CD16) is induced by ITAMi signaling

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Fc receptors are critical components of the innate immune system responsible for the recognition of cross-linked antibodies and the subsequent clearance of pathogens. However, in autoimmune diseases, these receptors play a role in the deleterious action of self-directed antibodies and as such are candidate targets for therapeutic approaches. Intravenous immunoglobulin (IVIg) has been used in the treatment of several autoimmune and inflammatory diseases. However, its mechanism of action remains incompletely understood. Here, we hypothesized that IVIg might induce its anti-inflammatory effects through inhibitory ITAM (ITAMi) signaling targeting activating Fc receptors bearing an ITAM in the FcR signaling adaptor. We demonstrated that interaction of FcR-associated mouse or human FcRIII with IgG1 or IVIg, or with bivalent anti-FcRIII $F(ab')_2$ reduced calcium responses, ROS production, endocytosis and phagocytosis, induced by heterologous activating receptors on monocytes/macrophages and $FcRIII^+$ transfectants. This inhibition required the ITAMi configuration of the FcRIII-associated FcR subunit and SHP-1 recruitment as shown by the formation of intracellular "inhibisome" clusters containing FcRIII and the targeted heterologous activating receptor. *In vivo,* anti-FcγRIII $F(ab')_2$ and IVIg lead to reduced renal inflammation and improved immune thrombocytopenic purpura in mice lacking FcRIIB. These results suggest that circulating IgG may continuously interact with FcRIII inducing ITAMi signaling to maintain immune homeostasis. These data support a new mechanism of action for IVIg and demonstrate the therapeutic potential of FcRIIIA targeting in inflammation and autoimmune diseases.

W1.13.04
Antibody opsonised pathogens profoundly alter secondary immune responses through cross-talk of TLRs and FcgammaRs

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Cell-mediated mechanisms of the host defense, involving innate immunity and adaptive immune responses, are of crucial importance for the control of infectious pathogens. Pathogen recognizing receptors (PRR) play an essential role during innate or primary responses. In contrast, the presence of pathogen-specific antibodies during re-encounter with the same pathogen induces rapid uptake through binding to Fc-receptors (FcR) with concomitantly rapid clearance. The individual importance of these different classes of receptors is well studied. However, as immune cells will encounter antibody-opsonized bacteria during secondary responses, it's a challenging thought that simultaneous binding of their respective ligand can evoke cross-talk between the two receptor types.
We investigated this hypothesis during infection of dendritic cells with antibody-opsonized pathogens and by determining protein and metabolite profiles of the differentially activated dendritic cells as well as T_H-cell polarization. We demonstrate that antibody-opsonised pathogens evoke cross-talk between TLRs and FcRs, which induced profoundly altered release of multiple inflammatory cytokines, as well as eicosanoids metabolites. We observed redirection of T_H-1-subset towards T_H-17-subtype and intriguingly skewing towards GM-CSF producing T_H-cells. Furthermore, altered inflammatory cytokine profile was regulated on both transcription and post-transcriptional levels depending on the distinct cytokine. Finally, cross-talk was promiscuous between several TLRs and FcRs and was not constrained to dendritic cells.
These findings generate novel insights how secondary infections are resolved and that antibody opsonisation of bacteria particularly induces an inflammatory profile that favours recruitment, activation and prolonged survival of neutrophils at inflammatory sites.

W1.13.05
Monocytes rather than NK-cells contribute to antibody mediated protection from MCMV infection

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Cytomegalovirus is a clinically important pathogen. Previously we could show using murine cytomegalovirus (MCMV) that the adoptive transfer of immune serum into B- and T-cell deficient Rag-/- mice was highly effective against MCMV-induced morbidity and mortality. Antibodies can act either by direct neutralization of extracellular virus and/or by Fcγ receptor (FcγR)-mediated effector functions e.g. antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC).
To analyze the contribution of the FcγRs we used animals lacking the FcRγ-chain (FcγRxRag-/-), which is the accessory chain of the activating murine IgG receptors FcγRI, FcγRIII and FcγRIV. Following

serum therapy, FcγRxRag-/- mice developed high viral titers and showed a significantly shorter survival compared to the Rag-/- control mice indicating a substantial contribution of the Fcγ receptors in antibody protection. Animals lacking FcγRI, FcγRIII or FcγRIV individually were completely protected after immune serum therapy. Thus, NK cells which express only the FcγRIII seem not to participate significantly in antibody-mediated protection from MCMV. The depletion of NK cells in recipient mice confirmed this assumption. Mice lacking both, the FcγRIV and FcγRIII receptor developed high viral titers following serotherapy indicating redundant functions of the various FcγRs. Interestingly, adoptively transferred CD115+ monocytes from Rag-/- donors, bearing all activating Fc-receptors, into FcγRxRag-/- mice several days after immune serum treatment resulted in reduced viral titers in the recipient animals compared to the FcγRxRag-/- control mice. These results suggest an important role of monocytes in antibody mediated protection from MCMV infection.

W1.13.06
Swiprosin-1/EFhd2 is involved in the initial reaction of the IgE Fc receptor signal

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To elucidate the initial mechanism of IgE Fc receptor signaling, we searched novel molecules which localization in lipid rafts was increased by antigen stimulation. As a result, we identified a protein, Swiprosin-1. In this study, we aimed to reveal the function of Swiprosin-1 in FcεRI signaling. We knocked down Swiprosin-1 in rat basophilic leukemia 2H3 (RBL-2H3) cells, and analyzed the effect to degranulation. As a result, It was suppressed in KD cells, whereas ionomycin, PMA stimulation was not. To examine the function of Swiprosin-1 in more detail, we measured Ca^{2+} response. As a result, antigen-induced Ca^{2+} transient was suppressed in KD cells. However, ionomycin-induced Ca^{2+} response pattern was the same as WT cells. To investigate the effect to the upstream signal, we examined tyrosine phosphorylation signaling induced by stimulation FcεRI. Total protein tyrosine phosphorylation induced by antigen stimulation was suppressed in KD cells. However, TNF-α production, downstream of tyrosine phosphorylation, was not affected by KD. We used immunoprecipitation method to detect Swiprosin-1 associate protein(s), and found Approximately 50kDa tyrosine phosphorylated protein that was significantly appeared in stimulated RBL-2H3. Besides, we analyzed Swiprosin-1 associated proteins by MALDI TOF-MS and detected Vimentin. This result raises the possibility that the interaction between Swiprosin-1 and Vimentin regulates allergic reaction including IgE-mediated degranulation. Thus, elucidation of associated proteins and interaction leads to reveal the mechanism of allergic regulation.

W1.14 Cytokines and other regulatory mediators of inflammation

IL1.14.01
Endotoxin tolerance as a novel defence strategy in infectious diseases

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Analysis of disease tolerance pathways could provide new approaches for treating infections and other inflammatory diseases. Candidate pathways include sensors of pathogen presence, host metabolism, and intrinsic danger- or damage-associated molecules. Typically, an initial exposure to bacterial Lipopolysaccharide (LPS) induces a state of protective tolerance to further LPS challenge ("endotoxin tolerance"). The complex events
underlying this phenomenon remain poorly understood. Here we demonstrate that endotoxin tolerance reprograms Toll-like receptor 4 signaling *via* the combined effects of tryptophan catabolism, the ligand-operated Transcription factor aryl hydrocarbon receptor, and the cytokine transforming growth factor β. The protective, LPS-triggered tolerant state is not restricted to LPS-or gram-negative bacteria-induced immunopathology, in that it also specifically targets the inflammasome in a *Streptococcus*-induced multifocal septic arthritis model. Thus interfering with the trade-offs that the defense systems in both pathogens and their hosts impose on host fitness could ultimately help to selectively enhance pathways responsible for strong and rapid inflammatory responses that lead to fast pathogen clearance, yet do not involve immunopathology.

W1.14.01
Expression and regulation of IL-35 in human tolerogenic dendritic cells and its role in regulating T cells responses

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IL-35 is a novel cytokine of the IL-12 family, existing as a heterodimer of IL-12p35 and EBV-induced gene 3 (Ebi3). IL-35 has regulatory properties and is produced by regulatory T cells in humans and mice, where it seems required for optimal suppression. Distinct from other IL-12 family members, expression of IL-35 has not been clearly described in antigen presenting cells. In view of its regulatory properties, we investigated the expression, regulation and function of IL-35 in human dendritic cells (DC) and tolerogenic DC (tolDC).
We generated tolDC by culturing monocyte-derived DCs in the presence of dexamethasone. These tolDCs did not produce detectable levels of bioactive IL-12p70 or the homodimer IL-12p40. In line with this, we demonstrate by Q-PCR that tolDC completely lack the expression of IL-12p40. However, tolDC maintain mRNA expression of IL-12p35 and Ebi3. Using intracellular FACS and western blot we confirm that tolDC maintain protein expression of both Ebi3 and IL-12-p35, which can be further enhanced upon stimulation with IFNγ, LPS and CD40 ligation. It was previously demonstrated that tolDC have the capacity to suppress T cell activation. We demonstrate that suppression can also be obtained with supernatant derived from tolDCs. Preliminary experiments using blocking antibodies and siRNA knockdown in tolDCs suggest that IL-35 contributes to regulatory function of tolDC.
Taken together, our results suggest that aside from increased expression of previously described tolerance inducing markers (B7-H1, ILT3) and increased IL-10 production, tolDC produce IL-35, providing an additional novel mechanism by which these cells elicit their tolerogenic potential.

W1.14.02
The BAFF/Th17 axis in Helicobacter pylori infection

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H. pylori (HP) infects the stomach and causes gastric pathologies such as chronic and autoimmune gastritis (AIG), ulcers, gastric MALT lymphoma and gastric cancer. Interestingly, studies carried out in humans and mice reveal that HP infection is followed by a mixed Th1/Th17 inflammatory response in gastric mucosa. Th17 cells are T lymphocytes with strong inflammatory effects that are involved in the pathogenesis of autoimmune diseases (AID).
B cells activating factor (BAFF), also is a crucial cytokine in B-cell development and survival but it is also up regulated in several AID. Recently, a relationship between the up regulation of BAFF and the rising of a Th17 response in AID in mice has been proposed, however the axis BAFF/Th17 in humans remains unknown.

In our study, we found that BAFF is up-regulated in macrophages infiltrating the gastric mucosa of HP infected patients; accordingly, IL-17, marker for the Th17 response, is similarly up-regulated in the presence of HP. We confirm that HP infected macrophages release BAFF in vitro. Other in vitro data show that BAFF could polarize human T cells toward the Th17 profile thorough an indirect action of monocytes; indeed, BAFF-treated monocytes release high amount of pro-Th17 cytokines (IL-6, TGF-β, IL-1β, and IL-23). These data are the first evidence for an involvement of BAFF in the Th17 cells differentiation in humans and suggest that the axis BAFF/Th17 inflammatory response in gastric mucosa could be the trigger of AIG in HP infected patient.

W1.14.03
Molecular basis of signaling through the IL-36 receptor

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The IL-36 subfamily of cytokines and receptors, members of the larger IL-1 family, have recently been described as potent activators of BMDCs and TH1 cells and play a key role in psoriasis, a common severe skin disease. The IL-36 receptor (IL-36R) uses the IL-1 receptor accessory protein (IL-1RAcP) as a secondary receptor for signaling, the same RAcP as do IL-1 family receptors (e.g., IL-1R itself and ST2 - the IL-33 receptor). As it is engaged by three agonist interleukins (IL-36alpha, IL-36beta and IL-36gamma) and two antagonist interleukins (IL-36Ra and IL-38), IL-36R represents the most diverse axis for agonism and antagonism within the IL-1 receptor family. This group of interleukins likely follows similar mechanisms as do IL-1 and IL-33 to promote or inhibit interaction with IL-1RAcP. We have now solved the high-resolution X-ray crystal structures of human IL-36gamma and IL-38, providing insights to molecular mechanisms of agonism and antagonism of signaling through IL-36R. We have further dissected the requirements for differential signaling through interleukin loop swapping and mutagenesis coupled with interaction and functional analyses. These results suggest strategies for developing novel psoriasis therapeutics and refine the general model of activating and inhibitory IL-1 family receptor complexes.

W1.14.04
Intracellular signalling involved in IL-22 release by Dendritic Cells

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The role of cytokines in immunoregulation and inflammation is well established and anticytokine terapies are now common in the treatment of chronic inflammatory diseases. However, it is necessary to define how cytokine function and how signalling pathways are regulated in different types of immune cells to understand underlying disease mechanisms. Here we show that dendritic cells (DCs) produce the cytokine IL-22 in response to Pattern Recognition Receptors (PRRs) stimulation and we characterize the signalling pathways that are engaged. Although it is known that innate lymphoid cells produce IL-22 under different conditions data on DCs are still unclear. The ability of DCs to produce IL-22 in response to TLRs and c-Type lectins receptors activation and their adaptor molecules has been characterized. We show that Jnk and Erk MAP kinases play a major role in IL-22 production whereas p38 is not implicated. By blocking NFkB with the inhibitor Bay11-7085, we confirmed that it takes part together with AP-1 in IL-22 production in DCs. Finally, we tested the activity of the aryl hydrocarbon (AhR) and RORgt receptors confirming a crucial role for AhR but not for RORgt. The data pointed out different intracellular IL-22 regulation mechanisms operating in DCs compared to innate lymphoid cells. In conclusion, our study provides the first evidence that DCs are able to produce IL-22 upon PRRs stimulation. Since IL-22 is highly dysregulated in chronic inflammatory conditions and correlated with disease activity, a better understanding of its regulation is important for development of IL-22 as a potential drug target.

W1.14.05
Environmental conditions perceived by the brain alter CD4+ T cell responses through an adiponectin-dependent mechanism

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We are interested in elucidating how the brain controls the immune system. To this aim, we have established and validated an environmental enriched (EE) paradigm in which C57BL/6 mice experience higher levels of sensory, motor, social, and cognitive stimuli, compared to animals housed in a standard environment (EE). In agreement with previous studies, we found that EE increased hippocampal neurogenesis and synaptogenesis at the cellular level, and decreased anxiety and increases exploratory activity at the behavioral level. EE housing did not result in gross alterations in the frequency of neutrophils, dendritic cells (DC), macrophages, Natural Killer (NK), CD4(+) and CD8(+) T cells and B lymphocytes in the lymphoid organs of immunologically naïve animals. However, both DC and NK cells expressed a more mature phenotype in EE animals. Furthermore, EE mice exhibited both a Th2-biaised T response upon immunization with OVA and a more robust memory T cell response. To elucidate the mechanisms that were responsible for these latter phenomena, we compared the levels of various metabolites in the serum of EE and SE mice. Compared to SE mice, EE animals exhibited increased levels of adiponectin, a cytokine that is mainly secreted by adipose tissue and that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation. Most importantly, adiponectin-deficient mice housed in EE did not exhibit the Th2-biaised immune response that was observed in wild-type (wt) mice therefore suggesting a critical role of this cytokine in EE-induced immune alterations.

W1.14.06
The non-specific and sex-differential immunological effects of the Bacillus Calmette-Guerin (BCG) vaccine in infants

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Introduction: Vaccine efficacy is almost exclusively evaluated based on specific effects. However, investigation of overall effects of vaccines is pivotal, as it can help optimizing current vaccine programs, especially in countries with high disease burden. The Bacillus Calmette-Guerin (BCG) vaccine against tuberculosis is one of the most widely used vaccines in the world. A recent randomized trial from Guinea-Bissau found that BCG provided at birth to low-birth-weight (LBW) neonates reduces neonatal mortality by 45%. Our observations cannot be explained by protection against tuberculosis; the main causes of death were septicaemia and pneumonia, and the effects occurred too quickly to be related to tuberculosis. Hence, BCG has beneficial non-specific effects on the immune system. Whereas the overall effect may be stronger in girls, the reduction in mortality is apparent already within 3 days after BCG particularly in boys, suggesting that non-adaptive mechanisms are involved.
Objective: The present study will shed light on how BCG modulates fundamental immunological functions in infants in a non-specific and possibly sex-differential manner.
Methods: 400 LBW infants in Guinea-Bissau were randomized to BCG at birth or the usual postponed BCG. The infants were bled at 4 weeks (before the control group received BCG) and at 10 weeks of age. Pro-inflammatory, Th1-, Th2-, Th17-related, and anti-inflammatory cytokine responses to common recall antigens and innate agonists are investigated in whole-blood assays. Moreover, common haematological and cytochemical values are analysed.
Results: The laboratory analyses are ongoing. Data will be presented at the congress.
Conclusion: Yet awaiting results of the analyses.

W1.15 Inflammasome

IL1.15.01
Role of inflammasomes for chronic inflammatory diseases

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Innate immunity evolved to recognize microbial infection and to respond to danger signals that appear under disease conditions. The most recently described innate immune receptor family is the Nod-like receptor (NLR) family. The NLR member NLRP3 and the adapter protein ASC form a multi-molecular complex termed the NLRP3 inflammasome. Inflammasomes control the activity of caspase-1, which cleaves and activates the pro-form of the inflammatory cytokines IL-1b and IL-18. The NLRP3 inflammasome can be activated by various membrane active bacterial toxins or after phagocytosis of crystalline materials. In addition, various microbes can activate the NLRP3 inflammasome. The mechanisms by which the NLRP3 inflammasome is activated by physico-chemical diverse activators are not well understood.
We demonstrated that crystals activate the NLRP3 inflammasome in a process that requires phagocytosis and we found that crystal uptake leads to lysosomal damage and rupture. Furthermore, sterile lysosomal damage was also sufficient to induce NLRP3 activation and inhibition of phagosomal acidification or inhibition or lack of cathepsins impaired NLRP3 activation. These results indicate that the NLRP3 inflammasome can sense lysosomal damage as an endogenous danger signal. Using different approaches including chemical biology screens, interactome analysis and RNA interference we are currently studying the mechanisms of NLRP3 activation.

IL1.15.02
Inflammasome activators induce the secretion of IL-1α via two distinct pathways displaying differential requirement for a protease activity-independent function of caspase-1

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Through their capacity to sense danger signals and to generate active IL-1β, inflammasomes occupy a central role in the inflammatory response. In contrast to IL-1β, little is known about the regulation of IL-1α activity. Unexpectedly, we find that inflammasome activators also induce the regulated secretion of IL-1α, thus leading to the co-secretion of both IL-1 cytokines. Depending on the type of inflammasome activator, release of IL-1α is inflammasome/caspase-1-dependent or -independent. Unlike for IL-1β, calcium influx, induced by the opening of cation channels such as TRPV2, suffices for the inflammasome-independent IL-1α activation. In both cases IL-1α is released primarily in a processed form, caused by a calpain-like protease. Remarkably, caspase-1-dependent release of IL-1α and IL-1β is independent of caspase-1 catalytic activity, defining a novel function for caspase-1. Considering the involvement of inflammasomes in chronic inflammatory diseases such as gout and diabetes, the use of IL-1α antagonists may be beneficial in the treatment of these disorders.

W1.15.01
Cytosolic cathepsin inhibitor Stefin B (Cystatin B) regulates NLRP3 inflammasome activation

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Pathogen-associated molecular patterns are recognized by innate immune receptors that are membrane bound Toll-like receptors (TLRs) and cytosolic Nod-like receptors (NLRs). Members of NLR family and adaptor ASC form inflammasomes, required for pro-inflammatory caspase-1 self activation and interleukin (IL)-1β maturation.
Cathepsins are proteases responsible for protein breakdown within lysosomes, recently cathepsin B was suggested to be involved in inflammasome activation. Cathepsin inhibitor stefin B (cystatin B) is located in the cytosol and nucleus, where it has protective role. Mutations in the gene of stefin B are associated with the neurodegenerative disease known as progressive myoclonus epilepsy (EPM1). Recently, it was reported that early microglial activation precedes neuronal loss in the brain of the stefin B deficient mice.
In our study, we demonstrate that Stefin B deficient mice secrete significantly higher amounts of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) upon LPS challenge. We further show that stefin B-/- bone marrow macrophages (BMM) exhibit increased caspase-1 and 11 activation and better IL-1β processing upon NLRP3 inflammasome activation. Moreover, in ATP stimulated BMM we observed lysosomal destabilization (but not disruption) and release of the lysosomal content. Cytosolic cathepsin B activity was higher in stefin B-/- BMM and cathepsin inhibition resulted in attenuated IL-1β levels.
We hypothesize that the same signalling pathways determined in macrophages are also functional in microglia that are important in the pathology of neurodegenerative disease. Elucidation of the stefin B role on cytokine synthesis in macrophage model will contribute to the better understanding of the pathology of the EPM1 disease.

W1.15.02
The inflammasome adaptor ASC forms a prion-like danger signal that perpetuates inflammation

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Upon activation, most of the inflammasome sensors interact with the adaptor molecule Apoptosis associated speck-like containing a CARD domain (ASC) which, in turn, recruits procaspase-1 leading to its autoproteolytic activation. ASC recruitment to the inflammasome sensors is followed by its rapid self-assembly into a large protein complex, the so-called “speck” or “pyroptosome”. Activation of caspase-1 in the ASC pyroptosome results in a special type of cell death, termed pyroptosis. Although it is well known that such cell death is accompanied by release of cellular content into the extracellular medium, the fate of the assembled ASC pyroptosome after cell demise remains unknown. Here we show that shortly after inflammasome activation, ASC specks containing a whole inflammasome assembly of ASC, NLRP3 and pro-caspase-1 are released from pyroptotic cells into the extracellular space. The released ASC specks remain biologically active in the extracellular medium and are capable of amplifying inflammation by processing pro-caspase-1 and pro-IL-1β into their mature forms. Furthermore, extracellular specks are sensed as danger signals and phagocyted by surrounding immune cells. Phagocyted ASC specks induce lysosomal rupture and potentiate production of IL-1β in recipient cells. Moreover, pre-aggregated ASC specks have prion-like properties and can recruit and nucleate endogenous ASC in recipient cells. Finally, extracellular specks are found in bronchoalveolar lavage from patients with chronic obstructive pulmonary disease (COPD). Together, our results reveal an important and unprecedented feature of the inflammasome adaptor ASC and extend our understanding of its function in innate immunity.

W1.15.03
The NLRP3 inflammasome links complement-mediated inflammation and IL-1β release

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The complement system is a potent component of the innate immune response, promoting inflammation and orchestrating defense against

pathogens. However, dysregulation of complement is critical to several autoimmune and inflammatory syndromes, including lupus, rheumatoid arthritis, glomerulonephritis, vasculitis and angioedema. Elevated expression of the pro-inflammatory cytokine IL-1β is often also linked to such diseases. In this study we reveal the mechanistic link between complement and IL-1β secretion using murine dendritic cells. IL-1β secretion occurs following intracellular caspase-1 activation by large multi-protein complexes, named inflammasomes. Here we show that complement elicits secretion of both IL-1β and IL-18 in vitro and in vivo via the NLRP3 inflammasome. This effect is dependent on the inflammasome components NLRP3 and ASC, and caspase-1 activity. Interestingly, sub-lethal complement membrane attack complex formation, but not the complement activation products C3a and C5a, activated the NLRP3 inflammasome and promoted IL-1β release by transient K+ efflux and production of reactive oxygen species. These findings provide insight into the molecular processes underlying complement-mediated inflammation and highlight the possibility of targeting IL-1β to control complement-induced disease and pathological inflammation.

W1.15.04
Fas-mediated inflammatory response in *Listeria monocytogenes* infection

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The molecular mechanisms of Fas (CD95/Apo-1)-mediated apoptosis are increasingly understood. However, the role of Fas-mediated production of pro-inflammatory cytokines such as IL-18 and IL-1β in bacterial infection is unclear. In this study, we demonstrate the importance of Fas-mediated signaling in IL-18/IL-1β production following infection with *Listeria monocytogenes* (LM). *Fas*$^{-/-}$ mice were more susceptible to LM infection than normal mice *in vivo*, and the elevation of IL-18 was lower in sera from *Fas*$^{-/-}$ mice infected with LM than from normal mice. In addition, IL-18/IL-1β production in LM-infected peritoneal exudate cells (PECs) from Fas-deficient mice was lower than those from Wt mice *in vitro*, indicating that Fas-signaling contributes to cytokine production in response to LM infection. LM infection induced Fas ligand (FasL) expression on NK cells, which stimulates Fas expressed on the infected macrophages, leading to the production of IL-18/IL-1β. This was independent of caspase-1, -11, and nucleotide-binding domain- and leucine-rich repeat-containing receptors (NLRs) such as Nlrp3 and Nlrc4, but dependent on apoptosis-associated speck-like protein containing a caspase recruitment domain (Asc). Upon infection with LM, Wt cells exhibited caspase-8 activation, while Fas deficient cells did not. LM-induced caspase-8 activation was abrogated by inhibitor for intracellular reactive oxygen species (ROS), *N*-acetyl-$_L$-cysteine (Nac). These results indicate the possibility that LM infection induced the activation of caspase-8 via Fas signaling in ROS-dependent manner, and the activated caspase-8 cleaved pro-IL-18/IL-1β to produce active cytokines. This study revealed a novel role of Fas in the induction of the inflammatory response against LM infection.

W1.15.05
TLRS-induced PAI-2 expression suppresses NLRP3-dependent caspase-1 activation and IL-1B processing

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The NLRP3 inflammasome, a multiprotein complex, triggers caspase-1 activation and maturation of proinflammatory cytokines IL-1β and IL-18 upon sensing a wide range of pathogen- and damage-associated molecules. Dysregulation of NLRP3 inflammasome activity contributes to the pathogenesis of many diseases, but its regulation remains poorly defined. Plasminogen activator inhibitor 2 (PAI-2), a member of a large group of proteins that inhibit serine proteases, is regulated by IKKβ-dependent activation of the transcription factor NF-κB. Previous studies demonstrated that mice with *Ikkβ* deletion in myeloid cells are hypersusceptible to endotoxin (LPS)-induced septic shock, and increased LPS susceptibility is associated with elevated plasma IL-1β. Reconstitution of *Ikkβ*-deficient macrophages with PAI-2 blocks IL-1β release after LPS stimulation. To further elucidate the physiological role of PAI-2 in LPS-induced septic shock, we generated transgenic mice that specifically express PAI-2 in myeloid cells, and then crossed this transgenic mice with myeloid-deleted *Ikkβ* mice. PAI-2 expression decreased plasma IL-1β in myeloid-deleted *Ikkβ* mic after LPS challenge. In addition, PAI-2 transgenic mice showed a decrease in circulating IL-1β level upon *Escherichia coli* infection. We also demonstrated that depletion of PAI-2 in macrophages resulted in NLRP3- and ASC-dependent caspase-1 activation and IL-1β secretion in macrophages in response to TLR activators and *E. coli* infection with no need of a second stimulus. Together, our data identify a new tier of TLR signaling in controlling NLRP3 inflammasome activation.
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W1.15.06
NLRP3 controls Trypanosoma cruzi infection through a caspase-1-dependent IL-1R-independent NO production.

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Introduction: *Trypanosoma cruzi* is an intracellular protozoan parasite and etiological agent of Chagas disease, a severe and chronic infectious illness that affects millions of people in the world. Although the role of TLR and Nod1 in controlling infection by *T. cruzi* is well established in the literature, there is no data about the involvement of inflammasomes. **Methods and Results:** In this study, we evaluated the participation of NLRP3 and caspase-1 in host response to *T. cruzi* infection and found that NLRP3$^{-/-}$ and caspase1$^{-/-}$ mice are susceptible to infection. Although inflammatory cytokines IL-6 and IFN-γ were found in spleen cells from NLRP3$^{-/-}$ and caspase1$^{-/-}$ infected mice, these mice displayed a defect in the production of nitric oxide (NO), which impairs the parasitic capacity of their macrophages. In fact, macrophages from NLRP3$^{-/-}$ and caspase-1$^{-/-}$ are more permissive to *T. cruzi* replication than the MyD88$^{-/-}$ ones. The inhibition of caspase-1 with z-YVAD-fmk, but not the neutralization of IL-1R abrogated the NO production by WT and MyD88$^{-/-}$ macrophages and renders them as susceptible as NLRP3$^{-/-}$ and caspase-1$^{-/-}$ cells to *T. cruzi* replication. **Conclusion:** Taken together our results demonstrate the role of iNLRP3 inflammasome for the control of *T. cruzi* and point out the caspase-1-dependent NO production as a new effector mechanism for these innate receptors.

W1.16 Complement system

IL1.16.01
Microbial manipulation of select host signaling pathways to disengage immune bacterial clearance from inflammation

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Porphyromonas gingivalis (Pg) is a keystone pathogen that enhances the pathogenicity of the multispecies periodontal community through the disruption of host-microbe homeostasis. In so doing, Pg manipulates select host signaling pathways to impair host immunity without significantly affecting the host inflammatory response, which serves the nutritional needs of the bacteria (inflammatory tissue breakdown products and hemin-derived iron). However, the molecular mechanism(s) by which Pg can selectively inhibit immune elimination without blocking inflammation has remained obscure. I will present our latest data that support a model according to which Pg can disarm and disassociate a host-protective TLR2-MyD88 pathway from a TLR2-PI3K proinflammatory pathway that promotes Pg survival. Specifically, our findings indicate that Pg causes MyD88 degradation in a C5a receptor- and TLR2-dependent manner, resulting in decreased killing of Pg by neutrophils. Moreover, the same Pg-

induced C5aR-TLR2 crosstalk induces PI3K signaling which inhibits Pg phagocytosis by neutrophils and promotes the induction of a non-host protective proinflammatory response. This subtle manipulation of host signaling pathways is a matter of life and death for Pg and co-habiting species. If Pg caused generalized immunosuppression, this would inhibit bacterial killing, but would also deprive the bacteria of critical nutrients derived from inflammatory tissue breakdown. These findings indicate that periodontal bacteria have evolved to evade host immunity in an inflammatory environment that is essential to their persistence but - at the same time- causes collateral damage to the tissues causing periodontitis.

W1.16.01
C3 opsonisation enhances apoptotic cell-associated antigen presentation by DCs by controlling the endocytic handling

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Apoptotic cells are considered a potential source of auto-antigens and can efficiently deliver an antigen load to dendritic cells (DCs). *In vivo* they are rapidly removed by macrophages and defective clearance may lead to autoimmunity. Complement has been shown to contribute to the uptake of apoptotic cells by macrophages but not by DCs. Complement C3-deficient (C3-/-) DCs have been shown to exhibit reduced potency to stimulate T cell responses but the underlying mechanism(s) remains unclear. Here, we investigated the role of C3 in the T cell response to apoptotic cell-associated antigens by feeding C3-deficient or C3-sufficient DCs from C57BL/6 mice ($H2\text{-}A^b$) with allogeneic $H2\text{-}E^+$ apoptotic B cells (from BALB/c mice) and assessing presentation and priming using the Y-Ae monoclonal antibody (specific for a peptide from the MHC molecule $H2\text{-}E^+$ molecule presented by $H2\text{-}A^b$) and 1H3 TCR transgenic T cells (same specificity as the Y-Ae mAb). Using this model we found that the lack of C3 did not affect the uptake of apoptotic cells by DCs but rather controlled the intracellular trafficking by accelerating the association of the apoptotic cargo with lysosomes. Consistent with this, C3 deficiency resulted in reduced antigen-specific T cell proliferation *in vitro* and *in vivo*. Notably, pre-opsonisation of apoptotic cells with exogenous C3 rectified the trafficking and T cell priming defects in C3-/- DCs. These data indicate that activated C3 may act as a "chaperone" in the intracellular processing of apoptotic cells and may modulate the fate of self-antigens displayed on dying cells.

W1.16.02
Follicular dendritic cells are essential for maintenance of autoreactive B cells

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Formation of germinal centers within secondary (and tertiary) lymphoid tissues is a hallmark of an ongoing B cell response to foreign and self-antigen. Recently, we identified a novel pathway by which FDC endocytose and cycle foreign antigen via complement receptors CD21/CD35 and periodically present it to cognate B cells. To test whether this pathway was required for maintenance of autoreactive B cells, we have used multiple approaches to disrupt FDC retention of self-antigen. For example, in 564 Igi knock-in mice (Berland et al 2006 *Immunity*), that are specific for nucleolar RNP such as SSB/LA, a small fraction of autoreactive B cells escape anergy, mature and form GC in spleen and lymph nodes in a TLR 7-dependent pathway. Strikingly, ablation of FDC, disruption of their maturation or blockade of the CD21 receptor in the 564 Igi mice leads to a rapid loss of mature autoreactive B cells relative to endogenous non-autoreactive B cells. We propose that autoreactive B cells are highly sensitive to the presence of self-antigen retained by FDC and that disruption of their contact leads to rapid elimination. Although not mutually exclusive, it is possible that FDC secretion of cytokines, *e.g.* IL-6, IFNγ, or growth factors such as BAFF are critical for survival of the autoreactive B cells. Current studies are in progress to dissect the role of FDC in maintenance of the lupus-antigen specific B cells.
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W1.16.03
Response gene to complement-32 mediates C5b-9 induced cell cycle activation and migration in endothelial cells

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Proliferation of endothelial cells (EC) and smooth muscle cells (SMC) are critical events involved in angiogenesis and atherosclerosis. We have previously shown that activation of complement and assembly of sublytic C5b-9 induced cell cycle activation in both aortic EC and SMC. We also found that sublytic C5b-9 induces the expression of Response Gene to Complement (RGC)-32 and that its overexpression leads to cell cycle activation. We investigated the role of endogenous RGC-32 in cell cycle activation by C5b-9. Silencing of RGC-32 expression, using siRNA, abolished C5b-9 induced DNA synthesis indicating that RGC-32 is required for cell cycle activation and S-phase entry in human aortic EC. In addition we found that RGC-32 is involved in C5b-9 induced EC migration. In order to identify the genes that are regulated by RGC-32 and involved in cell cycle activation we used gene array analysis and investigated the effect of RGC-32 knockdown on gene expression in human aortic EC. Of the 234 genes that were differentially expressed after RGC-32 knockdown, a group of genes involved in cell adhesion and cell cycle growth were the most significantly regulated by RGC-32. RGC-32 knockdown induced a decrease in the expression of cyclin D1, cyclin D3, Akt, ROCK1, Rho GDP dissociation inhibitor alpha and profilin. RGC-32 appears to mediate aortic EC migration through regulation of RhoA and ROCK1 expression. Since RGC-32 seems to play an important role in human aortic EC proliferation and migration, targeting RGC-32 is a potential therapeutic intervention in angiogenesis and atherosclerosis.

W1.16.04
Soluble GC1QR is an autocrine signal which induces bradykinin receptor 1 (B1R) expression on activated endothelial cells

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The endothelial cell (EC) receptor complex for high molecular weight kininogen (HK) comprises cytokeratin 1, urokinase-type plasminogen activator receptor (uPAR), and the receptor for the globular heads of C1q (gC1qR), and is essential for the assembly and activation of the kinin-kallikrein system (KKS). Of these, gC1qR serves as high affinity site for HK—the bradykinin (BK) precursor—and plays a critical role in triggering the KKS leading to the generation of bradykinin (BK). Bradykinin, which belongs to the kinin family of proinflammatory peptides, is one of the most potent vasodilator agonists known, and induces its activity via two G-protein-coupled receptors: bradykinin receptor 1 (B1R) and B2R. While B2R is constitutively expressed on many healthy cell types, IL-1 induces B1R after tissue damage. Because gC1qR is secreted by activated ECs, and soluble gC1qR (sgC1qR) binds to cells, we hypothesized that sgC1qR may serve as an autocrine/paracrine signal for the induction of B1R. Our results show that: 1) sgC1qR binds to microvascular ECs via a highly conserved gC1qR domain (aa174-180), and deletion of this domain abolishes sgC1qR binding as assessed by deconvolution fluorescence microscopy. 2) Incubation of ECs (2h and 24h) with sgC1qR resulted in a dose-dependent B1R expression (24h>2h) in a manner that suggested *de novo* synthesis. 3) More importantly, a

peptide corresponding to residues 174-180 also mimicked the effects of sgC1qR. Therefore at sites of EC activation, sgC1qR may provide an additional signal for B1R expression and could represent a potential target for therapy against BK-mediated inflammation and vascular permeability.

W1.16.05
Role of the long Pentraxin PTX3 in fibrosarcoma development and progression

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Pentraxins (PTXs) are prototypic components of the humoral arm of innate immunity and constitute a superfamily of multifunctional multimeric proteins phylogenetically conserved from arachnids to mammals. The prototypic long pentraxin PTX3 plays a fundamental role in innate immunity, in regulation of the inflammatory response and in tissue remodeling, through different molecular mechanisms, including Complement regulation, P-selectin-dependent leukocyte recruitment and FGF2-regulation.
Inflammatory responses play decisive roles at different stages of tumor development, including initiation, promotion, malignant conversion, invasion and metastasis. PTX3 is produced by diverse cell types present in the tumor stroma such as leukocytes, endothelial cells and fibroblasts. To define the role of PTX3 in tumor development, we investigated the susceptibility to 3-Methylcholanthrene-induced fibrosarcoma or transplanted fibrosarcomas in Ptx3-/- and wild type mice. PTX3-deficiency was associated with increased tumor incidence, fast tumor growth and higher inflammatory response (e.g leukocyte recruitment, inflammatory cytokines). Furthermore, the analysis of tumors reveled increased complement C3 and reduced Factor H deposition in PTX3-deficient mice, in agreement with the complement-regulatory function of PTX3. Finally, the genetic analysis of “hot” genes Tp53 and K-ras, classically mutated by 3-MCA, showed that PTX3-deficiency was associated with higher mutation frequency, possibly due to polyclonality of the lesions.
All together, these results suggest that the regulation of inflammation mediated by PTX3 could play a crucial role in fibrosarcoma development. Mechanisms potentially involved in the mentioned phenotypes, which range from regulation of angiogenesis, complement deposition, leukocyte recruitment and gene instability are currently under investigation.

W1.16.06
Dendritic cell alternative pathway production is differentially regulated by Interferons & IL-27

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Dendritic cells (DCs) and complement are both key members of the innate immune system, and recent experimental mouse models have shown that production of alternative pathway (AP) components by DCs strongly affects local T cell populations. The diverse functions of DCs are not only dependent on their mode of activation,but also strongly influenced by intrinsic differences between subtypes. In this study we investigated the production and regulation of factor P (fP), and H (fH), integral components of the AP, by both human DC and tolerogenic DCs (tolDCs).
We demonstrated by Q-PCR and ELISA that tolDCs expressed significantly elevated levels of fP and fH compared to DCs. Moreover FACS analysis and confocal imaging showed that DC, and particularly tolDC, derived fP was functionally active as shown by binding to necrotic cells and C3b.
We investigated the possible regulators of DC derived AP factors and found that IFNγ possesed a dual role, downregulating fP while also upregulating fH. Stimulation with TLR agonists including LPS did not effect either fP or fH.
IL-27 has been shown to share some functional characteristics with IFNγ. Interestingly while IFNγ (type II IFN) and IL-27 significantly increased production of fH in both DCs, IFNβ (type I IFN) significantly downregulated fH production. While fP production was significaty downregulated by IFNγ in both DC subsets this was not observed for IL-27 or IFNβ.
In summary, this study reveals a novel role of IFNs and IL-27 in regulating local complement production by DCs through differential affects on fP and fH.

W1.17 Allergic mediators

W1.17.01
Novel regulatory control of IgE antibody responses in vivo

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IgE antibodies bind to receptors on mast cells and basophils priming them to degranulate and release inflammatory mediators upon interaction with cognate antigen. IgE antibody responses offer protective immunity against parasite infection, though they are best known for their critical role in the acute and severe reactions suffered by patients with asthma and other allergies. Despite the central role of IgE in asthma pathogenesis, relatively little is known about the regulation of IgE production in vivo. Whilst investigations into the role of T helper (TH) cells and the innate immune system have shown some promise a detailed knowledge of the nature of the IgE-switched B cells and plasma cells together with the mechanism underlying their differentiation will be critical to the development of more effective strategies for asthma treatment and prevention. To gain greater insight into the biology of IgE-switched B cells we have developed and made use of a powerful in vivo mouse model to characterise antigen-specific IgE-switched B cells within the context of the local environmental and molecular cues that guide their differentiation. Using this experimental system our results reveal a novel role for a mutated protein previously not known to be involved in the regulation of IgE antibody production in vivo suggesting a new paradigm for considering the causes of allergies and the development of treatments for these diseases. Our ongoing studies to interrogate the unique function of this gene in tuning B cell responses to suppress IgE production within the germinal center microenvironment will be presented.

W1.17.02
Basophil-derived mouse mast cell protease 11 is involved in the development of IgE-mediated chronic allergic inflammation

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Basophils are the least common granulocytes, and had long been considered as minor relatives of mast cells. However, recent studies have illustrated non-redundant roles for basophils in various immune responses, including allergic reactions and protective immunity against parasites. We previously demonstrated that basophils but not mast cells play an important role in the development of IgE-mediated, delayed-onset allergic inflammation in the skin, designated IgE-CAI. However, it remains elusive how basophils trigger the skin inflammation. In this study, we focused on mast cell protease 11 (mMCP-11) that shows a tryptase activity, and is expressed by basophils rather than mast cells. In order to elucidate the role for mMCP-11 in IgE-CAI, we generated engineered mice deficient for mMCP-11, in that the number of basophils was comparable to that in wild-type mice. Their basophils expressed normal levels of other proteases including mMCP-8 and surface FcεRI, and normally degranulated upon stimulation with IgE plus antigens, as judged by β-hexosaminidase release assay. Of note, skin swelling elicited by the IgE-CAI reaction was reduced in mMCP-11-deficient mice to half of that in wild-type mice. Moreover, the number of proinflammatory cells accumulating skin lesions, including eosinophils and neutrophils, was significantly reduced in mMCP-11-deficient mice. Thus, mMCP-11

released by basophils is a key effector molecule in the development of IgE-CAI.

W1.17.03
Analysis of NK cell subsets in children with atopic dermatitis

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Atopic dermatitis (AD) is a frequent chronic inflammatory skin disease which is often complicated by recurrent microbial superinfections. Numerous studies demonstrated increased frequency of allergen-specific Th2 cells producing increased IL-4, IL-5 and IL-13 in the peripheral blood of AD patients, however little is known about the role of natural killer (NK) cells. In this study, NK1, NK2 and NK regulatory cytokine profiles, the expression of activatory receptors as well as the cytotoxic activity of NK cells in AD were investigated. The study group consists of children with AD (n=14, mean age=7 ± 3.8) and healthy subjects (n=14, mean age=10 ± 4). The patients were multisensitized to at least one aeroallergen and had high serum total IgE levels. Cytotoxic activity, expression of $CD16^{bright}CD56^{dim}$ and $CD16^{dim}CD56^{bright}$ NK cell subsets, NK cell activatory receptors and intracellular IL-4, IL-10 and IFN-γ levels were determined by flow cytometry. In AD patients the percentages of $CD3^{-}CD16^{+}CD56^{+}$, $CD16^{bright}CD56^{dim}$ NK cell subsets and expression of activatory receptors of NKG2D cells were significantly decreased compared to healthy subjects (p=0.000, p=0.000 and p=0.04, respectively). Although, IL-10 secreting regulatory and IL-4 secreting NK2 cells were increased, IFN-γ secreting NK1 cells and cytotoxic activity of NK cells were found to be decreased in AD patients (p=0.000, p=0.000, p=0.014 and p=0.016, respectively). Our results suggested that impaired NK cell functions such as decreased expression of $CD16^{bright}$ subset, NKG2D expression as well as cytotoxic activity, high secretion of L-10 & IL-4 might play a role in the pathogenesis of AD.

W1.17.04
Novel IL-9-producing Innate Helper Cells Promote Oral Antigen-induced Anaphylaxis

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IgE-mediated food allergy is manifested by an overactive T-helper 2 immune response to dietary antigens in the gastrointestinal (GI) tract. Interleukin (IL)-4, IL-13, and IL-9 promote IgE antibody production, intestinal mucus secretion, and mastocytosis, respectively, which allow the development of oral antigen-induced anaphylaxis in an experimental model. However, little is known about the primary cellular sources of IL-9 or the mechanisms that promote intestinal mastocytosis. Here, we report the identification of multifunctional IL-9-producing innate helper cells (IHC9) that promote intestinal TH2 inflammation and mastocytosis that drives oral antigen-induced anaphylaxis. Distinct from ILC2, IHC9 exhibit non-lymphoid cell attributes and can secrete prodigious amount of IL-9 and other TH2 cytokines, IL-4 and IL-13 in lesser amount. Histologic and electron microscopic analysis reveal that IHC9 display morphologic characteristics of innate helper cell lineages. Repeated intragastric antigen challenge induces accumulation of intestinal IHC9, which correlate positively with symptoms and susceptibility to oral antigen-induced anaphylaxis. The failure of irradiated mice reconstituted with STAT6-deficient bone marrow to generate IHC9 in response to challenge was accompanied by reduced serum MCPt-1, intestinal mastocytosis, and goblet cell hyperplasia and loss of allergic diarrhea. Thus, IHC9 represent a new type of innate helper cell lineage and play a pivotal role in the development of IgE-mediated food allergy. (This work is supported by grant NIH-P30 DK078392)

W1.17.05
Lack of PTX3 enhanced airway inflammation and hyperresponsiveness in a murine model of asthma

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Pentraxin-3 (PTX3) is a member of the long pentraxins family, which activates the innate and adaptive immune systems in a coordinated manner. It plays an important role in providing immunity against pulmonary infections such as aspergillosis, pneumonia, tuberculosis and Psuedomonas aeroginosa infection. Considering its role in fostering lung immunity, we examined the role of PTX3 in asthma. We have recently demonstrated an enhanced expression of PTX3 in bronchial biopsies of allergic asthmatics that correlated with the disease severity. In this report, we assessed the effect of PTX3 deficiency on OVA-induced experimental asthma in a murine model. The magnitude of increase in airway and tissue resistance of OVA sensitized/challenged mice in response to methacholine (MCh) is found to be greater in PTX3 KO mice in contrast to their WT counterparts. We observed an increase in the infiltration of inflammatory cells in BALF obtained from PTX3 KO mice upon OVA sensitization/challenge as compared to their WT littermates. However peribronchial and perivascular inflammation and goblet cells hyperplasia showed no significant difference in both mouse strains. Further we found an enhanced induction of IL-4, IFN-γ, IL-17A and IL-10 production in the lungs of PTX3 KO mice as compared to WT mice upon OVA challenge. In lymph node, IL-17A recall response was greater in PTX3 KO mice than WT mice but no difference was observed in term of IL-4, IFN-g and IL-10. Taken together, our data suggest that lack of PTX3 predisposes mice to airway hyperresponsiveness and an enhanced inflammation.

W1.17.06
MicroRNA-155 augments local Th2 response in a mouse model of allergic airway inflammation

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Background: Allergic inflammation and tissue remodeling in allergic asthma is driven by a type 2 immune response to inhaled allergens. The molecular mechanisms associated with the allergen induced immune response remain largely unclear. MicroRNAs **(**miRNAs) are important regulators of gene expression in the immune system and miR-155 has previously been shown to regulate T cell responses.
Aim: To investigate the regulatory role of miR-155 in a model of allergen induced airway inflammation.
Methods: Wild type (WT) and miR-155 knockout (miR-155 KO) mice were sensitized and airway challenged to OVA. Differential cell counts, cytokine and chemokine production were evaluated. T helper (Th) cell populations and co-expression of master regulatory transcription factors in lung Th cells were determined by flow cytometry.
Results: miR-155 deficiency resulted in a decreased eosinophilic inflammation in the lung, Th2 cytokine production and airway mucus hypersecretion and further virtually abolished allergen-induced airway eotaxin-2/CCL24 and periostin levels. These characteristics were accompanied by a significant reduction in Th2 ($CD4^{+}GATA\text{-}3^{+}$) cells as well as reduced number of Th cells co-expressing additional master regulatory transcription factors in the lung of allergen challenged miR-155 KO mice compared to WT mice.
Conclusions: Our data provides evidence that miR-155 is involved in the regulation of allergen-induced airway inflammation, possibly through the regulation of Th2 cells locally in the airways. Targeting miR-155 may be beneficial both in the acute phase response and the more long-term effect of remodelling.

W2.01 Leukocyte signalling

W2.01.01
The recruitment and activation of phosphatidylinositol 4-phosphate 5-kinases α critically regulate CD28-dependent signaling responses

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CD28 is one of the most relevant costimulatory receptors that delivers both TCR-dependent and TCR-independent signals regulating a wide range of signaling pathways crucial for cytokine and chemokine gene expressions, T cell survival and proliferation. Most of the CD28-dependent signaling functions are initiated by the recuitment and activation of class IA PI3Ks, which catalyze the conversion of phosphatidylinositol 4,5-biphosphate (PIP2) into phosphatidylinositol 3,4,5-triphosphate (PIP3), thus generating the docking sites for key signaling proteins. Hence, PIP2 is a crucial substrate in driving the PI3K downstream signaling pathways and PIP2 turnover may be an essential regulatory step to ensure the activation of PI3K following CD28 engagement. Despite some data evidence that CD28 augment TCR-induced turnover of PIP2, its direct role in regulating PIP2 metabolism has never been assessed. Here we show that CD28 regulates PIP2 turnover by recruiting and activating phosphatidylinositol 4-phosphate 5-kinases α (PIP5Kα) in human primary CD4+ T lymphocytes. This event leads to the neo-synthesis of PIP2 and to its consumption by CD28-activated PI3K. We also evidenced that PIP5Kα activation is required for both CD28 unique signals regulating IL-8 gene expression as well as for CD28/TCR-induced Ca2+ mobilization, NF-AT nuclear translocation and IL-2 gene transcription. Our findings elucidate a novel mechanism that involves PIP5Kα as a key modulator of CD28 costimulatory signals.

W2.01.02
Terminal transport of lytic granules to the immune synapse is mediated by the kinesin-1/Slp3/Rab27a complex

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Cytotoxic T lymphocytes (CTLs) kill target cells via the polarized secretion of cytotoxic granules at the immune synapse (IS). The lytic granules are initially recruited around the polarized microtubule-organizing center (MTOC). In a dynein-dependent transport process, the granules move along microtubules towards the MTOC in the minus-end direction. Here, we show that (i) polarized granule transport over the small remaining distance to the plasma membrane and (ii) delivery of the granules' contents at the IS depend on the conventional molecular motor kinesin-1. We found evidence to suggest that the Rab27a effector synaptotagmin-like protein 3 (Slp3) is expressed in CTLs and interacts with the tetratricopeptide repeat of the kinesin-1 light chain. We found that kinesin-1 regulates a very late step in the transport of Rab27a/Slp3-associated vesicles that is required for lytic granule secretion. Our data provide further molecular insights into the key functional and regulatory mechanisms underlying the terminal transport of cytotoxic granules and the latter's secretion at the IS.

W2.01.03
The potassium channel KCNK3 determines calcium signaling pathways in T lymphocytes

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Ion channels are known to influence the activation and effector functions of T lymphocytes. Recently, a role for members of the two-pore domain potassium channel family (K2P channels, e.g. KCNK3, KCNK5, KCNK9) could be described for autoimmune neuroinflammation. KCNK3-/- mice showed a significantly ameliorated disease course in myelin oligodendrocyte glycoprotein peptide induced experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. However, the underlying intracellular pathways in T cells have not yet been addressed. Therefore, we intended to investigate the influence of KCNK3 to intracellular T cell signaling.
Murine and human CD4+ T lymphocytes were stained with the calcium-sensitive dye Fura-2. Pharmacological blockade of KCNK3 with A293, a novel selective blocker, reduced the extracellular calcium influx upon T cell receptor (TCR) crosslink. These results could be confirmed with KCNK3-/- cells which displayed a significantly reduced calcium increase compared to wildtype mice. A293 had no additional effect on KCNK3-/- cells. The reduced calcium levels led to a reduced activation of calcineurin and therefore a reduced dephosphorylation and nuclear translocation of the calcium-dependent signaling protein nuclear factor of activated T-cells (NFAT) as detected by western blotting experiments. In contrast, upstream signaling proteins such as ZAP70 or LAT showed higher phosphorylation levels in KCNK3-/- mice than in wildtype mice both under basal and stimulated conditions.
In summary, we were able to demonstrate a crucial influence of KCNK3 on calcium signaling pathways in T cells. Further studies are needed to explore the molecular pathways in more detail.

W2.01.04
Molecular tuning of telomerase activity in highly differentiated human primary CD28−CD27− T cells

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Telomerase is a RNA-dependent DNA polymerase re-expressed by T cells mounting an immune response upon antigenic stimulation. Highly differentiated $CD28^{-}CD27^{-}$ (DN) T cells are an end-stage sub-population progressively accumulating in the elderly, in patients with autoimmune disorders, cancer and chronic viral diseases. These cells have short telomeres and show proliferative defects as a result of defective telomerase activity, due in part to active MAPK p38 signalling. Most interestingly telomerase activity and cell survival can be re-established following MAPK p38 blockade in DN T cells. Therefore key senescent features of the human memory T cell compartment are reversible. Although repeated antigenic stimulation is considered one of the main drivers of T cell senescence, metabolic dysfunctions and cytokine priming have been postulated to negatively impact the biochemical features of end-stage T cell sub-populations. However the molecular evidence for such a mechanism linking chronic inflammation, due to cytokines and/or metabolic impairment, and T cell senescence has not yet been elucidated. We report here an alternative mechanism regulating MAPK p38 activation in highly differentiated DN T cells. The mechanism described here provides a target for manipulation of telomerase activity in a T cell compartment specific manner, reducing the risk of malignancies. This may result in the design of novel immunotherapy strategies aimed at reversing T cell senescence and boosting T cell mediated immune responses.

W2.01.05
The expression and phosphorylation of HS1 protein are finely tuned in normal and leukemic B cells

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We demonstrated that HS1 is a pivotal molecule in the signal transduction pathway triggered by the B-cell receptor (BCR), serving as a central interactor of several cytoskeletal components and being involved in tissue trafficking and homing in neoplastic B lymphocytes, namely in Chronic Lymphocytic Leukemia (CLL). Interestingly, HS1

protein shows a differential expression in the bone marrow as compared to the peripheral blood of CLL patients. That parallels what we also observed in normal B cells subpopulations where HS1 is expressed at very low level in Pre and Pro-B cell lineages while it is expressed at a higher level in mature B cells.
Rather than the expression of the protein, HS1 phosphorylation appears to be important as it correlates with prognosis in CLL and is modulated in normal B cells. As we aimed at defining the nature of HS1 phosphorylation in B cells, we analyzed cells form CLL patients and we found that HS1 is constitutively phosphorylated at the activatory site Tyr397 in a sizable fraction of patients, while this site is phosphorylated in normal B cells only after BCR stimulation. By mass spectrometry analysis we found new additional HS1 phosphorylation sites on Serine and Threonine residues, in a subset of patients, probably mediating the inactivation of the protein, in clear contrast with normal B cells.
The modulation of the expression and activation of the molecule in CLL cells and infiltrated tissues can help to better characterize the function of HS1 in B-cell ontogeny and CLL development.

W2.01.06
The impact of arginine methylation on BCR signaling

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Signals through the B cell receptor (BCR) are transmitted by a multitude of molecules, many of which undergo post-translational modification. In BCR signaling, arginine methylation, a characterized post-translational modification, has been suggested to control B cell development. Specifically, Protein Arginine Methyl-Transferase 1 (PRMT1) has been shown to methylate Ig-α and consequently to modulate BCR signal.
We have examined the consequence of a conditional deletion of the prmt1 gene in B cells. We find that PRMT1 regulates B cell maturation by shaping the pre-B cell compartment and modulating the subsequent transition to the immature B cell stage. By deleting PRMT1 at the CD23+ stage of B cell development, we find that, while follicular B cells were not affected, marginal zone B cells were lost. When activated, PRMT1-deficient B cells displayed an altered proliferation and survival capacity. Arginine methylation mediated by PRMT1 is required in B cells for humoral immunity. Indeed, in response to protein antigen, B cells lacking PRMT1 failed to form germinal centre. Moreover, the T-independent immune response against a polysaccharide antigen was also severely impaired in mice lacking PRMT1 in B cells. Thus PRMT1 plays a critical, B-cell intrinsic role in specifying the outcome of humoral immune responses and in fine-tuning B cell development.

W2.02 Nuclear receptors

IL2.02.01
Modulation of TCR signaling and T cell activation by glucocorticoids: facts and hypotheses

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Glucocorticoids (GC) have the ability to regulate gene transcription through the glucocorticoid receptor, a cytosolic transcription factor that translocates to the nucleus upon ligand binding. GCs have long been known to inhibit T cell activation through the GC receptor mediated transcriptional regulation of several immunosuppressive genes. Recent evidence has however revealed that, in addition to this slow mode of action, GC also elicit rapid, transcription independent responses at the onset of T cell activation. These non-genomic activities, which are mediated by cytosolic and/or membrane bound GC receptors, involve suppression of T cell signaling independently of transcription. The unligated GC receptor has been indeed shown to interact with the activated TCR complex, which associates with the kinases Lck and Fyn. Upon GC short-term treatment this interaction is disrupted, leading to the dissociation of Lck and Fyn from the TCR and thereby suppressing TCR signaling. In my talk I shall summarize our current understanding of the cross-talk of GC receptors with the TCR, discussing the the immune synapse as a potential target of GC dependent immunosuppression.

W2.02.01
Antagonistic actions of Rcor proteins refine LSD1 activity and restrain cellular differentiation

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The histone demethylase LSD1/Kdm1a demethylates histone3-lysine4 residues on nucleosomal histones in collaboration with the adaptor protein CoREST/Rcor1. Both proteins are obligate co-factors of, and mediate repression of the gene targets of, the transcription factors Gfi1 and Gfi1b during hematopoiesis. Two other Rcor paralogs, Rcor2 and Rcor3, also interact with LSD1 and Gfi1/1b. However, while Rcor2 exhibits biochemical activities and physiological properties analogous to Rcor1 and facilitates LSD1 mediated processes, Rcor3 inhibits LSD1 enzymatic activity and antagonizes its cellular function. Appending the SANT2 domain of Rcor1 to Rcor3 transforms it into an activator of LSD1. Thus Rcor3 functions as a naturally occurring dominant-negative inhibitor of LSD1 function in vitro and in vivo owing to its lack of the critical SANT2 domain. Endogenous levels of Rcor3 rise sharply in maturing erythroid cells, while that of LSD1 and Rcor1/2 remain relatively uniform, likely enabling Rcor3 to dampen LSD1 activity during hematopoietic development. These results reveal that LSD1 together with Rcor1/2 "drive" hematopoietic differentiation, while Rcor3 "brakes" it, by opposing or disrupting LSD1-Rcor1/2 function. This study highlights a general mechanism of refining the enzymatic and physiological activity of a widespread epigenetic modifier (LSD1), and restraining its cellular differentiation potential both in hematopoiesis and other developmental contexts.

W2.02.02
Estradiol promotes functional responses in inflammatory and steady state dendritic cells through differential requirement of activation function-1 of estrogen receptor α

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Estradiol (E2) has been shown to regulate cytokine-driven dendritic cell (DC) differentiation through estrogen receptors (ER) -signaling in myeloid progenitors (MP). ER regulates transcription of target genes through two distinct activation functions (AFs), AF-1 and AF-2 which requirement for transcriptional activities is dependent on the cell-type and promoter context. We investigated the role of ER AFs in the development and effector functions of inflammatory DCs, steady state conventional DCs (cDCs) and plasmacytoid DCs (pDC), using mouse lacking either AF1 ($ERAF1^0$) or AF2 ($ERAF2^0$). In agreement with previous works, we showed that E2 fostered the differentiation and effector functions of inflammatory DC through ER-dependent up-regulation of IRF-4 in GM-CSF-stimulated MPs. Interestingly, whereas AF-1 was required for IRF-4 upregulation, it was dispensable for the E2-mediated increase in the differentiation of the more functional Ly6C- subsets of DC. By contrast, no specific response to E2 was seen with progenitors from either $ERAF2^0$ or $ER^{-/-}$ mice. These results suggest that AF-1-independent activation of ER genomic effects promotes additional pathways in MP that synergize with low levels of IRF-4 to sustain GM-DC differentiation. By contrast, in Flt3L-driven DC subsets, ER AF1 was required for the E2-mediated effects on the development and innate functions of both cDCs and pDCs. Moreover, lack of ER AF-1 blunted the TLR-7 mediated IFN-response of female pDCs *in vivo*. Thus, ER uses AF1 differently in steady state and inflammatory DC lineages to regulate their innate functions, suggesting that selective ER modulators could be used to target specific DC subsets.

W2.02.03
Identification of TREM-1 as a novel target of Nuclear Receptor ligands in Dendritic Cells

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Dendritic cells (DCs) are antigen-presenting cells playing a crucial role in the regulation of innate and adaptive immune responses. DCs initiate adaptive immune responses after their migration to secondary lymphoid organs, a process dependent on the expression of the chemokine receptor CCR7. Our group has recently demonstrated that tumors produce LXR ligands/oxysterols, which are able to dampen DCs migration to secondary lymphoid organs by inhibiting CCR7 expression. We have recently analyzed the transcriptome profile of DCs undergoing maturation in the presence of the oxysterol 22R-Hydroxycholesterol (22R-HC), identifying more than 1500 genes modulated upon the treatment. Among them the triggering receptor expressed on myeloid cells (TREM)-1 and some genes belonging to its pathway were found markedly up-regulated by oxysterol.
The expression of TREM-1, a strong amplifier of immune responses, induced by oxysterols on maturing DCs was confirmed by qRT-PCR and by flow cytometry. Since LXRs form heterodimers with RXR, we tested the effect of the combination of 22R-HC and 9-cis Retinoic Acid, and found an additive effect on TREM-1 induction. Moreover, also the ligands for the nuclear receptors RAR and VDR raised TREM-1 expression, while ligands for PPAR, FXR and PXR have no effect. This modulation was specific for DCs and was functional, as the triggering of TREM-1 strongly increased TNF release. Interestingly, several tumor-conditioned supernatants were able to induce TREM-1 expression. We are performing experiments *in vivo* to evaluate the biologic role of nuclear receptor ligand-induced TREM-1 up-regulation in DCs.

W2.02.04
Retinoic acid-rich microenvironment provides clonal survival cues for tumor and bacteria-specific CD8+ T cells

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While studies have implicated a role of vitamin A (VA) in host resistance to infectious disease, little is known as to the role of VA and its active metabolite, retinoic acid (RA) in host defense against cancer. Herein, it is shown for the first time that local RA production within the tumor microenvironment (TME) is increased up to 5-fold compared to naïve surrounding tissue, with a commensurate increase in RA signaling to regionally infiltrating tumor-reactive T cells. Tumor-specific CD8+ T cells deficient in RA signaling (by specific over-expression of dominant negative Retinoic Acid Receptor α in CD8+ T cells) failed to accumulate and control tumor growth. Specific deletion of RARα, RARβ and RARγ in CD8+ T cells further demonstrated that RARα is required for CD8+ T cell survival/homeostasis and RA-induced gut-homing receptor (α4β7 and CCR9) expression, whereas RARβ is indispensable for bacteria-specific CD8+ T cell expansion in response to Listeria monocytogenesis infection. These studies established a novel function of RA essential for both anti-tumor and infection CD8+ T cell immunity.

W2.02.05
Th17-mediated B cell activation contributes to splenomegaly in PPARγ hypomorphic mice

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PPARγ is a nuclear transcription factor that modulates diverse biofunctions, including lipid biosynthesis, glucose metabolism and inflammation. However, the specific role of PPARγ in immune function *in vivo* has not been fully explored. We have previously generated PPARγ hypomorphic mice with about 25% expression of PPARγ (*Pparg*$^{C/-}$ mice). Interestingly, we found that splenomegaly was observed from young to old of *Pparg*$^{C/-}$ mice, and young *Pparg*$^{C/-}$ mice with splenomegaly without relocation of hematopoiesis to the spleen. Moreover, old mice displayed gross splenomegaly accompanied by increased autoantibody production and renal immune complex deposition. We found that increased autoantibody production was caused by increased B cell activity. *In vitro* assay revealed splenic *Pparg*$^{C/-}$ CD4^{+} T cells showed increased proliferation and survival. Indeed, co-culture of wild type B cells with activated *Pparg*$^{C/-}$ CD4^{+} T cells promoted increased plasma cells and IgG production, compared that with wild type CD4^{+} T cells. Further evaluation of CD4^{+} subsets in *Pparg*$^{C/-}$ mice, the signature Th17-type factors, RORγt and IL-17A, were increased in the spleen of *Pparg*$^{C/-}$ mice. Also, *Pparg*$^{C/-}$ CD4^{+} T cells from *Pparg*$^{C/-}$ mice exhibited enhanced polarization toward Th17 phenotype in response to αCD3/CD28. Wild type B cells and CD4^{+} T cells co-culture system supplied with conditional medium from activated *Pparg*$^{C/-}$ CD4^{+} T cells with IL-17A depletion showed that lower IL-17A level decreased B cell activity. Our results indicated splenomegaly in young *Pparg*$^{C/-}$ mice was contributed by increased activation of B cells with the help of Th17, suggesting PPARγ has a new beneficial role in immunomodulation.

W2.02.06
Expression levels of mRNA for GRα, GRβ, 11βHSD1 and GRα/β ratio in peripheral blood mononuclear cells (PBMC) from patients with pulmonary tuberculosis, throughout the 6-month course of etiological treatment and 3 month following its completion

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Patients with pulmonary tuberculosis (TB) show several immune-endocrine alterations, like decreased plasma levels of dehydroepiandrosterone (DHEA) together with augmented concentrations of proinflammatory cytokines and cortisol. The latter continues to be increased throughout treatment, whereas IL-6 and IFN-γ fall to control values and DHEA concentrations normalize. Studies on the expression of mRNA for glucocorticoid receptor isoforms (GRα and β) and enzymes regulating cortisol availability (11beta-hydroxysteroid dehydrogenase type 1; 11βHSD1 and type 2; 11βHSD2) in PBMC indicate a lower GRα/β ratio and higher 11βHSD1 in severe pulmonary TB. Extending this analysis we now analyzed the expression of mRNA for GRα, GRβ, 11βHSD1 and GRα/β ratio in PBMC from 16 TB -HIV negative- patients, bled at diagnosis (T0), two (T2), four (T4) and 6 months (T6) of treatment as well as 3 months later (T9) and 19 age- and sex-matched healthy controls (HCo), for comparison purposes. Transcript levels for GRα at T0 remained within controls values, rose significantly throughout treatment ($p<0.05$) decreasing to normal levels by T9. Conversely, the GRβ expression, that was augmented at diagnosis ($p<0.03$), decreased significantly in the remaining time-point evaluations ($p<0.02$). This resulted in a significantly reduced GRα/β ratio at T0 ($p<0.03$), reaching values similar as those of HCo from T2 and

thereafter (p<0.02). Expression of 11βHSD1 was augmented at T0 (p<0.05), but further evaluations during and after specific treatment showed levels within the normal range (p<0.02). Clinical improvement and decreased inflammation resulting from specific treatment coexist with a milieu more suitable for the immunomodulatory effects of endogenous GC.

W2.03 Epigenetic control of immune responses

IL2.03.01
Epigenomic and transcriptional regulation of human Th cell differentiation

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T helper (Th) cell subsets with distinct cytokine secretion profiles and function play a crucial role in host defense as well as in pathological disorders such as allergy and chronic inflammatory and autoimmune diseases. To complement extensive knowledge on mouse Th cell differentiation, comprehensive studies in the human system are urgently needed for mechanistic understanding of human immune mediated diseases providing a key basis for translational research (Scand. J Immunol. 2013 doi: 10.1111/sji.12071. Aiming at identifying new regulators of Th cell differentiation we have analysed the epigenomic changes during the initiation of human Th cell differentiation.
The first human genome-wide maps of histone modifications that reveal enhancer elements at an early stage of polarization toward Th1 and Th2 lineages were generated (. Global Chromatin State Analysis Reveals Lineage-Specific Enhancers during the Initiation of Human T helper 1 and T helper 2 Cell Polarization, Immunity (2013), http://dx.doi.org/10.1016/ j.immuni. 2013.05.011). Our analysis indicates that even at this very early time point, cell-specific gene regulation and enhancers are at work directing lineage commitment. Further examination of lineage-specific enhancers identified known and novel transcription factors as putative drivers of lineage-specific gene expression. Lastly, an integrative analysis of immunopathogenic associated SNPs suggest a role for distal regulatory elements in the disease pathogenesis.

W2.03.01
Regulatory non-coding RNAs as modulators of human lymphocytes differentiation

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Most of the immune cell subsets can be purified by flow-cytometry, making it an interesting and powerful model system to study the role of non-coding RNAs in human primary cells. In particular we focused our studies on two classes of non-coding RNAs: microRNAs and large intergenic non-coding RNAs (lincRNAs). As little is known on expression of lincRNAs, in functional subsets of human immune system, we performed a comprehensive transcriptome analysis of 13 purified primary human cell subsets (belonging to T-CD4+, T-CD8+ and B lymphocytes) by RNA-seq using Illumina platform. We assembled ~2 billion RNA-seq reads across 63 cell samples, using both Cufflinks and Oases algorithms for Reference Annotation Based and de-novo assembly respectively. lincRNAs were identified taking into account length, structural, coding potential, and orthology features. Here we show that different lincRNAs are preferentially expressed in specific lymphocyte subsets and that their expression patterns change during the functional T cell differentiation. We identified lincRNA signatures of the selected lymphocytes subsets. Experiments of loss/gain of function suggest the role of these lincRNAs in lymphocytes' cell identity and maintenance. To investigate microRNAs role in human immune system, we have recently performed a microRNA profiling in seventeen lymphocyte subsets (Rossi et al. Nature Imm. 2011 12:796), providing an 'atlas' of microRNA expression in human lymphocytes, defining subset-specific signatures and their target genes, and indicating that T-cell naive state is enforced by microRNAs. We are exploring the interplay between microRNAs and lincRNAs as a new dynamic regulatory layer of non-coding RNAs controlling lymphocyte plasticity.

W2.03.02
Bromodomain inhibition enhances tolerogenic properties in dendritic cells

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Inhibition of acetylated-lysine reader enzymes, the bromodomain and extra-terminal domain (BET) proteins, is effective in specific downregulation of secondary response inflammatory genes and ameliorates lethal inflammatory states in several animal models. In this study we analyzed the effects of BET-inhibitor I-BET151 on dendritic cell function.
Bonemarrow derived dendritic cells (BMDC) were cultured from C57BL/6 mice by exposure to GM-CSF for 8 days. I-BET151 was tested at 1nM up to 1000nM against DMSO vehicle. Effect of I-BET151 on maturation, cytokine secretion and ovalbumin specific CD4+ CD62L+ naive T cell skewing was analyzed. Regulatory T-cell suppression assays were performed using 3-H thymidine incorporation.
I-BET151 treatment during maturation of BMDC resulted in lower expression of CD40, CD80, CD86 and MHC-II. Secretion of IL-6, IL-12 and IL-10 was abrogated (11%, 0.5% and 1.6% of vehicle, respectively; all p<0.001), whereas TNF-alpha secretion was still present (83% of vehicle). A 2 hour incubation of 1000nM I-BET151 to ovalbumin loaded matured BMDC, prior to addition of T naive T cells, did not affect IFN-gamma Th1 cell skewing, but led to a 3-fold increase in foxp3 expressing T cells (p<0.001). PMA/ionomycin stimulation of the T cells resulted in higher IL-10 levels in I-BET151 treated compared to the vehicle treated BMDC (p<0.01). These cells were able to suppress proliferation of naïve T cells.
We conclude that the epigenetic modifier I-BET151 histone mimic tolarizes DCs and enhances their potential to skew suppressive Treg by inhibiting maturation and inflammatory cytokine secretion.

W2.03.03
The Polycomb group protein Ezh2 regulates the germinal center B cell response

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In mammals, Polycomb group (PcG) proteins exert and essential function in the control of stem cell self-renewal, pluripotency, and cell lineage determination. PcG proteins are recruited within Polycomb Repressive Complexes (PRC) to promote transcriptional repression of target genes through post-translational modifications of lysine residues within histone tails. Ezh2, as part of PRC2, promotes the trimethylation of lysine-27 on Histone H3 (H3K27me3).
In mature B cells, Ezh2 is up regulated upon entry into the germinal center (GC) reaction, during a T-cell dependent immune response. Importantly, Ezh2 gain-of-function mutations are often identified in GC-derived Non Hodgkin B-cell lymphomas.
We used conditional gene targeting to study the role of Ezh2 in GC B cells. Inducible Ezh2 inactivation in GC B cells resulted in marked apoptosis of the cells, leading to poor antibody responses and fewer high-affinity memory B cells. Strikingly, Ezh2 protected GC B cells from genotoxic damage caused by AID. Global identification of

H3K27me3 targets in GC B cells revealed a significant enrichment for developmental regulators and BCL6 repressed genes. Ezh2 inactivation in GC B cells caused the up-regulation of H3K27me3 targets, including the plasma cell determinants IRF4 and Blimp1. This resulted in a significant enhancement of plasma cell differentiation in response to IL-21 stimulation. All together these results show that Ezh2 sustains AID-dependent antibody diversification and limits IL-21 dependent terminal differentiation of GC B cells, to sustain high affinity antibody responses. Our findings are relevant to understand how deregulated Ezh2 activity may contribute to lymphomagenesis.

W2.03.04
Loss of an Igκ gene enhancer in mature B cells results in rapid gene silencing and partial reversible de-differentiation

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We address here whether there is cellular memory of a transcriptional enhancer once it has served its purpose to establish an active chromatin state. We have previously shown that the mouse Igκ gene's downstream enhancers, E3′ and Ed, are essential but play redundant roles for establishing transcriptional activity in the locus during B cell development. To determine if these enhancers are also necessary for the maintenance of transcriptional activity, we conditionally deleted E3′ in mature B cells that possessed Ed -/- alleles. For these studies we bred F/F E3′ Ed -/- mice with either tamoxifen-inducible Cre mice, or with mice specifically expressing CD23-Cre in mature B cells. Successful deletion of E3′ in these cases generated E3′-/-Ed-/- mice, which have single LoxP and Frt sites in place of E3′ and Ed, respectively. Upon E3′ deletion, the locus became rapidly silenced, lost positive histone epigenetic marks, and the mature B cells partially de-differentiated, induced RAG-1 and -2 along with certain other pro-B cell makers, and then re-differentiated after triggering Igλ gene rearrangements. We conclude that the Igκ gene's downstream enhancers are essential for both the establishment and maintenance of transcriptional activity, and that there is no cellular memory of previous transcriptional activity in this locus. Furthermore, upon enhancer loss the mature B cells unexpectedly underwent reversible retrograde differentiation. This result establishes that receptor editing can occur in mature B cells, and raises the possibility that this may provide a tolerance mechanism for eliminating autoreactive B cells in the periphery.

W2.03.05
Regulation of CD4 T helper cell differentiation and plasticity by the histone methyltransferase Ezh2

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Upon encountering antigen, naïve CD4 T cells rapidly differentiate into discrete subsets of effector T helper (Th) cells that direct immune responses to different types of infection. Polarizing cytokines induce the expression of lineage-specifying genes (*Tbx21* for Th1 cells and *Gata3* for Th2 cells) that control differentiation. We found that the histone H3K27 methyltransferase Ezh2 controls differentiation of Th1 and Th2 cells, and that inactivation of Ezh2 enhances plasticity of both of these subsets. *Tbx21* and *Gata3* experienced large changes in Ezh2 occupancy during Th differentiation, and inactivation of Ezh2 depleted H3K27-trimethylation (H3K27-Me3) at these loci. Additionally, loss of Ezh2 enhanced the ability of Th2 cells to cause pathology in a mouse model of allergic asthma, and CD4 T cell-specific deletion of Ezh2 resulted in the progressive accumulation of memory phenotype Th2 cells *in vivo*. This study establishes a functional link between Ezh2 and transcriptional regulation of lineage-specifying genes in terminally differentiated peripheral T cells via H3K27-Me3. These data may therefore contribute to the development of therapies that target this pathway to enhance or repress immune responses in human disease.

W2.03.06
Unique epigenetic signatures are associated with the induction, silencing and re-expression of CD8 during T cell development and activation

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The CD8 co-receptor is a powerful modulator of T cell activation and function whose levels are regulated by various stimuli. Here we show that initiation, maintenance and silencing of CD8 expression during murine T cell development in the thymus were associated with complex changes in DNA methylation across the *Cd8a* locus. In peripheral $CD8^+$ T cells, CD8 down-regulation following activation in type 2-polarizing conditions was accompanied by a distinct pattern of *Cd8a* remethylation, which was stable long-term. A combination of activation and certain cytokine signals could induce a subset of apparently committed type 2 $CD8^{low}$ cells to re-express CD8; this reactivation correlated with reduced DNA methylation at specific regions of *Cd8a*. Thus, T cells display a unique epigenetic signature at the *Cd8a* locus at each stage in their development, polarization and reprogramming, suggesting complex, differential DNA modification in response to changing external signals. Notably, type 2-polarized $CD8^{low}$ cells induced to re-express CD8 switched to a type 1 effector phenotype. Therefore, CD8 co-receptor re-expression is one of a suite of functions that can be re-programmed after primary effector differentiation. We conclude that even strongly polarized effector $CD8^+$ T cells have the capacity to adapt to a new environment and tailor their phenotype and function accordingly. Such plasticity may be harnessed for treatment strategies targeting chronic diseases such as HIV or chronic B cell lymphocytic leukaemia, where $CD8^+$ T cells displaying type 2 cytokine production and low CD8 co-receptor expression have been reported and may be linked to poorer disease outcomes.

W2.04 MicroRNAs in immune regulation

W2.04.01
TCD8+ suppressor cells produce antigen-specific exosomes carrying miRNA-150 to inhibit contact sensitivity response

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Introduction: T suppressor cells (Ts) release the exosomal suppressor factor (TsF) that inhibit murine contact sensitivity reaction (CS). The antigen specificity of exosomes is warranted by coating with immunoglobulin light chains produced by B1 lymphocytes.
The research is aimed to define Ts phenotype and to determine the exact structure of exosome cargo conditioning suppressive activity of TsF.
Materials & Methods: Mice tolerized with high intravenous doses of antigen produce suppressive exosomes isolated in standard ultracentrifugation procedure. Their biological activity is estimated in adoptive transfer of CS effector cells or active hapten immunization and assessed as ear swelling response in CS test. To characterize Ts the negative and positive selection tests and DEREG mice depleted of FoxP3+ T regulatory cells (Tregs) were used. To define the RNA compound of TsF, appropriate miRNA inhibitors and knock-out (KO) mice were used.
Results: TsF exosomes from DEREG mice suppressed CS transfer similarly to wild-type mice. RNA cargo from chromatographically purified suppressive exosomes was sequenced which lead to the assumption that miR150 is the suppressive particle. This hypothesis was verified by using miR150 inhibitor and miR150 KO mice that produce non-suppressive exosomes. Supplementation of exosomes from miR150 KO mice with miR150 restored their suppressive activity.

Conclusions: TsF exosomes are produced by TCD3+CD8+ suppressor lymphocytes that are not Tregs. Their immunoregulatory activity is mediated by miR150 molecules equipped in antigen recognition system. To our knowledge, this is the first evidence of immunoregulation orchestrated in vivo by antigen-specific exosomes carrying inhibitory information coded in a short RNA particle.

W2.04.02
The role of microRNA-142-5P in experimental colitis

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The pivotal role of microRNAs in the regulation of gene expression, in particular genes involved in the immune response, indicates that they play a role in the pathogenesis of inflammatory bowel disease (IBD) as well. MicroRNAs, by their mechanism of action, are promising new therapeutic agents or targets.
Experimental colitis in severe combined immunodeficiency (SCID) mice shares many features of human inflammatory bowel disease (IBD). In this model in which transfer of CD4CD45RB^{high} T cells results in colitis we found a number of microRNAs upregulated during development of experimental colitis, among these microRNA-142-5p (miR-142-5P). The aim of this study is to determine the effect and the target genes of the upregulated microRNA.
We administered locked-nucleic-acid-modified (LNA) oligonucleotide anti-miR-142-5p or anti-scr-microRNA at the moment the mice demonstrated the first signs of disease 3 weeks after the transfer of the CD4CD45RB^{high} T cells. We determined the course of the disease and performed an mRNA analysis (Illumina, Service SX, Leiden) of the spleen and colon.
Blocking anti-miR-142-5p resulted in a higher survival rate compared to mice treated with anti-scr-microRNA (p=0.0026). In a separate experiment mRNA analysis demonstrated that four of the top ten genes that show upregulation in the spleen after blocking miR-142-5p are found in a pathway related to mast cell biology. In the colon the upregulated genes after blocking miR-142-5p are related to diverse signalling pathways (PPAR, TLR), early stage of cancer and metalloproteases.
Various potential interesting pathways are involved in the diminution of chronic experimental colitis after blocking miR-142-5P.

W2.04.03
Investigating the impact of individual miRNAs on Treg conversion

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T cell differentiation is imposed by transcriptional changes initiated by signaling events upon T cell activation. Induction of regulatory T cells (Treg) further depends on post-transcriptional mRNA regulation by Argonaute-loaded microRNAs, as shown by strongly impaired Treg differentiation in the absence of microRNAs in Dicer-knockout T cells.
Post-transcriptional gene regulation may be most important early after T cell activation before shortening of transcript 3'UTRs and downregulation of Argonaute protein expression occur. To elucidate the function of individual microRNAs on Treg induction during the initial activation phase, we employed adenoviral gene transfer into CD4 T cells, which transgenically express the coxsackie adenovirus receptor. This allowed overexpression of microRNAs in naive CD4 T cells before T cell activation. Based on a screen of 150 T cell-expressed microRNAs we identified a number of microRNAs that significantly affected Treg conversion. To discriminate specific effects of these microRNAs, we also determined their effect on Th17 differentiation. MicroRNA-99a promoted Treg and interfered with Th17 differentiation. Since mTOR is a known reciprocal regulator of Treg and Th17 differentiation we tested its regulation as a predicted miR-99a target. We show an inverse correlation between microRNA-99a overexpression and mTOR mRNA levels during Treg induction as well as a functional repression through the mTOR 3'UTR by microRNA-99a. In summary, our data suggest a role for microRNA-99a as part of a larger miRNA network, which specifically downregulates mRNAs like mTOR to control the reciprocal differentiation towards Treg and Th17 subsets early after antigen-recognition by naive T cells.

W2.04.04
IL-10-induced microRNA-187 negatively regulates TNFα, IL-6 and IL-12p40 production in TLR4-stimulated monocytes

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Interleukin (IL)-10 is a potent anti-inflammatory molecule that, in phagocytes, negatively targets cytokine expression at transcriptional and post-transcriptional levels. Post-transcriptional checkpoints also represent the specific target of a recently discovered, evolutionary conserved class of small silencing RNAs known as microRNAs (miRNAs), which display the peculiar function of negatively regulating mRNA processing, stability and translation. In this study, we report that activation of primary human monocytes upregulates the expression of miR-187, both *in vitro* and *in vivo*.
Accordingly, we identify miR-187 as the first IL-10-dependent miRNA playing a role in IL-10-mediated suppression of TNFα, IL-6 and IL-12p40 produced by primary human monocytes following TLR4 activation. Ectopic expression of miR-187 consistently and selectively reduces TNFα, IL-6 and IL-12p40 produced by LPS-activated monocytes. Conversely, the production of LPS-induced TNFα, IL-6 and IL-12p40 is significantly increased when miR-187 expression is silenced. Our data demonstrate that miR-187 directly targets TNFα mRNA stability and translation, and indirectly decreases IL-6 and IL-12p40 expression via downmodulation of IκBζ, a master regulator of the transcription of these latter two cytokines. These results uncover a novel miRNA-mediated pathway controlling cytokine expression and demonstrate a central role of miR-187 in the physiological regulation of IL-10-driven anti-inflammatory responses.
This study was supported by research grants from the Ministero dell'Istruzione dell'Università e della Ricerca, the University of Verona, Fondazione Cariverona, the Italian Association for Cancer Research and Regione Lombardia.

W2.04.05
MiR-146a promotes IVIg-mediated inhibition of NFκB activation in LPS-treated human monocytes

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Background: Monocytes are key players of the innate immune response and recent studies have shown that their differentiation and functions involve microRNA which act through the inhibition of protein translation. Among them, miR-146a has been shown to supress NF-κB activity and consequently, to inhibit NF-κB target genes. Therefore, miR-146a could lead to anti-inflammatory effects similar to those reported for IVIg, a therapeutic preparation of polyclonal human IgG isolated from the plasma of thousands of healthy donors. Indeed, IVIg has been shown to abrogate pro-inflammatory cytokines while increasing the expression of anti-inflammatory cytokines in monocytic cells.
Objectives: To evaluate whether miR-146a is involved in the anti-inflammatory effects of IVIg following LPS stimulation of human monocytes.
Methods: Human monocytes were obtained from the blood of healthy volunteers and treated with LPS (1 µg/mL) or IVIg (15 mg/mL) alone or alternatively, pretreated with LPS followed by addition of IVIg. Then, miR-146a, IRF3, IFN-β, TGF-β1 and IL-1Ra expression was measured by qPCR while NF-κB and IRAK1 expression was measured by Western blotting.
Results: Addition of IVIg to LPS-pretreated monocytes induced a significant up regulation of miR-146a expression associated with a significant reduction of its target IRAK1 and consequently of NF-κB activation. Furthermore the expression of IRF3 and IFN-β was reduced in LPS-pretreated monocytes following treatment with IVIg, whereas TGF-β1 and IL-1Ra expression were increased, thus

skewing the balance between proinflammatory vs anti-inflammatory cytokine towards an anti-inflammatory pathway.
Conclusion: Our data suggest that miR-146a may contribute to the anti-inflammatory effects of IVIg in human monocytes.

W2.04.06
MicroRNA regulation of bacterial infection in the lung

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RATIONALE MicroRNAs (miRNAs) are small non-coding RNA that can bind to multiple target mRNA to repress protein production. While the expressions of specific miRNAs are known to be dysregulated in multiple human diseases, the role of miRNAs during bacterial infection in the lung remains unknown. Lung pathogens, such as non-typeable Haemophilus Influenzae (NTHi), activate different TLR signalling pathways and inflammatory cytokines. Our aim is to investigate the roles of miRNA in regulating these processes.
METHODS Lung miRNAs that were differentially expressed following bacterial challenge were identified by microarray. miRNA in primary macrophages and neutrophils were knocked down using miRNA inhibitors (antagomirs) in vitro, to investigate the role of miRNA in regulating bacterial clearance by these immune cells. In vivo, antagomir-treated macrophages and neutrophils were adoptively transferred into naïve recipient mice. These mice were then challenged with bacterial. Following bacterial challenge, bacterial loads in lung were measured.
RESULTS Upon NTHi infection, 20 miRNAs were up-regulated while 52 were down-regulated by > 2.5 fold. Interestingly, knockdown of a specific miRNA in vitro resulted in significantly decreased bacterial load and increased phagocytosis by macrophages and neutrophils. Similarly, transfer of macrophages or neutrophils deficient in miRNA into naïve recipient mice leads to enhance clearance of the bacteria in vivo. This enhance clearance is independent of cellular infiltration. Inhibiting miRNA may play a protective role by enhancing the function of innate immune cells during bacterial infection.
CONCLUSION Our study suggests that miRNA may play important roles in regulating the innate immune response to bacterial infection.

W2.05 Immune responses in aging

IL2.05.01
Genetic Regulation of Longevity in Memory CD4 T cells in Aged mice

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While memory T-cells represent a hallmark of adaptive immunity, little is known about regulation of memory CD4 T-cell survival. A major challenge has been in finding and characterization sufficient numbers of antigen-specific CD4 memory T-cells that survive several months post immunization. Previously, we have shown that presentation of low doses of antigen to memory CD4+ T-cells induce a state of anergy. B cells specific for the protein antigen induce a dormant state in memory CD4 cells that is reversible upon re-infection. Here, by taking advantage of a sensitive technology, we have studied gene expression dynamic profile of specific CD4 cells in mice. We explored mechanisms that allow survival of memory DO11.10 CD4 T-cells throughout mouse life. We analyzed the dynamic patterns of gene expression at multiple time points post Vaccinia-OVA infection up to 11 months comparing it to the gene expressions in naïve cells in young mice, and to age-matched naïve cells in aging mice. The results demonstrated a striking pattern of changes in cellular metabolism as well as pro-survival and other factors that appear to work in synergy to achieve the biological goal of extending the longevity of memory CD4 T-cells. We show a pattern of gene expression that is specific for survival of memory, but not naïve cells in aging mice. Understanding this pattern of gene expression can be used in developing strategies for extending survival of memory CD4 T-cells, or repressing them for improved outcomes in transplantation.
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W2.05.01
Senescene associated cells in human CD8+ T-lymphocytes

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The expression of CD57 and the absence of CD28 on T-lymphocytes have been used as "senescence" markers. The association of these markers with characteristics of senescence that emerge during in vitro cellular aging is, however, not clear. Using flow cytometry, CD8+ T-cells were analyzed in old and young human subjects for their expression of CD57 and CD28. The subpopulations were tested for various markers of cellular senescence, apoptosis, homing and differentiation, and for their proliferation capacity and tendency for apoptosis. Significantly higher proportions of CD28+CD57+ and CD28-CD57+ cells were found in old subjects. CD28+CD57+ cells lacked proliferation, had the highest intensity of expression of p16, p21, Bcl-2 and CD95, and the highest frequency of Bcl-2 and CD95 expressing cells. The combined expression of these proteins favour their likely role in promoting senescence in CD28+CD57+ cells despite the significantly higher proportion of CD28+CD57+ cells positive for the apoptosis indicator, Annexin-V, compared with other subpopulations. The frequency of CD45RO and CCR5 expressing cells was also highest in CD28+CD57+ cells, and are highly expressed by T-cell populations believed to be dominated by senescent cells. Notably, our observation of a subpopulation of CXCR2+ cells among CD28-CD57+ cells indicates the possibility of a subpopulation of CD28-CD57+ cells that might represent a more pronounced senescent phenotype. We conclude that among CD8+ T-lymphocytes, characteristics that correspond to a senescent cell type are most prominent in CD28+CD57+ cells, thus providing further evidence for the existence of senescent cells in vivo and their possible importance in aging.
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W2.05.02
Characterization of the role of distinct plasma cell-free DNA (cf-DNA) species in age-associated inflammation and frailty

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Self-DNA that circulates in plasma as cell-free DNA (cf-DNA) has recently emerged as a potential biomarker of age-associated systemic inflammation and cell death. cf-DNA may also promote autoinflammation in certain cass. Because the total cf-DNA pool comprises different cf-DNA species, we quantified the plasma levels of gene-coding cf-DNA, Alu repeat cf-DNA, mitochondrial DNA (mtDNA) copy number and the amounts of unmethylated and total cf-DNAs. We identified the relationships between these cf-DNA species and age-associated inflammation, immunosenescence and frailty. In addition, we determined the cf-DNA species-specific genome-wide transcriptomic signatures in blood mononuclear cells to elucidate the age-linked leukocyte responses to cf-DNA. The study population consisted of n=144 nonagenarian participants of the Vitality 90+ Study and n=30 young controls. In the nonagenarians, higher levels of total and unmethylated cf-DNAs were associated with systemic inflammation and increased frailty. The plasma mtDNA copy number was also directly correlated with increased frailty but not with inflammation. None of the cf-DNA species were associated with immunosenescence. The transcriptomic pathway analysis revealed that higher levels of total and unmethylated cf-DNAs were associated with immunoinflammatory activation in the nonagenarians but not in the young controls. The plasma mtDNA appeared to be inert in terms of inflammatory activation in both the nonagenarians and young controls. These results demonstrate that the plasma levels of total and unmethylated cf-DNA could serve as biomarkers of age-associated systemic inflammation and frailty. We suggest that circulating self-DNA, assessed as total or unmethylated cf-DNA, might aggravate immunoinflammatory reactivity in very old individuals.

W2.05.03
Assessing human TCRB CDR3 repertoire by a RACE-PCR-sequencing method

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The T cell receptor (TCR) repertoire is a blueprint and record of the adaptive immune system in humans. The great diversity and proper distribution of the TCR repertoire is essential for a competent immune system that is capable of responding effectively to the vast universe of antigens/pathogens. However, neither the actual size of the diversity nor the distribution of specific TCR in man has been adequately determined. Here, we describe a RACE-PCR- next generation sequencing (RAP-Seq) method that allows a direct assessment of the size and distribution of the TCRβ CDR3 repertoire of T cells. We have also developed computational algorithms for data processing and analysis. From analysis of the TCRβ CDR3 repertoire of peripheral blood from 16 adults (21-94 years old), including 7 adults with longitudinal samples, we found that the size of the TCRβ CDR3 diversity of CD4+ T cells ranges from 2.6-3.7 x105, which was more than twice that of CD8+ T cells. With age, expansion of highly abundant TCRβ CDR3 clones as well as reduction in TCRβ CDR3 diversity size was observed in old adults (>late 60s). Remarkably, a significant number of TCRβ CDR3 retained over a decade including some public TCRβ CDR3 against known viral antigens. Together, these findings reveal for the first time the actual age-associated changes of the TCRβ CDR3 repertoire (reduced diversity and altered distribution) in humans, which could serve as a measure of immune competency and a guide for intervention in the elderly.

W2.05.04
Memory B cell repertoire changes with age.

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Diversity of repertoire is a necessity for effective function of the immune system. We have previously shown that repertoire diversity can be compromised in old age and that this correlates with poor health. We have also investigated the repertoire changes during vaccination in older people and have seen that age-related perturbations of the repertoire can be seen at different stages of the response. Closer analysis, following the expansion of Ig genes in samples of peripheral blood, show that the IgA response in particular seems to be much delayed, to the extent that there is not much expansion of IgA cells at day 7 after vaccination in old compared to young, and where the young group had resolved the IgA expansions by day 28, the old group still had significant Ig gene expansion. Since plasma cells have many more copies of Ig mRNA than B cells these studies likely reflect changes in peripheral blood plasma cells. Cell sorting to investigate age-related changes in different B cell subsets (by CD27 and IgD phenotype) has indicated that the repertoire of IgG memory B cells also changes with age, such that it acquires characteristics that we previously reported were distinguishing features of so-called IgM memory cells.

W2.05.05
Memory immune response to booster vaccination in old age depends on adequate priming earlier in life

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Regular booster vaccinations against tetanus and diphtheria are recommended for adults and older persons in many European countries. Vaccine-induced immune responses are generally weaker and antibody decline over time is more pronounced in the elderly. We therefore investigated immune responses to booster vaccinations against tetanus and diphtheria in older adults. 87 persons over 60 years received two consecutive booster vaccinations against tetanus and diphtheria and antibody concentrations were measured before and 4 weeks after the vaccinations. After the second boost tetanus- and diphtheria-specific circulating plasmablasts and cytokine secreting T cells were determined. The proportion of persons without protective antibody concentrations (0.1 IU/ml) against diphtheria was lower prior to the second compared to the first boost probably due to the shorter booster interval. Four weeks after vaccination all participants had protective antibodies against tetanus, but 14% or 7% had no protective antibodies against diphtheria after the first and the second vaccination, respectively. Individual antibody responses were very similar after the first and the second boost, but responses against tetanus and diphtheria were only weakly linked. Antibody responses were independent of gender, health status, CMV-status, inflammatory status and specific T cell responses. However, there was a strong correlation between antibody responses and the size of the memory B cell pool as indicated by the number of specific circulating IgG-plasmablasts 7 days after booster vaccination. Our results show that older persons respond similar to consecutive booster vaccinations and that vaccination history and primary responses are determining factors for the quality of booster responses.

W2.05.06
Human B cell response to the influenza vaccine is predicted by AID and decreased by inflammaging

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Deficits in immune responses in the elderly contribute to their mortality and morbidity. In order to develop predictive markers for a beneficial humoral immune response, we evaluated the in vivo and in vitro response to the seasonal influenza vaccine in young and elderly individuals. We measured serum antibody response and associated this with the in vitro B cell response to the vaccine, measured by AID (activation-induced cytidine deaminase). AID is a measure of immunoglobulin class switch recombination (CSR), the process that generates protective antibodies and memory B cells, and is a good measure of B cell function. Both responses decrease with age, are significantly correlated, and measures of AID in activated (mitogen) B cells at t0 (before vaccination) predict ability to respond to the vaccine. We hypothesized that the increased pro-inflammatory status of the elderly, called inflammaging, directly impacts B cell function, thus impairing the capacity of the individual to make protective antibodies and to respond to vaccination. Our data indicate that aged B lymphocytes themselves make TNF-α before vaccine challenge and this correlates with their impaired function, including reduced AID expression after B cell stimulation. Our results reveal new molecular mechanisms which contribute to reduced antibody responses in aging and suggest that these will have an impact for crucial development of effective vaccines to protect the elderly from infectious and other debilitating diseases.

W2.06 Leukocyte trafficking

W2.06.01
Clathrin light chain subunits control actin organization during infection and lymphocyte migration

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The self-assembly of clathrin into a lattice-like coat on membranes is mediated by the clathrin heavy chain (CHC) subunits, which form a triskelion-shaped molecule. The classical function of the clathrin coat is to sort proteins into vesicles during receptor-mediated endocytosis and organelle biogenesis. Our work now defines novel functions for

clathrin on both sides of host-pathogen interactions and these new roles are dependent on the clathrin light chain (CLC) subunits. While these subunits contribute to assembly regulation, they also provide a functional link between the clathrin lattice and the actin cytoskeleton. This link occurs via the Hip family proteins (Hip1R, Hip1 or Sla2p in yeast), which bind to CLC and actin. During infection by bacteria that co-opt actin for host uptake or adhesion, clathrin is exploited as a platform for actin organization and this requires CLC and Hip, as well as tyrosine phosphorylation of the CHC. CLC-Hip-actin interactions also contribute to cell migration and this is dramatically illustrated by a lymphocyte migration defect in mice that are genetically deleted for the CLCa isoform. Most tissues express both CLC isoforms, and the CLCb isoform can substitute for many CLCa functions. However, lymphocytes predominantly express CLCa and do not compensate by increased CLCb expression in the CLCa-deleted animals, so their lymphocytes are effectively CLC negative. In these animals, lymphocytes are defective in directional migration towards chemokine signals, demonstrating a major role for clathrin in lymphocyte response to infection. Thus, the clathrin-actin connection contributes to immune defense, as well as pathogen subversion.

W2.06.02
DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses

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To migrate efficiently through the interstitium, dendritic cells (DCs) constantly adapt their shape to the given structure of the extracellular matrix and follow the path of least resistance. It is known that this amoeboid migration of DCs requires Cdc42, yet the upstream regulators critical for localization and activation of Cdc42 remain to be determined. In this study, we show that DOCK8 is a Cdc42-specific guanine nucleotide exchange factor critical for interstitial DC migration. By generating the knockout mice, we found that in the absence of DOCK8, DCs failed to accumulate in the lymph node parenchyma for T-cell priming. Although DOCK8-deficient DCs migrated normally on 2-dimensional surfaces, DOCK8 was required for DCs to crawl within 3-dimensional fibrillar networks and to transmigrate through the subcapsular sinus floor. This function of DOCK8 depended on the DHR-2 domain mediating Cdc42 activation. DOCK8 deficiency did not affect global Cdc42 activity. However, Cdc42 activation at the leading edge membrane was impaired in DOCK8-deficient DCs, resulting in a severe defect in amoeboid polarization and migration. These results indicate that DOCK8 regulates interstitial DC migration by controlling Cdc42 activity spatially.

W2.06.03
All-trans retinoic acid and rapamycin synergize with transforming growth factor-β to induce regulatory T cells but confer distinct in vivo migratory capacities

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Regulatory T cells (Treg) hold great promise for immunoregulation. However, being the frequency of naturally-occurring Treg extremely low, inducing Treg (iTreg) from non-Treg is a viable alternative. We have then compared the functional and migratory properties of iTreg induced in presence of all-trans retinoic acid (RA) or rapamycin (Rapa) - two molecules that synergize with TGF-β to induce stable iTreg.
Addition of RA or Rapa during activation of mouse CD4+CD25- T cells increased the conversion to Foxp3+ cells over TGF-β alone. No significant differences were found in the expression of Treg-associated markers: CTLA-4, CD25, and GITR. All three iTreg types had similar in vitro suppressive capacity. Major differences were evident, however, in the expression of chemokine receptors and integrins: TGFβ-iTreg were CCR9loCD103hi; RA-iTreg were CCR9hiCD103hi, and Rapa-iTreg were CCR7hiCCR9loCD103lo - suggesting different migratory properties. Bioluminescent imaging of adoptively- transferred luciferase-expressing iTreg confirmed that Rapa-iTreg had a greater tendency to accumulate in lymph nodes, while RA-iTreg accumulated outside of lymphoid tissues. In a mouse model of inflammatory bowel disease (based on adoptive transfer of T cells with or without iTreg), Rapa-iTreg exerted the strongest protective effect, correlating with their ability to suppress the activation of colitogenic T cells in mesenteric lymph nodes.
Overall, although RA and Rapa are considered interchangeable in promoting stable iTreg, they confer a different migratory potential that translates in different abilities to control local immune responses. We then envision interventions that employ iTreg "optimized" for the specific type and stage of disease to be treated.

W2.06.04
The transporter Spns2 is required for secretion of lymph but not plasma sphingosine-1-phosphate

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Plasma sphingosine-1-phosphate (S1P) regulates vascular permeability, and plasma and lymph S1P guide lymphocyte egress from lymphoid organs. S1P is made intracellularly, and little is known about how S1P is delivered into circulatory fluids. Here, we find that mice without the major facilitator superfamily transporter Spns2 have a profound reduction in lymph S1P, but only a minor decrease in plasma S1P. Spns2-deficient mice have a redistribution of lymphocytes from the spleen to lymph nodes and a loss of circulating lymphocytes, consistent with normal egress from the spleen directed by plasma S1P and blocked egress from lymph nodes directed by lymph S1P. Spns2 is needed in lymphatic endothelial cells to supply lymph S1P and support lymphocyte circulation. As the first differential requirement for lymph and blood S1P to our knowledge, Spns2 may be an attractive target for immune suppressive drugs.

W2.06.05
D6 is a β-arrestin-biased signaling chemokine scavenger receptor

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The atypical chemokine receptor D6 is a scavenger for inflammatory CC chemokines with a non-redundant role in the control of inflammatory processes. Being unable to sustain cell migration and activate signal transduction pathways triggered by classical chemokine receptors, such as CCR5, D6 is currently classified as a "silent" receptor. Our previous data showed that, differently from CCR5, ligand induces D6 up-regulation on plasma membrane through a rapid mobilization of receptor from recycling endosomes, thus improving its scavenging performance. Here we report that in cells expressing D6 or CCR5, ligand engagement causes a massive actin cytoskeleton reorganization and changes receptors colocalization with actin filaments in a completely opposite fashion. Both receptors signal to actin cytoskeleton by phosphorylating cofilinA

through the Rac1-PAK1-LIMK1-dependent pathway. However, opposite to CCR5, D6 activates this pathway via a beta-arrestin-dependent, G protein-independent mechanism. Inhibition of each component of this signaling cascade completely abrogates D6 adaptive upregulation and scavenging activity. Altogether these results provide the first evidence that D6 scavenging activity relies on unique signaling properties and raise the intriguing possibility to extend our observations to the others members of atypical chemokine receptors subfamily, in order to understand if the beta-arrestin-dependent triggering of Rac1-PAK1-LIMK1-cofilin pathway represents a general signaling feature which specifically defines this particular subfamily and contributes to explain their peculiar activities aimed at chemokine gradient shaping instead of prompt cell migration.

W2.06.06
A mechanism of the skin-infiltration of basophils that is essential for the acquired protective immunity to tick infestation

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Blood-sucking ticks are ectoparasites that transmit a wide variety of microorganisms to humans and animals, leading to serious infectious disorders including Lyme disease. Many animal species show resistance to tick re-infestation, and thereby have less chance of pathogen transmission. We recently reported that basophils are recruited to tick-feeding sites during the 2nd infestation, and that basophil depletion abolishes the manifestation of tick resistance, illustrating a pivotal role for basophils in acquired protective immunity against tick infestation. However, it remains elusive how basophils are recruited to tick-feedingsites during the 2nd but not 1st infestation. We found that IL-3-deficient mice exhibited impairment in protective immunity against ticks and basophil infiltration to tick-feeding sites during the 2nd infestation. While Rag2-/- mice deficient for T and B cells, failed to accumulated basophils at tick-feeding sites during the 2nd infestation, adoptive transfer of T cells isolated from wild-type but not IL-3-deficient mice restored the basophil accumulatiuon. Of note, the-pretreatment with IL-3 but not TSLP conferred the ability to accumulate basophils on Rag2-/- mice even in the 1st infestation, in spite of the absence of T cells. These results suggest that in the 2nd infestation, T cells specific to tick antigen produce IL-3, which in turn promotes basophil recruitment to the tick-feeding sites.

W2.07 Peripheral tolerance and lymphocyte anergy

W2.07.01
Induction of Antigen-Specific Tolerance upon Infusion of Fc-fusion Proteins via The Materno-fetal Interface

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Regulatory T cells that developed *via* central tolerance mechanisms in the thymus play a major role in maintenance of peripheral tolerance. For inducing tolerance to an immunogenic protein therapeutic, we customized the immune repertoire during ontogeny of the immune system by exploiting the physiological process of maternal IgG transcytosis to fetal circulation *via* neonatal FcRn receptor. To this end, mouse Ig-Fc-fusion chimeric molecules were developed using domains of FVIII (A2-Fc, C2-Fc) and hemagglutinin (HA1-Fc), with biochemical and functional characterisation. ELISA and *in vivo* imaging validated the placental transfer of the proteins to the fetuses during gestation. The functional avidity, presentation by thymic APCs, time window and therapeutic dose were determined in HA-TcR transgenic mice, where $HA_{111-119}$ specific T cells can be identified using anti-clonotypic antibody. Intravenous injection of 100µg protein during gestational day 16 to 18 was sufficient to significantly induce HA1-specific Tregs and to delete autoreactive T cells in the progeny. These validated parameters were then applied to a mouse model of hemophilia A (FVIII $^{-/-}$) utilizing A2-Fc, C2-Fc and monoclonal IgG1 as control. The progeny of A2-Fc and C2-Fc treated mothers was challenged with therapeutic doses of the FVIII neo-antigen at 6 weeks of age. The data show an induction of FVIII-tolerance in the progeny with drastic reduction in the levels of total anti-FVIII IgG and abrogation of FVIII-specific T cell proliferation. Our study provides the first proof of concept towards exploiting the materno-fetal interface to shape the immune repertoire and to induce tolerance in allo-immune pathological conditions.

W2.07.02
Ly9 (CD229) cell surface receptor ameliorates autoimmune response

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Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by a major immunological stigmata, the production of antibodies against own cell nuclear components. Genetic predisposition is a central element for the development of SLE. A fine mapping of Sle1 region defined a gene interval of 0.9Mb, termed Sle1b, as the most potent segment implicated in the generation of autoantibodies. Interestingly, this segment includes seven signaling activation molecules family (SLAMF) cell surface receptor genes. Ly9 is a cell surface molecule that belongs to the SLAMF receptors. Here, we use *Ly9*-deficient mice backcrossed to B6 and BALB/c backgrounds to determine the role of Ly9 receptor in autoantibody development. Our findings revealed that the absence of Ly9 receptor *per se* initiates the spontaneous development of autoantibodies. By contrast, a chemically induced lupus model, injecting pristane, showed that both wt and *Ly9*-deficient strains (*BALB/c.129*) developed equally high titers of auto-antibodies. We further demonstrate that *Ly9* gene ablation in *BALB/c* background results in disturbed B and T cell subsets involved in autoimmunity, with major differences observed in Tfh cells and GC B cells. In summary, the data presented in this study shed light on the inhibitory function of Ly9 cell surface receptor, suggesting that this molecule is involved in maintenance of peripheral cell tolerance by serving as a negative regulator of immune response.

W2.07.03
An Agonistic Anti-BTLA mAb (3C10) Induced Generation of IL-10 Dependent Regulatory CD4$^+$ T Cells and Prolongation of Murine Cardiac Allograft

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Background: The co-inhibitory receptor B and T lymphocyte attenuator (BTLA) has been implicated in the regulation of autoimmunity and may potentially play an important role in allograft tolerance. We investigated the effect of an agonistic anti-BTLA mAb (3C10) in the fully MHC-mismatched murine cardiac transplantation.
Methods: CBA mice underwent transplantation of C57BL/6 hearts and received one dose of 3C10 on the day of transplantation (day 0) or four doses of 3C10 on day 0, 3, 6 and 9. Adoptive transfer studies were performed to determine whether regulatory cells were generated. Moreover, to confirm the requirement for regulatory T cell and Th-2 cytokines, we administrated anti-interleukin (IL)-2 receptor

alpha antibody (PC-61) or anti-IL-10 antibody (JES-2A5) to 3C10-treated CBA recipient.
Results: CBA mice treated with one and four doses of 3C10 prolonged allograft survival (median survival times [MSTs], 43 and >100 days, respectively). Secondary CBA recipients given whole splenocytes or $CD4^+$ cells from primary 3C10-treated CBA recipients had significantly prolonged survival of C57BL/6 hearts (MSTs, >100 in both). Also, flow cytometry studies showed an increased $CD4^+CD25^+Foxp3^+$ cell population in 3C10-treated mice. Additionally, IL-2 and interferon-γ production were suppressed in 3C10-treated mice, and IL-4 and IL-10 from 3C10-treated CBA mice increased. Moreover, 3C10 directly suppressed allo-proliferation in a mixed leukocyte culture. However, administration of PC-61 or JES-2A5 clearly attenuated prolonged survival of 3C10-treated mice (MSTs, 15.5 and 13.5 days, respectively).
Conclusion: 3C10 could control acute rejection by its suppressive effect on alloreactive T cells and induction of IL-10 dependent regulatory $CD4^+$ T cells.

W2.07.04
Upregulation of inhibitory molecules in T cells is associated with altered functions of dendritic cells by HIV-1 and activation of the P38MAPK/STAT3 pathway

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HIV-1 reportedly augments the expression of certain negative costimulatory and inhibitory molecules on T cells, leading to immune impairment. The signaling mechanisms underlying the induction of suppressor molecules and subsequent onset of T-cell impairment in HIV infection remain ambiguous. Our experiments with both autologous and allogeneic T cells exposed to HIV-pulsed dendritic cells showed increased expression of LAG-3, TIM-3, CD160 CTLA-4, TRAIL, and certain suppression-associated transcription factors, namely Blimp-1, DTX1 and FoxP3, whose recruitments were closely regulated by P38MAPK/STAT3 signal transduction pathways. Blockade of P38MAPK/STAT3 significantly decreased the expression of the inhibitory molecules studied and significantly restored T-cell proliferation. The P38MAPK/STAT3 proteins had a higher degree of phosphorylation in the HIV-1-primed cells. We also found that IL-6 and IL-10, and certain other growth factors commonly known to activate STAT3 signaling events were not responsible for STAT3 activation. Blockade of viral CD4 binding and fusion with DCs significantly reduced the negative effects DCs imposed on primed T cells. We concluded that HIV-1 negatively modulate DC functions, causing the activation of the P38MAPK/STAT3 pathway in T cells, leading to recruitment of inhibitory molecules and subsequent onset of T-cell impairment.

W2.07.05
Interleukin 15 reverses age-related CD8+ T cell inactivation with increasing interferon-gamma production and improves survival in aged septic mice

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Background: Aging plays an important role in sepsis, a systemic inflammatory response to infection with immunosuppression. Elderly patients with sepsis show reduced immunocompetent T cells. Interleukin 15 (IL-15) is a pluripotent antiapoptotic cytokine that promotes lymphocytes activation and proliferation. We examined whether IL-15 increases T-cell activation and improves survival of aged septic mice. Method: In vitro study Splenocytes from young (6-8 weeks) and aged (20-22 months) C57/B6 mice were stimulated overnight using an anti-CD3 antibody with/without recombinant mouse IL-15. We performed flow cytometric analysis for T]-cell activation and measured interferon-gamma (IFN-γ) levels in the supernatants. In vivo study Aged mice underwent cecal ligation puncture (CLP) or sham treatment. We subcutaneously injected 1.5 μg IL-15 or phosphate-buffered saline at 3 h after CLP. Mice were killed at 24 h after CLP for peritoneal lavage and blood collection. We measured cytokine levels, counted bacterial colonies in the peritoneal cavity, and performed a 7-day survival test. Results: In vitro stimulation of splenocytes showed that compared to young mice, aged mice showed impaired T-cell activation (68% reduction in both CD4+ and CD8+ T cells, $p < 0.01$). IL-15 reversed this impaired CD8+T cells l activation in aged mice and increased IFN-γ levels dose dependently. Furthermore, IL-15 increased bacterial clearance and IFN-γ levels in the peritoneal cavity in vivo. IL-15 also improved the 7-day survival after sepsis in aged mice (67% vs 0%, $p = 0.015$). Conclusion: IL-15 reverses age-related T-cell inactivation with increasing IFN-γ production and improves survival in aged septic mice.

W2.07.06
Upregulation of glucocorticoid-induced leucine zipper by hepatocyte growth factor promotes tolerogenic dendritic cells and inhibits experimental autoimmune encephalomyelitis

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Objective: To unravel the immunomodulatory mode of actions of hepatocyte growth factor (HGF) in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS).
Background: We previously showed that CNS-restricted expression of HGF, a factor known for both neuronal and oligodendrocytic protective properties, decreased EAE immunopathogenesis by promoting tolerogenic dendritic cells (tDCs) (Benkhoucha M. et al., *PNAS*, 2010). Expression of glucocorticoid-induced leucine zipper (GILZ), a transcriptional regulator, was reported to correlate with the regulatory activity of tDCs, conferring a possible mechanism by which HGF could exert regulatory activities on DCs.
Design/Methods: Systemic HGF (50 μg) treatment was applied s.c. two days before EAE induction (myelin oligodendrocyte glycoprotein p35-55). DC functions were evaluated *ex vivo*. Using DCs in which GILZ expression was selectively suppressed, we assessed the ability of HGF to induce tDCs.
Results: Mice treated with HGF developed reduced disease and had in the periphery decreased frequencies of both Th1 and Th17 cells but increased proportion of Tregs. DCs from HGF-treated mice produced high levels of GILZ. RNAi-mediated knock-down approach demonstrated that GILZ is critical for HGF in driving tDC differentiation. Using adoptive transfer experiments, we established that HGF treatment of DCs from wild-type, but not from GILZ knock-out mice, conferred significant protection to recipient mice with established EAE.
Conclusion/Relevance: These results indentify GILZ as a critical factor for effective suppression of T-cell-mediated CNS inflammation by HGF via the induction of tDCs, a mechanism that may be exploited for therapeutic benefit in pathology of autoimmune diseases such as MS.

W2.08 Antigen receptor signaling

IL2.08.01
Novel assignments for the CD43 molecule

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CD43 is the most abundant costimulatory molecule on the T cell surface; it transduces activation signals through its cytoplasmic domain, modulating the outcome of T cell responses. CD43 signals lower the threshold for TCR-dependent activation by controlling different regulatory loops. Particularly, CD43 engagement restricts the c-Cbl and Cbl-b inhibitory effects on TCR signaling as well as the participation of SHP-1, ultimately increasing the intensity and the duration of downstream signals. Recently, we undertook a series of projects aimed at identifying new signaling pathways and novel functions modulated by CD43. The analysis of protein expression profiles of normal human CD4+ T cells stimulated through TCR and CD43 uncovered changes in proteins involved in a variety of biological processes such as cell migration, cell signaling, apoptosis, cell cycle, gene expression and metabolism, with glucose metabolic processes significantly represented. Data will be shown supporting the participation of CD43 in glucose metabolism, as well as in activating moonlight functions of pyruvate kinase PKM2. In addition, we will show how a predictive analysis of the transcription factors (TFs) that would regulate the promoters of a panel of 42 cytokines, chemokines, and growth factors [the expression of which is upregulated in response to the combined signals of CD43 and the TCR] led us to identify a group of TFs as “global” regulators. Thus, in addition to strengthen TCR signals, CD43 modulates gene transcription and metabolism, two important processes for a cell to decide whether pursuing a differentiation program is wise.

W2.08.01
Antigen-specific TCR-pMHC catch bond enables force to trigger T cell signaling

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T cell receptor (TCR) binding to the peptide-major histocompatibility complex (pMHC) initiates adaptive immune responses. Mechanical force may be applied to TCR-pMHC bonds, thereby triggering T-cell signaling. However, it is not known how force regulates the stability of TCR-pMHC bonds and how such regulation impacts T cell triggering. Using a single-bond mechanical assay, here we show that force unexpectedly stabilizes TCR-pMHC interaction via an agonist-specific catch bond, where bond lifetime is prolonged by force. Concurrent Ca^{2+} imaging and binding measurement demonstrates that a 10-piconewton durable force applied via agonist pMHC to a TCR is sufficient and necessary to trigger intracellular Ca^{2+} flux. Single cell analysis revealed that Ca^{2+} signaling strength best correlates with total bond lifetime accumulated in the first minute during repeated force application through TCR-pMHC bond. For T cells with similar cumulative bond lifetimes, a greater number of short lifetimes corresponds to a lower level of Ca^{2+} flux. Therefore, our data support a T cell triggering model, where a mechanical force applied to the TCR delivers either an activating or inhibitory signal depending on the bond lifetime, such that agonists form catch bonds with the TCR to rapidly accumulate long lifetimes to activate the T cell, whereas antagonists form short-lived slip bonds that attenuate activation.

W2.08.02
Regulation of the Ras-Erk cascade during T-cell activation

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The duration and/or the magnitude of Erk activation are known to regulate cell-fate decisions. In lymphoid cell lines, the Ras-Erk cascade is activated by the action of two GEFs, the ubiquitously expressed Sos1 and the lymphoid-specific RasGRP1. Here, we have assessed how Ras-Erk is activated upon TCR triggering in primary human T cells. We used two different stimuli, CD3 mAbs cross-linked in solution (sAbs) or immobilized on microbeads (iAbs). sAbs induce a transient Erk signaling and an anergic-like, unresponsive state, whereas iAbs induce sustained Erk activation and T-cell proliferation. In order to evaluate the role of Sos1 and RasGRP1 in transient vs. sustained Erk activation, we suppressed Sos1 or RasGRP1 expression using siRNA. Surprisingly, we have found that transient Erk activation does not depend on Sos1 but requires RasGRP1. Conversely, sustained Erk signaling depends on both Sos1 and RasGRP1. In summary, our data show that the two GEFs expressed in T cells are differentially involved in the regulation of the duration of Erk activation and in the generation of T-cell responses (the work was supported by DFG SFB854 and GRK1167).

W2.08.03
Kidins220/ARMS associates with B-Raf and the TCR promoting sustained Erk signaling in T cells

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The activation kinetics of the mitogen activated protein kinase (MAPK) Erk is critical for T cell activation and development. In particular, sustained Erk signalling is required for T-cell activation, such as IL-2 production. While Raf-1 triggers transient Erk activation, B-Raf is implicated in sustained Erk signaling after TCR stimulation. However, how B-Raf activation is coupled to the TCR in unknown. Using mass spectrometry, we identified Kidins220/ARMS as novel B-Raf interaction partners. Kidins220, a protein so far studied only in neuronal and melanoma cells, was associated with the pre-TCR, αβTCR and γδTCR. In mature T cells, Kidins220 is required for TCR-induced sustained, but not transient, Erk activation. Consequently, expression of the transcription factors c-Fos and Erg-1 was impaired and upregulation of the activation markers CD69, IL-2 and IFN-γ was reduced, as we reported recently (1).
Next, we want to investigate the in vivo function of Kidins220 in T cell development. We generated chimeric mice in which a T cell-compromised host was reconstituted with fetal liver-derived cells from the embryonic lethal Kidins220-deficient mice. Thymocytes derived from Kidins220-/- cells exhibited a block at the double-positive stage. Moreover, the transition to CD4+ single-positive thymocytes, where sustained ERK is required, was drastically reduced.
In conclusion, we describe Kidins220 as a novel TCR-interacting protein that couples B-Raf to the TCR. Kidins220 is mandatory for sustained Erk signaling and thus, it is crucial for TCR-mediated T cell activation and development.
(1) Deswal et al., J Immunol., 2013 Jan. 28

W2.08.04
Dual-specificity phosphatase 14 negatively regulates TAK1 signaling and immune responses

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Dual-specificity phosphatase 14 (DUSP14, also named MKP-6) is a general mitogen-activated protein (MAP) kinase (MAPK) phosphatase *in vitro*. Here we show that overexpression of DUSP14, but not a phosphatase-dead DUSP14 mutant, reduced T-cell receptor (TCR) induced TAK1, JNK and IKKα/β activation; knockdown of DUSP14 by RNA interference enhanced these activations. To elucidate the *in vivo* function of DUSP14, we generated DUSP14-deficient mice. The subsets of thymic, spleen and lymph node T cells in DUSP14-deficient mice were indistinguishable from those in wild-type mice. DUSP14-deficient T cells displayed increased T-cell proliferation, enhanced cytokine production, and enhanced up-regulation of the activation markers upon anti-CD3 antibody stimulation. DUSP14 inactivated TAK1, leading to attenuation of IKK and JNK activation. Also, DUSP14-deficient mice exhibited enhanced *in vivo* T cell-mediated immunity and were more susceptible to experimental autoimmune encephalomyelitis (EAE) induction. The molecular mechanism of DUSP14 modification in TCR signaling will also be discussed. Taken together, our results indicate that DUSP14 is a negative regulator of TAK1 signaling and immune responses. (Supported by NHRI-98A1-IMPP01-014)

W2.08.05
Lysophosphatidic acid (LPA) receptor 5 inhibits B cell antigen receptor signaling and antibody response

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The development, trafficking and function of lymphocytes is dependent on signaling by sphingosine-1-phosphate G-protein coupled receptors (GPCR). Lysophosphatidic acid (LPA) is another major lysophospholipid found systemically that also signals via distinct GPCR expressed by B and T lymphocytes. Notably, LPA levels are found to be significantly elevated in certain pathological settings such as certain cancers and infections. We have found that BCR signal transduction by mature B cells is inhibited upon LPA engagement of the LPA5 receptor via a Gα12/13 - Arhgef1 signaling axis. The inhibition of BCR signaling by LPA5 manifests by impaired intracellular calcium store release and most likely by interfering with inositol 1,4,5-trisphosphate receptor activity. We further show that LPA5 also limits antigen-specific induction of CD69 and CD86 expression and that LPA5-deficient B cells display enhanced antibody responses. Thus, these data show that LPA engagement of LPA5 negatively regulates BCR signaling, B cell activation and immune response. These findings extend the influence of lysophospholipids on immune function beyond directing lymphocyte trafficking and suggest that alterations in LPA levels as found in certain pathological conditions likely influence adaptive humoral immunity.

W2.08.06
Negative regulation of chemokine receptor signaling and B cell chemotaxis by p66Shc

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Shc adaptors are ubiquitous components of the signaling pathways triggered by tyrosine kinase-coupled receptors. In lymphocytes, similar to other cell types, the p52 and p66 isoforms of ShcA/Shc participate in a self-limiting loop where p52Shc acts as a positive regulator of antigen receptor signaling by promoting Ras activation, while p66Shc limits this activity by competitively inhibiting p52Shc. Based on the fact that many signaling mediators are shared by antigen and chemokine receptors, including p52Shc, we have assessed the potential implication of p66Shc in the regulation of B cell responses to chemokines, focusing on the homing receptors CXCR4 and CXCR5. The results identify p66Shc as a negative regulator of the chemotactic responses triggered by these receptors, including adhesion, polarization and migration. We also provide evidence that this function is dependent on the ability of p66Shc to interact with the chemokine receptors and promote the assembly of an inhibitory complex that results in impaired Vav-dependent reorganization of the actin cytoskeleton, and map this function to the phosphorylatable tyrosine residues in the CH1 domain. The results identify p66Shc as a negative regulator of B cell chemotaxis and suggest a role for this adaptor in the control of B cell homing.

W2.09 MHC and other polymorphic genes in health and disease

IL2.09.01
MHC and other polymorphic genes in health and disease

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The Major Histocompatibility complex (MHC) is critical in the regulation of immune responses. This gene complex comprises about 4 Mbp in human and mouse. The MHC encodes genes that are critical to the generation and display of antigenic peptides on the cell surface for recognition by T cells. These include proteins that bind peptides. The are the class I genes that usually bind endogenously synthesized peptides and class II the bind exogenously derived peptides. The also include proteins in the immunoproteosome, LMP and in the transport pathway. In addition important innate effectors also map in the MHC. Finally MHC related proteins such as CD1 are also important in immune regulation. Because of its critical role in the presentation of antigen it impacts how the immune responses recognizes pathogens and how the pathogens can escape.
In the session here we have outstanding presentation that demonstrate the interplay between host immunity and pathogen immune evasion as well as the interaction between pathogens and the induction of autoimmunity.

W2.09.01
Tapasin facilitation of MHC-I separates closely related allomorphs, is strongly influenced by peptide length and depends on stability

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Only a small fraction of the peptides inside a cell are eventually presented by HLA-I on the cell surface. The presented peptides have HLA-I allomorph-specific motifs and length restrictions. Tapasin influences HLA-I antigen presentation both qualitatively and quantitatively to different degrees depending on both peptide sequence and HLA-I allomorph. The tapasin-dependence in cellular context has been shown to correspond to the facilitation of peptide-HLA-I complex formation by the first 87 amino acids of tapasin (Tpn1-87) (i.e., tapasin-facilitation = Bmax Tpn1-87/Bmax Ctrl) in a biochemical assay. Both peptide length and tapasin-facilitation are important for HLA-I antigen presentation and we here set out to study if these two parameters relate to each other. We used a luminescent oxygen channeling assay and seven different peptide libraries (X7-X13) to study 16 HLA-A and -B allomorphs and the results show a broad spectrum of tapasin-facilitation of HLA-I allomorphs and that HLA-A allomorphs were generally less restricted than -B allomorphs

to peptides of the classical lengths of 8-10 amino acids. Since both stability and tapasin-facilitation have been suggested as discriminators of immunogenic peptides we used a scintillation proximity based assay to study the stability of peptide-HLA-I complexes formed with peptides of different lengths. The results demonstrate an inverse correlation between tapasin-facilitation and stability valid for different peptide mixes of specific lengths but also on the level of HLA-I allomorphs, suggesting that molecules of poor stability are either not in a conformation that allows tapasin to interact or have a conformation where association has no effect.

W2.09.02
NLRC5 controls basal MHC class I gene expression in an MHC-enhanceosome dependent manner

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Nuclear binding domain, leucine rich repeat containing proteins (NLRs)play important roles in innate immune responses as pattern recognition receptors (PRRs). While most NLRs act in cell autonomous immune pathways, some do not function as classical PRRs. One such NLR is the MHC class II transactivator (CIITA), the master regulator of MHC class II gene transcription. Here we report that human NLRC5, which we recently showed to be involved in viral-mediated type I interferon responses, shuttles to the nucleus and activates MHC class I gene expression. Knock-down of NLRC5 in different human cell lines and primary dermal fibroblasts leads to reduced MHC class I expression, while introduction of NLRC5 into cell types with very low expression of MHC class I augments MHC class I expression to levels comparable to those found in lymphocytes. Expression of NLRC5 positively correlates with MHC class I expression in human tissues. Functionally, we show that both the N-terminal effector domain of NLRC5 and its C-terminal LRR (leucine rich repeat) domain are needed for activation of MHC class I expression. Moreover, nuclear shuttling and function depends on a functional Walker A motif. Finally, we identified a promoter sequence in the MHC class I promoter, the X1-box, to be involved in NLRC5-mediated MHC class I gene activation. Taken together this suggests that NLRC5 acts in a manner similar to CIITA to drive MHC expression and revealed NLRC5 as an important regulator of basal MHC class I expression.

W2.09.03
TAPBPR uses the same residues as tapasin to associate with MHC class I

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In order to induce an appropriate T cell response, MHC class I molecules must be loaded with high affinity peptide. The key player for peptide loading is the MHC class I dedicated chaperone tapasin. Recently we have found a tapasin related protein called TAPBPR which is an additional component of the antigen presentation system. Like tapasin, TAPBPR binds to a heterodimer of MHC class I heavy chain and β2m. In contrast to tapasin, TAPBPR is not a component of the peptide loading complex and is not required for peptide loading. However, it controls the ER export rate of MHC class I molecules.
Here we have characterised the interaction between TAPBPR and MHC class I. Residues in the N-terminal and IgC domain of tapasin which are important for its association with MHC class I are highly conserved in TAPBPR. Site-directed mutagenesis of these residues followed by immunoprecipitation of TAPBPR revealed that mutation of I282 or E226, R228, Q230, Q293 in the N-terminal domain and R335, Q336 and S337 in the IgC domain abolishes binding of TAPBPR to MHC class I. Tapasin is known to associate with specific residues on MHC class I such as T134, D227 and E229. We have identified that these residues are also essential for TAPBPR binding to MHC class I. The identification of TAPBPR as an additional MHC class I specific chaperon which binds to MHC class I in a similar way as tapasin has significant implications on our understanding of MHC class I biology.

W2.09.04
Usage of a genome-wide shRNA-based lentiviral screen and targeted siRNA knockdown to identify host cell proteins involved in US2- and US11-mediated degradation of major histocompatibility complex class I molecules

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Human cytomegalovirus (HCMV) utilizes multiple mechanisms to disrupt peptide presentation by major histocompatibility complex class I molecules (MHC I). US2 and US11 are two HCMV proteins that target MHC I for degradation through ER-associated degradation (ERAD), each dependent on different cellular factors to carry out this activity. To identify host proteins involved in US11 function, a genome-wide shRNA screen was used to identify knockdown targets that increased surface MHC I levels (as shown by fluorescence-assisted cell sorting) in cells expressing US11. Hits identified in the screen were filtered using gene ontology (GO) term associations, replication by independent shRNAs, and statistical approaches. Subsequent validation of hits by flow cytometry and western blotting identified new potential players in ERAD, and linked MHC I to these as an ERAD substrate. In addition to this screen-based approach, individual targets of interest were investigated. This led to the identification of cyclophilin C (cypC) as an ER-localized peptidyl-prolyl cis-trans isomerase involved in US2 mediated degradation of MHC I. Knockdown of cypC increased both surface and total expression of MHC I in cells expressing US2. This effect was not due to a general ER unfolded protein response (as measured by XBP-1 splicing), but more likely a direct effect on US2 itself, since an interaction between cypC and US2 could be detected. Our results suggest that cypC is involved early in US2 mediated-degradation of MHC I, and may act to bridge US2 to other proteins of the ERAD pathway.

W2.09.05
Comparative analysis of DQA1*05/DQB1*02 mRNAs and DQ2 heterodimer expression in relation to antigen presentation

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HLA is the main genetic risk factor in autoimmunity. The DQ2 heterodimer, coded by DQA*05/DQB*02 alleles, and located in cis conformation in DR3-DQ2 haplotype, restrict T cell response to gluten peptides in the great majority of celiac disease-(CD) patients. We have investigated the correlation between DQA1*05 and DQB1*02 mRNAs and the surface expression of DQ2 in respect to the ability to present immunodominant gluten peptides to intestinal T cells from CD patients carrying the DR3-DQ2 haplotype in homozygousity or in heterozygousity. We first evaluated DQA*05 and DQB1*02 mRNAs amount by RT-qPCR in EBV-transformed lymphoblastoid B cells (EBV-B), obtained by CD patients either DR3/DR3 or DR3/DR1 positive. Then, the surface expression of DQA1*05 and DQA1*01 chains was analysed by specific antibodies and flow cytometry. Our data showed that mRNAs of these alleles are more abundant compared to DQA1*01 and DQB1*05. Furthermore, flow cytometry analysis confirmed a greater surface expression of DQA1*05 respect to DQA1*01 chain, in agreement with RT-qPCR data. Preliminary results showed no statistical differences in the T cell activation, measured as IFNγ production, when DR3/3, DR3/5, and DR3/1 positive EBV-B were used to present suboptimal amount of DQ2-glia-γ1 peptide to celiac T cells, thus contrasting the expected T cell activation on the base of an unbiased combination of the alfa and beta chains in the APC, i.e: DR3/3 (100% DQ2), DR3/5 (50% DQ2) DR3/1 (25% DQ2), and DR1/1 (0% DQ2). Our results provide a molecular basis for the high penetrance of DR3-DQ2 haplotype in autoimmune diseases.

W2.09.06
Novel HLA-B27-restricted epitopes from Chlamydia trachomatis suggest a role of molecular mimicry in reactive arthritis

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The spondyloarthropathies are a group of rheumatic diseases strongly associated with HLA-B27, which include ankylosing spondylitis (AS) and reactive arthritis (ReA). This latter disease can be triggered by diverse bacteria, *Chlamydia trachomatis* being among the most prominent ones. However, its pathogenetic significance, autoimmune potential, and relevant epitopes are unknown. The purpose of this study was to directly identify *Chlamydia*-derived HLA-B27 ligands processed and presented *in vivo*, and to examine their potential as mediators of molecular mimicry. A methodology is described for studying the endogenous processing and presentation of chlamydial epitopes, which includes stable transfection of bacterial proteins and purification of the peptide-MHC complexes from HLA-B27-positive cells, followed by high-throughput comparative and targeted peptide sequencing for detecting specific bacterial ligands in the HLA-B27 peptidome. The use of mass spectrometry techniques with high resolution and sensitivity allowed us to detect peptides derived from the bacterial ClpC (CT286), NQRA (CT634) and DNA primase (C794) proteins, including some recognized by CTL of ReA patients. These peptides showed high homology to human protein sequences fulfilling the HLA-B27 binding motif. The DNAP(211-223) peptide was shown, through molecular dynamics simulation, to adopt a striking conformational similarity with a self-derived and highly flexible peptide, B27(309-320). The use of high-resolution and sensitivity MS techniques provided a major improvement in the detection of *Chlamydia*-derived antigens and shed new light about the pathogenetic role of molecular mimicry in this disease.

IL2.10.02
Regulation of inflammation and cell-death trough interactions of RHIM-domain protein kinases with caspase-8

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Caspase-8 was discovered as the proximal enzyme in the 'extrinsic cell-death pathway'- one of the main mechanisms by which immune killer cells impose apoptotic cell death. This pathway is restricted by signaling for activation of NF-κB - transcription factors that also control inflammatory genes. Cell vulnerability to death induction by the extrinsic pathway is therefore likely to decrease in association with induction of inflammation. Conversely, once the extrinsic death pathway does become activated various proteins that signal for NF-κB activation are cleaved and thus inactivated, resulting in arrest of induction of inflammation. The apoptotic process itself restricts inflammation by safeguarding against release of damage-associated molecular patterns from the dying cell and by immunosuppressive effects of apoptotic cell membrane structures. Studies of recent years revealed that caspase-8 also blocks inflammation by additional mechanisms. One common denominator to the signaling complexes mediating the induction of those mechanisms is that they all encompass the RHIM-domain containing protein kinase RIPK1. Some also contain the RHIM-domain kinase RIPK3. In all these complexes, the inhibitory effect of caspase-8 is associated with proteolytic cleavage of these kinases, which arrests signaling by these complexes and yields proteolytic fragments of the kinases that may act as signaling inhibitors. Consistent with these functions of caspase-8, in several transgenic mice models its deficiency was found to prompt severe inflammation. Recent advances in elucidating the mechanisms for the anti-inflammatory function of caspase-8 will be presented.

W2.10 Autophagy and cell death in the immune system

IL2.10.01
Understanding symptomatic infections in order to avoid initiation of autoimmunity

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Primary infection with the human herpesvirus Epstein Barr virus (EBV) can result in infectious mononucleosis, a self-limiting disease caused by massive lymphocyte expansion, which predisposes for the development of multiple sclerosis. It remains unclear why some individuals experience this symptomatic primary EBV infection, while the majority acquires the virus asymptomatically. Using a mouse model with reconstituted human immune system components, we could demonstrate that depletion of human natural killer (NK) cells enhances symptoms of infectious mononucleosis, mainly due to loss of immune control over lytic EBV infection. Particularly, an early NK cell differentiation stage expanded after EBV infection and loss of this particular NK cell subset increased viral load and CD8+ T cell expansion after infection with wild-type EBV, but not a recombinant virus, deficient in lytic replication. These data suggest that failure of innate immune control by human NK cells augments symptomatic lytic EBV infection, which drives lymphocyte expansion and features of infectious mononucleosis. Therefore, older EBV negative individuals with decreased NK cell reactivity against lytic EBV replication could benefit from vaccination to decrease their risk for infectious mononucleosis and the associated development of multiple sclerosis.

W2.10.01
Human and mouse mononuclear phagocytes are the only leukocyte subset expressing functional death receptors for Trail

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It is believed that TRAIL-induced apoptosis spares normal cells and selectively kills neoplastic cells. In a careful analysis of TRAIL-R expression in leukocyte subsets we found that monocytes express discrete levels of signaling TRAIL-Rs (DR4 and DR5) and lack the non-signaling decoy receptor DcR1. In contrast neutrophils and lymphocytes predominantly express the decoy receptor and have very low levels of signaling receptors. Accordingly, TRAIL-ligand induced the activation of caspase 8 exclusively in monocytes.
Macrophages differentiated in vitro with M-CSF, and in particular M2-polarized macrophages, were also confirmed to express functional TRAILRs. The expression of TRAIL receptors is modulated in monocytes stimulated with selected pro or anti-inflammatory stimuli.
Similar findings were observed in mouse mononuclear phagocytes, where inflammatory Ly6Chigh blood monocytes were found to express high DR5, and neutrophils and lymphocytes had low or negative expression. In murine fibrosarcoma, DR5 expression co-localized with F4/80+ macrophages and not with Gr1+ MDSC or CD31+ vessels. These results imply that monocytes and macrophages are selectively susceptible to this killing mechanism. In several tumor types, the presence of Tumor-Associated Macrophages (TAM) correlates with poor clinical outcome and resistance to conventional treatment and targeting of tumor macrophages is considered a promising therapeutic strategy. The differential susceptibility of mononuclear phagocytes to TRAIL-induced apoptosis could be exploited to selectively target monocytes/macrophages in the tumor micro-environment.

W2.10.02
Target cell death is necessary for cytotoxic T cell detachment and serial killing

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Cytotoxic CD8+ T lymphocytes (CTL) play a critical role in the cell-mediated immune response against virus-infected and cancerous cells. Following specific target cell (TC) recognition and formation of an immune synapse, CTLs release perforin and granzymes and the TC is destroyed through these synergistic actions. CTL can sequentially kill several TCs, a process that requires the periodic breaking of synapses. We can now visualise a functional synapse and delivery of perforin in real time, using live cell microscopy. For example, calcium signalling within the CTL and analysis of the subsequent kinetics and morphological changes associated with TC death, allows us to define the lethal hit for the first time. We demonstrate that upon delivery of the lethal hit, TC death is necessary for CTL to detachment. Therefore, the act of degranulation itself is not sufficient for a synapse breaking. Synapses formed with CTL from genetic-modified mice deficient in either perforin or both granzymes A and B, showed sustained calcium signalling and a striking delay in target cell detachment. One important consequence is elevated cytokine production by perforin-null CTL; this may serve as a model for the clinical manifestation of perforin deficiency in human patients with FHL2, who succumb to a 'cytokine storm' following vial challenge. We conclude that CTL/TC detachment is a regulated process and is dependent on feedback from the TC following effector molecule delivery. This study highlights the importance of a CTL rapidly detaching to facilitate further rounds of killing, and controlling an efficient and balanced immune response.

W2.10.03
The switch in T cell survival: Cross-regulation between homeostasis and antigen-induced activation

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The survival of mature T cells in the periphery is a tightly regulated process governed by two major processes: homeostasis of naïve T cells mediated by IL-7 and antigen-induced activation. How the transition between these two states is controlled is not well understood. Here we investigated the events controlling survival of T cells shortly after antigen activation before cells enter the proliferative state. We found that T cell survival is reprogrammed upon TCR-mediated stimulation by actively inhibiting homeostatic survival signals while initiating a new, dominant survival program. Activation through the TCR antagonised IL-7/IL-7R mediated STAT5 phosphorylation and Bcl-2 expression and induced pro-apoptotic molecules Bim and Bax, while simultaneously promoting survival through induction of alternative anti-apoptotic Bcl-2 family members A1 and Bcl-xL. Stimulation strength determined the kinetics of the transition between the survival programs. Calcineurin or MEK pathway inhibitors prevent the initiation of the new survival program while permitting the dominant repression of Bcl-2. Thus, in the presence of these drugs the response to antigen receptor ligation is cell death. Using calcineurin inhibitors Cyclosporin A or FK506 we were able to induce antigen specific loss of activated T cells in-vitro and in-vivo.Our results identify a molecular switch that can serve as an attractive target for inducing antigen-specific tolerance in treating autoimmune disease patients and transplant recipients.

W2.10.04
IFN-γ induces an autophagy-mediated antimicrobial response against Mycobacterium leprae in human monocytes

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Leprosy is a chronic infectious disease caused by the intracellular pathogen *Mycobacterium leprae* (ML). Previous data suggest that the establishment of different clinical forms is driven by host innate mechanisms. While in multibacillary (LL) patients there are highly infected macrophages, in paucibacillary (BT) rare or few bacilli are found. In the present study we evaluated the role of autophagy in the immune response to ML. Ultrastructural analysis showed a higher number of autophagosomes in isolated skin lesion cells from BT patients compared to LL patients. The increase of LC3-II expression observed in skin biopsies from BT patients was associated with higher gene expression of cathelicidin and β-defensin 2. Previous studies have demonstrated that IFN-γ induces autophagy in human monocytes and that the levels of IFN-γ were significantly raised in BT patients when compared to LL. Here, we demonstrated that IFN-γ treatment in ML-stimulated THP-1 cells decreased the interaction of the bacilli with the host cell and increased LC3-II and Atg3 expression. There was an increase on LC3-II expression in BT macrophages when compared to LL, in the presence or absence of IFN-γ. IFN-γ treatment promotes ML/LC3-II co-localization in THP-1 macrophages and leads to increased levels of IL-15 in the culture supernatants. The pre-treatment with wortmannin or 3-MA was able to reduce IFN-γ-induced LC3-II expression and led to decreased IL-15 levels after stimulation with IFN-γ and ML. These data indicate that IFN-γ induces IL-15 in ML-stimulated macrophages, which contributes to increase the microbicidal activity in host cells by autophagy induction.

W2.10.05
p53 contributes to T cell homeostasis through the induction of pro-apoptotic SAP

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Lack of functional SAP protein, due to gene deletion or mutation, is the cause of X-linked lymphoproliferative disease (XLP), characterized by functionally impaired T and NK cells and a high risk of lymphoma development. We have demonstrated earlier that SAP has a pro-apoptotic function in T and B cells. Deficiency of this function might contribute to the pathogenesis of XLP. We have also shown that SAP is a target of p53 in B cell lines. In the present study, we show that activated primary T cells express p53, which induces SAP expression. p53 is functional as a transcription factor in activated T cells and induces the expression of p21, PUMA and MDM2. PARP cleavage in the late phase of activation indicates that T cells expressing high levels of SAP undergo apoptosis. Modifying p53 levels using Nutlin-3, which specifically dissociates the MDM2-p53 interaction, was sufficient to upregulate SAP expression, indicating that SAP is a target of p53 in T cells. We also demonstrated p53's role as a transcription factor for SAP in activated T cells by ChIP assays. Our result suggests that p53 contributes to T cell homeostasis through the induction of the pro-apoptotic SAP. The involvement of p53 and SAP in activation induced T cell death (AICD) is poorly understood. High levels of p53 and SAP is necessary for the activation-induced cell death that is pivotal in termination of the T cell response.

W2.10.06
Autophagy facilitates TLR4,3-triggered migration and invasion of lung cancer cells by promoting chemokine and IL-6 production through maintaining TRAF6 ubiquitination

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Toll-like receptors (TLRs) expressed in cancer cells have been shown to function in tumor progression and immune escape. Autophagy is also known to exert effects on cancer cell survival and development under stress condition. However, the link between TLR signaling and autophagy as well as its role in tumor invasion remain largely undefined. Here we report that TLR4 and TLR3 both induced autophagy through TRIF adaptor, which promote migration and invasion of lung cancer cells by maintaining TLR4,3-triggered TRAF6

ubiquitination and TAK1 activation. Autophagy appeared essential for TLR4,3-induced production of chemokines and immunosuppressive factors including MCP-1, MIP-3α, IL-6, VEGF and MMP-2, in that inhibition of autophagy abolished release of these cytokines and the increased migration and invasion of lung cancer cells which mostly related to roles of these cytokines. Together with that IL-6 blockade significantly decreased the TLR4,3-induced release of VEGF and MMP-2, this seemed that IL-6 production was critical for enhanced invasion of lung cancer cells triggered by TLR4,3-induced autophagy. Strikingly, autophagy maintained TLR4, 3-triggered TRAF6 ubiquitination and TAK1 activation, which resulted in the enhanced activation of MAPK and NF-κB signaling, and inhibitor of MAPK and NF-κB signaling reversed the increased production of IL-6. The effect of autophagy on TLR4, 3-triggered activation of MAPK and NF-κB signaling were confirmed with siRNA knockdown of Atg5. Therefore, our study demonstrates that autophagy-dependent production of chemokines and IL-6 as a consequence of TRAF6 ubiquitination and downstream signaling activation are essential for TLR-triggered migration and invasion in lung
cancer cells.

W2.12 Signalling in immune cells

IL2.12.01
T-cell signaling via adaptor regulation of the nuclear pore complex (NPC)

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T cell activation is mediated by a complex array of intracellular signaling events that lead to transcription and effector functions. In addition to the CD4- and CD8-p56lck complexes and their initiation of the tyrosine phosphorylation cascade in T-cells involving ZAP-70, recent studies have identified adaptor proteins that integrate responses. Several of these such as LAT (Linker for the activation of T-cells) and SLP-76 (SH2 domain containing leukocyte protein of 76kDa) regulate calcium mobilization, while others such as ADAP (adhesion- and degranulation-promoting adapter protein) and SKAP1 (Src kinase-associated phosphoprotein 1) account of integrin adhesion of T-cells. SKAP1 binds to the Rap1 binding protein to ensure membrane localization and interaction with LFA-1 cytoplasmic tail. This pathway regulates both the activation of integrins as well as the 'stop' signal needed for T-cell interaction with dendritic cells. The talk will outline recent new findings on the role of adaptors in motility as well as in the modulation of nuclear pore complex (NPC) mediated transport by binding and regulation of exchange factor RanGAP.

W2.12.01
GRAIL targets CDC37 to maintain CD4 T cell unresponsiveness

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GRAIL (gene related to anergy in lymphocytes, also known as RNF128), an ubiquitin-protein ligase (E3), plays a central role in CD4 T cell unresponsiveness. We recently demonstrated that GRAIL is expressed in resting CD4 T cells as well as in anergic CD4 T cells suggesting that GRAIL might hold CD4 T cells in cell cycle arrest. Applying a novel E3 substrate screen we developed, we identified cdc37 (a G1-specific cyclin) as a potential target of GRAIL. Specifically, CDC37 can be ubiquitinated by GRAIL. CDC37 interacts with GRAIL in a co-immunoprecipitation assay and confocal microscopy shows both proteins co-localize predominantly in Rab7+ endosomes. Additional studies demonstrate that induced GRAIL expression leads to diminished CDC7 expression. Inversely, primary T cells lacking GRAIL have higher levels of CDC37 compared to wildtype T cells under resting as well as with TCR stimulation. These data together suggest that GRAIL ubiquinates and targets CDC37 for degradation and thus, maintains CD4 T cells in G1/S interphase arrest. This is consistent with published data demonstrating that GRAIL knock out T cells are hyperproliferative compared to wild-type T cells upon TCR engagement. Results from these studies highlight a role for CDC37 in GRAIL-mediated cell cycle regulation and CD4 T cell proliferation.

W2.12.02
TRAF6 regulates TCR signaling via interaction with and modification of LAT adapter

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TNF receptor-associated factor 6 (TRAF6) is an essential ubiquitin E3 ligase in immune responses, but its function in adaptive immunity is not well understood. Here we show that TRAF6 is recruited to the peripheral ring of the T cell immunological synapse in Jurkat T cells or human primary $CD4^+$ T cells conjugated with SEE-pulsed B cells. This recruitment depends on TRAF6 interacting with linker for activation of T cells (LAT) via its TRAF domain. Although LAT was indispensable for TCR/CD28-induced TRAF6 ubiquitination and its ligase activity, RNA interference-induced TRAF6 knockdown in T cells decreased TCR/CD28-induced LAT ubiquitination, tyrosine-phosphorylation and association with tyrosine kinase ZAP70. Overexpression of TRAF6 or its catalytically inactive form C70A promoted and decreased, respectively, LAT tyrosine phosphorylation upon stimulation. Moreover, LAT was ubiquitinated at Lysine-88 by TRAF6 via K63-linked chain. In addition, TRAF6 was required for and synergized with LAT to promote the TCR/CD28-induced activation of NFAT. These results reveal a novel function and mechanism of TRAF6 action in the TCR-LAT signaling pathway distinct from its role in TCR-induced NF-κB activation, indicate LAT also play an adapter role in TCR/CD28-induced activation of TRAF6.

W2.12.03
TNF reverse signaling induces translocation of CKIP-1

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When the transmembrane form of tumor necrosis factor (mTNF) interacts with its cognate receptors or agonistic antibodies signaling pathways are activated in the ligand expressing cells. This "reverse signaling" appears to be a fine-tuning control mechanism in the immune response. We have shown that ligation of mTNF leads to nuclear translocation of the N-terminal cytoplasmic fragment (Nterm) of TNF. We found that over-expression of Nterm is enough to elicit the reverse signal. Using the cytoplasmic domain of TNF as bait in a Y2H system TNF-interacting protein(TIP)/casein kinase-2 interacting protein-1 (CKIP-1) was identified. We found that CKIP-1 expression was elevated upon LPS challenge in THP-1 monocytes. Over-expression of CKIP-1 triggered activation of THP-1 cells and up-regulated the human TNF promoter, especially when co-expressed with c-Jun in HEK293 cells. TNF reverse signaling induced the translocation of CKIP-1 from the plasma membrane to intracellular compartments in THP-1 cells. Expression of the Nterm of mTNF also elicited the re-localization of CKIP-1 in HEK293 cells. CKIP-1-triggered activation of THP-1 cells was antagonized by TNF reverse signaling. Similarly, the presence of the Nterm of mTNF inhibited CKIP-1 mediated TNF promoter activation in HEK293 cells. Reverse signaling in THP-1 monocytes and expression of the Nterm of mTNF in HEK293 cells induced apoptosis that could be prevented by over-expression of CKIP-1. Our findings demonstrate that CKIP-1 activates pro-inflammatory pathways and interferes with TNF reverse signaling-induced apoptosis, while reverse signaling blocks CKIP-1-induced activation of the TNF promoter in human monocytes.

W2.12.04
PI 3-kinase sub-pathways in lymphocyte activation: regulation and functions of phosphatidylinositol (3,4)-bisphosphate

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Activation of PI3-kinase enzymes is essential for lymphocyte development and function. Active PI3Ks generate several types of D3 phosphoinositides (PI), including PI(3,4,5)P3 and PI(3,4)P2. These PIs are under distinct regulation by PI phosphatases, have distinct protein binding partners and distinct functions in cell biology. We are assessing the regulation of the PI(3,4)P2 sub-pathway via phosphatases SHIP1 and INPP4A, as well as the functions of PI(3,4)P2-binding proteins TAPP1, TAPP2, Bam32/DAPP1 and lamellipodin. Function of TAndem PH domain containing Proteins (TAPPs) have been assessed using knock-in mutant mice in which both TAPP1 and TAPP2 are uncoupled from PI(3,4)P2. We found that these TAPP KI mice exhibit elevated serum antibody levels and also develop autoantibodies and kidney pathology resembling that of lupus. TAPP KI B cells show increased responses to BCR stimulation associated with elevated Akt activation. TAPP KI mice show evidence of chronic lymphocyte activation in vivo and generated elevated germinal center (GC) responses upon immunization. We hypothesize that TAPP adaptor binding to PI(3,4)P2 contributes a novel regulatory mechanism controlling B cell activation. We further find that PI(3,4)P2 can positively contributes to some B cell functions, particularly migratory responses to chemokine stimulation. We found that selective degradation of PI(3,4)P2 by INPP4A impairs B cell migration and have implicated PI(3,4)P2-binding proteins TAPP2 and lamellipodin as important regulators of lymphocyte migration. Together these results indicate that distinct PI pathways activated by PI3K contribute to different aspects of B cell activation.
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W2.12.05
Interleukin-7 signaling is motorized in human CD4 T-cells

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Interleukin(IL)-7 is the main homeostatic regulator of CD4 T-lymphocytes (helper) at both central and peripheral levels. Upon activation by IL-7, several signalling pathways, mainly Jak/STAT, PI3K/Akt and MAPK, induce the expression of genes involved in T-cell differentiation, activation and proliferation. We have analyzed the early events of CD4 T-cell activation by IL-7. We have shown that IL-7 in the first few minutes induces the formation of cholesterol-enriched membrane microdomains that compartmentalize its activated receptor and initiate its anchoring to the cytoskeleton supporting the formation of the signalosome on the IL-7-receptor cytoplasmic-domains.
We describe by stimulated emission depletion (STED) microscopy, the key roles played by membrane microdomains and cytoskeleton transient organization in the IL-7-regulated Jak/STAT signalling pathway. We image phospho-STAT5 and cytoskeleton components along IL-7-activation kinetics using appropriate inhibitors. Lipid raft inhibitors delay and reduce IL-7-induced Jak1 and Jak3 phosphorylation. Drug-induced disassembly of cytoskeleton inhibits phospho-STAT5 formation, transport and translocation into the nucleus that controls the transcription of genes involved in T-cell activation and proliferation.
We fit together the results of these quantitative analyses and propose the following mechanism: activated IL-7-receptors embedded in membrane microdomains induce actin-microfilament meshwork formation, anchoring microtubules that grow radially from rafted receptors to the nuclear membrane. STAT5 phosphorylated by signalosomes are loaded on kinesins and glide along the microtubules across the cytoplasm to reach the nucleus two minutes after IL-7-stimulation. Radial microtubules disappear 15 minutes later while transversal microtubules, independent of phospho-STAT5 transport, begin to bud from the microtubule-organization-center.
Tamarit et al. 2013 JBiolChem 288(15).

W2.12.06
Molecular profiling of LFA-1 signalling in T-cells identifies novel genomic signatures implicated in Th1, Th17 and iTreg polarization

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Background: T-cell expression of functional phenotypes is dependent on a range of interacting signals. Here we demonstrate molecular mechanisms by which LFA-1-mediated signalling associated with lymphocyte migration modulates T-cell differentiation into Th1, Th17 or iTreg.
Methods: Primary human or mouse T-cells were stimulated via LFA-1 by incubating on immobilised recombinant ICAM-1. Affymetrix GeneChip® microarrays and Ingenuity Pathway Analysis were performed. Biochemical and imaging techniques including real-time PCR, Western-blotting, ELISA, siRNA-mediated gene silencing, confocal microscopy and High Content Analysis were utilised.
Results: Molecular profiling of LFA-1-stimulated T-cells identified genomic signatures defining both Notch and TGF-β signalling pathways. We further demonstrate that LFA-1/ICAM-1 interaction activates Notch signalling by nuclear translocation of its cleaved intracellular domain and up-regulation of target genes Hey1 and Hes1. This interaction also up-regulates a subset of molecules associated with reduced TGF-β responsiveness, including Smad7, Smurf2 and Ski. The increased expression of these molecules in T-cells significantly attenuates TGF-β-mediated phosphorylation of Smad2 and/or suppression of IL-2 secretion. While LFA-1/ICAM-1 favours Notch-dependent Tbet+ Th1 polarization, LFA-1-stimulated T-cells are refractory to TGF-β-mediated induction of Foxp3+ iTreg or RORγt+ Th17 differentiation. Pre-treatment of T-cells with blocking anti-LFA-1 antibody, specific inhibitors or siRNA against identified genes substantially antagonises LFA-1/ICAM-1-mediated effects on functional phenotypes and restores their TGF-β sensitivity.
Conclusion: This study establishes a crucial role of LFA-1-mediated signalling in immunoregulation concurrent with lymphocyte motility, involving both Notch and TGF-β pathways. Our findings suggest a rational basis for novel selective and “tunable” therapeutic approaches aimed to modulate immune functions in inflammatory diseases.

W3.01 Lymphoid organogenesis and lineage commitment

IL3.01.01
TNF and both soluble and membrane-bound lymphotoxins have distinct functions in GALT organogenesis and in intestinal immunity

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TNF and lymphotoxin are known to be involved both in the development and in the maintenance of lymphoid organs’ microstructure, and various cellular sources of these cytokines may contribute to these functions. In this study we addressed distinct contributions of TNF, membrane-bound LTα1β2 and soluble LTα3 produced by RORγt+ innate lymphoid cells (ILC) in the intestinal immune system. We generated novel mice with cell type-restricted ablation of TNF, LTβ and LTα in RORγt+ cells and found that ILC-derived TNF and both types of LT are distinctly required for GALT development. Furthermore, we found that ILC-derived soluble LTα3, but not TNF or heterotrimeric LTα1β2, is critical for IgA induction in the lamina propria. There appears to exist two distinct mechanisms of IgA induction by ILC: a T-cell-independent pathway that is critically dependent on ILC-derived LTα1β2 expression and a novel T cell-dependent pathway that relies on a non-redundant function of sLTα3 produced by ILC. Finally, ILC-derived sLTa3 is important for control of microbiota composition.

W3.01.01
The layered immune system: adult hematopoietic stem cells cannot fully reconstitute all of the functionally distinct immune cell subsets

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The mammalian immune system develops progressively during ontogeny. In both mice and humans, the types of immune cells that develop in fetuses/neonates differ from those that develop in adulthood. Among lymphocytes, B-1, γδT, and iNKT cells are the earliest to emerge and persist at low frequencies thereafter. In contrast, B-2, marginal zone B (MZB) and αβT cell emerge later but rapidly rise to predominance in adults. The accepted dogma has been that a single hematopoietic stem cell (HSC) from either neonatal or adult bone marrow (BM) can give rise to all of these developmentally distinct immune cell subsets.
However, in studies here, we finally demonstrate that individual HSC sorted from adult BM and transferred to lethally irradiated recipients clearly gives rise to B-2, MZB and B-1b but does not detectably reconstitute B-1a. These findings place B-1a in a separate lineage derived from HSC that are rare or missing in adults. In addition, we surprisingly show that HSC harvested from fetal/neonatal BM similarly fail to fully reconstitute B-1a in adoptive recipients, while E12 yolk sac cells fully reconstitute all B cell subsets.
In conclusion, we have now demonstrated that *certain lymphocytes can originate from an unsuspected developmental pathway that is independent of both neonatal and adult BM HSC*. This finding is poised to introduce dramatic changes into the current understanding of the developmental landscape of the immune system, and to impact human regenerative therapies in which HSC are used to restore hematopoiesis.

W3.01.02
Deletion of the atypical chemokine receptor CCX-CKR alters thymic stroma, impairs thymocyte development and promotes autoimmunity.

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The atypical chemokine receptor CCX-CKR regulates bioavailability of CCL19, CCL21 and CCL25, homeostatic chemokines that play crucial roles in thymic lymphopoiesis. Deletion of CCX-CKR results in accelerated experimental autoimmunity induced by immunization. Here we show that CCX-CKR-deletion also increases incidence of a spontaneous Sjögren's syndrome-like pathology, characterised by lymphocytic infiltrates in salivary glands and liver of CCX-CKR-/- mice, suggestive of a defect in self-tolerance when CCX-CKR is deleted. This prompted detailed examination of the thymus in CCX-CKR-/- mice. Negatively selected mature SP cells were less abundant in CCX-CKR-/- thymi, yet expansion of both DP and immature SP cells was apparent. Deletion of CCX-CKR also profoundly reduced frequencies of DN3 thymocyte precursors and caused DN2 cells to accumulate within the medulla. These effects are likely driven by alterations in thymic stroma as CCX-CKR-/- mice have impaired expansion of cTECs, the cell population that expresses the highest level of CCX-CKR in the thymus. A profound decrease in CCL25 within the thymic cortex was observed in CCX-CKR-/- thymi, likely accounting for their defects in thymocyte distribution and frequency. These findings identify a novel role for CCX-CKR in regulating cTEC expansion, which promotes optimal thymocyte development and selection important for self-tolerant adaptive immunity.

W3.01.03
Identification of thymic epithelial stem cells ensuring lifelong central T-cell tolerance

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The thymus consists of two distinct compartments, the cortex and the medulla, each containing functionally different thymic epithelial cells (cTECs and mTECs). mTECs are crucial for establishing immunological self-tolerance by promiscuous expression of tissue-specific self antigens (TSAs) in part via Autoimmune regulator (Aire). mTECs show high turnover rate, promoting cross-presentation of the TSAs by dendritic cells and effective deletion of autoreactive T cells. However, the mechanism for sustaining regeneration of functional mTECs to ensure lifelong T-cell self-tolerance remains unknown. Here we demonstrate that self-renewing mTEC stem cells capable of sustaining a functional thymic medulla throughout postnatal life are generated during early embryonic stage. Implantation of purified wild type embryonic Claudin-3,4^{+} mTEC progenitor fraction into *aly/aly* thymic microenvironment completely restored thymic medulla including Aire^{+} mTECs and suppressed the autoimmune manifestation for nearly life-long. A SSEA-1^{+} fraction within the embryonic Claudin-3,4^{+} mTEC progenitor population contained self-renewable clonogenic TECs in culture, which could specifically regenerate mature mTECs *in vivo*. While the mTEC stem cell activity was markedly diminished after birth in concordance with physiological thymic involution in normal mice, it was sustained at high levels in adult RAG2$^{-/-}$ mice. Our results demonstrate that life-long central T cell tolerance is ensured by the stem cells specified for a mTEC lineage, albeit the stem cell activity is declined postnatally under robust T cell development in the thymus. These findings provide an important clue for developing strategies for maintaining TEC stem cells and cell-based therapies against autoimmunity related to thymic dysfunction in diseases and aging.

W3.01.04
Dissecting the cellular and molecular requirement for Lymphotoxin in orchestrating B cell accumulation in ectopic follicle like structures in a rodent model of Multiple Sclerosis

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Multiple Sclerosis (MS) is a T cell dominated inflammatory disease of the central nervous system (CNS). Nevertheless, recent studies have implicated B cells as disease modifiers, including B cell depletion studies and the correlation of B cell rich, meningeal follicle like structures (FLS) with increased CNS neuropathology. While ectopic FLS are common to many chronic inflammatory diseases, the cellular and molecular instigators supporting FLS in the CNS remain unclear. Preliminary studies in the rodent model of MS, Experimental Autoimmune Encephalomyelitis (EAE) have implicated the Tumor Necrosis Factor family member Lymphotoxin (LT) in FLS formation, complementary to its role in orchestrating the architecture of secondary lymphoid organs. In this study we use the SJL model of EAE to evaluate the requirement of B cells and LT signaling as drivers of meningeal FLS in CNS inflammation. First, using a B cell depletion strategy we determined that despite their abundance in the follicles, meningeal FLS can form in the absence of B cells. Next, we used a combination of pharmacological and genetic approaches to dissected the requirement of LT on different cell populations to support FLS formation in the CNS. We consistently observed a pronounced decrease in B cell accumulation within the FLS as well as less compact morphology of meningeal follicles, dependent on LT receptor expression by a non-hematopoeitic cell population. Taken together these results shed light on the cellular requirements of lymphotoxin signaling in supporting meningeal FLS in a model of CNS autoimmunity.

W3.01.05
Pbx1 restrains myeloid maturation while preserving lymphoid potential in hematopoietic progenitors

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The capacity of the immune system to promptly respond to peripheral demands relies on adequate pools of progenitors able to transiently proliferate and differentiate in a regulated manner. However, little is known about factors that may restrain progenitor maturation to maintain their reservoirs. In addition to a profound defect in hematopoietic stem cell (HSC) self-renewal, conditional knockout mice for the Pbx1 proto-oncogene have a significant reduction in lineage-restricted progenitors, as well as aberrant size, cell number and microscopic structure of thymus and spleen. Through analysis of purified progenitor proliferation, differentiation capacity and transcriptional profiling, we demonstrate that Pbx1 regulates the lineage-specific output of multipotent and oligopotent progenitors. In the absence of Pbx1 multipotent progenitor (MPP), including lymphoid-primed multipotent progenitors (LMPPs), and common myeloid progenitor (CMP) pools are reduced due to aberrantly rapid myeloid maturation. This is associated with premature expression of myeloid differentiation genes and decreased maintenance of proto-oncogene transcriptional pathways including reduced expression of Meis1, a Pbx1 dimerization partner, and its subordinate transcriptional program. Conversely, Pbx1 maintains lymphoid differentiation potential of LMPPs and common lymphoid progenitors (CLPs), whose reduction in the absence of Pbx1 is associated with a defect in lymphoid priming that is also present in CMPs, which persistently express lymphoid and HSC genes underlying a previously unappreciated lineage promiscuity that is maintained by Pbx1. These results demonstrate a role for Pbx1 in restraining myeloid maturation while maintaining lymphoid potential to appropriately regulate progenitor reservoirs.

W3.01.06
Survivin inhibition disturbs Bcl-6 and Blimp-1 control of lymphocyte differentiation

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Survivin is a proto-oncogene and an inhibitor of apoptosis regulating cell division. In normal tissue survivin is critical for development of CD34+ stem cells and for beta-selection of early T cells. In the present study we assessed if inhibition of survivin affects antigen-induced maturation of T cells.
Survivin transcription was inhibited by shRNA-lentiviral construct (shSurv, 10^6-10^7 particles /mouse) provided as a single injection on the day of immunization. This resulted in significant survivin inhibition within T and B populations of bone marrow and spleen compared to mice received non-targeting construct (shNT).
Survivin inhibition was inversely correlated to an increase of transcription repressors Bcl-6 and Blimp-1. In consistence with overexpression of Blimp-1, shSurv mice had low levels of IL-2, suppressed proliferation response and increased populations of the effector (CD62l-CD44hi) CD4+ and CD8+ cells, followed by high IL-6 production. shSurv mice had increased populations of Tregs (CD4+Foxp3+) and Th2 cells supported by high mRNA of Foxp3 and GATA3, and high release of IL-10 and IL-4.
shSurv mice had small CXCR5+CD4+ and CXCR5+B220+ populations, suggesting insufficient Bcl-6 and poor Tfh development despite high expression of IL-21. Bcl-6 was sufficient to suppress production of TNFα, IFNγ, and IL-17A and to impair development of Th1, and Th17 subsets.
In conclusion, Survivin expression is an essential modulator of lineage commitments. Inhibition of survivin favours Blimp-1-driven development of Tregs and Th2 cells and impairs formation of Tfh.

W3.02 Role of the stroma in innate and adaptive immunity

IL3.02.01
Role of Lymph node structure in adaptive immunity to influenza virus

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In order to understand the dynamics of localization and uptake of an influenza vaccine within skin draining LNs, we have used a murine model in which mice are injected sub-cutaneously with fluorescent-labeled UV-inactive influenza virus (recombinant strain PR8 bearing the OT-II epitope). Combining two-photon live imaging with three-dimensional (3 -D) reconstruction of draining lymph nodes, viral particles were tracked within the afferent lymphatics over the first six hours. Results identified labeled virus resolving in medullary projections within inter-follicular regions (IFRs). These specialized sites, which are located adjacent to B cell follicles, are marked by a transition of sinus lining macrophages from sub-capsular macrophages to medullary macrophages. Further, 3-D analysis over a 6-12 hour period of LN isolated from immunized mice revealed large scale repositioning of the LN resident DC (LNDC) to the IFR. With a slightly delayed kinetics, viral specific CD4+ T cells are identified within IFRs where they make direct contact with LNDC and viral antigen. Strikingly, viral specific (but not naïve) CD4+ T cells express activation markers CD69 and CXCR3 and develop a Th 1 phenotype prior to skin draining DC arrival.
Together, we believe that these data elucidate the importance of LN structure and the localization of lymph borne viral antigen to the specialized IFR in the development of effective humoral immunity to influenza vaccination.
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IL3.02.02
Stroma-derived Hyaluronan induces CD44-dependent apoptosis in diabetic insulin-secreting cells

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We showed that the development of type 1 diabetes (T1D) in NOD mice is highly dependent on interaction between cell surface CD44 and its ligand hyaluronic acid (HA) (Proc. Natl. Acad. Sci. USA. 97, 285- 290,2000) generated by pancreatic islet stromal cells. Yet, the mechanism of action underlined this finding has not been elucidated. Here we show that the balance between CD44-dependent low mobility of inflammatory cells and CD44-dependent high susceptibility of insulin-secreting β cells to apoptosis dictates the activity of T1D in NOD mice. The reduced mobility of wild type (WT; $CD44^+$) diabetic inflammatory cells has been proven by the low rates of their *in vivo* islet invasion and *in vitro* cell migration, when compared with the CD44-deficient phenotype, indicating that the WT cells are firmly adhere to the HA substrate. This anti-diabetic effect is counter-balanced by the susceptibility of WT (but not CD44-null) insulin-secreting β cells to the autoimmune attack. The relative resistance of CD44-deficient β cells to pro-inflammatory cytokine attack *in vitro* or autoimmune attack *in vivo* has been evident by the decrease of their inducible nitric oxide (NO) synthase (iNOS) and caspase-3 signals, as well as by decline of their NO release and increase in glucose-stimulation insulin secretion, when compared with the WT phenotype. Hence, the balance between the reduced mobility of CD44-positive inflammatory cells and the enhanced susceptibility of β cells to programmed cell death dictates whether the mouse is diabetic or free of diabetes.

W3.02.01
Endocytosis and recycling of immune complexes by follicular dendritic cells enhances B cell binding and activation

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Follicular dendritic cells, which are stromal derived, are centrally located within B cell follicles of secondary lymphoid tissues, including the spleen and lymph nodes. Here they are a major reservoir for antigen that is essential for formation of germinal centers, the site where memory and effector B cells differentiate. A long-standing question is how the follicular dendritic cells retain antigen in its native form for extensive periods and how they display it to specific B cells. We find that follicular dendritic cells take-up complement-coated immune complexes directly from non-cognate B cells via complement receptors (CD21/CD35) and rapidly internalize them by an actin dependent pathway. Immune complexes are retained intact within a cycling compartment and are displayed periodically on the cell surface where they are accessible to antigen-specific B cells. After antigen-acquisition these cognate B cells are activated, as expected. Thus, internalization of immune complexes into a non- degradative compartment and periodic cycling of the intact immune complex to the cell surface would explain the long standing question how antigens are retained for extensive periods of time and made available to B cells.

W3.02.02
Dynamics of plasma cell survival niches in the bone marrow

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The maintenance of long-lived bone marrow PC is guaranteed by a special bone marrow microenvironment termed survival niche. This niche consists of stromal components as well as hematopoietic cells (e.g. eosinophils), which have been reported to secrete plasma cell survival factors such as APRIL and IL-6.
We have investigated the dynamics of reticular stroma cells and bone marrow eosinophils in the microanatomical context of the plasma cell survival niche. We studied the turnover of both stromal cells and accessory niche cells in T-dependent secondary immune responses. 30 days after secondary immunization of mice, we found 80% of bone marrow PC in direct contact to reticular bone marrow stroma cells. Studying the proliferation of reticular stroma cells by EdU pulse-chase labeling in vivo, we found that mesenchymal cells of the bone marrow do not respond to immunization by increased proliferation and that reticular stroma cells localized next to PC do not proliferate in the first 12 days after immunization. This indicates that reticular stroma cells are stable components of the plasma cell survival niche. In strong contrast to reticular stroma cells, eosinophils in the vicinity of PC have a high turnover, reflected by the incorporation of EdU during the pulse and complete loss of EdU within 18 days after the pulse. These findings support the idea of a dynamic PC survival niche, comprised of hematopoietic accessory cells with a high turnover like eosinophils, and stable organizers of the niche such as reticular stroma cells.

W3.02.03
Galectin-1-expressing stromal cells constitute a specific niche for pre-BII cell development in mouse bone marrow

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In the bone marrow (BM), stromal cells constitute a supportive tissue indispensable for the generation of pro-B/pre-BI, pre-BII, and immature B lymphocytes. IL-7-producing stromal cells constitute a cellular niche for pro-B/pre-BI cells, but no specific stromal cell microenvironment was identified for pre-BII cells expressing a functional pre-B cell receptor (pre-BCR). However expression of the pre-BCR represents a crucial checkpoint during B-cell development. We recently demonstrated that the stromal cell derived-galectin1 (GAL1) is a ligand for the pre-BCR, involved in the proliferation and differentiation of normal mouse pre-BII cells. Here we show that nonhematopoietic osteoblasts and reticular cells in the BM express GAL1. We observed that pre-BII cells, unlike the other B-cell subsets, were specifically localized in close contact with GAL1(+) reticular cells. We also determined that IL-7(+) and GAL1(+) cells represent 2 distinct mesenchymal populations with different BM localization. These results demonstrate the existence of a pre-BII specific stromal cell niche and indicate that early B cells move from IL-7(+) to GAL1(+) supportive BM niches during their development.

W3.02.04
Local Complement Activation Abrogates the Tumor-Endothelial Barrier and Mediates T Cell Homing and Tumor Immune Attack

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Given the survival advantage associated with T cell infiltration of tumor implants, this association has implications for clinical outcome. Cancer immune therapy does not only depend on the ability of the patients to mount an anti-cancer immuneresponse but also to the capability of the effector cells to infiltrate tumors through the endothelial barrier. Here, we show that tumor infiltration by antitumor T cells in vivo requires local endothelial complement activation and anaphylatoxin release. Th1 cytokines, released by tumor-reactive T cells, induce endothelial C3 expression, local complement activation, and C5a anaphylatoxin release. This promotes the upregulation of ICAM-1 and VCAM-1 and T-cell adhesion to the endothelium, circumventing the endothelial barrier in vivo and in vitro. We conclude that a sufficiently potent T cell response succeeds in overcoming the tumor endothelial barrier, and that local complement activation, acting via C5a, is a critical mechanism by which effector T cells disrupt this barrier.

W3.02.05
Alterations in stromal cell compartment during thymus atrophy induced by P. brasiliensis infection

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Thymus is the development site of T lymphocytes, which are important cells of the specific immune response. To perform its function, the thymic microenvironment must be preserved. Despite its importance, the thymus is a target organ for many infectious diseases, including paracoccidioidomycosis, the most prevalent systemic mycosis in Latin America, caused by the fungus *Paracoccidioides brasiliensis* (Pb). We have already demonstrated in experimental models that Pb invades the thymic microenvironment, inducing severe atrophy characterized by organ weight decrease and loss of corticomedullary delimitation. We have also reported that such atrophy is related to the increased death of immature lymphocytes and to the premature migration of these cells to the periphery. However, the possibility that thymic atrophy during the paracoccidioidomycosis infection is also associated to alterations in non-lymphoid cells, which include dendritic cells (DCs), thymic cortical (cTECs) and medullary epithelial cells (mTECs) cannot be discarded. In this study, we examined the stromal cell compartment of thymuses from BALB/c mice infected with the Pb virulent isolate (Pb18) during the acute phase of the disease. By immunofluorescence technique, our results show an altered pattern of distribution of DCs as well as mTECs (Keratin-5^{+} cells) and cTECs (Keratin-8^{+}) in thymuses from Pb-infected animals. Also, by flow cytometry technique, we observed phenotypic alterations in DCs and severe reduction in the number of mTECs and cTECs. Altogether, these findings show that the stromal compartment is also deeply

affected during thymic atrophy caused by Pb infection, complementing previous studies of our laboratory.
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W3.02.06
A quasi clone specific mechanism for the regulation of the size of the primary CD8 T cell response

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Previous studies suggested that the control of the size of the primary CD8 T cell response was clone specific (Kemp et al., 2004, J.Immunol. 173: 2923-27) and that regulation was achieved by controlling the number of progeny that survive. We now propose that the availability of the thymic positive selecting self peptide for recognition by a responding T cell clone is a factor in the control of clonal burst size. The recognition of the selecting peptide distinguishes members of the clone from members of other clones specific for the same epitope but which were selected on different thymic peptides. We have developed an in vivo - in vitro model in which the addition of CD45 negative stromal cells and the thymic selecting peptide can enhance, in a clone specific manor, the survival of CD8 T cells from transgenic mice responding to cognate peptide antigen. We hypothesize that T cells interact with Fibroblastic Reticular Cells in a selecting peptide dependent manor and receive a very locally available survival signal, such as IL-7, that increases the number of progeny that can survive. The mechanism proposed can serve to stabilize the repertoire of T cells responding to a particular epitope in the face of other selective pressures.

W3.03 Thymic selection and T cell development

IL3.03.01
Role of the affinity of TCR-self peptide interaction in commitment to $CD4^+Foxp3^+$ and $CD4^+Foxp3^-$ T cells and in shaping their TCR repertoires

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Binding of class II MHC presented peptides by TCRs on immature $CD4^+8^+$ thymocytes results in their deletion (negative selection) or in differentiation (positive selection) into two functionally different kinds of CD4+T cells: $Foxp3^+$ regulatory and $Foxp3^-$ non-regulatory effectors. Previous studies suggested that commitment towards $CD4^+Foxp3^+$ lineage requires higher affinity of TCR interaction with the selecting MHC/peptide complex than commitment to $CD4^+Foxp3^-$ lineage, which could explain differences in their TCR repertoires. To study the role of the affinity of TCR-self peptide interaction we analyzed the TCR repertoires of $CD4^+Foxp3^-$ and $CD4^+Foxp3^+$ thymocytes in mice with restricted TCR repertoire (TCR^{mini}) that expressed class II MHC molecules (A^b) bound exclusively with one peptide recognized with either low (Ep) or high (Ep63K) affinity by multiple TCRs from this repertoire. In "single peptide" $A^bEp\ TCR^{mini}$ mice, the TCR repertoires of $CD4^+Foxp3^+$ and $CD4^+Foxp3^-$ thymocytes are diverse but share large proportion of identical TCRs. In contrast, in "single petide" $A^bEp63KTCR^{mini}$ mice fewer TCRs are shared by corresponding repertoires, which are more dissimilar and less diverse as a consequence of negative selection of thymocytes recognizing self peptide with higher affinity. Our results indicate that intrathymic commitment into $CD4^+Foxp3^+$ or $CD4^+Foxp3^+$ lineage is not dictated by different affinity of TCR-self peptide interaction and that negative selection plays dominant role in separating their TCR repertoires.

IL3.03.02
Anti-cytokine autoantibodies in APECED correlate with chronic mucocutaneous candidiasis

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Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) is a monogenic autoimmune disease caused by mutations in the Autoimmune Regulator (AIRE). AIRE is a transcriptional regulator expressed in thymic epithelial cells where it promotes the expression of tissue-specific antigens. APECED patients commonly have chronic mucocutaneous candidiasis, hypoparathyroidism, Addison's disease and multiple other autoimmune disorders including type 1 diabetes. The autoantibodies in patient sera recognize many organ-specific targets. In particular, they are reactive to type I interferons; IFNalpha and IFNomega and to Th17 cell-associated cytokines; IL-17 and IL-22. These autoantibodies are neutralizing, occur in extremely high titers, are disease-specific and, in addition to APECED, have been only reported in patients with thymomas. We found that the immunoglobulins responsible for neutralizing type I IFNs and IL-22 belong to IgG with dominant subtypes of IgG1 and IgG4, the latter possibly having immune regulatory rather than inflammatory function. We also found neutralizing autoantibodies to IL-17 in aged Aire-deficient mice, which is the first antigen to which reactivity is seen in both human and mouse AIRE-deficiency states. We conclude that autoimmunization against IFN and Th17 cytokines in AIRE-deficiency is closely related to the disease initiation.

W3.03.01
Novel role for heparan sulfate in thymic development of CD8+ T cells

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A remarkable feature of adaptive immunity is the process of positive and negative selection of T cells in the thymus that results in self-MHC restricted T cells with minimal autoreactivity. Our recent studies have provided new insights into this important process. It has been known for decades that murine CD4+CD8+ (double positive (DP)) thymocytes form multicellular conjugates with autologous erythrocytes in vitro, a phenomenon termed autorosetting. We have recently identified CD8β as the thymocyte autorosetting receptor and heparan sulfate (HS) as the autorosetting ligand on erythrocytes. Furthermore, a subpopulation of thymic cells, probably of stromal origin, has been identified that expresses extraordinarily high levels of cell surface HS, exhibits very high MHC expression and rosettes with thymocytes. Immunohistochemical studies showed that these HShi cells are located in small 'islands' throughout the thymic cortex. Additional in vitro studies demonstrated that high molecular weight HS mimetics, such as dextran sulfate 500-kDa (DxS-500), are capable of inducing a strong and prolonged intracellular calcium flux in DP thymocytes that is CD8β and SLP76, but not Zap70, dependent and lowers the thymocyte TCR activation threshold. In contrast, sialylation of CD8β expressed by single positive thymocytes and peripheral CD8+ T cells results in a dramatic reduction in HS binding and loss of the DxS-500 induced calcium flux. Collectively these data imply that the interaction of CD8β on DP thymocytes with HS expressed by thymic HShi stromal cells triggers an intracellular calcium flux that aids the positive selection of thymocytes with low affinity for self-MHC.

W3.03.02
B cells engender thymic Tregs

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Although B cells have been reported in the thymus the exact role they play is unclear. Here we observe that B cells can modulate thymic production of $Foxp3^+$ $CD4^+$ T cells (Tregs). BAFF transgenic mice harbor increased numbers of peripheral-Tregs whose expansion is B

cell dependent. BAFF did not enhance Treg survival, proliferation or conversion in the periphery but promoted a ~3 fold increase in thymic Helios$^+$ Treg numbers. Interestingly BAFF also increased thymic B cells. Thymic-Treg expansion was lost in BAFF-Tg/µMT$^{-/-}$BM chimeras and B cell null µMT$^{-/-}$ mice exhibited decreased numbers of thymic Tregs. There was also a direct correlation between the amount of thymic B cells and thymic Tregs. Based on the surface markers IgM, CD21 and CD23, four distinct populations of intra-thymic B cells could be determined, reminiscent of splenic B cell populations. CD19$^+$ B cells accumulated in the thymic medulla region close to medullary thymic epithelial cells suggesting a role in thymic Treg selection. Further to this thymic B cells were observed in close proximity to Foxp3$^+$ T cells. In the absence of B cell surface MHC class II, thymic Treg expansion did not occur, indicating the possibility of direct B-Treg interactions in the thymus. Further, thymic Treg expansion did not occur in BAFF-Tg/IgHEL BCR BM chimeras demonstrating a requirement for antigen-specificity. Thus, we present a model whereby intra-thymic B cells provide a source of cognate signals to modulate thymic Treg production. This results in increased numbers of peripheral Tregs that allow for allograft acceptance.

W3.03.03
Involvement of commensal bacteria in thymic Aire expression

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Autoimmune regulator (Aire) expression in thymic epithelial cells (TECs) is involved in negative selection of self-antigen reactive T cells. However, little is known about how Aire expression is regulated. In this study, we found that Aire expression is reduced in TECs in germ-free (GF) mice in which commensal bacteria are absent, in comparison to specific pathogen-free (SPF) mice, allowing us to predict that components of commensal bacteria are involved in promoting Aire expression in TECs. Mutant mice lacking Nod1, an intracellular pattern recognition receptor of bacteria-derived molecules, also revealed reduced Aire expression in TECs. In the organ culture system of fetal thymus (FTOC) of wild type mice in the presence of synthetic ligand of Nod1, Aire expression was reciprocally increased in TECs. Staphylococcal enterotoxin B (SEB) produced from *Staphylococcus aureus* also induced Aire up-regulation in FTOC, which was reduced when thymic lobes were co-cultured with blocking antibody against the cytokine RANK ligand (RANKL). These observations indicate that intestinal bacterial components are involved in promoting Aire expression in TECs *in vivo* and *in vitro*, suggesting the novel possibility that commensal bacteria contribute to intrathymic negative selection through Aire induction.

W3.03.04
A Key Role for PTPN22 in Setting the Threshold for Natural and Inducible Regulatory T Cell Development

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Regulatory T cells (Tregs) represent a vital component of the adaptive immune response securing peripheral tolerance. During positive selection in the thymus, a stable population of FOXP3-expressing natural Tregs (nTregs) develops as a result of strong agonist recognition. In addition to nTreg development, a fraction of conventional CD4+ T cells can acquire de novo FOXP3 expression in the periphery developing into inducible Tregs (iTregs). For both natural and inducible Treg development combined signals through the TCR, CD28, IL-2 and TGF-beta promote the upregulation of FOXP3. How FOXP3 expression is induced and maintained during Treg development is poorly understood. Here we demonstrate that mice deficient for PTPN22, a protein tyrosine phosphatase that inhibits TCR signaling, had increased frequency and number of nTregs in thymus and secondary lymphoid organs. We found that in the absence of PTPN22, the induction of TGF-beta-driven FOXP3 expression by conventional CD4 T cells was dependent on the degree of TCR activation. While PTPN22 antagonized FOXP3 at low TCR-mediated activation, it augmented FOXP3-inducing signals at higher TCR activating conditions. Since iTregs can be elicited from conventional CD4 T cells in response to lymphopenia, we employed this model to study the contribution of PTPN22 in iTreg development in vivo. iTreg induction by PTPN22-deficient CD4 T cells was reduced at two weeks after transfer in lymphopenic hosts, which, however, was increased at six weeks post transfer. Altogether, our results identify PTPN22 as indispensable factor for setting the proper threshold for natural and inducible Treg development in vitro and in vivo.

W3.03.05
Aire-mediated central tolerance includes naïve but not post-translational modified self antigens

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The autoimmune regulator (Aire) controls ectopic expression of peripheral tissue-restricted antigens (TRAs) in medullary thymic epithelial cells, and is involved in mediating deletion of autoreactive T cells in the thymus. Despite the relevant role of Aire in regulating tolerance to TRAs, autoreactive T cells are frequently observed within the mature T cell repertoire. Many self-antigens are naturally subjected to post-translational modifications, such as glycosylation, and it is possible that such modified TRAs are not sufficiently presented to induce tolerance in the thymus. Here we have investigated the regulation of tolerance to type II collagen (CII), a self-antigen naturally subjected to glycosylation. Using an autologous collagen-induced arthritis model, where the transgenic expression of CII is Aire-dependent, we show that tolerance to CII is restricted to the non-modified version of the autoantigen, whereas T cells specific for the glycosylated version escape central tolerance. This finding explains earlier observations that the glycosylated form of CII constitutes the immunodominant epitope, despite that peripherally expressed self-CII appears uniformly glycoslylated. However, as many self-antigens are naturally subjected to post-translational modifications or may become altered over time either spontaneously or due to traumas, our data points out a possible general mechanism whereby T cells may escape an otherwise efficient tolerogenic mechanism.

W3.03.06
Functional study of WW domain-containing oxidoreductase in T cells

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WW domain-containing oxidoreductase (known as WOX1) has been shown to promote stress-induced cancer cell death and function as a tumor suppressor. Previous studies indicated that WOX1-deficient mice exhibit preweaning lethality, abnormal bone formation and growth retardation. However, whether WOX1 plays a role in the immune system is unknown. In this study, we investigated the functional role of WOX1 in lymphoid organs and found thymus atrophy in WOX1-deficient mice. Using cleaved caspase-3 staining and TUNEL assay, we examined higher levels of caspase activation and apoptosis in WOX1-deficient thymus. After ex vivo culture for 24 hours, WOX1-deficient thymocytes showed higher percentages of cell death than control, as determined by annexin-V and propidium iodide staining. The presence of a pan-caspase inhibitor, Z-VAD, in cultures partially prevented apoptosis in WOX1-deficient thymocytes. These results suggest that WOX1-deficient thymocytes are prone to cell death. To explore the intrinsic or extrinsic defect that causes extensive apoptosis in WOX1-deficient thymocytes, wild-type or WOX1-deficient bone marrow cells carrying CD45.2 were transferred into CD45.1+ NOD.SCID mice. Our results demonstrated that wild-type bone marrow cells differentiated into mature T cells and showed normal distribution of thymocyte subpopulations in NOD.SCID recipients 6 weeks after adoptive transfer. However, WOX1-deficient bone marrow cells failed to differentiate into mature T cells in recipient mice. In summary, we show here that WOX1-deficient

thymocytes exhibit intrinsic defects in sustaining cell differentiation and survival. These in vivo findings run against the role of WOX1 as a pro-apoptotic protein and unravel a novel function of WOX1 in thymocyte development.

W3.04 Genetic rearrangements of lymphocyte receptors

IL3.04.01
Combinatorial H3K9acS10ph histone modifications in IgH locus S regions target 14-3-3 adaptors and AID to specify antibody class switch DNA recombination

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Class switch DNA recombination (CSR) is central to effective antibody responses. By recombining an upstream (donor) S region and a downstream (acceptor) S region, it changes an antibody IgH constant region and endows it with news biological effector functions. 14-3-3 adaptors recruited/stabilize to/on S regions AID, which is critical for CSR. 14-3-3 a high affinity for 5'-AGCT-3' repeats, which recur at a high density in all S regions. How 14-3-3 and the AID-centered CSR machinery target exclusively the S regions that will undergo recombination is poorly understood. We hypothesized that histone posttranslational modifications play an important role in CSR targeting. We showed that "primary" (CD154 or LPS) CSR-inducing stimuli induced histone methyltransferases and acetyltransferases, which catalyzed H3K4me3 and H3K9ac/K14ac modifications in S regions as well as germline IH-S-CH transcription (specified by cytokines) were associated with but did not precisely predict the S region targets of CSR. By contrast, the combinatorial H3K9acS10ph histone modification marked only the donor Sμ and acceptor S regions that were set to recombine. H3K9acS10ph was introduced into the recombining S regions by CD154- or LPS-induced pGcn5 and Pcaf histone acetyltransferases and specifically recruited 14-3-3 (and AID), as determined by free H3K9acS10ph peptide competition. Thus, the combinatorial H3K9acS10ph histone code is "written" by primary stimuli-induced histone-modifying enzymes, specifically marks the S regions that will undergo recombination and is "read" by 14-3-3 adaptors, thereby targeting the AID-centered CSR machinery to "transduce" CSR as specific functional outcome. Supported by NIH AI079705, AI105813, AI045011 and AI060573 grants to P.C.

IL3.04.02
Proteostasis in the exocytic compartment: synthesis, folding and transport of antibodies as intracellular signals

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Immunoglobulin subunits act as powerful signaling devices during the entire B cell developmental program. At the B cell level, for instance, the antigen receptor is essential for survival also in the absence of nominal antigen. Upon encounter with antigen in a suitable context, however, the B cell receptor delivers signals that induce B lymphocytes to differentiate into antibody secreting plasma cells. Most of these undergo apoptosis after a few days of intense immunoglobulin production, so as to limit antibody responses. Real professional secretors, plasma cells allow dissecting the mechanisms that maintain protein homeostasis (proteostasis) in the exocytic compartment, the stresses that the exuberant production and transport of antibodies along the exocytic route entail, as well as their roles in signaling. Our data reveal how proteotoxic stresses physiologically contribute to regulate the biogenesis, function and lifespan of B and plasma cells, and explain in part why multiple myeloma cells are exquisitely sensitive to proteasome inhibitors.

W3.04.01
A novel DNA break and phosphorylation-dependent positive feedback loop promotes class switch recombination

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Activation induced cytidine deaminase (AID) is essential for class switch recombination (CSR), a deletional-recombination reaction that occurs between repetitive DNA elements, termed switch (S) regions, in the immunoglobulin heavy chain (*Igh*) locus. The ability of AID to mediate CSR is significantly dependent on its phosphorylation at serine-38 (S38); however, the trigger that induces AID phosphorylation and the mechanism by which phosphorylated AID drives CSR have not been elucidated. We have used catalytically-inactive AID and mice impaired in processing deaminated DNA to demonstrate that AID phosphorylation at S regions is induced by DNA breaks. Conversely, in the absence of AID phosphorylation, DNA breaks are not efficiently generated at S regions due to a failure of AID to interact with the apurinic/apyrimidinic endonuclease APE1. Additionally, Ataxia telangiectasia mutated (ATM) deficiency impairs AID phosphorylation and interaction with APE1. Our results uncover a novel positive feedback loop for amplification of DNA breaks at S regions through a phosphorylation- and ATM-dependent interaction of AID with APE1.

W3.04.02
AID-dependent IgV hypermutation requires a splice isoform of the SR protein SRSF1

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Somatic hypermutation (SHM) of IgV genes in B cells is crucial to generate high-affinity antibodies during immune responses. Both IgV transcription and activation-induced cytidine deaminase (AID) are required for SHM. However, it remains unclear how IgV genes are much more sensitive to AID-induced mutagenesis than other genes and what links the action of AID with IgV transcription in SHM. We found an essential role for a splice isoform of the prototypical serine/arginine-rich (SR) protein splicing factor SRSF1, termed SRSF1-3, in AID-induced SHM in a hypermutating chicken B cell line DT40. We unexpectedly found that both SHM and gene conversion (GCV) did not occur in a DT40 line lacking SRSF1-3 expression (DT40-ASF), while they are readily detectable in parental wild-type DT40 cells. In DT40-ASF cells, the endogenous SRSF1 gene is disrupted and a tet-regulated SRSF1 cDNA is introduced. Importantly, reconstitution of SRSF1-3 in DT40-ASF cells completely restored SHM and GCV without increasing off-target mutations. In contrast, overexpression of AID in DT40-ASF cells led to a large increase in off-target mutations, while marginally affected IgV mutation. ChIP analysis showed that SRSF1-3 bound preferentially to the IgV gene and inhibited processing of the Ig transcript, but reconstitution of SRSF1-3 did not enhance the recruitment of AID on the IgV gene. Our findings define an unexpected and important role for SRSF1, specifically for its splice variant, in enabling AID to specifically attack its natural substrate during IgV hypermutation.

W3.04.03
Effect of the 3'Regulatory Region insulation on B cell development and DNA rearrangements at the Immunoglobulin heavy chain locus

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The Immunoglobulin heavy chain locus (IgH) undergoes a series of developmentally-regulated DNA rearrangements, named V(D)J recombination.
Regulation of these events is effected by various cis-acting elements among which the Eμ enhancer plays a major role. Another important

cis-element is the 3' Regulatory Region, located downstream of the IgH locus, and which was suggested to interact with Eμ enhancer. CTCF-binding elements have been described at the IgH locus and are thought to mediate the formation of large loops that may be important for various processes linked to V(D)J recombination.
In this study, we have adopted an insulation approach by inserting the well-characterized cHS4 insulator of the chicken β-globin locus, upstream of the IgH 3'RR. We generated two mouse lines in which either the wild-type, CTCF-binding, cHS4 insulator was used or a mutated version of it devoid of CTCF-binding site.
We will present our recent results on the long-range effect of 3'RR insulation on B cell development, V(D)J recombination, allelic exclusion and IgH expression.

W3.04.04
Genetic variation and positional biases influence rearrangement of lymphocyte receptor genes

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An important source of the diversity of lymphocyte receptors is combinatorial diversity - the joining of gene segments to produce functional lymphocyte receptor genes. Estimates of the size of the primary human immunoglobulin repertoire generally assume that functional genes of the same type (V, D or J) are equally likely to be present in any V(D)J rearrangement. However, there are clear biases in gene usage, and some studies have even suggested that there are weak positional biases in the pairings of different DJ and V(DJ) heavy chain genes. Using 454 pyrosequencing, we have studied repertoires of V(D)J rearrangement in 30 individuals. Each dataset included at least 5000 sequences. The data show that the frequencies with which different IGHD genes partner with each IGHJ gene are subject to strong, though complex, positional biases. Techniques that allow the inference of both chromosomal sets of germline genes in an individual have allowed us to separately analyze rearrangements of each chromosome. IGHD/IGHJ haplotypes associated with different IGHD and IGHJ alleles show stronger or weaker but still predictable positional biases. Furthermore, individuals who carry IGHD gene deletion polymorphisms on one chromosome show a predictable overutilization of DJ pairings with those D genes on the second chromosome. Incorporating these positional biases into models of repertoire development will be essential if we are to understand individual variation in immunocompetence.
The primary repertoire may be more comprehensible and predictable than previously reports have suggested, and genetic variation may account for individual differences in the primary antibody repertoire.

W3.04.05
Secondary immunoglobulin heavy chain rearrangements in IgA cloned mice

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The generation of a diversified repertoire of Immunoglobulin Heavy (IgH) chains requires RAG-mediated VDJ recombination. Recent reports have suggested that secondary IgH rearrangements both in humans and mice contribute to the complexity of the primary antibody repertoire. Secondary IgH rearrangements have been preferentially studied under conditions where B cells express either non-functional or autoreactive IgH chains, raising the question of the physiological relevance of this process. This study estimated the contribution of secondary IgH rearrangements to primary antibody diversification under physiological conditions. We employed a novel mouse strain generated through the reprogramming of terminally differentiated intestinal IgA plasma expressing a non-autoreactive BCR. Heterozygous IgA (IgAH/+) mice allowed us to easily track secondary VH rearrangements. Surprisingly, almost 20% of peripheral B cells in IgAH/+ cloned mice expressed IgM instead of IgA. Sequencing of IgA rearrangements in sorted IgM+ B cells of IgAH/+ mice indicated that both VH replacement and direct VH-to-JH joining contributed to the disruption of the original VH rearrangement. The presence of n-nucleotides in the CDR3 region of secondary VH rearrangements indicated that these events occurred in pro-B cells. Interestingly, analysis of V-gene usage in secondary IgH rearrangements revealed a strong bias for V-genes proximal to the pre-rearranged VH gene. The preferential use of proximal VH genes for secondary VH rearrangements correlated with higher levels of germline transcripts detected in pro-B cells. Altogether these results reveal a major contribution of secondary IgH rearrangements to the diversification of the primary antibody repertoire under conditions of unperturbed B-cell development.

W3.04.06
The conservation of immunoglobulin genes: an evolutionary link between autoreactivity and innate response against pathogens

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Selection and physiologic production of protective natural antibodies (NAbs) have been associated with exposure to endogenous antigens. The extent to which this association depends on germline NAb sequence is uncertain. To test the role of natural selection of germline sequence in this cross-protection, in mice with gene-targeted alterations of DH sequence we evaluated responses against a hazardous self antigen, oxidized low density lipoprotein (OxLDL), and against phosphorylcholine (PC), a bacterial cell wall component. Here, we show that alterations in germline DH sequence can sever the association between the production of self-reactive NAbs and NAbs that afford protection against a pathogen. In unmanipulated hosts, the availability of the evolutionarily conserved DFL16.1 gene segment sequence profoundly affected the serum levels of NAbs against bacterial phosphorylcholine (PC), but not OxLDL. Mice with partially altered DFL16.1 sequence could use N nucleotides to recreate the amino acid sequence associated with the classical protective T15 idiotype positive NAbs, whereas those without DFL16.1 could not. DFL16.1 gene deficient mice proved more susceptible to challenge with live Streptococcus pneumoniae. We present data that support the moderating concept that natural selection of conserved DH sequence, and self-antigen driven somatic selection operate in concert to create a protective, functional NAb repertoire reactive with a bacterial cell wall component. In remarkable contrast, NAb reactivity and function in the case of an endogenous antigen representative of molecular debris does not show this dependence on evolutionarily conserved DH sequences. The potential relevance of these findings for the rational design of vaccines is discussed.

W3.05 Antigen processing and presentation

IL3.05.01
Compartment cross-talk controlling phagosome maturation and exogenous antigen presentation in dendritic cells

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Dendritic cells (DC) process internalized material in a manner optimized for simultaneous extraction of antigenic information for stimulation of adaptive immune responses and controlled activation of innate signaling. This optimization is thought to depend on DC-specific attenuated phagosome maturation due to fusion events with the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) on the one hand, and with inhibitory lysosome-related organelles on the other hand. Here we report a critical role of a specific slowly recycling endosomal compartment in DC-specific phagosome maturation. Formation and stability of the compartment requires a number of proteins including the aminopeptidase IRAP and Rab14.

Upon destabilization of the compartment by IRAP deletion or expression of Rab14 mutants, phagosome maturation is accelerated, associated with functional consequences described in the accompanying abstract by Saveanu et al. Interestingly, knockdown of Sec22b, described to mediate ERGIC-fusion with phagosomes, profoundly affects formation of the compartment. Our results point to a central role of a specific endosomal storage compartment in triggering of adaptive and innate immune responses by DCs.

W3.05.01
The true story of how MHC peptides are produced

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The cellular proteolytic pathways leading to the formation of the MHC peptidome were analyzed by dynamic metabolic labeling of cultured cells with stable heavy isotope heavy amino acids, followed by large-scale immunopeptidome and proteome analyses. The MHC molecules were immunoaffinity purified and the bound peptides were analyzed by capillary chromatography and mass spectrometry (dynamic-SILAC immunopeptidome analysis). Specific proteolysis inhibitors were used to define the relative contribution of the proteolytic pathways that produce the immunopeptidomes. Surprisingly, the proteasomes inhibitors, epoxomicin and bortezomib, affected the rate of synthesis of the cellular proteins and of their degradation products, the MHC peptides in a complex manner. While (as expected) the proteasome inhibitors reduced the rates of degradation of many cellular proteins, they increased the degradation (and synthesis) rates of others. Correlating between the rates of production of the source proteins and their derived HLA peptides suggests that the contribution of the proteasomal proteolysis to the production of the HLA peptidome should be reevaluated. It is possible that the proteasomal proteolysis contributes more significantly to the production of HLA peptides derived from newly synthesized proteins, possibly including defective ribosome products (DRiPs) and short lived proteins (SLiPs), while non-proteasomal pathway (such as the autophagosomal pathway) contribute more to the production of HLA peptides derived from stable, long-lived proteins.

W3.05.02
ER-resident editors optimize distinct features of the peptide cargo loaded onto MHC class I molecules

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Effective CD8+ T cell responses depend upon presentation of a stable peptide repertoire by MHC I molecules on the cell surface. The overall quality of pMHC I is determined by poorly understood mechanisms that generate and load peptides with appropriate consensus motifs onto MHC I. Here we show that both tapasin, a key component of the peptide loading complex, and ERAAP, the ER aminopeptidase associated with antigen processing, are quintessential editors of distinct structural features of the peptide repertoire. The T cell responses elicited by the pMHC I showed that absence of tapasin or ERAAP independently altered the peptide repertoire by causing loss as well as gain of new pMHC I. Changes in amino acid sequences of MHC bound-peptides revealed that ERAAP and tapasin respectively defined the characteristic amino and carboxy termini of canonical MHC I peptides. Thus, the optimal pMHC I repertoire is produced by two distinct peptide editing steps in the ER..

W3.05.03
A CD74-dependent MHC class I endolysosomal cross-presentation pathway

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Immune responses are initiated and primed by dendritic cells (DCs) that cross-present exogenous antigen. The chaperone CD74 (invariant chain) is thought to promote DC priming exclusively in the context of major histocompatibility complex (MHC) class II. However, we demonstrate here a CD74-dependent MHC class I cross-presentation pathway in DCs that had a major role in the generation of MHC class I–restricted, cytolytic T lymphocyte (CTL) responses to viral protein– and cell-associated antigens. CD74 associated with MHC class I in the endoplasmic reticulum of DCs and mediated the trafficking of MHC class I to endolysosomal compartments for loading with exogenous peptides. We conclude that CD74 has a previously undiscovered physiological function in endolysosomal DC cross-presentation for priming MHC class I–mediated CTL responses.
Our observations have defined a previously unknown pathway for the priming of immune responses; future studies should completely elucidate this process. Our results are of considerable clinical relevance and suggest that targeting vaccine candidates to the endolysosomes of DCs would enhance priming for both MHC class I and MHC class II antigens and thereby improve the immunogenicity and efficacy of vaccines.

W3.05.04
IRAP endosomes are an intracellular crossroad of innate and adaptive immunity

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An efficient immune response depends on tight cooperation between innate and adaptive immunity. Such cooperation requires dendritic cells (DCs) that detect pathogens via innate immune receptors including TLRs, and present antigens to specific T cells. Coupling TLR signaling to antigen presentation depends on cellular mechanisms that are still poorly understood. Here, we demonstrate that these mechanisms implicate a previously uncharacterized endosomal DC compartment bearing the features of storage endosomes. These endosomes contain, and are formed in the presence of the antigen trimming aminopeptidase IRAP (Insulin Responsive AminoPeptidase). We show that they are important for regulating the dynamics of phagosomal maturation and TLR9 signaling in DCs, in addition to the previously described role of IRAP in antigen trimming during cross-presentation. Destabilization of the endosomal storage compartment by IRAP deletion led to accelerated phagosome maturation, as documented by premature loss of early endosomal markers and accelerated acquisition of phagolysosome markers such as Lamp1 and V-ATPase. This was associated with faster degradation of phagocytosed antigen, enhanced acidification and increased killing of internalized microbes. IRAP deletion also affected the trafficking of TLR9, as illustrated by steady state mis-localization of the receptor to lysosomes. Altered TLR9 trafficking was correlated with a hyper-inflammatory phenotype of IRAP deficient mice, which displayed increased mortality during Pseudomonas aeruginosa infection. These data suggest a major role of IRAP in maintaining a functional storage endosome compartment required both for innate and adaptive immunity.

W3.05.05
CD4 T cell receptor transgenic mice recognizing an unstable peptide of insulin are directly recruited into islets bypassing local lymph nodes

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In the NOD model of autoimmune diabetes, a predominant component of the islet autoreactive CD4 T cell repertoire is directed against the B:9-23 segment of the insulin beta chain. Previous studies have established that the overwhelming majority of insulin reactive T cells specifically recognize a weak peptide-MHC binding register within the B:9-23 peptide. These T cells are uniquely stimulated when the B:9-23 peptide but not the insulin protein is offered to APC. Strong evidence suggests that these T cells are intimately involved in the autoimmune response leading to diabetes. Within the islets, resident APC routinely take up free peptides derived from the insulin protein found in the secretory granules of beta cells. As a result, islet APC constitutively present insulin peptides and have the capacity to trigger these T cells during disease development. To study these unconventional insulin reactive T cells in greater detail we generated a TCR transgenic (8F10) mouse. The peptide specific CD4 T cells in 8F10 mice are not negatively selected despite being actively recruited to the islets and being highly pathogenic. Additionally, we have found that the local lymph nodes are dispensable for the migration of 8F10 T cells into islets, indicating that these T cells can bypass an initial priming stage in the pancreatic lymph node previously thought to be critical prior to islet T cell entry. We believe these findings are highly relevant to the study of autoimmunity and provide new insight into the anatomical location of disease initiation in autoimmune diabetes.

W3.05.06
Structural basis of human beta-cell killing by CD8+ T cells in Type 1 diabetes

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Autoreactive T-cells play a role in type 1 diabetes (T1D) by killing insulin producing beta cells in the pancreas, although the mechanism has not been fully resolved. The antigen specific interaction between the T-cell receptor (TCR), on the T-cell surface, and peptide-major histocompatibility complexes (pMHCs) on the target cell surface, govern T-cell mediated immunity. Thus, we examined how T-cells kill human islet beta cells by solving the atomic structure of an autoreactive-TCR (1E6) specific for a MHC-restricted glucose-sensitive preproinsulin peptide (A2-ALW).
Rigid 'lock-and-key' binding underpinned the 1E6-A2-ALW interaction, whereby 1E6 docked similarly to most MHCI-restricted TCRs. However, this interaction was extraordinarily weak, due to limited contacts with MHCI. Thus, highly focused peptide-centric interactions associated with suboptimal TCR-pMHCI binding affinities might lead to thymic escape and potential T-cell-mediated autoreactivity.
This first sight of how T-cells target insulin producing beta cells has increased our understanding of how T1D arises. This knowledge will be used in the future to help predict who might get the disease, and also to develop new approaches to prevent it. Our aim is to catch the disease early before too many insulin-producing beta cells have been damaged.
This study has been recently published in Nature Immunology (Nature Immunology. 2012 Jan 15;13(3):283-9) and was featured in the News and Views section of the same issue.

W3.06 Costimulation

IL3.06.01
Autonomous CD28 signalling pathways in the regulation of T lymphocyte survival and pro-inflammatory functions

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CD28 costimulatory receptor is a crucial determinant of the outcome of T lymphocyte activation. The engagement of CD28 by its natural ligands, B7.1/CD80 or B7.2/CD86, expressed on the surface of professional APC, lowers TCR activation threshold, thus leading to the enhancement of early signalling events necessary for efficient cytokine production, cell cycle progression, survival and regulation of T cells effector responses. CD28 is also able to act as a unique signalling receptor and to deliver TCR-independent autonomous signals, which account for its critical role in the regulation of pro-inflammatory cytokine/chemokine production and T cell survival.
In the last years, we extensively worked on the characterization of CD28 autonomous signalling and we found that CD28 stimulation by B7 expressed on APCs, in the absence of TCR engagement, is able to recruits and activates IKKα and a non-canonical NF-κB2-like cascade leading to both the production of pro-inflammatory cytokine/chemokines and the activation of survival genes, which protect cells from apoptosis and ensure their long-term survival. The mechanisms and molecules coupling CD28 to NF-κB signaling pathway as well as their functional relevance in immune diseases will be discussed.

IL3.06.02
Viruses and type 1 diabetes - the good and the bad

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We have been studying the histopathology of human type 1 diabetes as part of the nPOD consortium (www.jdrf.nPOD.org). This has offered important insight into the development of human type 1 diabetes including some surprises, which could make us rethink therapeutic priorities in some cases. Three interesting, yet unexplained, findings indicate the potential involvement of viral infections in triggering or re-igniting insulitis that then leads progressively to beta-cell loss: First, insulitis cannot be consistently documented prior to the onset of diabetes clinically, raising the possibility that human diabetes in its development is a relapsing-remitting disorder. Second, many human islet over-express uniformly MHC class I after diagnosis of diabetes (a phenomenon not present in healthy controls or type 2 diabetes), which is not seen that pronounced and uniformly in mouse models, the cause for this being unknown. Last, human diabetes appears develop in a lobular fashion and not randomly affecting islets throughout the pancreas, as this is usually seen in rodent models. Thus, we have to consider that enteroviral (or possibly even herpes virus) infections could constitute at least an additional factor that contributes to diabetes pathogenesis. On the other hand, studies in experimental animal models clearly show that viral infections can also prevent diabetes (enteroviruses, rotaviruses). This occurs through mechanisms that 'reset' the immune system, eliminate autoreactivity and enhance immune regulation, which are byproducts of successfully overcome viral infections. Systematic studies in humans such as the ones initiated by nPOD-V, VIDIS and PEVNET will be critical in resolving this important dilemma.

W3.06.01
Mechanical Insights into the functional impacts of interactions between antigen-presenting cells and T cells

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Antigen recognition and discrimination by T lymphocyte are essential in initiating appropriate immune responses. The mechanisms underlying exquisite sensitivity and specificity of antigen discrimination are not fully elucidated but involved physical intercellular interactions between T cell and antigen-presenting cell (APC) including dendritic cells (DCs) and B cells. Using single cell force spectroscopy (SCFS), we have successfully probe mechanical interactions between DC and T cells in response to a panel of altered peptide ligands (APLs). In the presence of different type of APLs, DC:T conjugate displays different ranges of mechanical forces that match their corresponding T cell responsiveness. Strong cell-cell interaction forces are contributed not only by surface adhesion molecules including TCR:pMHC, CD28:B7 and ICAM-1:LFA-1 interaction pairs, but also dependent on the integrity of membrane cholesterol and cytoskeleton dynamics. As compared to B:T interactions, stronger DC:T cell-cell interactions are likely to provide a mechanically stable environment that induces potent functional T cell activation, suggesting that the measurement of mechanical forces could be a significant predictor of T cell functional activation efficacy. Our SCFS studies provide mechanical insights into the adhesive roles of cell surface proteins in regulating APC:T cell-cell interactions. Functional roles of TLR ligands in regulating APC:T interactions and T-cell responses will be discussed.

W3.06.02
Basilic, a lymphoid-specific protein essential for CD28 costimulation and regulatory T cell development

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Background: Early studies have led to a "two-signal model" of T cell activation in which the first signal is delivered via the T cell antigen receptor following recognition of antigenic peptides bound to class I and II molecules of the major histocompatibility complex. This signal provides antigen specificity to T cell responses. The second signal is known as the costimulatory signal and is delivered by the CD28 molecule upon recognition of the CD80 and CD86 ligands that are expressed on antigen presenting cells.
Methods: By using an N-ethyl-N-nitrosourea-mutagenesis screen aiming at discovering effectors of CD28-mediated costimulatory signals, we identified a mutation in the functionally uncharacterized Basilic gene.
Results: We showed that Basilic is a T cell-specific protein that is essential for CD28 costimulation and regulatory T cell development. TCR-CD28 engagement at the immune synapse resulted in the spatial colocalization of CD28 with wild-type and mutant Basilic forms. However, the Basilic mutant form prevented CD28 signaling by abrogating the connection between CD28 and protein kinase C-θ and Carma1, two key effectors of CD28 costimulation.
Conclusions: Our findings provide a more complete model of CD28 costimulation in which Basilic plays an essential role.

W3.06.03
Human memory T-cell subsets require distinct costimulatory signals to efficiently expand ex vivo

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Costimulation plays a critical role for activation of T cells in response to pathogens, cancer and in alloreactive immune responses. However, costimulatory requirements of memory T cell responses have been much less studied than those for primary response generation, especially in humans. In the present work, we evaluated the ability of several costimulatory molecules (the CD28-family member CD28 and ICOS, and the TNFR-family members 4-1BB, OX40, GITR, CD27, CD30, HVEM) in expanding ex-vivo human memory T-cell subsets. We assessed the effect of these costimulatory molecules on central memory (TCM), effector memory (TEM) and on the recently identified memory stem T cells (TSCM). Memory T-cell subsets were FACS-sorted, according to the expression of CD45RA/CD62L and CD95, activated by coated anti-CD3 antibody in the presence of anti-costimulatory molecules antibodies and cultured with low dose homeostatic cytokines. The efficacy of costimulatory molecules was measured in terms of T-cell expansion and preservation of the original surface phenotype. Strikingly, we found that each memory T-cell subset best responded to distinct costimulatory signals. TSCM lymphocytes expanded, while maintaining a higher fraction of cells with the original and less-differentiated phenotype, when stimulated in the presence of OX40- and GITR-mediated signals, while TCM best performed when CD28- and 4-1BB-costimulated. Finally, TEM cells expanded to higher numbers in response to CD27 costimulation. We are currently evaluating the cytokine secretion profile of differentially costimulated T-cell subsets. Altogether, these findings may help in designing new strategies to boost immunity against immune-evading pathogens and tumors, as well as to increase vaccine efficacy.

W3.06.04
Differential usage of costimulatory/coinhhibitory pathways by antigen-specific CD8+ T cells in patients with chronic hepatitis C

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The function of antigen-specific T cells is regulated by a sophisticated network of co-stimulatory/inhibitory receptors. Particularly, hepatitis C virus (HCV), Epstein-Bar virus (EBV) and cytomegalovirus (CMV) that establish lifelong infections show upregulation of an expanding repertoire of distinct coinhibitory receptors on virus-specific CD8+ T cells. The differential usage of costimulatory pathways by CMV, EBV and HCV-specific CD8+ T cells has not been studied thus far. It also remains unknown if antigen-specific CD8+ T cell responses to CMV and EBV are altered in the face of secondary infections such as chronic hepatitis C. To investigate these, we assessed the proliferation, degranulation and cytokine production of CMV-, EBV- and HCV-specific CD8+ T cells after *in vitro* blockade of PD-1, CTLA-4, Tim-3 and 2B4 in varying combinations in PBMC of healthy blood donors and chronic HCV patients. We also analysed expression of different costimulatory molecules by virus-specific CD8+ T cells *ex vivo* by flow cytometry. We found that at the individual level, a 'private pattern' of co-stimulatory receptor usage determines the functionality of CMV-, EBV- and HCV-specific CD8+ T cells in chronic HCV patients. Notwithstanding, we observed that regulation of virus-specific CD8+ T cell responses by costimulatory molecules is similar between CMV and EBV but distinctly different from HCV at the level of proliferation and interferon-γ production. Finally we report that CMV or EBV-specific CD8+ T cells in chronic HCV patients and healthy individuals use similar patterns and hierarchies of costimulatory molecules.

W3.06.05
PD-1 controls effectors but not the generation or function of natural or induced regulatory T-cells

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During T cell activation, the balance of co-stimulatory and co-inhibitory interactions plays an important role in shaping the resulting T cell response. Co-inhibitory signals help maintain peripheral immune tolerance, and thus animals with defective co-inhibitory molecules, such as PD-1 KO mice, are predisposed to autoimmunity. Unlike PD-1 KO mice, which develop mild autoimmunity, adult Rag KO recipients of PD-1 KO hematopoietic stem cells (HSC) succumb

to rapid and severe multi-organ autoimmune disease soon after newly generated T cells emerge from the thymus, implicating PD-1 as an especially critical controller of T cell self-reactivity during lymphopenia induced homeostatic proliferation (LIP). While we have found no reduction in absolute numbers of FoxP3+ regulatory T cells (Treg) in diseased PD-1 KO vs. wild type (WT) HSC recipients, some studies have indirectly suggested that PD-1 deficiency may inhibit the conversion of conventional T cells to induced Treg (iTreg) in the periphery. We therefore investigated whether PD-1 deficiency results in an intrinsic inability of T cells to convert to iTreg in vivo, which could underlie disease in PD-1 KO HSC recipients. Surprisingly, our findings show that PD-1 deficiency increases the numbers of iTreg and does not reduce Treg suppressive function. In the context of LIP, co-inhibitor deficient effector T cells cause disease primarily by expanding beyond the ability of Treg to control them. The finding that PD-1 restrains the effector response rather than promoting iTreg may guide application of immunotherapeutics currently under investigation to block human PD-1 in cancer.

W3.06.06
Immunogenic, but not steady-state, antigen presentation permits Treg control of CD8+ T-cell effector differentiation through IL-2 modulation

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One proposed mechanisms of Treg suppression is absorption of IL-2 by Treg and in vivo experimental evidence for this has recently been obtained. While modulation of IL-2 bioavailability controls CD8+ effector differentiation under strongly immunogenic conditions it is not known whether this modulates CD8+ T cell responses under steady-state conditions. Here we assess this using a model in which dendritic cells (DC) are manipulated to present cognate antigen to CD8+ T cells either in the steady-state or when activated. Our observations show Treg exert a check on expansion and effector differentiation of CD8+ T cells, under strongly immunogenic conditions associated with TLR ligand activation of DC and this is mediated by limiting IL-2 availability. In contrast, when DC remained unactivated, depletion of Treg had little apparent effect on effector differentiation or IL-2 homeostasis. We conclude that while modulation of IL-2 homeostasis is an important mechanism by which Treg control CD8+ effector differentiation under immunogenic conditions, this mechanism plays little role in modulating CD8+ T-cell differentiation under non-immunogenic or tolerogenic conditions.

W3.07 B cell development and plasma cell differentiation

W3.07.01
The Ras/Erk/PI3K pathways during positive selection of primary B cells

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Expression and signaling of the B cell antigen receptor (BCR) guide the differentiation and selection of immature B cells. The BCR is also able to signal in the absence of ligand binding, a response called "tonic". We have found that tonic BCR signaling in immature B cells propagates via Erk and PI3K through a pathway that can be activated by Ras. These pathways promote the differentiation of non-autoreactive immature B cells and their selection into the peripheral B cell pool. Here, we propose that alterations of the Ras/Erk/PI3K pathways can modify the selection of immature B cells and the composition of the peripheral B cell pool, potentially increasing the frequency of autoreactive B cells. We demonstrate that these pathways are less active in autoreactive cells than in non-autoreactive cells. Morevover, we show that activation of the tonic BCR signaling cascade within autoreactive immature B cells breaks central tolerance and leads to the differentiation into transitional B cells via the same Erk and PI3K pathways. Our findings support a model whereby arrest in differentiation and central B cell tolerance are caused by the absence of tonic BCR signaling rather than the presence of self-antigen-mediated signals.
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W3.07.02
Characterization of Umbilical Cord Blood CD34+CD43+CD19+CD38lo/int Cells Suggests a Common Progenitor for Human B1 And B2 Cells

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Recently Vuyyuru et. al. reported that human Umbilical Cord Blood hematopoietic stem cells (UCBHSC) engrafted NSG (HIS) mice generate protection against Borrelia hermsii, which causes relapsing fever in infected humans, and is counteracted by murine B1b cells in WT mice. A substantial number of HIS B cells displayed the human B1-phenotype, CD19+CD27+CD43+CD70-. Importantly, we confirmed that only these B cells spontaneously secreted IgM antibody, a characteristic of B1 cell function. Together, we found that CD34+ UCBHSC can give rise to both B1 and B2 cells. To further identify human B1 potential progenitors, we characterized the phenotype of human CD34+ UCBHSC utilizing multi-color flow cytometry. Ex vivo analysis of CD34+ UCBHSC cells showed that 90% of CD34+CD38lo/int cells express CD43. As cells differentiated into B cell lineage, identified by the dual expression of CD19 and CD10, most early B cells lost CD43 while gaining higher CD38 and surface Ig expression. Interestingly, a small fraction of B lineage committed cells retained CD43 while gaining CD38 and surface Ig expression. To investigate the progenitor potential of CD34+CD38lo/int and CD34+CD38int/hi, we co-cultured sorted CD34+CD38lo/int and CD34+CD38int/hi cells with human primary stromal cells. We found that sorted CD34+CD38lo/int cells gave rise to both CD34+CD19+10+CD43+CD38hi and CD34+CD19+CD10+CD43-CD38hi cells, whereas sorted CD34+CD38hi cells generated mostly CD34+CD19+CD10+CD43-CD38hi B cells. Our findings suggested CD34+CD43+CD19+CD38lo/int as the common progenitors for both human B1 and B2 cells, and as these cells developed, B2 cells lost both CD34 and CD43 expression, whereas B1 cells only lost CD34 expression while sustaining CD43 expression.

W3.07.03
A human marginal zone B cell precursor

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In mice, marginal zone B cells (MZB) represent a distinct B cell lineage which arises in the spleen where marginal zone precursor cells (MZP) differentiate under the control of the Notch2 pathway. In humans, the existence of such a lineage is still controversial. We propose here that in humans, blood IgM+IgD+CD27+ B cells represent circulating splenic MZB cells with a differentiation pathway that could be conserved at least in part between mice and humans. We identified in human spleen a putative MZP subset, characterized by its capacity to differentiate into MZB-like cells through Notch2 activation *in vitro*. This subset which is IgM^{high} IgD^{high} $CD27^{neg}$ $ABCB1^{pos}$ $CD24^{int}$ and $CD1c^{int}$, can be distinguished from naive cells by the presence of a glycosylated variant of the CD45RB molecule. It accounts on average for 5% of total splenic B cells in children and decreases throughout life. A transcriptomic analysis confirmed that MZP represent an intermediate differentiation stage between naive and MZB cells. A Notch induction signature predominated among genes discriminating MZP from naive B cells, a signature further developed among MZB cells. We next analyzed the blood of three patients with an inactivating mutation in one Notch2 allele (a subset of Allagille syndrome) and observed a specific decrease of IgM+IgD+CD27+ MZB cells whereas switched memory B cells were not impacted, similarly to the phenotype seen in Notch2-

haploinsufficient mice Altogether, our results suggest that the early development of human blood and splenic MZB cells, proceeds, as in the mouse, through a Notch2-dependent differentiation pathway..

W3.07.04
The ELL-associated factor 2 negatively regulates germinal center B cell survival and humoral immune responses

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The germinal center (GC) B cells undergo Ig gene hypermutation and those with increased affinity for antigen are selected to survive and differentiate into plasma or memory cells whereas those with deleterious/nonsense mutations are eliminated by apoptosis. It is not well understood how GC B cell survival is regulated. We found that the ELL-associated factor 2 (EAF2) is selectively and highly expressed in GC B cells. Mice deficient in EAF2 contained a higher frequency of GC B cells than did WT mice after immunization with the T-dependent (TD) antigen NP-CGG (4-hydroxy-3-nitrophenyl-acetyl coupled to chicken gamma-globulin). Immunohistochemical staining of spleen sections revealed that EAF2-deficient mice had a 2-fold increase in the size of GC and decreased $TUNEL^+$ apoptotic cells compared with WT mice. In addition, GC B cells isolated from EAF2-deficient mice exhibited enhanced survival compared with WT GC B cells under *in vitro* culture conditions. These results collectively suggest that EAF2 promotes the apoptosis of GC B cells. Consistently, EAF2-deficient mice produced increased levels of NP-specific antibodies in response to NP-CGG than WT mice. Furthermore, the mutant mice also showed elevated antibody production against the T-independent (TI) antigen NP-Ficoll than did WT mice. These results demonstrate that EAF2 mediates apoptosis of GC B cells and negatively regulates humoral immune responses against both T-D and T-I antigens.

W3.07.05
Epstein-Barr virus-encoded Latent membrane protein 2A impairs B cell selection in germinal centers (GCs) but not GC formation

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Germinal center (GC) reaction is a critical process for generation of high-affinity B cells. It is known that Epstein-Barr virus (EBV) infect GC B cells and persist in memory B cells and is associated with lymphoid malignancies derived from GC B cell origin. EBV-infected GC B cells express EBV-encoded latent membrane protein 2A (LMP2A) that is known to mimic B-cell antigen receptor signals. To determine whether LMP2A affects GC reaction, we generated knock-in mice expressing LMP2A in GC B cells ($LMP2a^{GCB}$). Normal GC formation and increased numbers of $CD138^+$ plasma cells were observed in spleen of $LMP2a^{GCB}$ mice after immunization with NP-CGG. However, $LMP2a^{GCB}$ mice had markedly reduced number of NP-specific B cells and significantly lower serum levels of NP-specific IgG. Sequencing analysis of Ig heavy chain from NP-specific B cells revealed the significantly reduced frequency of $V_H186.2$ gene usage but not of somatic hypermutation in $LMP2a^{GCB}$ mice. *In vitro* Ig production assay demonstrated that LMP2A inhibited neither Ig production nor Ig class switching. In addition, elevated serum levels of autoantibodies were observed in $LMP2a^{GCB}$ mice. These results suggest that LMP2A-mediated signals perturb GC selection in which low affinity B cell clones such as autoreactive B cells would be generated. We discuss the molecular mechanism by which LMP2A contributes to latent infection of EBV in GC and memory B cells.

W3.07.06
IgE-expressing B cells are restrained by an intrinsic cell fate predisposition

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IgE antibodies are best known for their role in allergic disease. The pathological hypersensitivity reactions mediated by IgE, coupled with the extremely low abundance of IgE in serum, suggest that IgE+ B cell responses are normally tightly regulated. However, direct studies of IgE+ B cells have been hindered by substantial technical challenges in the detection of these rare cells. To overcome these limitations, we have generated IgE reporter mice, in which membrane IgE expression is translationally linked to the expression of the yellow fluorescent protein Venus. In these mice, IgE+ B cells can be readily detected by flow cytometry and microscopy. In addition, we have developed an improved flow cytometry procedure to detect IgE+ B cells in any strains of mice.
We have used these new tools to study the genesis and fate of IgE+ B cells during primary immune responses. Initially, IgE+ B cells differentiated into both plasma cells and germinal center B cells, similar to IgG1+ B cells generated in parallel. However, IgE+ B cells showed an atypical propensity to upregulate the transcription factor Blimp-1 and to undergo accelerated differentiation into short-lived plasma cells, which contributed to the loss of IgE+ B cells from the germinal center. As a result, the duration and affinity maturation of the IgE antibody response were limited. We also observed that the differentiation of activated B cells into plasma cells is affected both intrinsically by the isotype of the B cell receptor that is expressed and extrinsically by the concomitant activation signals.

W3.08 Th1 and Th2 cells

W3.08.01
Crucial role of IL-18R1 signaling in Th1 cell differentiation in mice infected with Trypanosoma cruzi

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The myeloid differentiation protein 88 (MyD88) has been implicated in the polarization to Th1 response through the induction of IL-12 in dendritic cells (DC), following TLR activation. As expected, MyD88-deficient mice are highly susceptible to infection with different intracellular parasites, including the human parasite *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. In fact, IFN-gamma production by $CD4^+$ T cells is severely affected in $MyD88^{-/-}$ mice infected with *T. cruzi.* However, we have shown that CD8 T cell-mediated responses are intact in MyD88-deficient mice, suggesting that DC function is not completely abolished in MyD88-deficient mice. As MyD88 is also an essential adaptor for signaling through IL-1R and IL-18R, we aimed to investigate the intrinsic role of MyD88 expression in CD4 and CD8 T cells during infection with this parasite. In order to answer these questions, we analyzed the production of IFN-gamma by spleen CD4+ and CD8+ T cells in mixed (WT + MyD88KO → WT) bone marrow chimeric mice infected with the Y strain, by intracellular staining and flow cytometry. We also analyzed mixed (WT + $IL\text{-}1R1^{-/-}$ → WT) and mixed (WT + $IL\text{-}18R1^{-/-}$ → WT) bone marrow chimeras and measured CD4+ T cell *in vivo* proliferation by BrDU incorporation. Our results show that MyD88 expression in CD4+ T cells, by signaling through IL-18R1, is necessary for the expansion of the Th1 subset induced during infection with *T. cruzi*.
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W3.08.02

Pro- and anti-inflammatory cytokine signals control the balance between Th1 and Tfh differentiation during the first few days of viral infection

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During viral infections, anti-viral CD4 T cells receive multiple sometimes opposing cytokine signals, but how these cells interpret and respond to all of these signals is not well understood. We have profiled the T_H1 and T_{FH} responses during acute viral infection from the earliest activation events through memory cell formation. During acute LCMV infection, $Ly6C^{lo}$ effector T_H1 cells gave rise to functional T_H1 memory cells and within the first 2 days of infection, and the earliest activated T cells had already bifurcated into $Ly6C^{hi}$, $CD25^{hi}$, $PSGL1^{hi}$, $T\text{-}bet^{hi}$, $BCL6^{lo}$ (T_H1 precursor) and $Ly6C^{lo}$, $CD25^{lo}$, $PSGL1^{lo}$, $T\text{-}bet^{lo}$, $Bcl6^{hi}$ (T_{FH} precursor) subsets. The T_H1 precursor population was also the greatest IL-2 producer at these very early time points, prior to elaboration of IFN-gamma. Furthermore, the first anti-viral CD4 T cells to exit the T zone into the inflammatory red pulp were T_H1 precursors. Direct IFN-I was not required for T_H1 precursor formation, but was sufficient to promote IL-2 responsiveness and was required for the migration of T_H1 precursor cells out of the white pulp. Gene expression profiling of T_H1 and T_{FH} effector cells revealed a profound TGF-beta signature in anti-viral $FoxP3^-$ T_{FH} cells and indeed, we found that direct TGF-beta signals promoted the differentiation of T_{FH} precursor cells. Together, these studies identify novel pro- and anti-inflammatory signals that control the balance between anti-viral T_H1 and T_{FH} differentiation during the first few days of infection and have a profound impact on the overall effector and memory cells generated.

W3.08.03

CD4+ T cells are both trigger and target of the glucocorticoid response that prevents lethal immunopathology in toxoplasma infection

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While synthetic glucocorticoids (GC) are commonly used in the treatment of inflammatory diseases, the role of endogenous GC in the regulation of host-protective immune responses is poorly understood. Here we show that GC are induced during acute *Toxoplasma gondii* infection and directly control the T cell response to the parasite. When infected with toxoplasma, $GR^{lck\text{-}Cre}$ mice that selectively lack GC receptor expression in T cells undergo acute mortality despite displaying parasite burdens indistinguishable from control animals and unaltered levels of the innate cytokines IL-12 and IL-27. Mechanistically, the excessive immunopathology of infected $GR^{lck\text{-}Cre}$ animals was associated with hyperactive Th1 cell function *in vivo*, but not *in vitro*, as revealed by enhanced IFN-g and TNF production. Unexpectedly, these $CD4^+$ T lymphocytes also overexpressed IL-10. Importantly, $CD4^+$ T cell depletion in either wild-type or $GR^{lck\text{-}Cre}$ mice led to ablation of the GC response to infection. Moreover, in toxoplasma infected $RAG2^{-/-}$ animals, adoptive transfer of $CD4^+$ T cells was required for GC induction. These findings establish a novel IL-10-independent immunomodulatory circuit in which Th1 cells trigger a GC response that in turn dampens their own effector function. In the case of *T. gondii* infection, this self-regulatory pathway is critical for preventing collateral tissue damage.

W3.08.04

Blimp-1 triggers the formation of immunoregulatory IL-10 producing Th1 cells during chronic viral infection

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During many chronic viral infections, the anti-viral T cell response becomes attenuated in a process that is partly host regulated. While elevated expression of the immunosuppressive cytokine IL-10 is involved in this process, the relevant cellular sources of IL-10, as well as the pathways responsible for IL-10 induction, remain unclear. In this study, we trace IL-10 production over the course of chronic lymphocytic choriomeningitis virus (LCMV) infection using an IL-10 reporter mouse line. We demonstrate that “exhausted” virus-specific T cells, particularly virus-specific Th1 cells, display elevated IL-10 expression during chronic LCMV infection and that ablation of IL-10 from the T cell compartment can partially restore T cell function and reduce viral loads. We find that Blimp-1 is required for IL-10 expression by Th1 cells and implicate antigen as the likely Blimp-1/IL-10 induction signal. Thus, effector T cells self-limit their responsiveness during persistent viral infection via an IL-10-dependent negative feedback loop.

W3.08.05

RNA-binding protein HuR coordinately regulates GATA-3 and CD4+ Th2 cytokine gene expression in dose-dependent manner

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Posttranscriptional control by RNA binding proteins (RBPs) and microRNAs of CD4+ T cells is poorly understood. Asthma is driven by GATA-3, IL-4 and IL-13. The RBP, HuR, posttranscriptionally regulates IL-4 and IL-13. GATA-3 3’ UTR contains AU-rich elements (ARE) which are binding sites for HuR. We identified GATA-3, IL-4 and IL-13 as HuR targets using RNA immunoprecipitation applied to microarrays. We hypothesized that HuR may be coordinately regulating Th2 differentiation. We used a HuR over-expression transgenic mouse, as well as a HuR conditional mouse ($HuR^{fl/fl}$) to ablate HuR in activated T cells, as well as siRNA and lentiviral shRNA to knock-down HuR. HuR over-expression stabilized GATA-3, IL-4 and IL-13 mRNAs, leading to increases in mRNA and protein. Conversely, HuR RNAi produced opposite results. These findings were confirmed in human lymphocytes. We verified GATA-3 is a HuR target by IP and biotin pull-downs and defined binding sites. Th2 polarized cells with reduced HuR levels (26%) from $HuR^{fl/+}$ mice, had significant decreases in IL-4, IL-13 and GATA-3 mRNA but not protein. Surprisingly, Th2 cells from $HuR^{fl/fl}$ mice with HuR knockdown (93%) showed significantly increased IL-4, IL-13 mRNA and protein levels but no changes in GATA-3 or IFNg. We measured mRNA transcription, stability and translation for cytokine mRNAs. IL-4 transcription was increased but not its stability. In contrast, IL-2 and IL-13 transcription were unchanged but their transcripts were stabilized. Polysomal gradient analysis revealed equal rates of translation. Further studies defining how RBPs regulate GATA-3 and Th2 cytokines in asthma will be critical.

W3.08.06
Modifications of the bone marrow microenvironment in the transition from monoclonal gammopathy of undetermined significance to multiple myeloma in Vk*MYC mice

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Multiple myeloma (MM) is a neoplastic plasma cell (PC) disorder of the oldest, and it is characterized by clonal proliferation of PC in the bone marrow (BM), monoclonal protein in biological fluids and organ dysfunction. While monoclonal gammopathy of undetermined significance (MGUS) often anticipates MM, the role of the cellular components of the BM microenvironment in the shift from MGUS to MM is unknown. To this aim, we have investigated disease development and progression in the Vk*MYC mouse model of spontaneous MM.
We have found that the BM of Vk*MYC mice is the primary organ of PC accumulation and based on the frequency of PC in the BM and M-spike quantification, we were able to distinguish between MGUS and MM in the Vk*MYC mice. The transition from MGUS to MM in these mice was characterized by increased vascularization of the BM. A comparison between age-matched Vk*MYC and WT mice affected by MGUS revealed precocious alteration of the CD4/CD8 T cell ratio, increased percentage of IL2 and IL4 producing CD4+ T cells, and CD8+ T cells more prone to produce IFNγ in the BM of the former.
In summary, our data suggest that early in disease development, yet unknown factors elicit an inflammatory reaction that significantly modifies the cellular composition of the BM and associates with neoangiogenesis and PC accumulation. Being the pathology in the Vk*MYC mouse similar to the human disease, our data might be relevant to understand the mechanisms of progression from MGUS to MM in humans.

W3.10 Th17 cells

IL3.10.01
Contribution of IL-17 to the chronicity of inflammatory diseases

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IL-17 was first identified as a T cell derived cytokine with effects on inflammation and neutrophil activation. Using the example of Rheumatoid arthritis (RA), it was next shown that its inhibition induced a significant reduction in the production of inflammatory mediators by explants of inflamed synovitis. This effect resulted from synergistic interactions between IL-17 and other proinflammatory cytokines such as TNF or IL-1. In addition to its effect on inflammation and related destruction, the role of IL-17 was shown in RA chronicity through an effect on reduced apoptosis of synoviocytes. Exposure of synoviocytes to IL-17 reduced NO-induced apoptosis. At the same time, enhanced expression of anti-apoptotic molecules such as synoviolin was observed in the blood of RA patients, specifically those not responding well to the TNF inhibitor infliximab. Extension to a mouse model indicated that mice lacking the IL-17R had reduced arthritis, reduced synovium infiltrate but increased local apoptosis. Increased local production of IL-17 may result from local cell-cell interactions. Indeed, interaction between activated T cells with various mesenchymal cells, either from bone marrow, synovium, or skin was found to favor the switch towards the Th17 pathway through a caspase-1 monocyte-driven mechanism. Taken together, these results support the early targeting of IL-17 in chronic inflammation associated with matrix destruction. Reduced effect of IL-17 inhibition can be expected when inflammation had a long term effect on mesenchymal cells. Recent clinical results are in line with these observations.

W3.10.01
Phosphorylation status determines the divergent roles of Smad2 and Smad3 as STAT3 cofactors in Th17 differentiation

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Transforming growth factor-β (TGF-β) and interleukin (IL)-6 are the pivotal cytokines to induce IL-17-producing $CD4^+$ T helper cells (T_H17), which play pathogenic roles in inflammatory diseases. Although crucial roles of IL-6 signaling through STAT3 in T_H17 differentiation have been well demonstrated, roles of TGF-β signaling through Smads are still controversial. Here we show that distinct phosphorylation status of the highly homologous TGF-β receptor-regulated Smads (R-Smads) divergently regulate STAT3-induced T_H17 differentiation. Smad2 deficiency ameliorated murine collagen-induced arthritis (CIA) with decreased T_H17 cells, whereas Smad3 haploinsufficiency exacerbated CIA with increased T_H17 cells. Smad2 and Smad3 exerted the opposite effects on STAT3-induced transcription of IL-17A and the retinoic acid receptor-related orphan nuclear receptor, RORγt (encoded by *Rorc*), an essential transcription factor for induction of IL-17. Smad2 phosphorylated at the linker region, serine 255 interacted with STAT3 to enhance transcription of RORγt and IL-17A. By contrast, Smad3 MH2 domain interacted with STAT3 to suppress STAT3-induced T_H17 differentiation by regulating different sets of genes through distinct C-terminal phosphorylation status: C-terminally unphosphorylated Smad3 recruited protein inhibitor of activated STAT3 (PIAS3) to repress STAT3-induced transcription of RORγt and IL-17A, while C-terminally phosphorylated Smad3 enhanced STAT3-induced transcription of the JAK/STAT inhibitors: SOCS3, SHP1/2. Thus, cite-specific phosphorylation status of R-Smads determines their divergent functions as transcription cofactors of STAT3 to regulate T_H17 differentiation.

W3.10.02
Transcriptional regulation of human Th17 cell differentiation

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Dissecting the molecular mechanisms by which naïve CD4+ T cells differentiate to effector Th17 cells is important for understanding the pathogenesis of inflammatory and autoimmune diseases. Most of our current understanding on Th17 cell differentiation relies on studies done in mouse, whereas the molecular mechanisms controlling Th17 cell differentiation program in human are not well defined.
We aim at identifying gene regulatory networks and molecular machinery regulating early stages of human Th17 cell differentiation program through combined RNA interference, genome-wide experimental and computational approaches. As a first step, naïve CD4+ T cells isolated from umbilical cord blood were used to construct detailed kinetics of gene expression after initiation of Th17 differentiation (Tuomela et al. Blood 2012, 119:e151-60). Genome-wide expression analysis of the cells polarizing towards Th17 phenotype, demonstrated differential regulation of several novel and known genes associated with Th17 differentiation. The differential expression of selected candidate genes were further validated at protein level and analyzed for their specificity in initiation of Th17 as compared to initiation of other T helper subsets. Using this dataset as a starting point for constructing the gene regulatory network, we selected to study further the role of STAT3, a transcription factor influencing Th17 polarisation. We have combined RNAi and ChIP sequencing to identify direct and indirect STAT3 targets during Th17 cell differentiation. Further analysis is in progress. Finally, this integrated data set on STAT3-mediated transcriptional network will

provide a basis for modulating Th17 mediated pathogenic and autoimmune immune responses in human.

W3.10.03
Protein C receptor (PROCR) regulates pathogenic phenotype of Th17 cells

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We performed a temporal microarray analysis of developing Th17 cells to identify cell surface molecules, which are differentially expressed in Th17 cells and regulate the development of Th17 cells. We found protein C receptor (PROCR) to be specifically expressed in Th17 cells. Focusing on the regulation of PROCR expression, we demonstrated that the Th17-specific transcription factors Rorγt, IRF4, and STAT3 bound to the promoter of *PROCR* by chromatin immunoprecipitation (ChIP)-PCR. Furthermore, we found PROCR expression was dramatically reduced in Th17 cells from Rorγt KO, IRF4 KO, or STAT3 KO mice, indicating these Th17-specific transcription factors induce PROCR expression. To elucidate the biological function of PROCR in Th17 cells, we overexpressed PROCR in T cells and observed that PROCR decreased the expression of genes identified as part of the pathogenic signature of Th17 cells, including CXCL3, IL-3, and CCL4. To specifically address the role of PROCR in Th17 cells *in vivo*, we overexpressed PROCR in myelin oligodendrocyte glycoprotein-specific Th17 cells *in vitro* and adoptively transferred these cells to induce experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis. In line with our *in vitro* data, PROCR reduced the pathogenicity of Th17 cells as recipients of PROCR expressing Th17 cells displayed ameliorated disease. In conclusion, we identified PROCR as a receptor that is specifically expressed in Th17 cells and regulates the pathogenic phenotype in Th17 cells. Furthermore, we found Rorγt, IRF4, and STAT3 regulated PROCR expression in Th17 cells. Finally, we demonstrated PROCR could reduce Th17 pathogenicity *in vitro* and *in vivo*.

W3.10.04
IL-17A secretion by CD8+ T cells supports Th17-mediated autoimmune encephalomyelitis

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IL-17-producing $CD8^+$ T (Tc17) cells are detectible in multiple sclerosis (MS) lesions; however their contribution to the disease is unknown. To identify functions of Tc17 cells, we induced experimental autoimmune encephalitis (EAE), a murine model of MS, in mice lacking interferon regulatory factor 4 (IRF4). IRF4-deficient mice failed to generate Tc17 and Th17 cells and were resistant to EAE. After adoptive transfer of wild-type $CD8^+$ T cells and subsequent immunization for EAE induction in these mice, the $CD8^+$ T cells developed a Tc17 phenotype in the periphery but could not infiltrate the CNS. Similarly, transfer of small numbers of wild-type $CD4^+$ T cells alone did not evoke EAE, but when transferred together with $CD8^+$ T cells, IL-17-producing $CD4^+$ (Th17) T cells accumulated in the CNS and mice developed severe disease. Th17 accumulation and development of EAE required IL-17A production by $CD8^+$ T cells, suggesting that Tc17 cells are required to promote $CD4^+$ T cell-mediated induction of EAE. Accordingly, patients with early-stage MS harbored a greater number of Tc17 cells in the cerebrospinal fluid than in peripheral blood. Our results reveal that Tc17 cells contribute to the initiation of CNS autoimmunity in mice and humans by supporting Th17-cell pathogenicity.

W3.10.05
Development and stability of human Th17 cells require endogenous NOS2 and cGMPc/GK-dependent NO signaling

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Nitric oxide (NO), a mediator of inflammation and immunity, is involved in the pathogenesis of and resistance to cancer, autoimmunity and infectious diseases. We observed that the local markers of Th17 responses in ovarian patients cancer positively correlated with the intarumoral expression of nitric oxide synthase-2 (NOS2/iNOS). While high concentrations of exogenous NO indiscriminately suppressed Th1, Th2, and Th17 responses, the physiologic NO concentrations produced by patients' myeloid-derived suppressor cells (MDSCs) promoted the development of $ROR\gamma t(Rorc)^+IL\text{-}23R^+IL\text{-}17^+$ Th17 cells.
In addition to the positive impact of the exogenous (MDSC-produced) NO, we observed that the development of Th17 cells from naive-, memory-, or tumor-infiltrating $CD4^+$ T cells, driven by IL-1β/IL-6/IL-23/NO-producing MDSCs or by recombinant cytokines (IL-1β/IL-6/IL-23), is associated with the induction of endogenous NOS2 and NO production, and critically depends on NOS2 activity within $CD4^+$ T cells.
Inhibition of NOS2 activity or the canonical cGMP/cGK pathway of NO signaling abolishes the *de novo* induction of Th17 cells and selectively suppresses IL-17 production by established Th17 cells isolated from ovarian cancer patients. Our data indicate that, apart from its previously recognized role as an effector mediator of Th17-associated inflammation, NO is also critically required for the induction and stability of human Th17 responses. The current results provide rationale for targeting NO, NOS2, cGMP, and cGK as new targets to manipulate Th17 responses in cancer, autoimmunity and inflammatory diseases.

W3.10.06
Evidence of a transient nature of the Th17 phenotype of CD4+CD161+ T cells in the synovial fluid of juvenile idiopathic arthritis patients

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Objective: To investigate the phenotype and function of CD4+ T cells in synovial fluid (SF) from the affected joints ofchildren with oligoarticular-onset juvenile idiopathic arthritis (JIA) and to establish a possible link with disease activity. Methods: CD4+ T cells were obtained from the peripheral blood (PB) and SF of 23 children with oligoarticular-onset JIA, as well as from the PB of 15 healthy children and were analyzed for CXCR3, CCR6, CD161 expression and for IFN(interferon)-γ and interleukin-17A (IL-17A) production. Results: The numbers of CD4+CD161+ cells, showing either the type 1 T helper (Th1) or Th17/Th1 phenotype, were higher in SF than in PB of children with JIA. The few Th17 cells from SF of JIA underwent a spontaneous shift to the Th1 phenotype in vitro, whereas Th17 cells from PB of healthy children shifted only in presence of JIA SF; this effect was neutralized by antibody blocked of IL-12 activity. The frequencies of CD4+CD161+ cells, particularly the Th17/Th1 cells, in the JIA SF positively correlated with levels of erythrocyte sedimentation rate and C-reactive protein. Conclusion: These findings suggest that a shifting of CD4+CD161+ T cells from Th17 to the Th17/Th1 or Th1 phenotype can occur in the SF of oligoarticular-onset JIA, and indicate that the accumulation of these cells is correlated with parameters of inflammation. Thus, the results support the hypothesis that these cells may play a role in JIA disease activity.

W3.11 Gamma-delta T cells

IL3.11.01
Regulatory interactions between granulocytes and gamma/delta T cells in response to aminobisphosphonates

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When Ficoll-Hypaque-separated human peripheral blood mononuclear cells (PBMC) are activated by aminobisphosphonates (n-BP) in the presence of exogenous IL-2, a strong and selective proliferation of Vgamma9/Vdelta2 T-cells ensues. This is commonly attributed to the uptake of n-BP by monocytes and subsequent endogenous production of pyrophosphates due to the inhibitory effect of n-BP on farnesylpyrophosphate synthase. To study the uptake of n-BP in various cell populations, we chemically coupled the n-BP zoledronic acid to CFSE ("FluorZOL"), and analyzed cellular ingestion of FluorZOL by flow cytometry. When using RBC-lysed whole blood (containing all leukocytes) we observed strong uptake of FluoZOL not only in monocytes but also in granulocytes and very little, if any, in lymphocytes. gamma/delta T-cell proliferation was drastically inhibited when total leukocytes rather than PBMC were stimulated with n-BP or FluorZOL, despite highly efficient uptake. The inhibitory effect of granulocytes on gamma/delta T-cell proliferation in response to n-BP could be fully prevented by neutralizing three inhibitory factors of granulocytes. These observations could explain the observed disappearance of gamma/delta T-cells from blood upon prolonged treatment with n-BP (Kalyan et al, J Bone Miner Res 28:728,2013); and, furthermore, these findings may be relevant for the future exploitation of gamma/delta T-cells for immunotherapeutic application.

W3.11.01
Heterogeneity and prognostic influence of tumor- infiltrating γδ T lymphocytes in colon cancer patients

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Tumors grow in a complex and intricated network of epithelial and mesenchymal cells, inflammatory and immune cells and the characterization of the immune contexture is a major prognostic factor for patients survival and represent a target for innovative cancer therapies.
We characterized colon cancer-infiltrating γδ T cells in a cohort of 70 patients in terms of phenotype and effector functions and correlated the immunological analysis with clinicopathological features of colon cancer. Results show that Vδ1 T cells were the predominant population in the vast majority of specimens and upon short term mitogen *in vitro* stimulation produced IL-10, while Vγ9Vδ2 T cells were found uniformly at lower proportion in most of the patients, had a predominant terminally-differentiated effector memory (TEMRA) $CD45RA^{+}CD27^{-}$ phenotype, expressed cytotoxic molecules as perforin and granzyme B and upon short term *in vitro* stimulation with phosphoantigen produced pro-inflammatory cytokines as IL-17 and IFNγ in different combinations.
Correlation with different clinicopathologic features demonstrates that higher percentage of tumor-infiltrating Vγ9Vδ2 T cells are found in well differentiated tumors and in patients with early-stage disease and absence of metastasis; moreover, intratumoral γδ T cell numbers are positively correlated with overall survival of colon cancer patients.
In conclusion, our results highlight the role of γδ T cells against colorectal cancer cells and suggest that immune response mediated by γδ T lymphocytes may contribute to the immunosurveillance of colon cancer. These findings may foster the development of novel alternative or adjuvant therapies targeting γδ T cells for the treatment of colon cancer patients.

W3.11.02
Key role of Free Heavy Chain of HLA class I molecules in HCMV and tumor stress sensing by gamma-delta TCR

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Non-Vδ2 γδ T cell clones isolated from Cytomegalovirus (HCMV)-infected individuals show dual TCR dependent reactivity against tumor cell lines and HCMV-infected cells suggesting recognition of stress-associated self antigens by non-Vδ2 TCRs. In order to identify these uncharacterized ligands, we immunize mice with tumor cells targeted by HCMV-reactive non-Vδ2 TCRs and select mAbs able to specifically abrogate the reactivity of γδ TCR transductants against these targets.
In this study, we focused on a Vγ9Vδ3 TCR, which confers to transductants a restricted reactivity against three tumor B cell lines only. By contrast, several blocking mAbs (LSM) selected to recognize Vγ9Vδ3 antigen, ubiquitously targeted HLA class I (HLA-I) molecules expressed by a wide panel of cells. These results suggested the Vγ9Vδ3 TCR recognition of HLA-I molecules expressed in a particular shape or environment in the three B cell targets. Interestingly, B cell targets and HCMV-infected cells expressed β-2-microglobulin-free HLA-I heavy chains (FHC). Silencing β-2-microglobulin in B cell targets abrogated both HLA-I FHC cell surface expression and Vγ9Vδ3 TCR reactivity, suggesting the recognition of HLA-I FHC by the Vγ9Vδ3 TCR. Moreover, the original Vγ9Vδ3 T clone expressed CD85j and CD158b inhibitory receptors specific for HLA-I, that do not bind FHC, suggesting a regulation of TCR-mediated HLA-I recognition in physiological context. Blocking these receptors markedly increased the Vγ9Vδ3 T clone reactivity against B cell targets and HCMV-infected cells.
Altogether, our results identify HLA-I FHC as a potential stress-associated antigen common to HCMV-infection or cellular transformation that mediate stress surveillance by γδ T cells.

W3.11.03
Implication of gamma delta T cells in the immune response against murine CMV

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We previously showed that gamma delta T cells are key contributors to the immune response against CMV in humans, and participate to CMV riddance partially through interferon gamma (IFNgamma) release. The aim of the present study was to use the mouse model of CMV infection in order to dissect in vivo the implication of murine γδ T cells in the control of mouse CMV (MCMV). Using C57BL/6 mice deficient for alpha beta and/or gamma delta T cells, we showed that whichever subpopulation is sufficient to confer protection against MCMV. Viral loads increased with time in all organs tested from CD3epsilon-/- (alpha beta- gamma delta-) infected mice, and the presence of high levels of transaminases in blood was suggestive of liver damage. CD3epsilon-/- ultimately died unlike TCRalpha-/- (alpha beta- gamma delta+) mice that survived with much lower viral loads. In TCRalpha-/- mice, CD27+ gamma delta T cells were increased in the liver 7 days post MCMV-infection suggesting an implication of IFNgamma-secreting gamma delta T cells in the clearance of MCMV in this organ. Finally, bone marrow transfer experiments using TCRalpha-/- donors rendered CD3epsilon-/- mice resistant to MCMV in accordance with a protective anti-viral role for gamma delta T cells. These results which suggest that gamma delta T cells could compensate for the absence of alpha beta T cells during MCMV infection could be of particular relevance in immune-suppressive contexts where alpha beta T cells are more specifically compromised.

W3.11.04
HDAC7 plays a significant role in γδ T-cell development and function

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In many cellular systems Histone Deacetylases (HDACs) play important roles in the regulation of cell differentiation, proliferation and survival. HDACs regulate chromatin structure and thus gene transcription by reversing histone lysine acetylation. As such they been identified as key players in the development of the immune system with evidence suggesting HDAC7 acts as a gene expression switch regulating T-cell development and function. A small subset of T-cells express TCR chains encoded by the gamma and delta gene loci. A role for HDAC7 in these γδT cell's development and function is yet to be identified. We have bred an HDAC7 floxed/floxed mouse with a vav-cre transgenic mouse in order to delete HDAC7 from all haematopoietic cells. γδ T-cell number in the Thymus of these mice is normal however we have identified a 10-fold increase in γδ T-cell number in the peripheral tissues (spleen and lymph nodes) compared to wild type. Splenocytes from these mice stimulated in-vitro with CD3 and cultured in the presence of IL2 for 6 days preferentially proliferate γδ T-cells to a higher degree than αβ T-cells. Interestingly these activated HDAC7 KO γδ T-cells are functionally competent for producing IFNγ, but not IL-17. Preliminary data also suggests an impairment of γδ T-cells ability to home to epithelial compartments as shown by a 50% reduction of γδ T-cells in the ear epithelia. We propose HDAC7 plays a significant role in the proliferation of γδ T-cells and their ability to produce IL-17 with a further role in γδ T-cell homing.

W3.11.05
A network of High Mobility Group box transcription factors programs innate IL-17 production

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All lymphocytes, whether innate or adaptive, can be segregated into effector subsets, based on distinct gene regulatory networks. Innate or innate-like lymphocytes become effectors in their tissue of origin such as thymus (γδ T and iNKT cells) and gut (Lymphoid Tissue inducer-like, LTi, cells) while adaptive lymphocytes acquire specialized functions upon pathogen encounters. Unique gene regulatory networks responsible for the generation of innate lymphocytes were unknown. γδ T cells are the major source of IL-17 and IL-22 (Tγδ17) at the early phases of immune response and are also responsible for inflammatory disorders in the skin. We demonstrate that a transcription factor (TF) network consisting of a quartet of High Mobility Group box TFs, SOX4, SOX13, TCF1 and LEF1, programs the intrathymic differentiation of Tγδ17 cells. While SOX4 and SOX13 positively regulate the two requisite Tγδ17 cell-specific genes, *Rorc* and *Blk*, TCF1 and LEF1 interact with SOX proteins and promote alternate effector subset differentiation. Furthermore, we show that T cell lineage specification factor TCF1 is indispensable for the generation of IL-22 producing gut NKp46$^+$ innate lymphoid cells and restrains cytokine production by LTi-like effectors. These results predict that a shared gene network architecture programs all innate sources of IL-17 and IL-22, independent of anatomical origins and antigen receptor expression.

W3.11.06
Major contribution of gamma delta T cells to IL-17A production and ovarian cancer growth in vivo

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A significant body of evidence implicates γδ T cells in tumour immunosurveillance. The anti-tumour function of γδ cells stems from their potent cytotoxicity and their capacity to produce high levels of IFN-γ. However, γδ cells were also recently implicated in promoting tumour growth, possibly as a result of their ability to secrete IL-17A. Considering the ultimate goal of manipulating γδ cells for cancer treatment, we aim to further dissect the pro-tumour mechanisms of γδ cells. We use a transplantable ID8 ovarian cancer cell line, that grows slower upon IL-17A neutralization. Our data show that γδ cells infiltrate ID8 tumour foci and are a major source of IL-17A in the tumour microenvironment. Furthermore, γδ cell-deficient mice (TCRδ-/-) show reduced ID8 tumour burden, which strongly suggests that the pro-tumour role of IL-17A is mediated by γδ cells. A significant fraction of γδ cells is proliferating in situ and accounts for a 2.3-fold increase in γδ cell numbers in the peritoneal cavity, 6 weeks after ID8 cell injection. The majority of these γδ cells display a Vγ1- Vγ4- phenotype, indicating they belong to the Vγ6+ subset which is naturally enriched in the peritoneal cavity. Interestingly, both IFN-γ+ and IL-17A+ γδ cells accumulate in the tumour microenvironment, potentially suggesting a dominance of the pro-tumour effects of IL-17A over the anti-tumour functions of IFN-γ. We are currently generating TCRδ-/- IL-17A-/- mice to further assess the contribution of γδ cells to the IL-17A-mediated tumour growth in this ovarian cancer model.

W3.12 Follicular helper T cells

IL3.12.01
Interleukin-21 directs T follicular helper cell differentiation

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T helper cell derived cytokines such as interleukin (IL)-21 play an important role in the generation of antibody forming cells during the germinal center reaction following T dependent immunization. However, several recent studies have questioned whether IL-21 has a CD4$^+$ T cell intrinsic role. Using a variety of approaches including MHC II tetramer-based detection of endogenous antigen specific Tfh cells, we provide evidence that IL-21 acts on antigen specific CD4$^+$ T helper cells to support their survival and differentiation. IL-21 receptor deficient (*Il21r-/-*) "Tfh-like" cells exhibited phenotypic similarities with Tfh cells, but had abnormally high expression of programmed cell death protein 1 (PD-1), selectively differentiated into FoxP3+ regulatory Tfh cells and were functionally deficient as B cell helpers. The propensity for *Il21r-/-* T cells to differentiate into T regulatory cells may reflect the altered balance between responsiveness to IL-2 and IL-21 (Stat5/Stat3) and reinforces the important role for IL-21 in Tfh cell differentiation.

W3.12.01
Functional overlap of ROQUIN-1 and ROQUIN-2 in the repression of mRNAs controlling Tfh cell accumulation and systemic inflammation

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Accumulation of Tfh cells and excessive production of pro-inflammatory cytokines by myeloid cells lead to autoantibody-driven diseases. ROQUIN-1 (Rc3h1) has been previously shown to repress ICOS and IFNγ and prevent Tfh cell accumulation. Unlike Rc3h1san mice that carry a single mutation in the RNA binding ROQ domain of ROQUIN-1, mice lacking the protein within T cells paradoxically do not display increased T follicular helper (Tfh) cells. We analyzed mice with mutations that eliminate the RING domain from ROQUIN-1 (Rc3h1RING) or its paralog, ROQUIN-2 (Rc3h2). We find that the RING deletion impairs Icos mRNA regulation by ROQUIN-1, but not as dramatically as the ROQ mutation. We propose that in RING-deficient ROQUIN-1 mice, ROQUIN-2 can compensate and largely repress ICOS expression and Tfh cells accumulation. These paralogs, whose common ancestral gene preceded adaptive immunity, also target TNF in non-lymphoid cells. We found that mice carrying the combined mutations Rc3h1san and Rc3h1RING show early lethality, have heightened sensitivity to endotoxic shock and

increased susceptibilty to TNF-dependent autoantibody-induced arthritis. Thus the ROQUIN family emerges as a post-transcriptional brake for both the adaptive and innate immune systems to curtail antibody responses and inflammation.

W3.12.02
Identification of human Tfh-specific miRNAs and investigation of their role in Tfh differentiation and function

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Follicular helper T cells (Tfh) are a separate CD4+ T helper subset specialized in provision of help to B cells. They develop independently from other T cell subsets and are critical for humoral immunity, including the generation of long-lived and high affinity plasma cells and memory B cells. Alteration of Tfh-specific genes leads to defects in germinal centers (GCs) formation, with consequent autoimmune or immunodeficiency disorders. The molecular mechanisms underlying Tfh cells differentiation are poorly understood. MicroRNAs (miRNAs) are highly conserved non-coding single-stranded small RNA molecules that control gene expression post-transcriptionally by binding the 3'untranslated region of target mRNA. To investigate the role of miRNAs in Tfh cells biology we performed RT-qPCR and deep sequencing analysis on Tfh and Naive CD4+ T cells sorted from human adenoids. We identified Tfh-specific miRNAs and in vitro validated their predicted targets. We found that Tfh-specific miRNAs regulate transcripts specifically expressed in Tfh cell subset and known to be essential for their function. We are performing gain or loss of function experiments using lentiviral vectors aimed at modulating miRNA expression in human Tfh cells. Through these experiments we will assess how these Tfh specific-miRNAs influence the ability of Tfh cells to help B cells, and elucidate their role in Tfh biology.

W3.12.03
Follicular regulatory T cells migrate to germinal centers via NFAT2-mediated signaling

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Maturation of high affinity B lymphocytes is precisely controlled during the germinal center reaction. This is dependent on $CD4^+CXCR5^+$ follicular helper T cells (T_{FH}) and inhibited by $CD4^+CXCR5^+Foxp3^+$ follicular regulatory T cells (T_{FR}). Since NFAT2 was found to be highly expressed and activated in follicular T cells we addressed its function herein. Unexpectedly, ablation of NFAT2 in T cells caused an augmented GC reaction upon immunization. Consistently, however, T_{FR} cells were clearly reduced in the follicular T cell population due to impaired homing to B cell follicles. This was T_{FR}-intrinsic. The overall frequency of nTreg cells remained unaltered in mice lacking NFAT2 or even NFAT1 plus NFAT2. Furthermore, the suppressive function of both nTreg and iTreg cells was independent on robust NFAT levels, as absence of one or two NFAT members did not alter suppressor activity *in vitro* or during colitis and transplantation *in vivo*. However, the physiological relevance of NFAT2 for humoral (auto-) immunity was corroborated by exacerbated lupus-like disease in the presence of NFAT2-deficient T_{FR}.
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W3.12.04
The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut

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Immunoglobulin A (IgA) is essential to maintain the symbiotic balance between gut bacterial communities and the host immune system. Intestinal IgA production occurs via both T helper cell-dependent and independent pathways. The diversification of IgA repertoire by somatic hypermutation takes place mostly in specialized microenvironments called germinal centers (GCs), in which B cell interaction with T follicular helper (T_{FH}) cells induces the expression of activation-induced cytidine deaminase (AID). T_{FH} cells express high amounts of the inhibitory co-receptor programmed cell death-1 (PD-1), a key receptor for shutting down the ineffective immune responses and maintaining the tolerance. We found that PD-1 regulates the gut microbiota through the appropriate selection of IgA plasma cell repertoires. PD-1-deficiency generates an excess number of T_{FH} cells with pro-inflammatory phenotypes, which affect the selection of IgA precursor cells in GCs of Peyer's patches. Consequently, the IgAs produced in PD-1-deficient mice have reduced bacteria-binding capacity causing alterations of microbial communities in the gut. Interestingly, the altered bacterial composition in PD-1-deficient mice induces systemic activation and induction of GCs where poly- /auto-reactive antibodies could be generated. Thus, we revealed that PD-1 plays a critical role in regulation of antibody diversification required for the homeostasis and prevention of autoimmune diseases.

W3.12.05
Regulation of T Helper Cell-Dependent Antibody Response in Lymphopenic Hosts

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Cognate interactions between T helper cells and B cells are required for the production of high-affinity antibodies and for the generation of memory B cells and long-lived plasma cells. To gain insight into the nature of signals regulating these different aspects of B cell responses, we set up an experimental system in which CD3e-deficient mice - which lack T cells but have normal B cell development - are reconstituted with small number of TCR-transgenic T cells. We found that adoptively transferred OVA-specific CD4+ T cells induced effective primary antibody responses following immunization with OVA and adjuvant; however, serum antibody levels were not sustained. Further analysis revealed that upon immunization, T cells proliferated extensively and preferentially developed into T follicular helper (Tfh). Although initiation of germinal center (GC) reaction and differentiation of B cells into antibody-secreting short-lived plasma blasts were normal, Tfh cells became dysfunctional and failed to provide help to OVA-specific B cells during late GC events, resulting in impaired affinity-maturation and lack of long lived plasma cells. Instead, hypergammaglobulinemia, hyper-IgE, and production of autoantibodies were observed in the recipients. These data provide a link between overstimulation of Tfh cells and the development of dysregulated humoral immune responses and may have implications for treatment of patients with congenital defects in lymphocyte homeostasis or suffering from diseases involving lymphopenia, such as HIV infection or certain tumors.

W3.12.06
Fate decision of germinal centre B cells is based on asymmetric division

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Evolution of germinal centre (GC) B cells involves somatic hypermutation and selection. Multi-photon imaging of GC B cells has revealed the migration path of B cells in the GC environment and allowed localisation of critical selection steps. These data are analysed in silico in the framework of a theory of GC B cell selection, divsion, and exit. The analysis unravels so far unappreciated implications of the multi-photon data sets for B cell differentation to plasma cells: B cells, after having received selection signals from T follicular helper cells, always return to the GC dark zone. Thus, the pathway of recylcing, which was not even proven until 3 years ago, turns out to be the exclusive fate of positively selected B cells. In the dark zone, B cells divide and distribute the previously collected antigen asymmetrically onto their daughters. After two divisions one of the four B cells still carries the antigen, enters final differentiation into a plasma cell and leaves the GC in direction of the adjacent T zone. The evidence for this new GC theory, called GC LEDA model, is presented and the implications for the success of the GC reaction is illustrated: Compared to a classical recycling model, the number of plasma cells derived from the GC is ten-fold and affinity maturation is achieved one day earlier. This is of major relevance in time critical immune responses.

W3.13 Regulatory T cells: basic aspects

IL3.13.01
Regulatory T cells fine-tune DC costimulation in vivo to set the threshold for T cell activation

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Despite extensive research into their functional properties, the mechanism(s) of regulatory T (Treg) cell action remains controversial. Based on preliminary data indicating that lymph node and spleen dendritic cells (DCs) express abnormally high levels of costimulatory molecules in mice deficient in Tregs, we hypothesised that Tregs exert real-time control over the immunostimulatory function of DCs.
To test whether Tregs reduce the expression of costimulatory molecules in vivo, we set up a mouse model in which immunodeficient animals are reconstituted with pure Tregs, supported by exogenous IL-2. Only when the total number of Tregs reached the number present in normal mice was DC costimulation reduced to normal levels. Using CFSE labelling, we showed that Treg reconstitution prevented fast-phase lymphopaenia-induced proliferation, which is a necessary step in the induction of autoimmune disease in lymphopenic mice. In contrast, reconstitution with conventional CD4 T cells led to an increase in DC costimulation and enhanced lymphopenia-induced proliferation. Treg expression of CTLA-4 was necessary for their ability to normalise DC costimulation and to inhibit lymphopaenia-induced proliferation. Treg expression of CTLA-4 was also responsible for controlling recruitment of antigen-specific CD4 T cells into division.
These data indicate that the activation of self-reactive T cells in lymphopenic mice is a specific result of Treg deficiency, independent of conventional T cells, and that the effect is mediated via control of DC costimulation.

IL3.13.02
Impaired peripheral tolerance as a consequence of disturbed regulatory T cells - dendritic cell interactions

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Dendritic Cells (DC) are professional antigen presenting cells (APC) that play a dual role in the control of adaptive immune responses. DC that have been activated through recognition of pathogen- or danger associated molecules can prime naïve T cells. In contrast, non-activated DC in the steady state induce peripheral T cell tolerance. We have recently shown that CD4+Foxp3+ regulatory T cells (Treg) are important for tolerance induction by steady state DC. Depletion of Treg results in an activated phenotype of steady state DC and these DC induce priming instead of tolerance. However, whether DC activation and the concomitant loss of tolerizing capacity is a result of general autoimmunity that develops in the absence of regulatory T cells, or whether direct interactions between DC and Treg are necessary to allow steady state DC to induce tolerance has remained unclear. To address this question in vivo, we have generated mouse models in which cognate DC-Treg interactions or previously reported suppressive Treg-mechanisms are impaired.
We find that in those case where DCs cannot be controlled by regulatory T cells induction and maintenance of peripheral CD8+ T cell tolerance is compromised.

W3.13.01
Low/ negative expression of CD6 in natural T-regulatory cells

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Natural T-regulatory cells, nTreg, are responsible for the maintenance of dominant self tolerance and play an important role in the prevention of autoimmune disorders, allergy, and in the maintenance of fetal maternal tolerance and organ graft tolerance. While the nuclear transcription factor, FOXP3, uniquely defines nTreg, there is a need to identify convenient surface markers that can be used to isolate and expand viable highly enriched nTreg. Several surface markers have been already identified. Among them, CD4, CD25 and CD127 are the most commonly used. In our study healthy blood samples were obtained with informed consent. PBMC were isolated and a 10 color multicolor staining was performed and analyzed on Gallios™ flow cytometer. In addition, labeled Treg cells were isolated using a MoFlo® XDP cell sorter and plated for use in a Treg-suppression assay with allogeneic PBMC stimulated with anti-CD3/CD28 mAbs in a CFSE proliferative assay. Our results show that $CD4^+CD25^{hi}FOXP3^+$ nTreg exhibit low/ negative CD6 expression ($CD6^{lo/-}$). We show that $CD4^+CD25^{hi}CD6^{lo/-}$ and $CD4^+CD25^{hi}CD127^{lo/-}$ cells exhibit similar nTreg-associated marker expression and similar *in vitro* suppression activity on $CD8^+$ T-cell proliferation. Moreover, two major nTreg subpopulations were identified from the analysis of CD6 and CD127 expression on $CD4^+CD25^{hi}$ cells: a $CD6^{lo/-}CD127^{lo/-}$ population, with high expression levels of FOXP3, and a $CD6^+CD127^+$ population, with low FOXP3, high CCR4 and low CD45RA, HLA-Dr and CD39 markers. Lack of CD6 on nTreg cells could participate to Treg anergy response to antigenic stimulus and can contribute to better understanding of biology of nTreg immune regulation.

W3.13.02
Regulatory T cells increase the avidity of CD8+ T cell responses to non-self antigens and promote memory

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Regulatory T cells (Tregs) suppress autoreactive T cells through multiple effector mechanisms, acting both during the priming and the effector phases of immune responses. In healthy individuals, Treg-mediated suppression does not always compromise T cell responses to infectious, non-self antigens. We show that Tregs play a critical

role during the priming of immune responses in mice. We observed that Treg depletion induced the activation and expansion of an endogenous population of low avidity CD8+ T cells. To study the mechanisms involved in the suppression of low avidity CD8+ T cells, we took advantage of two peptides N4 vs. T4, that are recognized with different affinities by the OT-I TCR. We visualized the early interactions between DCs, OT-I cells and Tregs using dynamic 2-photon microscopy. During the early phases of T cell priming, Tregs established an affinity threshold for CD8+ T cell activation that prevented the activation of low-avidity T cells. We investigated the effects of Treg depletion and the role of CCL-3/4/5 chemokines, on DC-T cell interactions. In the absence of Tregs the over production of CCL-3/4/5 chemokines, stabilized the dynamic interactions between antigen presenting dendritic cells and low avidity CD8+ T cells.
In the absence of Tregs, the avidity of the endogenous primary immune response to Listeria monocytogenes -OVA infection was also impaired, resulting in reduced memory protection.
These results suggest that Tregs are important regulators of CD8+ T cell priming and play a critical role in the induction of high avidity primary responses and effective memory protection.

W3.13.03
Costimulatory requirements for Treg homeostasis and function

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We have previously shown that blockade of the CTLA-4 pathway significantly enhances Treg proliferation, presumably by enhancing signaling through the CD28 pathway. We now show using CD28-deficient animals that the effects of anti-CTLA-4 are entirely dependent on CD28 signaling. Analysis of bone marrow chimeric mice, comprising wildtype and CD28-deficient cells, has allowed us to demonstrate that intrinsic CD28 signals are necessary for anti-CTLA-4 induced Treg proliferation. By blocking CD86 and CD80, independently or together, we have been able to dissect the relative requirements for each ligand in maintaining Treg homeostasis. In addition to regulating the strength of CD28 signaling, we have previously shown that Treg-expressed CTLA-4 reduces expression of CD86 and CD80 by a process of trans-endocytosis. Together our data suggest a model in which peripheral Treg control their own pool size by regulation of costimulatory ligand expression.
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W3.13.04
Continuous interactions with self are required for maintaining regulatory CD4 T-cell numbers and suppressive capacities in the periphery

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Thymic development of regulatory Foxp3$^+$ CD4 T cells (Tregs) is instructed by TCR with high avidity for self-peptides bound to self MHC class II molecules (self). In the periphery, Tregs are important for the maintenance of self-tolerance but how their self-reactivity impacts on their homeostasis and suppressive capacities is poorly understood. In this study, we show that, in young mice, peripheral Tregs can be subdivided into two subsets according to Ly-6C expression. Interestingly, almost all thymic Tregs were Ly-6C$^-$ but they gave rise to both Ly-6C$^-$ and Ly-6C$^+$ Tregs after migrating to the periphery. Phenotypic analysis and adoptive transfer experiments of peripheral Ly-6C$^-$ and Ly-6C$^+$ Tregs revealed that the non-expression of Ly-6C by about 60% of peripheral Tregs was dependent on self-recognition. According to their higher ability to interact with self, Ly-6C$^-$ Tregs expressed higher surface amounts of key immunosuppressive molecules such as CD25, CTLA-4, CD39 and CD73 than Ly-6C$^+$ Tregs and were the only ones to produce constitutively anti-inflammatory cytokines. In line with their phenotype, only Ly-6C$^-$ Tregs displayed suppressive capacities both *in vitro* and *in vivo*. Finally, whereas Ly-6C$^-$ Tregs maintained their numbers with age, Ly-6C$^+$ Tregs gradually disappeared. Altogether, our data suggest that both the survival and the function of Tregs rely on continuous interactions with self in the periphery.

W3.13.05
TGF-b signalling is required for CD4+ T cell homeostasis but dispensable for regulatory T cell function

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TGF-β is widely held to be critical for the maintenance and function of regulatory T cells and thus peripheral tolerance. Here we show that inducible deletion of the TGF-β receptor II (TR2) from mature CD4$^+$ T cells does not result in the collapse of the T_{reg} cell population as observed in constitutive models. Instead, it leads to a pronounced enlargement of both Nrp-1$^+$ thymic regulatory and effector memory T cell pools. This T cell expansion is a cell intrinsic process caused by increased T cell receptor sensitivity. Further, the expression of Foxp3 and other regulatory T cells markers was not dependent on TGF-β signalling and the TR2–deficient T_{reg} cells retained their suppressive function both *in vitro* and *in vivo*. We also show that peripheral TR2 ablation from CD4$^+$ T cells does not lead to lethal autoinflammation and transfer of theses T helper cells to lymphopenic recipients results in colitis, but not overt autoimmunity. In contrast, thymic ablation of TR2 receptor in combination with lymphopenia leads to lethal multi-organ inflammation. In summary, our findings indicate that TGF-β regulates the homeostasis of mature CD4$^+$ T cells but is dispensable for peripheral tolerance and T_{reg} function in the adult animal.

W3.13.06
Essential role for Bcl11b in the FoxP3 transcriptional complex and T regulatory cells suppressive function

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Naturally occurring T regulatory cells (Treg cells) engage in the maintenance of immunological self-tolerance and homeostasis, by expression or repression of lineage specific molecules. Foxp3, which is exclusively expressed by Treg cells, critically controls the development and function of Treg cells; however, the molecular mechanisms by which Foxp3 controls its target genes are still remain elusive. We characterized constituents of the Foxp3 transcriptional complex, and found that the T cell lineage specific transcriptional factor, Bcl11b, was a novel constituent of the Foxp3 transcriptional complex. Treg specific gene regulation and molecule expression, especially CTLA-4 expression, and immune suppressive activity were impaired in Treg cells lacking Bcl11b. Consequently, lethal autoimmunity was observed in Treg-specific Bcl11b conditional knockout mice. Impaired immunesupressive activity in Bcl11b-null Treg cells was rescued by forced expression of CTLA-4 Taken together, Bcl11b is an essential component of the Foxp3 transcriptional complex, which regulates Treg specific gene expression. This model explains how transcriptional complex controls expression of lineage specific gene.

W3.13.07
Human regulatory T cells rapidly suppress T cell receptor-induced calcium, NF-κB, and NFAT signaling in conventional T cells

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CD4+CD25hiFoxp3+ regulatory T cells (Tregs) are critical mediators of self-tolerance, which is crucial for the prevention of autoimmune disease, but Tregs can also dampen antitumor immunity. Tregs inhibit the proliferation of CD4+CD25− conventional T cells (Tcons), as well as the ability of these cells to produce effector cytokines; however, the molecular mechanisms of suppression remain incompletely understood. We show that human Tregs rapidly suppressed the release of calcium ions (Ca2+) from intracellular stores in response to T cell receptor (TCR) activation in Tcons. The inhibition of Ca2+ signaling resulted in decreased dephosphorylation, and thus decreased activation, of the transcription factor nuclear factor of activated T cells 1 (NFAT1) and reduced the activation of nuclear factor kB (NF-kB). In contrast, Ca2+-independent events in Tcons, such as TCR-proximal signaling and activation of activator protein 1 (AP-1), were not affected during coculture with Tregs. Despite suppressing intracellular Ca2+ mobilization, coculture with Tregs did not block the generation of inositol 1,4,5-trisphosphate in TCR-stimulated Tcons. The Treg-induced suppression of the activity of NFAT and NF-kB and of the expression of IL-2 was reversed in Tcons by increasing the concentration of intracellular Ca2+. Our results elucidate a previously unrecognized and rapid mechanism of Treg-mediated suppression. Ongoing and future studies aim at deciphering new molecules causative for this rapid suppression by next-generation proteomics studies, pathway analyses and computational data integration. This increased understanding of Treg function and Tcon resistance to suppression may be exploited to generate possible therapies for the treatment of autoimmune diseases and cancer.

W3.13.08
Selective inhibition of pro-inflammatory T cell responses by TIGIT+ Tregs

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Foxp3$^+$ regulatory T cells (Tregs) are a subset of CD4$^+$ T cells that is key in regulating immune responses and maintaining self-tolerance. Recently the concept has emerged that Tregs are comprised of many specialized subpopulations with distinct regulatory functions. Although the transcription factors that differentially induce the effectors that are required for this specialized suppression have been identified, the molecules that mediate these selective effector functions remain largely unknown. Our results indicate that the co-inhibitory molecule TIGIT, which is expressed on about 30% of nTregs, represents such a mediator.
TIGIT marks a functionally distinct Treg subset that displays increased suppressive capacity and an activated phenotype. Using *in vivo* models of experimental autoimmune encephalomyelitis (EAE) and colitis, we demonstrate that TIGIT$^+$ Tregs specifically suppress pro-inflammatory Th1/Th17 responses yet spare or even enhance Th2 responses. This selective suppression of pro-inflammatory responses (IFN-gamma and IL-17) is mediated by secretion of a soluble Treg specific effector molecule, which is a key factor contributing to the higher suppressive capacity of TIGIT$^+$ Treg and the selective inhibition of Th1/Th17 repsonses. We have identified a transcription factor that is induced by TIGIT that drives the expression of this effector molecule and thereby enables TIGIT$^+$ Tregs to selectively suppress pro-inflammatory effector T cell responses, while sparing or even promoting Th2 effector T cell responses. TIGIT$^+$ Tregs are thus likely to be a specialized Treg subset equipped to specifically suppress inflammatory immune responses and tissue inflammation mediated by Th1/Th17 cells.

W3.13.09
Regulatory T cells suppress the late phase of the immune response in lymph nodes through P-selectin glycoprotein ligand-1

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Regulatory T (Treg) cells maintain tolerance towards self-antigens and suppress autoimmune diseases, although the underlying molecular mechanisms are unclear. In this study we investigated the role of the mucin P-selectin glycoprotein ligand (PSGL)-1 in the suppressor activity exerted by Treg during autoimmune disease development.
RESULTS. We initially found that mice deficient for PSGL-1 develop a more severe form of experimental autoimmune encephalomyelitis (EAE) than wild-type animals, suggesting that PSGL-1 has a role in the negative regulation of autoimmunity. Moreover, Treg cells lacking PSGL-1 were unable to suppress EAE and failed to inhibit T-cell proliferation *in vivo* in the lymph nodes. Using two-photon laser-scanning microscopy in the lymph node, we found that PSGL-1 expression on Treg cells had no role in the suppression of early T-cell priming (day +1 post-immunization) after immunization with antigen. Instead, PSGL-1 deficient Treg cells lost the ability to modulate T-cell movement and failed to inhibit T cell-dendritic cell contacts and T-cell clustering essential for sustained T-cell activation during the late phase of the immune response (day +7 post-immunization). Notably, PSGL-1 expression on myelin-specific effector T cells had no role in T-cell locomotion in the lymph node. In conclusion, our data show that PSGL-1 represents a previously unknown phase-specific mechanism for Treg cell-mediated suppression of the persistence of immune responses and autoimmunity induction.
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W3.13.10
Visualizing tolerance induction: behavior and function of regulatory T cells during the establishment of materno/fetal tolerance

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A fetus is inherently antigenic to its mother and yet is not rejected. Our understanding of the mechanisms that regulate materno/fetal tolerance is fragmentary, yet these mechanisms have important therapeutic implications in abnormal pregnancies, organ transplantation and autoimmune diseases.
Recently, regulatory T cells (Tregs) have been suggested to play a pivotal role in preventing the rejection of the fetus during pregnancy, as ablation of Tregs results in increased resorption of the embryos in allogeneic matings in mice. Furthermore, women with repeated spontaneous abortions and preeclampsia were found to display decreased numbers of CD25$^+$CD4$^+$ Tregs.
We have undertaken a comprehensive analysis of the role, activation status and function of Tregs, their spatial and temporal distribution and their interactions with other immune system cells (DCs, Teffs and NK cells) during pregnancy. We found that embryo implantation triggers the early recruitment and proliferation of activated/memory Tregs in the uterine draining lymph nodes. Moreover, Treg proliferation is antigen-driven and self-specific. Finally, low-dose IL-2 treatment prevents abortion in an abortion prone model.
Furthermore, we have developed two-photon *in vivo* imaging approaches to directly visualize T cells at the materno/fetal interface, the decidua. Our data suggest that Tregs are recruited to the decidua upon implantation. Here, they are either motile or big and sessile and these latter are able of long lasting interactions with DCs.

Our results establish novel *in vivo* approaches to study the dynamic interactions of immune cells at the materno/fetal interface and shed light on the establishment of immune tolerance.
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W3.13.11
Migratory, and not lymphoid-resident, dendritic cells maintain peripheral self-tolerance and prevent autoimmunity via induction of iTreg cells

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There is evidence that dendritic cells (DCs) induce peripheral tolerance. Nevertheless, it is not known whether immature DCs in general are able to tolerize CD4 (+) T cells or if this is a prerogative of specialized subtypes. Here we show that, when autoantigen presentation is extended to all conventional mouse DCs, immature lymphoid tissue resident DCs are unable to induce autoantigen-specific regulatory T (iTreg) cell conversion. In contrast, this is an exclusive prerogative of steady-state migratory DCs that are able to induce antigen-specific iTreg cells in an RA-dependent manner. Because only lymph nodes host migratory DCs, iTreg cells develop and are retained solely in lymph nodes, and not in the spleen. Mechanistically, in cutaneous lymph nodes, DC-derived CCL22 contributes to the retention of iTreg cells. The importance of the local generation and retention of iTreg cells is emphasized by their essential role in protecting mice from autoimmunity in a model of autoimmune Herpes Stromal Keratitis.

W3.13.12
Der P 1 induced CD4+FOXP3+GATA3+ T cells have suppressive properties while contributing to the polarization of the Th2 response

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Functionally distinct T helper cell subsets, such as regulatory T cells (Treg) and T helper 2 (Th2) cells play an important role in allergy. Cumulating evidence indicates that FOXP3+ Treg can be functionally regulated by transcription factors previously thought to be T helper lineage specific, but little data is available for humans. Here, we functionally characterize allergen-specific human T helper cell responses in vitro. We stimulated peripheral blood mononuclear cells (PBMC) of allergic children and non-sensitized healthy controls with purified Der P 1 allergen and analyzed responding cells for GATA3 and FOXP3 transcription factor expression, associated cell surface phenotypes and cytokines production.
In allergic individuals, a typical Th2 response with high GATA3, IL4, IL5 and IL13 expression was observed. Interestingly, a fraction of cells co-expressed GATA3 and FOXP3. These double positive cells were highly proliferative and produced typical Th2 cytokines, but also potently suppressed T cell proliferation and production of non-Th2 cytokines such as IFN-g and TNF-a.
Our data indicate that allergen-specific T helper cells co-expressing GATA3 and FOXP3 have functional features of Th2 and Treg cells. These data indicate that GATA3 and FOXP3 are not exclusive human T helper cell markers and suggest that GATA3+/FOXP3+ T helper cells may contribute to allergic polarization by producing Th2 cytokines while suppressing non-Th2 pathways.

W3.13.13
Highly pure human antigen-specific Tregs with superior function in preventing allograft rejection

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Adoptive transfer of CD4+FOXP3+ regulatory T cells (Tregs) might be an alternative option to achieve tissue specific tolerance without perturbance of general immunocompetence. Polyspecific Tregs can control graft versus host diseases under lymphopenic conditions. However, under non-lymphopenic conditions as in patients after organ transplantation polyspecific Tregs were so far largely ineffective in controlling immune responses. Herein, we describe that the surface molecules latency associated peptide (LAP) and glycoprotein A repetitions predominant (GARP) can specifically identify Tregs activated by their T cell receptor and not in bystander fashion. Using these markers we could show for the first time that the human natural Treg repertoire contains about 10% of alloreactive Tregs. In addition we show that CD154 is neither expressed on resting nor on activated Tregs and can therefore be used to increase the purity of isolated Tregs. The combination of CD154-LAP+ or CD154-GARP+ markers allowed the isolation of highly pure antigen-specific Tregs. The purity, assessed by TSDR methylation analysis, exceeds all other published Treg isolations and identifies furthermore just antigen-specific Tregs. Furthermore, we demonstrated that those LAP+ allospecific Tregs are highly capable in the prevention of potent allospecific DTH responses in humanized mice and in the prevention of rejection of allogeneic cells in immune reconstituted humanized mice.
These results represent a major step forward for the use of adoptively transferred Tregs in non-lymphopenic conditions such as autoimmunity and transplant rejection. As current clinical studies are using CD4+CD25high Tregs, the findings could be of major importance for future clinical trials.

W3.13.14
TCR-independent CD4+CD25+ regulatory T cell activation during immune responses

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To clarify the precise behavior of $CD4^+CD25^+$ regulatory T (Treg) cells during immune responses is important to realize whole truth of immunity. Since activated T cells express CD25 on their surface, it is very hard to distinguish between activated conventional T (Tconv) cells and Treg cells. However, we found that activated Tconv cells were distinguishable from Treg cells by cell-size and the expression levels of CD25. By using this discrimination method, action of Treg cells in mixed lymphocyte culture (MLC) was analyzed in mice. Surprisingly, cell-sizes of the majority of Treg cells enlarged in MLC, although the majority of Tconv cells were still small-sized. TCR-transgenic system indicated that Treg enlargement was not dependent on antigen recognition. Furthermore, a trans-well culture system showed that Treg enlargement required cytokines rather than cell-cell contact. To characterize the large-sized Treg cells in MLC, expression levels of proliferation- and apoptosis-related molecules were assessed. The large-sized Treg cells in MLC expressed Ki-67, a cell-cycle related antigen, and incorporated BrdU as much as Tconv cells. In addition, the large-sized Treg cells expressed Bim, a pro-apoptotic factor, higher than activated Tconv cells. In fact, much apoptotic large-sized Treg cells were found. Taken together, the large-sized Treg cells, which were majority of Treg cells, in MLC were in the situation of both proliferation and apoptosis.
Now we are trying to clarify the trigger by which Treg cells were enlarged, and the regulatory function of large-sized Treg cells in MLC.

W3.14 B cells in autoimmunity and regulatory B cells

IL3.14.01
TACI regulates T-independent marginal zone B cell responses through innate activation-induced cell death

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Activation-Induced Cell Death (AICD) plays a critical role in immune homeostasis and tolerance. In T cell-dependent humoral responses, AICD of B cells is initiated by Fas ligand (FasL) on T cells, stimulating Fas on B cells. In contrast, T cell-independent B cell responses involve innate-type B-lymphocytes, such as marginal zone (MZ) B cells and only little is known about the mechanisms that control AICD during innate B cell responses to TLR activation. Here, we show that MZ B cells undergo AICD in response to TLR4 activation *in vivo*. This process requires engagement of the BAFF/APRIL receptor, TACI, which cooperates with TLR4 signaling to up-regulate expression of both FasL and Fas on MZ B cells and to also repress inhibitors of Fas-induced apoptosis signaling. This process is also dependent on Mal downstream of TLR4. These findings demonstrate an unappreciated role for TACI and its ligands in the regulation of AICD during T-independent B cell responses.

IL3.14.02
Regulatory B and iNKT cells interaction: novel mechanism of Suppression

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Currently, dendritic cells (DCs) are believed to be the antigen presenting cells that predominantly present glycolipid antigen and as such are responsible for iNKT cell activation/proliferation. Immunotherapy using lipid presenting cytokine-pretreated dendritic cells to activate iNKT cells has been proposed as a strategy to enhance tumor immunity, infectious diseases or to dampen autoimmune diseases. Given the role for CD19+CD24hiCD38hiCD1dhi B cells in the activation of iNKT cells, and the powerful immunoregulatory properties of these B cells, we propose that lipid presenting Bregs may strongly influence the outcome of iNKT cell based immunotherapy. We have shown that, in healthy individuals, Bregs activate invariant Natural Killer T (iNKT) cells via presentation of lipid antigen, which results in iNKT cell proliferation and cytokine production. We reported also that both CD19+CD24hiCD38hi B cells and iNKT cells are functionally and numerically, deficient in patients with Systemic lupus erythematosus (SLE). In SLE patients, B cells are unable to induce iNKT proliferation or cytokine production, defects that were associated with the rapid internalization and reduced surface expression of CD1d on CD19+CD24hiCD38hi B cells. CD1d expression and iNKT cell function were restored in B cell repopulated patients responding to treatment with rituximab. It is therefore important to further explore the nature of iNKT-Breg interactions during homeostasis, and whether the consequences of these interactions are different during inflammation or following immunotherapy.

W3.14.01
Dual-reactive and autoreactive B cell selection and function in autoimmunity

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Rare dual-reactive B cells expressing two types of Ig light or heavy chains have been shown to participate in immune responses in healthy mice. Using mice bearing Igκ allotypic markers and a wild-type Ig repertoire, we demonstrate that the generation of dual-κ B cells increases with age and disease progression in autoimmune-prone. These dual-reactive cells express markers of activation and are more frequently autoreactive than single-reactive B cells. Moreover, dual-κ B cells represent up to half of plasmablasts and memory B cells in autoimmune mice, whereas they remain infrequent in healthy mice. Furthermore, dual-κ B cells that differentiate into plasmablasts retain the capacity to secrete autoantibodies indicating that they contribute to the autoimmune process. We hypothesize that dual-κ autoreactive B cells mature because an activation of the tonic BCR signaling pathway mediated by the nonautoreactive BCR promotes the differentiation of autoreactive B cells despite the presence of self-antigen-mediated BCR signals. We show that activation of the tonic BCR signaling cascade in autoreactive immature B cells leads to the inhibition of receptor editing via the PI3K pathway and to the differentiation into transitional B cells via the activities of both Erk1/2 and PI3K. Our findings demonstrate that antigen-mediated BCR signals do not block the tonic BCR signaling pathways that travel via Erk and PI3K in immature B cells, and support a model whereby sufficient levels of tonic BCR signaling lead to the inhibition of tolerance and the maturation of low avidity autoreactive B cells.
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W3.14.02
The atypical IκB protein IκBNS is important for TLR-induced IL-10 production in B cells

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Recent studies have revealed an important immune suppressive function of B cells, which is mainly mediated by secretion of the anti-inflammatory cytokine, IL-10. However, the mechanism by which IL-10 is induced in B cells has not been elucidated. Here we report that the atypical IκB protein IκBNS is important for the development of IL-10-producing B cells. We previously reported that IκBNS expression is transiently induced in B cells upon signaling through the BCR, CD40 or TLR and that IκBNS plays a key role in B cell development and antigen-specific antibody production. We also observed that the number of IL-10-producing B cells is reduced in the spleens of IκBNS knockout mice. Analysis of B cells in bone marrow chimeric mice constructed by transferring wild type or IκBNS-deficient bone marrow cells into μMT mice revealed that IκBNS intrinsically controls IL-10 production in B cells. In addition, TLR-stimulated induction of cytoplasmic IL-10-positive cells and IL-10 secretion is significantly reduced in IκBNS-deficient B cells. LPS-induced expression of Il10 is reduced in the absence of IκBNS during the first 24 hours of induction and IL-10 production in IκBNS-deficient B cells is impaired for at least several days, suggesting a role for IκBNS in transcriptional and posttranscriptional regulation of IL-10 production in B cells. These results indicate that IκBNS is required for the development of IL-10-producing regulatory B cells, thus defining an additional important role for IκBNS in control of the immune response.

W3.14.03
Aicda expression in immature B cells, but not in mature B cells is critical for IgG autoantibody production in a model of systemic lupus erythematosus (SLE)

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Systemic lupus erythematosus (SLE) is a debilitating autoimmune disorder, which is characterized by anti-nuclear antibody. The mechanisms by which B cells produce autoantibodies and avoid tolerance to cause disease are yet to be fully elucidated. 564Igi is a mouse model of SLE that has a heavy and light chain gene for a nucleic acid specific autoantibody knocked-in to the H and L chain loci. On a 564Igi activation induced cytidine-deaminase (*Aicda*) deficient background we conditionally expressed an *Aicda* transgene in all B cells in one mouse model (Mb-1-cre) and only in mature B

cells in another model CD21-cre. CD21-cre mice express no auto-reactive IgG antibodies, while Mb-1-cre mice have high levels of pathogenic IgG anti-RNA antibodies in their sera. Our data demonstrate that AID expression in developing B cells through class switch recombination (CSR) is critical for the production of pathogenic IgG autoantibody. These cells undergo CSR to IgG, avoiding mechanisms of IgM-mediated central tolerance. However, AID in mature B cells does not contribute to CSR of auto-reactive antibody genes. The production of IgG autoantibody is crucial for the etiopathology of SLE.

W3.14.04
Analysis of a wild mouse promoter variant reveals a novel role for FcgammaRIIb in the control of the germinal center and autoimmunity

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Genetic variants of the inhibitory Fc receptor FcgammaRIIb have been associated with systemic lupus Erythematosus (SLE) in humans and mice. The mechanism by which *Fcgr2b* variants contribute to the development of autoimmunity is unknown and was investigated by "knocking-in" the most commonly conserved wild mouse *Fcgr2b* promoter haplotype, also associated with autoimmune-prone mouse strains, into the C57BL/6 background. We found that in the absence of an AP-1 binding site in its promoter, FcgammaRIIb failed to be up-regulated on activated and germinal center B cells. This resulted in enhanced germinal center responses, increased affinity maturation and autoantibody production. Accordingly, in absence of FcgammaRIIb activation-induced up-regulation, mice developed more severe collagen-induced arthritis and spontaneous glomerular immune-complex deposition. Our data highlight how natural variation in *Fcgr2b* drive the development of autoimmune diseases and show how the study of such variants using a knock-in approach can provide insight into immune mechanisms not possible using conventional genetic manipulation, in this case demonstrating an unexpected critical role for the activation-induced up-regulation of FcgammaRIIb in controlling affinity maturation, autoantibody production and autoimmunity.

W3.14.05
Contribution of IL-10 competent Regulatory B cells (Bregs, CD19+CD24hiCD38hi) to cytotoxic T lymphocyte dysfunction after reactivation of HIV latent reservoirs

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HIV-infected "controllers" (HIV_{CON}) often exhibit robust anti-HIV cytotoxic T-lymphocyte (CTL) activity and control viral load without antiretroviral therapy (ART). In HIV-infected typical viremic non-controllers (HIV_{VIR}), CTL responses are impaired, persisting during ART, preventing clearance of the reservoir during curative interventions. During viral infections, Bregs attenuate CTL responses. We therefore investigated Breg-mediated CTL impairment during HIV infection. In HIV-peptides stimulated Breg-depleted or total PBMCs of ART-treated HIV^+ subjects (HIV_{ART}), Breg-depletion led to significant proliferation of cytotoxic $CD8^+$ T cells, total $CD4^+$ T cells and increased frequency of HIV-specific $CD8^+$ T cells. Breg-inhibition of $CD8^+$ T cell proliferation was partially IL-10-dependent. HIV_{CON} had higher Breg-frequencies than HIV_{ART} and HIV_{VIR} subjects. However, HIV_{VIR} Bregs expressed significantly more IL-10 than all other subjects; HIV_{ART} Bregs expressed more IL-10 than those of HIV_{CON} and HIV_{NEG} subjects. To evaluate if Bregs attenuate CTL activity after latent reservoirs reactivation, we assessed CTL-modulating factors in suberoylanilide hydroxamic acid (SAHA) supplemented Breg-depleted or total PBMCs of HIV_{CON} and HIV_{ART} subjects. Breg-depletion led to significant upregulation of MHC-II on B cells and MHC-II/I on dendritic cells ($LIN^-HLA\text{-}DR^+CD11c^+$). Finally, Breg-depletion was associated with significant clearance of infected cells $CD4^+$ T cells. In summary, we find that increased Breg function (IL-10 production) might contribute to CTL impairment and HIV control during untreated and treated infection. Further, Breg-depletion enhances anti-HIV CTL activity and increases *ex vivo* clearance of latently infected cells. Understanding Breg contributing to impairment of anti-HIV CTL effectiveness might be crucial in designing vaccines against HIV and immunotherapies towards viral eradication.

W3.14.06
Human innate-like B cells with regulatory properties in response to viruses

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Newborns and very young infants are highly susceptible to infections and poorly responsive to vaccines with a Th2 bias. We identified in cord blood a population of innately responsive B lymphocytes with regulatory properties. This neonatal Breg population showed a biased immunoglobulin repertoire and an innate-like phenotype, and is not found in adult blood. Gene expression microarray analysis revealed an immunosuppressive signature for this Breg population. These innate-like Bregs can modulate neonatal CD4 Th1 development through the production of a high level of IL-10 upon TLR activation. This regulatory loop can be engaged following stimulation by viruses including mainly pediatric disease associated viruses. Plasmacytoid dendritic cells contributed to this regulatory mechanism through the production of type I IFNs that further enhance the production of antiinflammatory IL-10. Thus, we uncovered a novel mechanism by which innate and adaptive inflammatory responses to pathogens are controlled in early life through a population of innate B cells.

W3.15 B and T cell memory

IL3.15.01
Regulation of human CD8+ T cell effector functions

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Our recent work has revealed the existence of a yet unrecognized transcription factor that is specifically expressed in human cytolytic CD8+ lymphocytes, a population that accumulates significantly in HCMV infected people (*J. Clin. Invest,* 2010). The factor, which we termed HOBIT, is highly related to BLIMP-1, a transcriptional repressor known to regulate terminal differentiation of B and T lymphocytes. An antibody that was raised against the unique N-Terminal part of HOBIT specifically stained cytolytic subsets of human lymphocytes. Knockdown of HOBIT in NK cell lines via siRNA reduced IFNγ production and increased cell survival but did not modulate Granzyme B expression. Interestingly, although structurally highly conserved, HOBIT expression in mice is very different from humans. Mouse HOBIT appear to be redundant for the formation and function of cytolytic T cells and NK cells, but is essential for the generation and function of NKT cells (*Nature Immunology,* 2012). Based on the combined human and mouse findings, we postulate that human HOBIT is essential for controlling formation, maintenance and function of resting cytolytic CD8+ T cells.

W3.15.01
Disparate individual fates compose robust CD8+ T cell immunity

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A core feature of protective T cell responses to infection is the robust expansion and diversification of naïve antigen-specific T cell populations into short-lived effector and long-lived memory subsets. We have developed a novel approach in which we combined high sensitivity flow cytometry and multiplexed single cell adoptive transfer to simultaneously map the fate of multiple individual T cells and their descendants in vivo during the course of bacterial and viral infection. Using this approach we find that in contrast to uniform expansion and diversification of CD8+ T cell populations single cell-derived responses are characterized by the expansion of very few effector-differentiated "giant" progenies dominating in overall size many memory phenotype "dwarfs". We identify the initial recruitment of multiple precursors as essential for robust acute and recall immunity and show that memory capacity of single cell-derived progenies is not predicted by their primary expansion size but - once established - robustly maintained during repetitive recall responses. The unique single cell origin or our data allowed us to implement novel unbiased mathematical modeling strategies. These identify the random integration of multiple differentiation and division events as the driving force behind single cell-derived variability. Reminiscent of recently described developmental processes in tissue stem cells, cell fate is specified within this probabilistic framework, along a linear developmental path that progresses from slowly proliferating long-lived to rapidly expanding short-lived subsets. Together, these data provide novel insights into how complex biological systems implement population-based developmental strategies to guarantee that stochastic processes generate robust results.

W3.15.02
Late IL-2 signals limit effector CD4 T cell contraction and serve as a checkpoint for transition to memory

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Pathogen challenge causes the dramatic expansion of responding T cells resulting in large populations of activated effector cells at both secondary lymphoid sites where they originate, and at sites of infection and inflammation to which they migrate. Following the resolution of infection, most effector cells undergo apoptosis in the process known as 'contraction', while a cohort escapes death, transitions to a resting memory state, and persists for extended periods to provide long-term immunity. The factors and mechanisms determining the extent of T cell contraction and the efficiency of memory generation are unclear. We find that late, autocrine IL-2 during a narrow timeframe is required to limit the contraction of highly-differentiated CD4 T cell effectors generated by influenza A virus (IAV). We validate key findings by restoring IL-2 signals to IL-2-deficient CD4 T cells responding in wild type (WT) hosts, and by short-term neutralization of IL-2 in recipients of WT IAV-specific donor cells. Interestingly, late IL-2 is required to rescue effectors generated from both naïve and memory precursors from enhanced contraction. One of the few differences that distinguish WT and IL-2-deficient effectors at the peak of their response is higher expression of IL-7 receptor by WT cells. We find that late IL-2 is required for effectors to increase IL-7 receptor expression, allowing ambient IL-7 to limit excessive contraction and to sustain their long-term persistence in the lung and in secondary lymphoid organs. This establishes a late IL-2-dependent checkpoint for memory CD4 T cell generation, with important implications for vaccine design.

W3.15.03
Id2-mediated inhibition of E2A represses memory CD8 T cell differentiation*

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The transcription factor Inhibitor of DNA binding 2 (Id2) modulates T cell fate decisions but the molecular mechanism underpinning this regulation is unclear. Here we show that loss of Id2 cripples effector CD8 T cell differentiation and instead programs CD8 T cells to adopt a memory fate with increased expression of the transcriptional regulators Eomesodermin and Tcf7 expression. We demonstrate that Id2 restrains the differentiation of CD8 T cells into memory cells by inhibiting E2A-mediated direct activation of Tcf7 and that Id2 expression level correlates with T cell memory recall capacity. As a result of the defective effector differentiation, Id2-deficient CD8 T cells fail to induce sufficient level of Tbx21 expression to generate short-lived effector CD8 T cells. Indeed, enforced expression of Tbx21 in Id2-deficient CD8 T cells rescues the differentiation of short-lived effector CD8 T cells. Our findings reveal that the Id2-E2A axis orchestrates T cell differentiation through the induction or repression of downstream transcription factors essential for effector and memory T cell differentiation.

W3.15.04
The transcription factor Zeb2 plays a role in promoting terminal differentiation of CD8+ T-cells through repression of the miR-200 family

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CD8+ T-cells play an important role in the immune response against intracellular pathogens, preventing the spread of infection. Yet, the mechanisms involved in the development of a robust response and its resolution into a protective immune memory population remain to be fully elucidated. We are interested in how differentiation of the effector CD8+ T-cell response is regulated. Expression profiling revealed the transcription factor Zeb2 is highly enriched in short lived effector CD8+ T-cells (SLECs). To further investigate what role Zeb2 might play in promoting SLECs we knocked out Zeb2 in activated CD8+ T-cells. Upon infection with lymphocytic choriomeningitis virus, we found the Zeb2 KO CD8+ T-cells were phenotypically skewed towards a memory precursor effector CD8+ T-cell (MPEC) phenotype, with low expression of KLRG1, and the gain of IL-7R, CD27, CXCR3, and IL-2 production. The Zeb2 KO SLECs also had altered transcriptional profiles, with reduced levels of T-BET and Prdm-1 but elevated TCF-7, EOMES, and BCL-6, genes known to be critical for memory formation. In many cancer cell lines undergoing EMT Zeb2 functions in part through repression microRNAs. We found that miR-200a, miR-200b, and mir200c where all inversely expressed with Zeb2. Furthermore, overexpression of these microRNA's largely phenocopied the Zeb2 KO, and all 3 microRNAs are upregulated in the Zeb2 KO. Our work has revealed a role for the miR-200 family in inhibiting terminal differentiation in CD8+ T-cells and a role for Zeb2 in regulating the miR-200 family to promote differentiation.

W3.15.05
IL-12-mediated STAT4 signalling and T-cell receptor (TCR) signal strength cooperate in induction of CD40L in human and mouse CD8+ T cells

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CD40L is one of the key molecules bridging the activation of specific T cells and the maturation of professional and non-professional antigen-presenting cells (APCs) including B cells. Originally, CD4+ T

cells have been regarded as the major T-cell subset that expresses CD40L upon cognate activation. We could demonstrate that a putative CD8+ helper T-cell subset expressing CD40L is generated in human and mice. To analyse which conditions during priming of CD8+ T cells lead to the induction of CD40L on CD8+ T cells we cultured naïve human or mouse CD8+ T cells with a variety of cytokines produced by dendritic cells (DCs) early after activation. STAT4-mediated IL-12 signalling proved to be the major instructive cytokine signal to boost the ability of CD8+ T cells to express CD40L not only in vitro but also in vivo after immunisation with peptide loaded DCs. Furthermore, modulation of T-cell receptor (TCR) signalling strength using stimulation of OT1 CD8+ T cells with SIINFEKL or altered peptide ligand loaded APCs revealed a correlation of TCR signalling strength during priming with CD40L induction in CD8+ T cells in vitro as well as in vivo. The induction of CD40L in CD8+ T cells regulated by IL-12 and TCR signalling may enable CD8+ T cells to respond autonomously of CD4+ T cells. Thus, we propose that under proinflammatory conditions a self-sustaining positive feedback loop could facilitate the efficient priming of T cells stimulated by high affinity peptide-displaying APCs.

W3.15.06
Survival of CD8 memory T cell precursors depends on NKG2D mediated PI3K signaling and Mcl-1 induction

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Memory formation of activated CD8 T cells is the result of a specific combination of signals that promote long-term survival and inhibit differentiation into effector cells. Much is known about initial cues that drive memory formation. Which signals are essential during the intermediate stages before terminal differentiation, however, is poorly understood. We show that the activating co-receptor NKG2D controls formation of CD8 memory T cells by promoting survival of precursor cells. We demonstrate that NKG2D enhances PI3K signaling in a specific phase of memory cell commitment. This phase characterizes a transition stage from IL-2 to IL-7 dependency, during which activated cells highly rely on NKG2D-signaling for their survival. NKG2D-induced PI3K signaling is essential for the induction of pro-survival protein Mcl-1 and precursor cell survival. NKG2D-deficiency results in reduced memory cell formation and impaired protection against re-infection. Our findings show a new role for PI3K and NKG2D in an underappreciated stage of effector to memory transition that is essential for the generation of efficient long-term anti-viral protection.

W4.01 Immunity to virus infection

IL4.01.01
Genomic polymorphisms in 3β-hydroxysterol Δ24-reductase promoter sequences

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It was recently reported by the present team that 3β-hydroxysterol Δ24-reductase (DHCR24) is induced by hepatitis C virus (HCV) infection. In addition, upregulation of DHCR24 impairs p53 activity. In human hepatoma HuH-7 cells, the degree of DHCR24 expression is higher than in normal hepatic cell lines (WRL68) at the transcriptional level. The genomic promoter sequence of DHCR24 was characterized and nucleotide substitutions were observed in HuH-7 cells at nucleotide numbers -1453 (G to A), -1420 (G to T), - 488 (A to C) and -200 (G to C). The mutations of these sequences from HuH-7 cell types to WRL68 cell types suppressed DHCR24 gene promoter activity. The sequences were further characterized in hepatocytes from patient tissues. Four tissues from HCV-positive patients with cirrhosis or hepatocellular carcinoma (#1, 2, 3, and 5) possessed HuH-7 cell type sequences. Interestingly, one patient with liver cirrhosis (#4) possessed WRL68 cell-type sequences; this patient had been infected with HCV and was HCV negative for 17 years after interferon therapy. Next, the effect of HCV infection on these polymorphisms was examined in humanized chimeric mouse liver and HuH-7 cells.
The human hepatocytes possess WRL68 cell type and did not show the nucleotide substitution after HCV infection. The HCV-replicon was removed by interferon treatment and established the cured K4 cells. These cells possess HuH-7 cell type sequences. Thus, this study showed the genomic polymorphism in DHCR24 promoter is not directly influenced by HCV infection.

W4.01.01
Suppressor of cytokine signalling (SOCS) 4 is a critical regulator of the anti-viral immune response

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The suppressors of cytokine signalling (SOCS) proteins are negative regulators of cytokine signalling, immune cell development and function, and the inflammatory response. SOCS1-3, CIS and to some extent SOCS5-7 have defined roles in controlling the magnitude of response of various cytokines including interferons, IL-12, IL-10, gp130, G-CSF, prolactin, growth hormone, insulin, IL-4, and IL-2. SOCS4 is the last remaining SOCS protein for which there is no described biological role or intracellular target. Given the important role of other SOCS proteins in controlling the immune response and the expression of SOCS4 in the haematopoietic system, we have investigated its role during infection. Using a murine influenza model

we have demonstrated that mice lacking functional SOCS4 protein (*socs4*$^{108/108}$) show greater susceptibility to viral infection. *socs4*$^{108/108}$ mice have exacerbated symptoms of infection including substantially increased weight loss, linked to dysregulated cytokine and chemokine production in the lungs. SOCS4-deficeint mice also have delayed viral clearance associated with impaired trafficking of influenza specific CD8$^+$ T cells. *In vitro*, *socs4*$^{108/108}$ CD8$^+$ T cells display defective proliferation upon T cell receptor stimulation indicating defective TCR signalling. Investigation of candidate SOCS4 interacting partners by affinity purification and mass spectrometry identified an adapter protein involved in TCR signalling as a putative target, further implicating SOCS4 in regulating viral responses. This is the first demonstration of a functional phenotype for SOCS4-deficient mice and suggests that SOCS4 plays a role in immune regulation during infection. We postulate that SOCS4 is a critical regulator of the anti-viral response.

W4.01.02
Timing and quality of the type-I interferon response to viral infection is tuned by stochastic cellular decisions at multiple levels

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Viral infection triggers IFN-β gene induction upon recognition of viral RNA and subsequent activation of transcription factors IRF-3/7 and NF-κB. Secreted IFN-β stimulates antiviral gene expression via the IFN-α receptor. IFN-β expression is heterogeneous, however, the contribution of cellular signalling is unknown. Here, we visualize dynamics of IFN signalling at cellular and subcellular resolution with BAC-encoded reporter genes and chimeric transcription factor-fluorescent proteins. We find stochasticity on multiple layers of the IFN system. While viral dose impacts on IFN expression frequency, variation of expression timing appears stochastic and largely independent of viral influence. Sister cells reveal quick randomization of response timing to viral infection. To elucidate underlying mechanisms of stochastic IFN gene expression we quantitatively analyze signalling dynamics of IRF-3, NF-κB and the mitochondrial adaptor MAVS.
The IFN-stimulated response within a cell population exhibits bimodality at suboptimal concentrations as revealed by IRF-7 reporter gene expression and validated for other IFN-stimulated genes. We find the frequency of IFN-stimulated expression increasing in a dose-dependent manner. Consequently, upon viral infection the fraction of cells responding to IFN is protected whilst non-responders are not.
Our multi-scale mathematical model suggests that despite temporal variability of IFN-β production, the signal amplifies by paracrine stimulation into an all-or-nothing protective state of the population.
Thus, we hypothesize antiviral response control by tuning expression frequency of IFN-β and IFN-stimulated genes coupled with multiple stochastic signalling components controlling timing of IFN-β. These findings deepen our understanding how cell-to-cell variability can regulate innate immunity at the population level.

W4.01.03
Serological diagnosis of Pepino mosaic virus with new generated monoclonal antibodies

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Pepino Mosaic Virus (PepMV), monopartite RNA virus, belonging to Flexiviridae and potexvirus group is highly infectious and easily transmissible. Its economic impact is major for the tomato producer's countries. Prevention, based on early virus detection is the only effective control measure. Monoclonal antibodies appeared to be very useful tools. We used hybridomas technique to generate monoclonal antibodies, by fusing B cells of immunized BALB/C mice to PepMV and myeloma cells.
The aim of this work is to produce in Morocco hybridomas producers of Mab that could be used for ELISA. In the same time, these efforts will serve to decrease expenses of producers concerning phytosanitory control. We obtained 16 hybridomas lines producers of Mab specific for PepMV. They were tested for efficiencies in ELISA and 2 lines were retained for production of Mab on large scale 1B11-G10 and 5A1- G5. Isotyping of these 2 lines was carried out using Ouchterlony double diffusion; both clones are of the IgG1 isotype. The conjugation of these two antibodies to alkaline phosphatase has been verified by DAS-ELISA.
These antibodies will enable to diagnose the disease from infected tomato plants, integrating several serological tests to control it in field and target the actions of struggles.

W4.01.04
An identical miRNA of the human JC and BK polyoma viruses targets the stress-induced ligand ULBP3 to escape immune elimination

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The human polyoma viruses JC virus and BK virus establish asymptomatic persistent infection in 65%-90% of humans but can cause severe illness under immunosuppressive conditions. The mechanisms by which these viruses evade immune recognition are unknown. Here we show that a viral miRNA identical in sequence between JCV and BKV targets the stress-induced ligand ULBP3, which is a protein recognized by the killer receptor NKG2D. Consequently, viral miRNA-mediated ULBP3 downregulation results in reduced NKG2D-mediated killing of virus-infected cells by natural killer (NK) cells. Importantly, when the activity of the viral miRNA was inhibited during infection, NK cells killed the infected cells more efficiently. Because NKG2D is also expressed by various T cell subsets, we propose that JCV and BKV use an identical miRNA that targets ULBP3 to escape detection by both the innate and adaptive immune systems, explaining how these viruses remain latent without being eliminated by the immune system.

W4.01.05
CD4+T cells modulate Epstein-Barr virus (EBV) latency types

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Following infection with EBV, the virus is carried for lifetime in the memory B cell compartment in a silent state (latency I/0). These cells do not resemble the proliferating lymphoblastoid cells (LCL, latency III) that are generated after infection.
It is of fundamental significance to identify how the different EBV latencies are established. In view of the prompt response of CD4+T cells in primary EBV infection and their role in B cell differentiation, we studied the involvement of CD4+T cells in the regulation of EBV latency.
Co-culture of LCLs with activated CD4+T cells led to changes in viral protein expression, indicating a shift from latency III to the less proliferating latency II. This effect was mediated by soluble factors. IL21 and soluble CD40L are two of the responsible cytokines.
To study the in vivo establishment of latency, we infected humanized mice (NOD/SCID/IL2R-/- mice engrafted with human CD34+ HSC) with EBV. Q-PCR analysis of viral promoters confirmed that besides latency III, other latencies were also present in the splenocytes of the infected mice. Depletion of CD8+T cells (leading to higher numbers of CD4+T cells) lead to about 4 times higher relative Q promoter activity (used in latency II and I) compared to mice with unmanipulated T cells. On the other hand, depletion of CD4+T cells was associated with the lack of Qp activity in the majority of mice. Thus, both our in vitro and in vivo experiments suggest that CD4+T cells contribute to the establishment of type II/I latency.

W4.01.06
HSV-2 regulates inflammatory response in the vaginal epithelium via the Fas/FasL pathway

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Introduction. The role of Fas/FasL in the development of mucosal epithelium lesions and regulation of the early immune response during sexually transmitted diseases, such as HSV-2 infection has been poorly investigated. Here we define the role of Fas/FasL in maintaining the mucosal epithelium integrity and launching local immune response.
Materials and methods. Fas (lpr) and FasL (gld) knock-out mice with C57BL background were infected intravaginally with HSV-2. The vaginal tissues were used for detection of apoptosis, phenotyping of immune competent cells, determination of virus titers, and expression of selected cytokines and chemokines as well as anti- and proapoptotic proteins.
Results. Infection of Fas and FasL-deficient mice resulted in the increased accumulation of inflammatory cells, but also Tregs within the infected vaginal epithelium, followed by decreased recruitment of monocytes, NK and CD8+ T cells, in parallel with decreased production of CXCL9 and TNF-α. Fas (-) and FasL (-) mice showed delayed virus clearance from the infected vaginal tissue. Furthermore, lack of Fas or FasL increased the percentage of apoptotic, HSV-2 infected cells due to the activation of caspase-9 in comparison to HSV-2 infected C57BL6 wild type strain at early stage of infection. Additionally, Fas and FasL-deficient mice showed a significant decrease in the anti-apoptotic Bcl-2 protein expression.
Conclusion. Fas/FasL pathway in the HSV-2 infected vaginal epithelium participates in regulation of inflammatory response and further recruitment of immune competent cells by influencing production of chemokines and cytokines

W4.01.07
Evolution of neutralizing antibodies against HIV-1C gp160 molecular envelope clones from acute heterosexually acquired HIV-1C infections in Botswana

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Thirty years after the discovery of HIV, there is still no potent and efficacious vaccine against the infection. The recent discovery of potent and broad neutralizing antibodies against HIV-1 has revived the interest in the search for more of these antibodies and to investigate their possible role in preventing the infection. A cohort of eight HIV-1C acutely infected individuals were enrolled and followed for a period more than 24 months. Plasma samples were collected at different time points during the follow-up and stored at -80ºC. Viral RNA was extracted from the plasma of these individuals collected at or around baseline as well as 6 months post enrolment and envelope protein, glycoprotein 160, amplified. The HIV-1C envelope gene from these individuals was cloned into pcDNA3.1D/V5-His expression vector. The envelope clones were subsequently used to co-transfect 293 T cells along with a backbone vectors to produce pseudoviruses. A standardised neutralization assay based on TZM-bl cells was used to determine the autologous neutralizing capacity. All but one individual's envelope pseudotyped viruses were neutralised by some of the autologous plasma albeit very late. Viruses generated for individuals displayed varying neutralization sensitivities towards autologous plasma. Potency of the neutralization increased with time. Neutralizing antibody development lags behind as evidenced by the lack of neutralizing capacity of the contemporaneous plasma. HIV-1C neutralizing antibodies develop much later, around 5 months, with significant potency increasing at around 2 years post infection. This gives interesting pointers to HIV vaccine development and design for prevention.

W4.01.08
Dual deletion of type I and type II interferon-binding proteins in NYVAC-HIV-1 Env-Gag-Pol-Nef poxvirus vaccine vectors markedly improves vector immunogenicity

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Background: NYVAC, a replication deficient derivative of the Copenhagen vaccinia virus used, is a safe and immunogenic HIV vaccine vector. Deletion of immune evasion genes is an attractive strategy for improving NYVAC immunogenicity. Our aim was to characterize the innate immunological profile of NYVAC expressing the HIV-1 clade C env-gag-pol-nef genes (NYVAC-C) with single or double deletion of genes encoding type I (ΔB19R) or type II (ΔB8R) IFN-binding proteins. Methods: Human monocytes were infected with NYVAC-C, ΔB19R or ΔB8RB19R. Gene arrays and biological pathways were analyzed using the Illumina BeadChips and Ingenuity Pathway Analysis. Cytokines were quantified by RT-PCR, Western blot and ELISA. Proliferation of allogenic CD4-T cells was measured by H3-incorporation. Results: Transcriptomic analyses of monocytes infected with NYVAC-C, ΔB19R and ΔB8RB19R revealed a concerted up-regulation of innate immune pathways (IFN-stimulated genes-ISGs) of increasing magnitude with ΔB19R and ΔB8RB19R relative to NYVAC-C. Deletion of *B8R* or *B19R* enhanced activation of IRF3, IRF7 and STAT-1 and production of IFNs whose expressions were inhibited by anti-type I IFN antibodies. ΔB8RB19R induced the production of much higher levels of pro-inflammatory cytokines than NYVAC-C or ΔB19R and a strong inflammasome response. Consistent with these findings, ΔB8RB19R-infected monocytes induced stronger type I IFN-dependent and IL-1b-dependent allogenic CD4-T cell responses than NYVAC-C or ΔB19R. Conclusions: Deletion of type I and II IFN evasion genes in NYVAC markedly enhanced its immunogenic properties via an increased expression of type I IFNs and IL-1b and make it an attractive candidate HIV vaccine vector.

W4.01.09
A novel mechanism of HTLV-1-associated mylopathy/tropical spastic paraparesis (HAM/TSP) pathogenesis

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Human T cell leukemia virus type 1 (HTLV-1), is etiologic agent of HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), a slowly progressive neurologic disease. Soluble form of Fas (sFas) molecule implicate in Fas signaling, suggesting a role in some pathogenesis. We hypothesized that existence of sFas might cause partial resistance against Fas-mediated apoptotsis seen in HAM/TSP patients.
Twenty people of each HAM/TSP patient, asymptomatic HTLV-1 carrier (AC) and healthy volunteer groups, who were followed at HTLV-1-Clinic of Ghaem Hospital, were chosen. Gene expression (using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)) and serum protein level (using a highly sensitive enzyme-linked immunosorbent assay (ELISA)) of sFas in each three subject groups were evaluated.
The Mean sFas serum level in patients (0.389 ng/ml) was significantly higher than healthy individuals (0.131 ng/ml) ($p<0.05$). There were no significant differences between HAM/TSP patients and ACs (0.253 ng/ml) ($p=0.17$) and also no significant differences between AC and healthy groups ($p=0.16$).
Although the mean sFas gene expression in patients (0.456) was higher than both carriers (0.304) ($p=0.35$) and control individuals (0.221) ($p=0.15$), and also in carriers was higher than controls ($p=0.6$), but these differences were not statistically significant. There

was no significant difference and correlation between sFas serum level and gene expression in each three subject groups.
These results suggest that sFas may play an important role in the pathogenesis of HAM/TSP and that serum sFas may be related to clinical activity in HAM/TSP patients, but the source of the elevated serum sFas in HTLV-1 infected patients remains unclear.

W4.01.10
Molecular characterization of immunoglobulin (Ig) genes reveals that plasmablasts generated during heterologous secondary dengue infections and circulating memory B cells after recovery are of distinct origin

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Memory B cells generated after exposure to dengue viral (DENV) infection are a central component in shaping immune memory. However, re-exposure to dengue virus of a different serotype is often associated with an increased disease pathogenesis. During repeated infection, the rapid activation of memory B cells leads to the generation of massive amounts of cross-reactive antibodies. In the acute phase of a secondary infection, this B cell memory pool probably outcompetes the newly generated virus specific B cells. To characterize the specificity of plasmablasts and memory B cells generated during and after acute infection, we performed single cell RT-PCR for sequence analysis of Ig variable regions. Surprisingly, we found that memory B cells isolated one month after disease were of different genetic composition than acute phase plasmablasts. Our data reveal preferential usage of the Ig heavy chain variable region gene VH1 in DENV binding plasmablasts. In contrast, VH3 family repertoires were noted in specific long lasting circulating memory cells isolated over a month after infection. The antibodies isolated from these memory B cells had a broad reactivity, with only 30% being specific to DENV envelope (E) protein, whereas 70-80% plasmablasts were specific to E protein. Whether VH gene usage differs in primary and secondary infected patients and reflects a specific antigen/epitope selection is currently under investigation. This study can help to understand immune mechanism of protection for potential application in vaccination.

W4.01.11
Chronic viral infection alters subnuclear localization of PD-1 and L-selectin loci linked to transcriptional programming in LCMV-specific CD8 T-cells

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Mechanisms that mediate changes in transcriptional regulation during differentiation of long-lived memory T-cells during infection remain poorly understood. During chronic viral infection, CD8 T-cell differentiation yields a unique transcriptional profile: T-cell exhaustion. The suboptimal effector function of exhausted cells is partly due to sustained expression of the inhibitory receptor PD-1. Using the lymphocytic choriomeningitis virus mouse model of infection, we examined pdcd1 (PD-1) and sell (L-selectin) gene regulation at different stages of T-cell differentiation. We hypothesized that epigenetic programming and nuclear localization of these genes were coupled to viral persistence and T-cell differentiation. Changes in gene expression are accompanied by patterns of locus conformation and positioning of genes relative to specific compartments such as nuclear lamina (silencing). Using DNA-FISH, we report a significant decrease in pdcd1 alleles associated to nuclear lamina in exhausted cells relative to other CD8 T-cell subsets (permissive). Dissociation from nuclear lamina was consistent with upregulated expression of PD-1 mRNA and protein, and with loss of PD-1 DNA methylation in exhausted cells. In contrast, we observed a significant increase in sell alleles associated to nuclear lamina with exhaustion (repressive), which mirrored changes in sell gene expression. CD8 T-cells from Blimp-1-deficient chronically infected mice exhibited an impaired ability to regulate pdcd1 and sell association to lamin, thus implicating Blimp-1 in modulating gene expression of both PD-1 and L-selectin during chronic infection. Our findings contribute to the understanding of the molecular pathways mediating T-cell memory and exhaustion, and may provide insight into implementing therapeutic strategies to resolve chronic infections.

W4.01.12
The anti-apoptotic protein Mcl-1 is essential for the CD8 T cell response upon chronic viral LCMV infection

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Chronic infections with viruses such as hepatitis B virus, hepatitis C virus or HIV are in endemic steady state in the world population, constituting a major global public health problem. Studies of chronic viral infections in humans and mice show that persistent antigenic stimulation induces deregulation of T cell responses by a process called T cell exhaustion that culminates in deletion of virus-specific T cells. The ability to generate and retain sufficient numbers of functionally competent T cells relays on the balance between T cell proliferation and apoptosis. Apoptosis is regulated by the interplay of pro-survival and pro-apoptotic molecules of the Bcl-2 family. In particular a pro-apoptotic protein of the family, Bim, has been implicated in the deletion of virus-specific CD8 T. However is still unknown which of the pro-survival Bcl-2 family members is neutralized by Bim to mediate deletion of cytotoxic T cells.
The aim of this project is to determine the role of the anti-apoptotic protein Mcl-1 in promoting CD8+ T cell responses upon chronic viral infection. To address this question we infected Mcl-1 haploinsufficient mice with LCMV docile, which causes chronic infection in mice. Indeed infected Mcl-1+/- mice show a reduction of viral-specific CD8+ T cells and reduced viral elimination in Mcl-1+/- mice. These data indicate that Mcl-1 is essential for the CD8+ T cell response upon chronic viral infection.
We propose that pharmacological targeting of Mcl-1, e.g. during anti-cancer therapy, may cause enhanced susceptibility to viral infection as one possible side effect.

W4.01.13
Pregnancy-induced deviation of chemokine/cytokine serological levels during influenza virus AH1N1 infection

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Introduction: For seasonal and pandemic influenza, pregnancy is one of the major risk factors for severe complications. It has been hypothesized this is related with the "tolerant" state needed for pregnancy maintenance, which include diminished inflammatory cytokine production. Interestingly, for high virulence influenza virus, fatal complications among young people seem to be related with hypercytokinemia, which may not occur in pregnant women. We decided to establish if there were differences in serological cytokine-chemokine levels from pregnant and non-pregnant women infected with influenza virus pdm2009.
Methods: Serum was obtained from pregnant (n=10) or non-pregnant women(n=14) infected with influenza virus AH1N1 pdm2009. None of the patients required hospitalization. As control, serum samples from clinically healthy women(n=7) were included. chemokines/cytokines (TNFa, IL-6, IL-8, MCP-1, MIG, IP-10)quantification was made using cytometric bead array system for flow cytometry.
Results: When pregnant and non-pregnant healthy women are compared with influenza infected pregnant and non-pregnant women, non-pregnant patients showed diminished TNFa and IL-10 serological levels (TNF: 54.1+/-13.6pg/mL vs 3.5+/-2.6pg/mL; IL-10: 37.5+/-

13.6pg/mL vs 7.4+/-7.3pg/mL). For chemokines, pregnant women showed diminished, MCP-1, MIG and IP-10 concentrations compared with non-pregnant infected women (MCP-1: 153.0+/-160.2pg/mL vs 974.7+/-1256.2pg/mL); (MIG: 243.5+/-208.9pg/ml vs. 2748.2+/-1665.8pg/mL) (IP-10: 308.4+/-223.0pg/mL vs. 4270.8+/-1371.8pg/mL).
Conclusion: Pregnancy deviates cytokines/chemokines responses induced by AH1N1 pdm2009 influenza infection, with exacerbated inflammatory responses but less leucocyte migration capabilities to infected tissues.

W4.01.14
Spred-2 deficiency exacebates Influenza A Virus (H1N1)-induced Pneumonia

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Influenza A (H1N1) viral infections have been identified as the etiologic agents for historic pandemics, and contribute to the annual mortality associated with acute viral pneumonia. Many studies have elucidated the functions of H1N1-viral proteins of this negative-strand RNA virus have been well elucidated. However, the virus-induced intracellular signaling events remain unknown. The Raf/MEK/ERK cascade is the prototype of mitogen-activated protein (MAP) kinase cascades and has an important role in cell growth, differentiation, and survival. The Sprouty-related Ena/VASP homology 1-domain-containing protein (Spreds) has recently been identified as a negative regulator of Raf-dependent ERK activation. Here, we demonstrated that Spred-2-knockout (KO) mice led to higher mortality compared with wild-type (WT) mice during influenza A (H1N1) viral infection. Spred-2 KO mice showed more severe pneumonia with increased number of neutrophils in bronchoalveolar lavage. Whole lungs from Spred-2 KO mice had significantly higher levels of Type-I interferon (IFN-α and IFN-β) and pro-inflammatory cytokine (IL-6) in comparison to WT mice. Moreover, the administration of U0126, MEK inhibitor, improved the survival with decreased virus titers and cytokines in mice during influenza A (H1N1) viral infection. Together, the results of this study show that Spred-2 negatively regulates the development of anti-viral immunity and has an effect on abrogated cytokine balance through Raf/MEK/ERK pathway. We propose these regulations of intracellular signaling through Spred-2 during influenza viral infection might provide mechanistic approaches for modifying and controlling the immune response against influenza H1N1 virus infection.

W4.02 Liver viral infections

IL4.02.01
Innate Sensing A Virus Designed Not To Be Seen

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Viral nucleic acids often trigger an innate immune response in infected cells. Many viruses, including hepatitis C virus (HCV), have evolved mechanisms to evade intracellular recognition. Despite this, HCV triggers a robust interferon-stimulated gene (ISG) response in the infected liver. In an attempt to reconcile these apparently contradictory observations, we discovered that HCV-infected cells and HCV subgenomic replicon cells can trigger a viral RNA-, TLR7- and cell contact-dependent interferon response in nonpermissive plasmacytoid dendritic cells (pDCs). Here we report that these events are mediated by transfer of HCV RNA-containing exosomes from infected cells and subgenomic replicon cells to pDCs. In contrast, pDCs are not stimulated by infectious virus particles, even after very high multiplicity inoculation. Exosomal viral RNA transfer is dependent on the endosomal sorting complex (ESCRT) machinery and on Annexin A2, an RNA-binding protein involved in membrane vesicle trafficking, and it is suppressed by exosome release inhibitors. Further, purified concentrated HCV RNA-containing exosomes are sufficient to activate pDCs. Thus, vesicular sequestration and exosomal export of viral RNA may serve both as a viral strategy to evade pathogen-sensing within infected cells and as a host strategy to induce an unopposed innate response in replication-nonpermissive by-stander cells.

IL4.02.02
NK cells and HCV: a complex and controversial relationship

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NK cells are important antiviral effectors of innate immunity either via direct killing of infected cells or cytokine (namely IFN- and TNF-) release, and are controlled by a complex network of signals which interact with membrane-expressed, germline-encoded, inhibitory and activating receptors. NK cell responses are readily generated during acute HCV infection and persist through chronic infection. Despite the availability of standardized reagents, studies on NK cells in HCV infection have generated controversial findings in the past recent years. Current evidence suggests that peripheral NK cells are polarized towards cytotoxicity and that interferon-gamma secretion is deficient in chronic HCV infection, consistent with a functional dichotomy and a consequent inability to control the virus through non-cytolytic mechanisms. However, intrahepatic NK cells display an exhausted phenotype characterized by reduced TRAIL expression and degranulation ability which may also contribute to viral persistence. Consistent with this view, exposure of healthy donors' NK cells to culture-derived HCV resulted in significant TRAIL upregulation and degranulation, whereas this was not observed using NK cells from patients with chronic HCV infection. Recent findings also suggest that early NK cell activation following administration of IFN- based therapies is associated with sustained virological response and that the NK perforin content play a role in response to antiviral treatment. Collectively, this data underlines the importance of innate immune cells in controlling HCV infection and provides new translational applications in this setting.

W4.02.01
Dynamic changes in the liver upon persistent viral infection

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Persistent viral infections such as hepatitis C and hepatitis B pose a great challenge for human health. Yet, the molecular mechanisms of viral pathogenesis remain poorly understood. This is in part due to the inherent complexity of the involved multi-layered host-pathogen interactions as well as due to experimental limitations. Here we adopted the lymphocytic choriomeningitis virus mouse model, which provides a pathophysiologically relevant and versatile experimental benchmark system, to study virus-induced changes and chronic inflammation in the liver upon persistent infection. Using RNAseq and iTRAQ protein mass spectrometry we identified extensive dynamic changes in the liver transcriptome and proteome over a period of more than 60 days after infection. We integrated these systems-wide data with virological readouts, immunohistopathology and bioinformatical network analysis in order to delineate key molecular events and pathways that govern and precede virus-induced liver immunopathology. This model approach shall contribute to the identification of novel general principles of host-virus interactions relevant to persistent viral hepatitis in man.

W4.02.02
Reactive oxygen species delay control of lymphocytic choriomeningitis virus

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CD8+ T cells are of critical importance to prevent chronic viral infections because they not only promote virus elimination, but also induce virus mediated immunopathology. Elevated levels of reactive oxygen species (ROS) have been reported during virus infections. However, the role of ROS in T cell mediated immunopathology remains unclear. Here we used the murine lymphocytic choriomeningitis virus to explore the role of ROS during the processes of virus elimination and induction of immunopathology. We found that virus infection led to elevated levels of ROS producing granulocytes and macrophages in virus infected liver and spleen tissue that was triggered by the NADPH oxidase. Lack of the regulatory subunit p47phox of the NADPH oxidase diminished ROS production in these cells. While CD8+ T cells exhibited ROS production that was independent of NADPH oxidase expression, survival and T cell function was elevated in p47phox deficient (Ncf1-/-) mice. In the absence of p47phox, enhanced T cell immunity promoted virus elimination and blunted corresponding immunopathology. In conclusion, we find that NADPH-mediated production of ROS critically impairs the immune response, impacting elimination of virus and outcome of liver cell damage.

W4.02.03
Cellular inhibitors of apoptosis (IAPs) play a critical role in the host response to hepatitis B virus and their repression promotes viral clearance

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Background: Pattern recognition receptors, TNF-alpha (and other death receptors), and IL-1 are involved in inflammation, apoptosis, necroptosis and pyroptosis signalling. These processes are critical regulators of innate and adaptive immune responses to infectious diseases. IAPs form the central hub that modulate inflammatory and death signalling and we hypothesised that they determine the host responses to hepatitis B infection (HBV). HBV uses several strategies to antagonise immunity and cell death allowing persistence of its transcriptional matrices called covalently closed circular (ccc) DNA within hepatocytes. The aim of our study was to investigate the role of IAPs in HBV infection.
Design: We used a combination of gene-targeted mice and compound mutant mice, small drug molecules that target IAPs, and a highly novel mouse model of HBV. The model recapitulated HBV chronicity with HBV viremia persisting beyond 8 weeks and innate and adaptive immunity were critically required to control infection.
Results: Modulation of IAP function, through gene-targeting or the use of small molecule drugs profoundly affected host control of HBV and its elimination. As early as three days post drug treatment, mice showed a substantial log reduction in viral loads.
Conclusion: Using a novel mouse model we show that IAPs are central regulators of host responses to HBV and they are critical determinants of infectious outcomes.

W4.02.04
Imaging the innate immune response to viral infection: neutrophils, platelets, and NETs

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Through the use of intravital spinning-disk confocal microscopy, we have visualized the immune response to viral infection of the liver within live mice. Following intravenous injection of myxoma virus, we observe infection of host cells and a rapid accumulation of neutrophils in the mouse liver. These neutrophils adhere in the liver sinusoids, crawl within the vessels, and appear to probe the liver microvasculature. Neutrophils are not specifically recruited to virally infected cells, but rather, appear throughout the liver vasculature. We also observe recruitment of platelets to liver sinusoids, which associate with neutrophils, forming large aggregates. These platelet aggregates are dynamic structures, continually expanding and sloughing off, re-entering the circulation.
Using various blocking antibodies, and mice deficient for specific immune receptors and adhesion molecules we have characterized, in detail, many aspects of this early innate immune response to virus. Surprisingly, the observed platelet-neutrophil interactions led to the formation of neutrophil extracellular traps (NETs) within the liver. NETs are diffuse, web-like structures comprised of sticky, extracellular DNA decorated with histones and antimicrobial proteins. Importantly, we have demonstrated, for the first time using an *in vivo* model, the presence of NETs within the vasculature protects host cells from viral infection.
This work will provide a better understanding of the roles of, and interactions between, immune cells within the liver in response to virus and will allow for the better design of treatments to optimize the host response to viral infections.
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W4.02.05
Tim-3/galecin-9 pathway modulates the cross talk between NK cells and CD8+ T cells in chronic hepatitis B

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T cell immunoglobulin and mucin domain-3 (Tim-3) has been widely known as immune regulator which plays important roles in various disease. We previously reported the repression of Tim-3 on NK cells in patients with chronic hepatitis B (CHB). In this study, we characterized the role of Tim-3 on NK-CD8+ T cells cross talk in HBV infection. We found that Tim-3 expression was significantly up-regulated on CD8+ T cells which significantly correlated with HBV viral load. Tim-3 expressing CD8+ T cells had a higher expression of activated maker CD69 but failed to produce IFN-gamma, Granzyme B, perforin or proliferate in response to HBs-antigen peptide or anti-CD3 and anti-CD28, indicating the negative role of Tim-3 on CD8+T cells. Consistently, blocking Tim-3 pathway in PBMCs enhanced HBV specific IFN-γ expression and proliferation in CD8+ T cells of CHB patients. However, blocking Tim-3 pathway didn't change HBs-stimulated IFN-γ expression of purified CD8 T cells from CHB patients. Depletion of NK cells destroyed the Tim-3 mediated suppression on HBV specific IFN-γ expression and proliferation in CD8+ T cells. Pretreatment of lactose which blocks galectins significantly increased the activity of CD8 T cells from CHB patients. In conclusion, the Tim-3-mediated suppression on CD8+T cells needs the presence of NK cells. All these findings have an implication that Tim-3 represents a new therapeutic target in chronic hepatitis B infection.
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W4.02.06
Breaking T-cell tolerance to viral antigens in a model of chronic HBV infection

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Chronic HBV infection is characterized by a weak and exhausted T cell response. To promote sustained viral clearance it is necessary to boost a viral specific immune response of the host. HBV transgenic mice are immunologically tolerant to HBV antigens, representing a good model to test strategies aiming at breaking T cell tolerance. Gene delivery of cytokines to the liver may represent an interesting strategy to maximize its antiviral efficacy and reduce side effects. We now provide a novel strategy to induce functional T cell in HBV transgenic mouse model based on the genetic transfer of immunostimulatory cytokines. Sustained liver specific IL-15 expression expands the population of HBV-specific CD8 T cells in spleen and the liver, as shown by pentamer lymphocyte staining. However, IL-15 expanded T cells failed to kill peptide loaded cells in an in vivo killing assay, indicating that they remained functionally silent. When IL-15 expression is combined with IFN-α, we observed an increase in the number of HBV specific T cell but more importantly those cells are functional, since they were able to kill antigen loaded cells. Furthermore a strong antiviral effect was observed. Thus, our study defines a potent new approach to brake the tolerance in a model of chronic viral infection by 1.- expanding virus-specific CD8 T cell with IL-15 and 2.- blocking negative signals with IFN-α. Such an approach may have broad applications in developing treatment strategies for chronic infections in general, and perhaps also for tumors.

W4.03 HIV pathogenesis and immunity

IL4.03.01
The Fight Between HIV Infection and Macrophage Polarization

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HIV-1 infects CD4+ T lymphocytes and cells of the mononuclear phagocyte lineage, including dendritic cells, circulating monocytes and tissue macrophages. Unlike T cells, macrophages are resistant to the cytopathic effects of the virus and accumulate virions in intracellular compartments of debated origin. We have described already that functional polarization of human monocyte-derived macrophages (MDM) as either M1 or M2a cells (after 18 h stimulation with TNF-a and IFN-g or IL-4, respectively) results in a restricted replication of HIV-1 when compared to nonpolarized MDM. The underlying mechanisms of restriction appear to be different for M1- and M2a-MDM and involve the upregulation of factors either known or suspected to play a role in controlling virus replication and transmission to other cells. These factors include the upregulation of CCR5-binding chemokines and of the intracellular factor known as APOBEC3A in M1-MDM and of DC-SIGN expression on M2a cell surface. Overall, macrophage polarization is a transient process that likely aims at containing or eliminating HIV-1 infection either directly or by potentiating adaptive recognition of infected cells via upregulation of MHC Class II antigens on the cell surface.

W4.03.01
Endoplasmic reticulum aminopeptidase 2 (ERAP2) haplotypes play a role in antigen presentation and resistance to HIV-1 infection

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Background: Haplotype-specific alternative splicing of the endoplasmic reticulum aminopeptidase type 2 (*ERAP2*) gene results in either full-length (FL, haplotype A) or alternatively spliced (AS, haplotype B) mRNA. As this protein trims peptides loaded on MHC class I and CD8+ T lymphocytes play an important role in protection against viral infections, we analysed the role of this gene in resistance to HIV-infection. Methods: *ERAP2* was genotyped in a cohort of 104 Spanish HESN individuals exposed to HIV through injection drug use (IDU-HESN). PBMC isolated from 139 healthy controls (HC) grouped according to their *ERAP2* genotype were infected with HIV-1_{Ba-L} and analysed for: 1) mRNA specific for *ERAP2* and 96 genes involved in the antigen presentation pathway; 2) MHC class I MFI on CD45+ cells; 3) susceptibility to HIV infection; 4) ERAP2 protein expression. Finally, we analysed the co-segregation of *HLAB*57* with *ERAP2* haplotype in a cohort of Italian HESN. Results: Genotype analysis indicated that *ERAP2* haplotype A is associated with protection from HIV infection (meta-analysis *p* value of 7.6 x10^{-5}) and that *HLAB*57* allele is significantly more common among HESN homozygous for haplotype A (homoA). In vitro HIV-1 infection mRNA for ERAP2-FL and a number of genes involved in antigen presentation as well as of MHC class I on CD45+ cells were significantly increased in homoA cells from HC; notably, these same cells, but not isolated CD4+ cells, were less susceptible to HIV-1 infection.
Conclusions:*ERAP2* haplotype A confers resistance to HIV infection secondarily to its effect on antigen processing and presentation.

W4.03.02
Molecular characterization of high avidity CD4+ T cells in HIV Controllers

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Background: HIV Controllers spontaneously control HIV replication to levels undetectable by standard assays in the absence of antiretroviral treatment. We previously reported that HIV Controllers harbour a pool of memory CD4+ T cells able to respond to the immunodominant Gag293 peptide with particularly high TCR avidity. We set to functionally analyze high avidity CD4+ T cells and to characterize their TCRs at the molecular level.
Methods: HIV Controllers from the ANRS CODEX CO21 cohort (n=8) were compared to efficiently treated patients (HAART group, n=8). Primary CD4+ T cell lines were generated by stimulating PBMCs with decreasing doses of Gag293 peptide. Next, Gag293-specific CD4+ T cells were analyzed for cytokine production by IFNγ ELISPOT and for Gag293/MHC-classII tetramer binding ability. TCR diversity in sorted Gag293/Tetramer+ cells was evaluated by immunoscope analysis.
Results: Stimulation with low Gag293 peptide doses generated IFNγ-positive CD4+ T cell lines in HIV Controllers (response rate: 6/8 at 10-9M, 2/8 at 10-11M), but not in HAART patients, confirming the presence of high avidity Gag-293-specific cells in the HIV Controller group. The immunoscope analysis revealed major amplifications of certain TCR Vα and Vβ chains in the sorted tetramer+ Gag293-specific population, indicating the presence of dominant clonotypes.
Conclusion: We identified a number of TCR Vα and Vβ chains preferentially expressed by the high avidity population of Gag293-specific CD4+ T cells. Transfer of these TCRs to heterologous cells will help determine whether they are sufficient to confer the efficient Gag-specific responses characteristic of HIV Controllers.

W4.03.03
Characterization of HIV-1 specific T-cell response in an Italian cohort of HIV-1 Natural Controllers

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HIV-1 infection is associated with a progressive decrease of CD4 T-cell count and an increase in viral load. However, Natural Controllers

(NC) maintain high level of CD4 and a low viral load for several years in the absence of therapy. Our study characterized the HIV-1 specific CD8 T-cells response, an important player in controlling virus replication, in three Italian cohorts with a different story of progression of HIV-1 infection. Immune-dominant Gag- and Nef-specific responses as well as Tat-response, important in the early phase of infection, have been investigated.
Chronic HIV-infected patients under therapy (CHIwT) show a significant reduction of Gag- and Nef-specific response ($p<0.05$). NCs (56.4%) show a higher frequency of Tat-responder than CHIwT (20%, $p<0.01$) and subjects naïve for therapy (Naïve, 16%, $p<0.01$), with a significant increase of Tat-specific IFN-γ producing T-cells (median 80, IQR 13-457, $p<0.05$), that correlate inversely with plasma viral load ($\rho=-0.3231$, $p=0.0346$). Fully differentiated Tat-specific CD8 T-cells (expressing CD45RA) are higher in NCs (median 0.29% of total CD8, IQR 0.2-0.54%) than Progressors (median 0.17%, IQR 0.11-0.38%, $p<0.01$), correlating inversely with viral load ($\rho=-0.4202$, $p=0.0208$). Interestingly, Tat-specific response shows a peculiar immune response patterns in NCs. CD45RA+ GzB+ IFN-γ+ MIP-1β+ CD8+ (present in 89% of NCs) and CD45RA+ GzB- IFN-γ+ MIP-1β+ CD8+ (present in 67% of NCs) subsets are absent in Progressors and cover the 10% of the total Tat-specific CD8 T-cell response ($p<0.01$).
In conclusion, Tat-specific response shows unique features in NCs: a higher magnitude and two polyfunctional CD8 subsets exclusively present in NCs.

W4.03.04
NK cells in HIV controller patients express an activated effector phenotype and do display unique NKp46,NKp30,NKp44 modulation on IL-2 stimulation

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Control of HIV replication in Elite Controllers (EC) and Long-Term Non-Progressor (LTNP) patients has been associated with features of efficient CD8+CTL function. However, innate immunity, in particular NK cells, may play a role in HIV control.
We studied the expression of Natural Cytotoxicity Receptors (NCR, NKp46,NKp30,NKp44) and their induction over a short time-frame (2-4 days) upon activation of purified NK cells in a cohort of 31 HIV-controller patients (15EC,16LTNP), 10 HIV-infected patients on ART and 10 Healthy Donors (HD).
In EC/LTNP, induction of NKp46 expression was normal but shortlasting (2days) and NKp30 was induced to lower levels vs. HD. In antiretroviral-treated aviremic progressor patients (TAPP) no induction of NKp46 or NKp30 expression occurred. EC/LTNP failed to induce expression of NKp44, a receptor efficiently induced in activated NK cells in TAPP. The specific lack of NKp44 expression resulted in sharply decreased capability of killing target cells via NKp44, while TAPP had conserved NKp44-mediated lysis. In addition, peripheral NK cells from EC/LTNP were activated as shown by significant HLA-DR expression and a mature effector phenotype (NKG2A-CD57+KIR+CD85j+), with conserved cytolytic function also against immature dendritic cells. Importantly, conserved NK cell responses, accompanied by a selective defect in the NKp44-activating pathway may result in lack of killing of uninfected CD4+NKp44Ligand+ cells that may be induced by an HIVgp41 peptide.Thus, NK cells in EC/LTNP can maintain substantially unchanged functional capabilities and actively control virus replication, while the lack of NKp44 induction may be related to CD4 maintenance, representing a hallmark of these patients.

W4.03.05
Inhibition of human immunodeficiency virus type-1 replication in CD4+ natural killer T cells by gamma-delta T lymphocytes

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It has widely been accepted that human immunodeficiency virus type-1 (HIV-1) in the circulating blood in HIV-1-infected patients can be controlled by highly active anti-retroviral treatment (HAART). However, shortly after the termination of HAART, HIV-1 virions emerged in the circulating blood. The fact suggest the existence of reservoir even after the HAART. In addition, it has turned out that the majority of emerged HIV-1 is macrophage-tropic that will infect CD4+ CCR5-expressing cells. On the basis of these observations, we focused on R5-HIV-1-susceptible cells as the reservoirs during HAART. There are three distinct cell populations are known as R5-HIV-1 susceptible, such as CD4+ natural killer T (NKT) cells, dendritic cells (DC) and macrophages, and tissue-associated T cells mainly arranged at surface mucosal areas. After the extensive analysis, we found that CD4+ NKT, one of the main components of innate immunity, efficiently expanded HIV-1 in vitro and also confirmed the existence of HIV-1-infected CD4+NKT cells in the small intestine of persistently infected patients with HAART. These findings indicate that CD4+ NKT cells play an important role in HIV-1 persistency even during HAART. Here, we show that gamma-delta T lymphocytes, as innate effctors, may suppress R5-type HIV-1 replication in CD4+ NKT cells through secreting CC chemokines like macrophage inflammatory protein (MIP)-1α. These results suggest the importance of innate immunity to control HIV-1 persistency during HAART.

W4.03.06
Immune exposure to a specific sequence of antibody escape variants could program long-term potential for neutralization breadth in subtype A HIV-1 infection

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Approximately one quarter of HIV-1 infected individuals will generate broadly neutralizing antibodies, but the exact mechanisms for triggering and maturation of these responses are presently undefined. We consequently investigated the roots of neutralization breadth. In a subtype A HIV-1 infected Rwandan seroconverter, we pinpointed the primary neutralizing antibody target and the successive routes of viral escape using envelope glycoproteins from the transmitted/founder virus and longitudinal escape variants along with autologous plasma and monoclonal antibodies. Initially, a single mutation at one of three clustered residues proximal to gp120's third hypervariable loop conferred viral escape. This putative epitope subsequently elicited at least two somatically related monoclonal antibodies, which bound and neutralized the established escape mutations. Resistance to this secondary wave of immune pressure then arose in later viral envelopes through introduction of two glycans that obscured this consistently targeted space. At 16-months post-infection, what had been a narrow, regional response evolved to force recognition and neutralization of distinct envelope portions, which resulted in moderate cross-clade humoral breadth. Our data suggest that unveiling a certain chain of envelope mutations could drive B cells toward the production of broadly neutralizing antibodies. Appreciating this knowledge during immunogen construction could positively impact HIV-1 vaccine design.

W4.03.07
Decrease of CD73 expressing CD8 T cells in the course of HIV infection correlates with immune activation and T cell exhaustion

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In murine models, the ectonucleotidases CD39 and CD73 play an important in-tandem role for T regulatory cell (Tregs) mediated T effector cell suppression by adenosine production. For human Tregs, an elevated expression of CD39 was shown in HIV infection. However, no studies concern the expression of CD73 on T cells in HIV infection so far.
In our study, we analyzed the expression of CD73 on peripheral and lymph nodal T effector cells and Tregs in a cohort of 101 HIV patients at different stages of disease, including long term nonprogressors (LTNP) and elite controllers (EC) and 27 healthy controls. In contrast to murine Tregs, CD73 was only expressed on a minority (ca 10%) of peripheral Tregs. In contrast, we see a high expression of CD73 on peripheral CD8+ T cells. In HIV infection CD73 is markedly reduced on all T effector and Treg cells regardless of their memory status. On CD8+ T cells, a positive correlation between CD73 expression and CD4 counts (p=0.0003) was detected. CD73 expression on CD8 T cells negatively correlated with HLA DR (<0.0001) and PD1 (p=0.0312) expression. The lower CD73 expression on CD8+ T cells was partially reversible after initiation of antiretroviral therapy (ART). Functionally, we observed that CD8+CD73+ T cells produce more IL-2 upon HIV-specific and unspecific stimulation than their CD73- counterparts and show a higher proliferative capacity. These data indicate that down regulation of CD73 on CD8+ T cells correlates with immune activation and leads to functional T cell deficits in HIV infection.

W4.03.08
Telomere length and CD38 expression on monocytes and CD8 T cells of HIV infected people

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Background: Infection with HIV leads to persistent inflammation and premature immune senescence.Progression of the disease is predicted by expression of CD38 on CD8 T cells which are also known to undergo premature senescence. Evidence shows that these processes,characterized by shortening of telomeres also take place in monocytes. We sought to determine the relation between the expression of CD38 as a marker of the disease progression and the telomere length as an indicator of immunosenescence.Methods:We enrolled 38 HIV infected patients and 53 healthy patients in the study. Analyses of CD38 expression were performed on CD8+T cells and CD14+ monocytes by means of cytofluorimetry. Telomere lengths were assessed in PBMCs by RT-PCR. Results: Regardless of the clinical status of HIV infected patients,percentage of CD38+CD8+T cells and CD38+CD14+ monocytes was higher than in uninfected people(10,5 vs. 2,1%;p<0.001; 61,6 vs. 35,5%; p<0,001 respectively) and in the latter group telomere length was inversely corelated with age (R2=0,17; p=0.002). In HIV infected patients the correlation with age was not present. We show that telomere length is inversely correlated with the frequency of CD38+CD14+monocytes (R2=0,18; p=0,015),but such correlation was not found in CD8+T cells. Conclusions: We show that CD38 expression may differently mark monocytes and CD8+T cells in terms of senescence and that the percent of CD38+ monocytes is indicative of these cells undergoing senescence. We confirm recent data showing HIV induced monocyte senescence and its persistance despite antiretroviral therapy and suggest that events taking place in these cells can have different timing from those characteristic of CD8+ T cells.

W4.03.09
HIV-1 infection impairs CD34+ hematopoietic progenitor cells differentiation toward Dendritic Cells

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The impairment of dendritic cells (DCs) homeostasis during HIV-1 infection is central to both the spread of the infection and the progression of the immunodeficiency. Given previous indirect evidences that DCs maturation and function are impaired by HIV, we explored the possibility that HIV infection impairs CD34+ hematopoietic progenitor cells (HPC) differentiation toward DCs. In the present work, we used a culture model which allows CD34+ HPC differentiation toward monocytic lineage, and upon treatment with GM-CSF and IL-4, further switching to functional DCs. We showed that both CD34+ HPC infected in vitro by HIV, and CD34+ HPC from HIV patients undergoing cART, were found unable to generate DCs, suggesting that an HIV-1-induced impairment of CD34+ differentiation may play a key role in decreased DCs frequency observed in vivo. The absence of replicating HIV-1 in differentiation studies of CD34+ HPC from chronic patients underlies the need to further investigations, in order to clarify the direct or indirect mechanism through which HIV may block DC differentiation.

W4.03.10
Over-production of BAFF by dendritic cells and inflammatory monocytes during primary HIV-1 infection

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Recent studies indicate that early activation of the immune system bears a major role in shaping B-cell repertoire and virus-specific antibody (Ab) response during primary HIV-1/SIV infection. The B cell–activating factor belonging to the TNF family (BAFF) is an inflammatory cytokine involved in Ab response and Ig class switching. However, BAFF excess also promotes the abnormal survival of self-reactive B-cell clones. We have thus evaluated the potential contribution of BAFF/APRIL impaired B-cell responses in 35 untreated primary HIV-1-infected patients (PHI) (PRIMO-ANRS Co06). Serum BAFF levels were strongly increased at enrolment, which occurs between 20 and 45 days post-infection (pi), and rapidly decreased after one month. At six months pi, the average BAFF level was similar to that of healthy donors in most patients. BAFF levels correlate directly with plasma viral load and inversely with $CD4^+$ T-cell counts. Moreover, human monocytes and myeloid dendritic cells released BAFF when exposed *in vitro* to R5 HIV-1, TLR3 agonist or type I IFN. Plasmacytoid dendritic cells were unable to secrete BAFF but exhibited its enhanced membrane expression in response to HIV-1 and CpG. However, preliminary data indicated that blood $CD16^+$ monocytes might be a major source of BAFF in PHI patients. Altogether, our data suggest that HIV-1 triggers BAFF over-production by dendritic cells and inflammatory monocytes, which likely contributes to local inflammation after their rapid relocation in various tissues, including gut mucosa. BAFF over-production might thus contribute to early inflammation and to the imbalance between polyclonal and virus-specific Ab responses.

W4.03.11
HIV-infected T cells are migratory vehicles for viral dissemination

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The mechanisms by which HIV spreads among permissive target cells locally during early stages of transmission, and systemically during subsequent dissemination are not known. In vitro studies suggest that formation of virological synapses (VSs) during stable contacts between infected and uninfected T cells greatly increases the efficiency of viral transfer. It is unclear, however, if T cell contacts are sufficiently stable in vivo to allow for functional synapse formation under the conditions of perpetual cell motility in epithelial and lymphoid tissues. Here, using multiphoton intravital microscopy (MP-IVM), we examined the dynamic behavior of HIV-infected T cells in lymph nodes (LNs) of humanized mice. Unexpectedly, we found that most productively infected T cells migrated robustly, resulting in their even distribution throughout the LN cortex. A subset of infected cells formed multinucleated syncytia through HIV envelope (Env)-dependent cell fusion. Both dyssynchronous motility of syncytia as well as adhesion to CD4+ LN cells led to the formation of long membrane tethers, increasing cell lengths to up to 10 times that of migrating uninfected T cells. Blocking the egress of migratory T cells from LNs into efferent lymph, and thus interrupting T cell recirculation, limited HIV dissemination and strongly reduced plasma viremia. Thus, we have found that HIV-infected T cells are motile, form syncytia, and establish tethering interactions that may facilitate cell-to-cell transmission through VS. While their migration in LNs spreads infection locally, T cell recirculation through tissues is important for efficient systemic viral spread, suggesting new molecular targets to antagonize HIV infection.

W4.03.12
TLR3 activation on dendritic cells causes complete suppression of HIV-1 infected dendritic cells by inducing up-regulation of miR-155 and APOBEC-3G

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Human Immunodeficiency Virus type-1 (HIV-1) is transmitted predominantly through the vaginal or rectal mucosa where dendritic cells (DCs) are among the first cells to interact with the virus. In addition to supporting viral replication, DCs facilitate viral dissemination and contribute to HIV-1 pathogenesis by migrating to the lymph nodes and contributing to trans-infection of CD4+T cells. However, upon maturation, DCs show reduced susceptibility to HIV-1 infection, concurrently with the up-regulation of various cellular host factors such as microRNA-155 (miR-155) and Apolipoprotein B mRNA editing enzyme 3G (APOBEC-3G). Our goal was to investigate the mechanism(s) by which maturation of DCs causes suppression of HIV-1 infection in vitro. Immature DCs were activated with different Toll-like receptor ligands to obtain a mature phenotype and we then determined the expression levels of miR-155, APOBEC-3G, and their susceptibility to infection by a HIV-1 recombinant virus. We observed an up-regulation on the expression of both miR-155 and APOBEC-3G upon TLR3 stimulation in comparison to unstimulated cells ($p=0.003$) and those stimulated with other TLR ligands. This up-regulation correlated with increased expression of the maturation markers CD80, CD86, HLA-DR and CD83 ($p=0.03$), and with the extent of inhibition of HIV-1 infection ($p=0.031$). By using a miR-155 inhibitor and silencing APOBEC-3G, infectivity was restored to 60% of that observed in unstimulated cells. This suggests that other factors are also involved in suppressing infection upon TLR3 stimulation. Future studies will address other mechanism(s) involved in these processes.

W4.03.13
Trogocytosis between germinal center B cells and TFH cells during acute SIV infection of macaques

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$CD4^+$ T follicular helper cells (T_{FH}) play a critical role in the development of class-switched and affinity-matured antibodies by supporting germinal center (GC) B-cell responses. Studies of passive antibody transfer in macaques demonstrated that virus-specific neutralizing antibodies prevent Simian-Human Immunodeficiency Virus (SHIV) acquisition. However, these antibodies are only rarely produced during natural infection. Defining novel aspects of the interaction between T_{FH} and GC B-cells may improve our understanding of HIV immunology and pathogenesis.
This study describes a novel lymphocyte $CD3^+CD20^+$ "double positive" (DP) population in lymph nodes and spleen of healthy macaques. Image stream and immunohistochemistry experiments confirmed co-expression of these two molecules on the same cell, and flow cytometric and microarray analysis demonstrated that a significant subset of these "double positive" cells have a T_{FH} phenotype ($CD4^+PD1^{bright}CXCR5^+ICOS^+Bcl\text{-}6^+$), function ($IL\text{-}21^+IL\text{-}17^-IL\text{-}2^{+/-}IFN\text{-}g^-$) and profile of gene expression. In addition to CD20, DP cells also express B-cell markers such as CD79, CD21, and surface immunoglobulins. Importantly, expression of CD40L upon brief in vitro stimulation identifies DP cells that are T_{FH} in origin vs. those of B-cell lineage. Longitudinal analysis of lymphoid tissues has shown a marked increase in DP cells during acute SIV/SHIV infections of macaques. We propose that (i) DP cells arise as a result of membrane exchange in active germinal centers after high-affinity contact between T_{FH} and GC B-cells, through a process defined as trogocytosis, and (ii) the DP phenotype may identify T_{FH} and GC B-cells that have recently undergone high affinity interactions during acute HIV and SIV infections.

W4.03.14
Characterization of XCR1+ dendritic cells in healthy and SIV-infected Rhesus Macaques

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In mice, the $CD8^+$ myeloid dendritic cells (mDC) optimally cross-present antigens to $CD8^+$ T cells and respond strongly to TLR3 ligands, which are often expressed by viruses. An equivalent DC subset has been identified by comparative genomic analysis and functional studies in human $CD141(BDCA\text{-}3)^+$ mDCs, and in sheep $SIPR^{neg}CD26^+$ mDCs. In all three species, the XCR1 chemokine receptor and the adhesion molecule CADM1 were found to be characteristic membrane markers for this DC subset as compared to other myeloid cells. Here we identified a new $XCR1^+$ mDC population in Rhesus Macaques, with a strong phenotypic and transcriptional homology to human $CD141^+$ mDC and murine $CD8^+$ mDC. Like their human and murine homologs, simian $XCR1^+$ mDC had much higher responses to TLR3 stimulation than other myeloid and dendritic cells. Taking this novel DC subset into account, the frequencies of all DC and monocyte subsets were monitored in 17 rhesus macaques after SIV_{mac251} infection in vivo, the most relevant animal model for pathogenic HIV-1 infection. $XCR1^+$ mDC increased sharply, transiently and in inverse correlation with viral loads during acute infection, and were reduced in blood and spleen during advanced disease. The identification and characterization of macaque $XCR1^+$ mDC opens new avenues for future preclinical vaccinal studies.
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W4.04 Immunity to bacterial infection (excluding mycobacteria)

IL4.04.01
Lesson from Leishmania Infection to Combat Bacterial-Mediated Inflammatory Response

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Bacterial infection often conducts to exacerbate inflammatory disorder such as septic shock leading to death if not properly controlled. However, parasites of Leishmania genus can infect and survive within macrophages by inhibiting several microbicidal molecules, such as NO and by taming down pro-inflammatory cytokines. For instance, Leishmania has been reported to inhibit or significantly reduced the production of IL-1β in vitro and in vivo. However, the mechanism whereby Leishmania parasite can influence IL-1β production by macrophages is still not fully understood. Recent reports have been proposed that IL-1β production is importantly regulated by the NLRP3 inflammasome complex. In the present work we investigated whether Leishmania parasites could modulate inflammasome activation. Using PMA-differentiated THP-1 cells we demonstrated that Leishmania infection effectively inhibit macrophage IL-1β production, and to involve the expression of the zinc metalloprotease GP63, a critical virulence factor produced by all infectious Leishmania sps. GP63 can be secreted, membrane bound, as well as to be released with Leishmania exosomes vesicles. Both L. mexicana purified GP63 and GP63-containing exosomes inhibited macrophage IL-1β production and caspase-1 cleavage, suggesting their effect upstream of caspase-1. Some known mechanisms to regulate the inflammasome activation involve ROS production, cathepsin B release and signaling pathway. Indeed ROS production is inhibited by Leishmania infection and could confer in part the explanation whereby Leishmania can interfere with NLRP3 complex. Collectively, we report herein the first observation that Leishmania surface metalloprotease can strongly affect NLRP3 inflammasome complex and therefore could be envisaged as a new therapy to abolish IL-1β-mediated inflammation.

IL4.04.02
Fooling phagocytes and delivering T cell vaccines: The 'yin and yang' of Bordetella adenylate cyclase toxin

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Bordetella adenylate cyclase toxin (ACT) targets myeloid phagocytes bearing the $\alpha_M\beta_2$ integrin CD11b/CD18 (Mac-1 or CR3), such as neutrophil, macrophage or dendritic cells (DC, $CD11b^{high}$). ACT penetrates across cell membrane, promotes influx of calcium ions, binds cytosolic calmodulin and converts ATP to cAMP, thus causing phagocyte impotence. We showed that ACT mobilizes its receptor into membrane lipid rafts, where translocation of the AC domain across cell membrane is completed. AC next produces subversive phagocyte ruffling through cAMP signaling that inhibits complement-mediated opsonophagocytosis. In DCs, partial maturation is induced that compromises their capacity to stimulate T cells. Influx of calcium ions allows ACT to escape endocytic removal from cell surface and oligomerize into cation-selective pores, causing potassium efflux and NALP3 inflammasome activation.
The AC domain of detoxified ACT, having the enzyme activity ablated genetically (dACT), in turn exhibits an amazing capacity to accommodate foreign T cell antigens and entrain them into the cytosol of dendritic cells both *in vitro* and *in vivo*. This allowed development of dACT toxoids into a particularly efficient tool for antigen delivery for cytosolic processing and MHC class I-restricted presentation to cytotoxic $CD8^+$ T lymphocytes. Moreover, a fraction of dACT molecules is taken up by clathrin-dependent uptake, which enables endosomal antigen delivery for presentation on MHC II molecules. dACT thus promotes efficient induction of prophylactic, as well as therapeutic antigen-specific $CD4^+$ and $CD8^+$ T cell immune responses. I will, hence, conclude by reviewing the recent applications of ACT technology for antigen delivery and tumor immunotherapy.

W4.04.01
Orchestration of inflammation and adaptive immunity in Borrelia burgdorferi-induced arthritis by NapA

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Lyme arthritis (LA) is characterised by infiltration into the joints of inflammatory cells, mainly neutrophils (PMNs) and T cells. Traditionally, LA inflammation has been defined as a Th1-mediated response; however, the paradigm of the disease has been extended to include the Th17 cells: we demonstrated that T cells from synovial fluid of patients with LA produce IL-17 in response to NapA, a *Borrelia burgdorferi* major antigen.
Accordingly to the fact that two major types of T cells are involved in LA pathogenesis, there is a prominence of some chemokines crucial for their recruitment into the synovial fluids of patients: CXCL10 and CCL2. Moreover, although not evaluated yet in LA, CCL20-mediated recruitment of Th17 cells into the joints of rheumatoid arthritis patients has been described. Macrophages contribute in creating a T cells chemoattractants enriched milieu in the joint fluid of LA patients has been established; however, the possibility that PMNs are also involved exists.
In this study, we demonstrate that the protein NapA, which accumulates in the joint cavity of LA patients, recruits PMNs in the early stage of the disease and, subsequently, T lymphocytes. We show that NapA acts with the contribution of chemokines released by PMNs in recruiting T cells. The latter are Th1 or Th17 cells, but include also a subset producing both IFN-γ and IL-17. Our results show that NapA is crucial in driving the generation of a pro-inflammatory T cell response responsible for clinical onset and histopathological changes in LA.

W4.04.02
Imaging Bacillus anthracis edema toxin effects on normalized murine dendritic cells and macrophages

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Virulence of *Bacillus anthracis*, the agent of anthrax is caused by two main factors, the capsule and two toxins. One of these toxins, edema toxin (ET) is an adenylate cyclase, producing a rise in intracellular cAMP concentrations. ET deregulates the immune system but little is known on its effects on immune cell cytoskeleton. Using adhesive micropattern array, initially designed for cells capable of stretching on micropatterns and forming stress fibers, we have developed a method of depositing immune cells to normalize their morphology. First, we present an innovative, robust and sensitive model to study murine bone-marrow-dendritic cells (BMDCs) and macrophages (BMDMs) *in vitro*, based on their adhesion to micro-patterns to standardize their morphology. This model reduces the variability of shape inherent in our cell cultures and gain sensitivity and reproducibility. Thus, the quantification of sub-cellular phenomena has become possible for studying many functions of immune cells. Second, our micro-patterning model has been used to identify and quantify the effects of ET on cytoskeleton of BMDCs and BMDMs. We observed that ET caused a time-dependent retraction of the actin cytoskeleton on both cells. This retraction begins with the formation of actin-rich cortical protrusions, appearing like membranes ruffles. This reorganization is accompanied by a perinuclear accumulation of actin, responsible of a deformation of the nucleus. We thus infer that our imaging model on immune cells opens new perspectives for high throughput analysis of morphology and functions of BMDCs and BMDMs *in vitro*.

W4.04.03
Role for coagulation factor XI during pneumococcal pneumonia independent of factor XII activation

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Background: Pneumococcal pneumonia is associated with activation of coagulation.
Coagulation is initiated via the tissue factor (extrinsic) pathway or via the intrinsic pathway both converging in the formation of thrombin. Factor XI (FXI), the key component of the intrinsic pathway, can be activated either via factor XII (FXII) or via thrombin.
Aim: To determine whether intrinsic coagulation is involved in pneumococcal pneumonia and whether this is dependent on FXII activation.
Methods: Pneumonia was induced by intranasal inoculation with *S. pneumoniae* serotype 2 in male wildtype (WT), FXI-KO and FXII-KO mice. Mice were sacrificed 48 hours following infection, blood, organs and bronchoalveolar lavage fluid (BALF) were collected to determine bacterial loads, inflammatory markers and pathology.
Results: Bacterial loads in lung, blood and distant organs were significantly higher in FXI-KO mice. In line, increased lung cytokine and chemokine levels were detected in FXI-KO mice. Lung damage in FXI-KO mice was increased, mainly caused by presence of endothelialitis. Enhanced plasma and lung E-selectin levels confirmed endothelialitis. No differences in any of the examined parameters were observed between WT and FXII-KO mice. In a separate experiment BALF was collected. Although FXI-KO mice again showed higher bacterial counts in blood and distant organs, BALF bacterial loads and leukocyte influx were comparable between WT and FXI-KO or FXII-KO mice.
Conclusion: FXI deficiency increased bacterial dissemination during *S. pneumoniae* induced pulmonary infection, while FXII deficiency did not influence the host response. These results indicate that FXI protects against pneumococcal pneumonia via a mechanism independent of FXII activation.

W4.04.04
The macrophage serves as a gate-keeper for the induction of protective host immunity and bacterial dissemination during Chlamydia infection

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The obligate intracellular pathogen, Chlamydia, is a common sexually transmitted bacterial infection and a leading cause of infectious blindness. Here we demonstrate the central importance of the macrophage in directing a protective immune response to Chlamydia.
To deplete host macrophages at the site of infection, clodronate-liposomes were injected intra-peritoneally with PBS-liposomes as the control. Subsequent to macrophage depletion mice were infected IP with Chlamydia muridarum and examined at days 2, 7 and 14 post-infection. Significant weight loss was associated with infection of control mice, but unexpectedly macrophage depletion was protective against this early weight loss. During early infection, robust IFN-γ production by peritoneal NK and NKT cells was observed using flow cytometry, with macrophages being indispensable for this host innate immune response. During the ontogeny of adaptive immunity, IFN-γ by CD4 and CD8 T cells also proved to be dependent on an intact macrophage population. Chlamydia growth was controlled in the presence of macrophages, yet in the absence of macrophages, persistent growth was observed using qPCR. Indeed, macrophage depleted mice had greater dissemination of Chlamydia beyond the portal of entry, as reflected by Chlamydia 16sRNA in circulating leukocytes. Adoptive transfers of infected macrophages confirmed that these cells are necessary and sufficient for the IFN-γ surge by lymphocytes at the site of infection.
These data identify the macrophage as the central orchestrator of innate and adaptive immunity to Chlamydia. These studies suggest that quantitative or qualitative alteration in macrophages may play a key role in the development of Chlamydia-associated diseases.

W4.04.05
B cells participate on the early stages of immune response against intracellular bacterial pathogen *Francisella tularensis*

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Francisella tularensis, as an intracellular bacterial pathogen, adhere, interact, and enter the range of phagocytic and non-phagocytic cells. Recently, we have demonstrated, using *in vitro* model, that both, vaccine strain *F. tularensis* LVS and virulent *F. tularensis* strain FSC200 are able to adhere and even to enter the human (Ramos) and mouse (A20) B cells where survive in non-replicative state. The entrance of *F. tularensis* into B cells required active participation of bacterium and engagement of B cell receptor. The *in vivo* analysis of the cellular response during early stages of *F. tularensis* FSC200 i.p. infection on murine model demonstrated that the cells responding early are the B1b cells and macrophages expressing CD11c marker. In parallel the monocyte-derived CD123+ F4/80+ dendritic cells started to express CD11b and disappeared from the site of infection. During this stage of infection *F. tularensis* induces increased expression of MHC class II molecules on B1a and B1b cells in peritoneum and activates genes for IL-12, TNF alpha, TGF beta, IFN-gamma, and iNOS at splenic B cells. Twenty-four hours after infection murine splenic B cells produce the specific anti-*F. tularensis* antibodies. Moreover, the co-cultivation of A20 B cells with the *F. tularensis* virulent strain FSC200 rendered them to be potent antigen presenting cells presenting *F. tularensis* antigens. Thus, considering the protective efficacy of circulating antibodies, production of cytokines, and potent antigen-presenting function of B cells, B cell-mediated, as well as T cell-mediated immunity plays an equivalent role in control of *F. tularensis* infection in mice.

W4.04.06
Immune evasion in *Leptospira*: the secretion of proteases that cleave complement proteins

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Leptospirosis is a neglected infectious disease of public health importance. The complement system is a major arm of innate immunity. However, pathogenic leptospires have evolved multiple strategies to escape complement attack. In this work we analyzed the ability of leptospires to secrete proteases that cleave and inactivate complement proteins, which could constitute an immune evasion strategy. We demonstrated that the culture supernatant of pathogenic, but not saprophytic leptospires, was able to inhibit the activation of the three complement pathways. Moreover, the supernatant of seven pathogenic strains presented proteolytic activity against C3, C3b and iC3b, proteins from the alternative (Factor B), classical and lectin pathways (C4b and C2). The cleavages were also observed when normal human serum was used. Interestingly, the proteases act together with the host regulators Factor I and Factor H, promoting an efficient inactivation of C3b. Proteolytic fragments of C3 had their N-terminal portion sequenced by Edman degradation. The α-chain cleavage produced 43 kDa (RSSKI-), 47 kDa (YGGGYG-) and 68 kDa bands and the β-chain degradation generates 28 kDa (HPSQKPL-) and 46 kDa fragments. Furthermore, the proteolytic

activity was inhibited by 1,10-phenanthroline, indicating the participation of metalloproteases. A recombinant leptospiral metalloprotease from the thermolysin family was produced and cleaved C3 in human serum, suggesting that this protein could be one of the proteases responsible for the effect observed with the whole supernatant. In conclusion, pathogenic leptospiral proteases can deactivate immune effector molecules, and represent potential targets for the development of new therapies and prophylactic approaches in leptospirosis.

W4.05 Immunity to mycobacterial infection

IL4.05.01
Ex vivo chemokines and cytokines measrurements as biological markers in the immuno-diagnosis of active pulmonary tuberculosis

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Background: Early and accurate diagnosis of active tuberculosis (TB) is important for the effective management diagnosis, and control of active TB. Improved diagnostic methods are also crucial in clinical trials especially with innovative therapies and vaccine studies. In extrapulmonary tuberculosis, paucibacillary and smear negative disease, and pediatric TB, immunodiagnosis would improve outcomes. Newer research for TB biomarkers often employs technology which may be unfeasible in resource limited countries.
Methods: To determine the role of ex vivo biomarkers in diagnosis of active tuberculosis, patients with clinical features of TB, non TB controls with or without HIV positive had plasma collected. Patients had chest XRay and sputum microscopy and culture. Controls also had tuberculin test. Plasma was measured for selected cytokines and chemokines by either ELISA or LUMINEX.
Results: Ex vivo MIP-1-alpha and RANTES, TGFα, sCD40L, MMP2, sCD40L, MMP2, MMP9, IFNg, IP-10 and IP-10 and MMP9 exhibited higher ability to differentiate active TB from no infection or latent infection. Most had an area under the curve AUC 0.70 with sensitivity between 66% and 88%, and specificity 63% and 75%. Combinations of some cytokines/chemokines had higher sensitivity and specificity, and had potential for more accurate detection of active tuberculosis
Conclusions:
Ex vivo chemokine and cytokine levels have a potential for timely serodiagnosis of active tuberculosis , and would be especially beneficial in areas where advanced technology may not be available.

W4.05.01
Antigen transfer drives CD4 T cell activation in tuberculosis

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CD4 T cells are essential for immunity to tuberculosis in humans and mice, but the mechanisms underlying their initial activation and limited efficacy during chronic infection in tuberculosis are incompletely understood. Our previous finding that CD11bhiDC (the heavily infected cell-type) are poor at stimulating antigen-specific CD4+T cells even after addition of exogenous antigenic peptide, suggests that CD11bhiDC may not directly prime CD4+T cells during M. tuberculosis-infection.
In our studies, intra-tracheal transfer of M. tuberculosis-infected MHC-II-/- migratory DC into MHC-II+/+ mice resulted in activation of M. tuberculosis antigen (Ag85B)-specific naïve CD4+T cells in MLNs, indicating that migratory DC transfer bacterial antigen to other DC. The antigen transferred from infected to uninfected DC was full-length, in unprocessed form and involved a vesicular pathway independent of apoptosis or exosomes. Uninfected lung CD11b+CD11chiDC flow-sorted from mice infected with GFP-expressing M. tuberculosis, activated Ag85B-specific CD4+ effector T cells without addition of exogenous peptide at a better efficiency than the infected DC. However, studies with MHC-II-/- mixed bone-marrow chimeras showed that direct recognition of infected DC and macrophages by CD4 effector T cells lead to control of intracellular M. tuberculosis invivo.
Our results provide evidence that M. tuberculosis-infected DC transfer antigen to uninfected cells in lungs and lymph nodes. While antigen-loaded uninfected DC activate CD4 effector T cells efficiently, direct interaction between infected cells and CD4 effector T cells leads to intracellular bacterial control implying that in tuberculosis, activation of effector T cells by bystander antigen-loaded uninfected cells may not contribute to better control of infection.

W4.05.02
Accumulation of Gr-1^{dim} myeloid-derived suppressor cells during experimental TB infection in mice

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Severe TB infection is characterized by excessive inflammation and impaired T-cell responses. How these two features are interrelated is poorly understood. Earlier we revealed, that experimental TB in mice is associated with the accumulation of unusual Gr-1^{dim} CD11b^{+} cells. The role of these cells during TB is unknown. However, in cancer researches it was established, that similar cell population can be induced under the influence of pro-inflammatory cytokines, suppress T-cell responses and is named myeloid-derived suppressor cells (MDSC). Considering the peculiarities of TB progression, we suggested that Gr-1^{dim} cells, accumulating during TB may represent MDSC and influence TB outcome.
To test this assumption we studied dynamics, characteristics and possible role of Gr-1^{dim} cells during TB. We found out that Gr-1^{dim} cells were rare in un-infected mice, but accumulated in lungs, bone marrow and blood of infected mice during TB progression reaching their maximum (30 - 40%) at the prelethal stage of infection. These cells co-expressed monocytic (F4/80^{+}) and granulocytic (Ly-6G^{dim}) markers and had mononuclear morphology, thus, displaying the phenotype of immature myeloid cells. Further we revealed that magnetically sorted Gr-1^{dim} cells suppressed proliferation of anti-CD3-stimulated T-cells and their IFN-γ production. The suppressive activity of Gr-1^{dim} cells was mediated by NO-dependent mechanism and required cell-to-cell interactions with target cells.
Thus, here we report the accumulation of MDSC during TB infection. This may represent the new mechanism of TB progression and may also be helpful in disease monitoring or therapy development in future.

W4.05.03
Annexin1 plays a critical role in dendritic cells cross-presentation and protection against *Mycobacterium tuberculosis*

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Annexin1 is a 37kDa protein with a wide spectrum of biological activity. Although there are emerging data emphasizing the anti-inflammatory role of Annexin1 in inflammatory diseases, little is known about its role in pathogenesis of tuberculosis. Recently, Annexin1 has been described to be involved in different stages of immune response including: (1) formation of apoptotic envelope; (2) generation of “eat me” signal that mediates recognition, tethering, and internalization of apoptotic cells/vesicles; and (3) activation of T cells. As phagocytosis of apoptotic vesicles from *Mycobacterium tuberculosis* (*Mtb*) infected macrophages is one of the main source of antigens for CD8 T cells via cross-presentation, we hypothesized that impaired capacity of Annexin1-deficient DC in cross-presentation will increase host susceptibility to pulmonary *Mtb* infection. We found that Annexin1$^{-/-}$ mice were highly susceptible to pulmonary *Mtb* infection. The high levels of pulmonary bacterial burden and mortality in Annexin1$^{-/-}$ mice were associated with reduced antigen-specific CD8+T cell response (H-2K^{b}-32c$_{309-318}$) in the lungs. This reduced

CD8+ T cell response was not due to an intrinsic role of Annexin1 in macrophages apoptosis or T cell activation as the generation of chimeric mice in which the T cells were only deficient in Annexin1 showed no sign of impairment in T cell mediated immunity or protection against *Mtb* infection. Interestingly, Annexin1-deficient DC demonstrated a significantly reduced capacity to cross-present antigens to CD8 T cells. Collectively, these data identify Annexin1 as a central player in protective immunity against *Mtb* infection by primarily regulating the power of DC in cross-presentation.

W4.05.04
Analysis of the global CD8 T cell response during *Mycobacterium tuberculosis* infection

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Tuberculosis is a world-wide re-emerging infectious disease caused by *Mycobacterium tuberculosis* (Mtb) and both BCG vaccine and therapy are not fully effective in containing its spreading.
We investigated the frequency of Mtb epitope-specific HLA-E-restricted CD8 T cells in adult patients affected by tuberculosis (TB) before and after 6 months of anti-mycobacterial therapy and in healthy subjects. Using peptides/HLA-E tetramers, we found a high frequency of peptide-specific CD8 T cells in peripheral blood of patients with active TB, which consistently decrease after therapy. HLA-E-specific T cells produced *in vitro* high levels TNF, but very poor IFN- and/or IL-2 and exerted cytotoxic activities toward peptide-pulsed HLA-E transfected target cells. Monitoring of epitope-specific HLA-E-restricted CD8 T cells by the use of tetramers in PBMC of Mtb patients during chemotherapeutic treatment, showed their frequency decreased, a pattern opposite to HLA-A*0201-restricted CD8 T cells, whose frequency instead increased after therapy. Our results indicate that HLA-E-restricted CD8 T cells behave and function differently than HLA-A-restricted CD8 T cells and could be an useful parameter to check the efficacy of therapy.
Moreover, since HLA-E is an highly conserved molecule with a very limited polymorphism and it's not down-regulated by HIV infection, often associated to TB reactivation, the evaluation of *ex-vivo* frequencies of peptides-specific HLA-E CD8 T cells could be useful to predict subjects that are at high risk to develop the disease and these peptides could be suitable candidates for a more effective vaccine against Mtb in HIV subjects.

W4.05.05
Cytokine polymorphisms, their influence and levels in Brazilian patients with pulmonary tuberculosis during anti-tuberculosis treatment

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Cytokines play an essential role during active tuberculosis disease and cytokine genes have been described in association with altered cytokine levels. Therefore, the aim of this study was to verify if *IFNG, IL12B, TNF, IL17A, IL10 and TGFB1* gene polymorphisms (SNP) influence the immune response of Brazilian patients with pulmonary tuberculosis (PTB) at different time points of anti-tuberculosis treatment: T1: beginning; T2: three months; and T3: six months. Our results showed that the *IFNG*+874 T allele and the *IFNG*+2109 A allele were associated with higher IFN-gamma levels. The *IL12B*+1188 C allele was associated with higher IL-12 levels. A *TNF*-308 A allele was associated with higher TNF-alpha plasma levels in controls and mRNA levels in PTB patients at T1. The *IL17A* A allele at rs7747909 was associated with higher IL-17 levels. The *IL10*-819 T allele was associated with higher IL-10 levels. A *TGFB1*+29 CC genotype was associated with higher TGF-beta plasma levels in PTB patients at T2. The present study suggests that *IFNG*+874T/A, *IFNG*+2109A/G, *IL12*+1188A/C, *IL10*-819C/T and *TGFB1*+21C/T are associated with different cytokine levels in PTB patients and may play a role in the initiation and maintenance of acquired cellular immunity to TB and in the outcome of the active disease and anti-tuberculosis treatment. Cytokine profile determined by the respective functional SNPs could demonstrate the actual pattern of the cytokine response against the mycobacteria and may provide a better understanding of active TB disease progression and response to the anti-TB treatment and may serve as genetic risk markers of TB susceptibility.

W4.05.06
Mycobacterium Tuberculosis-specific lung innate and adaptive immunity in close contacts of TB index cases

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Background: Despite exposure, almost half of the close contacts of smear-positive tuberculosis (TB) patients remain TST/IGRA-negative. We hypothesised that a lack of TB sensitization is associated with a specific innate immune biomarker signature.
Methods: Phlebotomy and bronchoscopy were performed on 22 close contacts of smear-positive index cases with cavitatory TB. Pattern recognition receptors (TLRs, MMR) and innate (autophagy, cathelicidin, perforin, granulysin, granzyme A) immune profiles were investigated with flow cytometry on unstimulated and PPD-stimulated PBMCs and broncho-alveolar lavage (BAL) cells. TST- and IGRA-positive (converters) contacts were compared with TST- and IGRA-negative (non-converters) contacts.
Results: The biomarker-specific immune profiles are different in BAL and blood reflecting compartmentalisation of the infection. In the BAL compartment, both for unstimulated and PPD-stimulated macrophages, TLR-9 expression was significantly higher in non-converters versus converters (p=0.04). However, no differences were found between groups for other pattern recognition receptors including TLR-2, TLR-4, DC-SIGN and Mannose-receptor. In the blood and BAL compartment, both for unstimulated and PPD-stimulated CD4+ T-cells, LC3BII expression was significantly higher in non-converters versus converters (p=0.02). No differences in the numbers of NK, NKT or γδ T cells or the expression of granulysin (NK-T), granzyme A (INK) and perforin (NK-T) was found between groups.
Conclusion: A differential innate immune signature between converters and non-converters was found. These patterns and on-going work may reveal important insights into the mechanisms underlying protective pulmonary TB immunity and may have therapeutic and diagnostic implications.

W4.06 Immunity to fungal infection

IL4.06.01
The crucial role of indoleamine 2,3-dioxygenase (IDO) in the tolerogenic and immunogenic mechanisms against Paracoccidioides brasiliensis infection

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IDO is an enzyme that catalyzes the oxidative degradation of tryptophan along the kynurenine pathway. IDO is mainly expressed by dendritic cells, is regulated IFN-gamma, and controls pathogen growth by inducing tryptophan starvation. However, this enzyme also plays a robust suppressive activity on the innate and adaptive mechanisms of immunity. Importantly, in fungal infections IDO was shown to control the tolerogenic function of DCs, the intensity of inflammatory reactions and pathogen persistence. We developed a murine model of paracoccidioidomycosis where susceptibility was associated to immunosuppression mediated by excessive proinflammatory responses and resistance with initial TGF-beta mediated tolerance followed by tightly regulated T cell responses. Using this model, we verified that P. brasiliensis infection induces an

increased expression of IDO that controls fungal growth but inhibits inflammation and T cell responses. In addition, IDO was involved in the tolerogenic activity of plasmacytoid DCs developed by resistant mice, and in the early control of fungal growth that, unexpectedly, characterizes the innate immunity of susceptible mice. Furthermore, the mutual regulation of Th17/Treg cells was also controlled by IDO expression. Interestingly, IDO inhibition by 1-methyl tryptophan had a detrimental effect to B10.A mice whose susceptibility is linked to excessive pro-inflammatory immunity, whereas a neglected effect was observed in resistant mice because the increased fungal burdens were compensated by impaired tolerance and precocious development of adaptive immunity. Interestingly, our data indicate that in susceptible mice IDO plays a dominant catalytic function whereas in resistant mice this enzyme appears to play both, enzymatic and signaling functions.

IL4.06.02
Dectin-2 and NLRP3 Are Involved in Inflammasome Activation in Dendritic Cell Response to *Histoplamsa* Stimulation

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Histoplasma capsulatum is an opportunistic dimorphic fungal pathogen. Dendritic cells can take up *Histoplasma* yeasts and present antigens to activate both CD4 and CD8 T cells. Therefore, the interaction of dendritic cells with *Histoplasma* is critical to host defense against *Histoplasma* infection. Here, we investigated the receptors and signals that are involved in the activation of inflammasome in dendritic cells after stimulation by *Histoplasma*.
We found that bone-marrow-derived dendritic cells (BMDC) responded to *Histoplasma* stimulation by producing IL-1β. Flow cytometric analysis revealed that high percentages of BMDC expressed CR3, Dectin-1, Dectin-2 and TLR-2. However, only by blocking Dectin-2 and not other receptors reduced pro-IL-1β synthesis and caspase-1 activation. While blocking Dectin-2 inhibited the phosphorylation of JNK, ERK and p38, inhibiting the phosphorylation of ERK and JNK but not p38 suppressed pro-IL-1β synthesis and caspase-1 activation. NLRP3-deficient BMDC failed to activate caspase-1 and to process pro-IL-1β. Our results showed that both Dectin-2 and NLRP3 are important in the production of IL-1β in response to *Histoplasma* stimulation. Dectin-2 activation triggered both the first (transcription of pro-IL-1β) and the second (activation of caspase-1) signals and NLRP3 was involved in triggering the second signal.

W4.06.01
IL-17 regulates fungal immunity by controlling the functional competence of NK cells

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IL-17-mediated immunity plays a key role in host protection from fungal infections, which is generally believed to be mediated by Th17 cells. Although IL-17RA-deficient mice are highly susceptible to acute systemic infection with Candida albicans, we found that blocking IL-17RA in wild type mice during the course of infection did not interfere with fungal control, suggesting that IL-17RA signaling may have unanticipated indirect effects on the immune system. Indeed, we could show that IL-17RA is essential for the development of functional NK cells and that these cells have a non-redundant role in protection from acute systemic candidiasis. Adoptive transfer of wild type NK cells was sufficient to restore normal fungal clearance in IL-17RA-deficient mice. Moreover, RAGγc-/- mice, which lack NK cells, could be rendered resistant to C. albicans by transferring wild-type (but not IL-17RA-deficient) NK cells prior to infection, confirming that NK cells act as novel key players in fungal immunity. They critically enhance the viability and fungicidal activity of neutrophils in infected mice in a GM-CSF-dependent, but completely IFN-γ-independent manner. Together, these data reveal a new role of IL-17 signaling: In addition to its well-known pro-inflammatory and protective functions during infection at mucocutaneous surfaces, IL-17 regulates NK development and thereby impacts critically on the control of systemic infections. These findings have implications for NK-mediated immunity not only against fungi, but also against bacteria, viruses and tumors.

W4.06.02
LAB/NTAL facilitates fungal/PAMP-induced IL-12 production and Th1 responses by repressing β-catenin activation in dendritic cells

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Fungal pathogens elicit cytokine responses downstream of immunoreceptor tyrosine-based activation motif (ITAM)-coupled or hemiITAM-containing receptors and TLRs. The Linker for Activation of B cells/Non-T cell Activating Linker (LAB/NTAL) encoded by *Lat2*, is a known regulator of ITAM-coupled receptors and TLR-associated cytokine responses. Here we demonstrate that LAB is involved in anti-fungal immunity. We show that *Lat2*$^{-/-}$ mice are more susceptible to *C. albicans* infection than WT mice. Dendritic cells (DCs) express LAB and we show that it is basally phosphorylated by the growth factor M-CSF or following engagement of Dectin-2, but not Dectin-1 or TLRs 2/4. Our data revealed a unique mechanism whereby LAB controls basal and fungal/PAMP-induced nuclear beta-catenin levels. This in turn is important for controlling fungal/PAMP-induced cytokine production in DCs. *C. albicans*- and LPS-induced IL-12 and IL-23 production was blunted in *Lat2*$^{-/-}$ DCs. Accordingly, *Lat2*$^{-/-}$ DCs directed reduced Th1 polarization *in vitro* and *Lat2*$^{-/-}$ mice displayed reduced Natural Killer cell (NK)/Th1-mediated IFN-gamma production *in vivo/ex vivo*. Thus our data define a novel link between LAB and beta-catenin nuclear accumulation in DCs that facilitates NK/Th1-mediated IFN-gamma responses during anti-fungal immunity. In addition, these findings are likely to be relevant to other infectious diseases that require IL-12 family cytokines and an NK/Th1 response for pathogen clearance.

W4.06.03
IL-33-dependent signaling regulates innate and adaptive immunity following pulmonary Cryptococcus neoformans infection

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The opportunistic fungus *Cryptococcus neoformans* (*Cn*) is a major cause of death among immune compromised individuals. Susceptibility to *Cn* is associated with an allergic (Th2) pattern of lung inflammation while resistance is mediated by a Th1 pro-inflammatory response. The epithelial polarizing cytokine IL-33 has been implicated in Th2 mucosal inflammation caused by allergens and helminths; however, its role in defense to fungal infection is not well characterized.
To investigate the regulation of host immunity by IL-33, Balb/c mice were intratracheally infected with the virulent *Cn* strain H99 that is known to provoke a Th2 response. These mice had a significant time-dependent induction of lung *Il33* mRNA. Following *Cn* H99 infection, mice deficient for the IL-33-receptor subunit T1/ST2 had a prolonged survival time with decreased fungal burdens in the lung, spleen, and brain.
T1/ST2 signaling during *Cn* H99 infection was required for the early pulmonary accumulation of recently characterized CD25^{+}CD127^{+}Sca-1^{+}cKit^{low} type-2 innate lymphoid cells (ILC2) and for the concurrent production of the Th2 cytokines IL-5 and IL-13. T1/ST2$^{-/-}$ mouse lungs also exhibited an increased number of CD11b^{+}CD11c^{+}MHCII^{Int} fungicidal exudate macrophages that correlated with a decrease in lung mRNA expression of the alternative activation markers *retnla* and *chi3l3*. T1/ST2-deficiency also prevented Th2 lymphocyte polarization in lung-associated lymph nodes, airway eosinophilia, and serum immunoglobulin E production.

These observations establish a detrimental role for IL-33-dependent innate and adaptive immunity during invasive *Cn* infection and identify a novel pathway that may be amenable to future therapeutic intervention.

W4.06.04
Pathogen recognition by the long pentraxin PTX3: fine tuning by protein glycosylation

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Background. The long pentraxin PTX3 is a soluble Pattern Recognition Molecule (sPRM) with non-redundant protective roles against the opportunistic fungus *A. fumigatus* (AF). PTX3 is a functional ancestor of antibodies: it has opsonic activity towards AF, and enhances recognition, phagocytosis and killing of fungal conidia by immune cells, mainly polymorphonuclear neutrophils (PMNs) [1]. The human PTX3 protein contains a single N-glycosylation site that carries complex type sialylated oligosaccharides. Here we report on the role of this post translational modification in the PTX3-depedent opsono-phagocytosis of AF.
Results. Natural and recombinant PTX3 both contain complex type sugars, which however are heterogeneous with regard to content of terminal sialic acid (SA) and glycan branching, depending on cellular source. Enzymatic removal of SA causes ~50% reduction of PTX3 binding to AF, with no further change upon additional deglycosylation. Conversely, saccharide hydrolysis has no effect on PTX3 interaction with PMNs, both producer and binder of this long pentraxin. Desialylated PTX3 shows reduced potentiation of conidia phagocytosis by PMNs as compared to the unmodified protein.
Conclusions. Different pathogens express neuraminidases on their surface and innate immunity cells, such as PMNs, mobilize intracellular neuraminidases to the plasma membrane during infection. Here, we report that the glycosylation status of PTX3 changes, with respect to SA, depending on cellular source and inducing stimuli. We describe that SA modulates the PTX3-dependent recognition and phagocytosis of AF, which points to glycosylation as a strategy to tune PTX3 functions in innate immunity.
References.
1. Inforzato A, et al (2012) *Front Immunol* **3**:407

W4.06.05
Enhancement of innate immune response to *Penicillium marneffei* conidia by cooperative interaction of pattern recogntion receptors

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Toll-like receptors (TLRs) and C-type lectin receptors have been identified as a family of pattern recognition receptors (PRRs). They recognize conserved molecules called pathogen-associated molecular patterns (PAMPs) that presented by microbes, i.e., bacteria, fungi, parasites and viruses. Recent studies have demonstrated a crucial involvement of TLR members and the C-type lectin receptor, Dectin-1, in the recognition of several fungal pathogens such as *Candida albicans*, *Aspergillus fumigatus*, *Histoplasma capsulatum* and *Cryptococcus neoformans*. In this study, cytokine inhibition assay and cell transfection system were utilized to verify the interaction of the thermal dimorphic *Penicillium marneffei* conidia to TLR1, 2, 4, CD14 and Dectin-1 that leads to NF-B activation and subsequently TNF- secretion. Here, we demonstrated by blocking experiments that TLR2 and Dectin-1 play role in *P. marneffei* conidia recognition and TNF- production by human monocyte-derived macrophages. TLR4 also involves in sensing of *P. marneffei* conidia depicted by an increase in IL-8 mRNA expression in *P. marneffei* conidia-stimulated TLR4/MD2 transfected HEK293 cells. In cell co-transfected with both TLR4/MD4 and CD14 stimulated with *P. marneffei* conidia, an enhanced expression of IL-8 has been observed compared to the stimulated TLR4/MD2 transfected cells indicating the collaborative function between TLR4 and CD14 in *P. marneffei* conidia sensing. Furthermore, the cooperation between TLR4 and Dectin-1 in the recognition of conidia of *P. marneffei* has also been demonstrated in this study by luciferase reporter system. Recognition of the fungal conidia by a glycoreceptor and PRRs on skin cells will also be demonstrated.

W4.06.06
Are lipid rafts involved in the immune response to Candida albicans?

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Several pathogens have been described as entering host cells via cholesterol enriched lipid rafts. Here we show that the antifungal drug Amphotericin B (AmB), that binds and sequesters ergosterol and cholesterol of fungal and mammalian cell membranes respectively, strongly impairs *Candida albicans* uptake by human monocytes. The cholesterol-removing agent methyl-beta-cyclodextrin (beta-mD), that is known to cause disruption of lipid rafts, similarly inhibits fungus uptake, suggesting a role of these membrane microdomains in *Candida albicans* phagocytosis.
Dectin-1 is the C-type lectin receptor that recognizes beta-glucan in the cell wall of fungi. Here we show by video-lapse microscopy that Dectin-1 localizes to lipid rafts upon *C.albicans* uptake by monocytes, supporting the hypothesis that pathogen recognition receptors specific for Candida albicans may exploit lipid rafts as an entry and signaling platform.
Interestingly disruption of lipid raft integrity by AmB modulates proinflammatory cytokine production by monocytes in response to *Candida albicans* and preliminary data indicate also an impact on antigen specific T cell response.
In conclusion we raise the question regarding the role of host cell lipid rafts in the innate and adaptive T cell response to *C. albicans* and highlight a new and unexpected immunomodulatory function of the antifungal drug Amphotericin B.

W4.07 Immunity to helmint infection

IL4.07.01
Driving disease outcome in schistosomiasis: correlating antibody to disease severity

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Approximately 85 million people worldwide are infected with the parasitic helminth, Schistosoma mansoni, and of these, 10% will develop severe heptaosplenic disease. Hepatosplenic schistosomiasis is characterized by significant enlargement of the spleen and liver (heptosplenomegaly) and periportal fibrosis leading to portal hypertension and vascular shunting. Using an experimental model of this disease, we compared how the proteomic and immunomic signatures of male CBA/J mice changed over time and correlated these changes to liver and spleen pathology to identify early markers of the development of severe disease. Specific signature molecules including cytokeratin 18 were validated by determining their abundance in the serum of patients with moderate or hepatosplenic schistosomiasis. Additionally, we explored the recognition of unique parasite proteins by host antibodies and found specific parasite molecules that correlated closely with severe pathology. Together these studies reveal valuable biomarkers for the early detection of hepatosplenic schistosomiasis and shed light on the early events that dictate and drive disease outcome.

W4.07.01
CD209a expression on dendritic cells is critical for the development of pathogenic Th17 cell responses in murine schistosomiasis

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In murine schistosomiasis, immunopathology and cytokine production in response to schistosome eggs is uneven and strain dependent. Infected CBA mice develop severe hepatic egg-induced granulomatous inflammation associated with prominent Th17 and Th1 cytokine responses, whereas in BL/6 mice milder lesions develop in a Th2-dominant cytokine environment. The pathogenic Th17 response in CBA mice is largely dependent on IL-1β and IL-23 produced by schistosome egg-stimulated dendritic cells (DC); by comparison, this pro-inflammatory cytokine pathway fails to materialize in low-pathology BL/6 mice. The requirements for Th17 cell differentiation induced by CBA DC have been elucidated; however, the reason for strain-dependent difference in APC reactivity to live eggs is not known. Initial gene profiling disclosed a significant difference in C-type lectin receptor (CLR) expression between CBA and BL/6 bone marrow derived DC (BMDC). CLR are pattern recognition receptors capable of binding carbohydrates, including those secreted by schistosome eggs. A dramatic increase in CD209a, a murine homologue of human DC-specific ICAM-3-grabbing non-integrin (DC-SIGN), was documented by real-time PCR and flow cytometry on tissues from infected CBA mice, including liver, spleen and granuloma cells. Functional assays determined that CBA DC, but not macrophages, B cells, or granulocytes, elicit Th17 cell differentiation in response to schistosome eggs. Gene silencing in CBA DC, and over-expression in BL/6 DC, demonstrated CD209a to be essential for IL-1β and IL-23 production and subsequent Th17 cell differentiation. These findings reveal a novel role for CD209a in mediating pathogenic pro-inflammatory Th17 responses in helminthic disease.

W4.07.02
Immunological changes in epidermal keratinocytes following infection with schistosome cercariae

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The epidermis is the site of initial interaction of schistosome parasites with their mammalian host. Infective larval schistosomes (cercariae) actively penetrate the skin and release excretory/secretory (E/S) products containing proteolytic enzymes and glycans. Infection promotes angiogenesis and differentiation of 'wound healing' leukocytes but it is unclear how this process is orchestrated in the skin. Since epidermal keratinocytes play a prominent role in repairing cutaneous tissues, we hypothesised that these cells become activated early during schistosome infection leading to changes in the cutaneous immune responses.
The pinnae of C57BL/6 mice were infected with cercariae and populations of non-haematopoietic (CD45-) cells were isolated from the infection site and phenotyped at 6h, 24h and 96h post-infection via flow cytometry. Relative to un-infected skin, a population of epidermal keratinocytes (CD45- CD326- CD34+) were identified that increased in proportion following infection. The expansion of this population coincided with up-regulation of keratinocyte activation- and wound healing-associated molecular markers in skin explants. Exposure of primary keratinocyte cultures to cercariae E/S material also demonstrated that parasite antigens activate these cells in vitro. The functional relevance of changes in the activation state and proportions of epidermal keratinocytes was explored via analysis of parallel changes in skin-infiltrating antigen presenting cells and tissue inflammation.
These results suggest that cutaneous non-haematopoietic cells, particularly keratinocytes, may be important mediators of the early innate immune responses to schistosomiasis in situ.

W4.07.03
Basophils contribute to the Th2-type protective immunity against re-infection with intestinal helminths

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Basophils had long been considered as minor relatives of tissue-resident mast cell owing to their paucity and phenotypic similarity to mast cells. However, recent studies using newly-developed analytical tools have illustrated critical and non-redundant roles for basophils in a variety of immune responses. We previously demonstrated that basophils are involved in the acquired protective immunity to tick infestation. In the present study, we explored the possible role for basophils in protection against intestinal helminth *Nippostrongylus brasiliensis* (Nb) in mice. Nb shows a complex, multi-stage life cycle: infected larvae enter mice through skin penetration, and migrate into the small intestine through the lung. We found that many larvae were retained in the skin, and therefore restrained from migrating toward the lung during the second but not first Nb infection. Many eosinophils and neutrophils as well as smaller numbers of basophils accumulated in the skin lesions during the second but not first infection. IL-4-deficient mice failed to inhibit the migration of Nb larvae to the lung during the second infection. We found that basophils were the major source of IL-4 in the skin lesions. Basophil ablation before the second infection significantly reduced the number of larvae trapped in the skin during the second infection. These results suggest that basophils contribute to the acquired protective immunity through trapping larvae in the skin of penetration sites in an IL-4 dependent manner.

W4.07.04
Innate and adaptive IL-9 orchestrate type 2 immunity

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Type 2 responses induced during helminth infections and allergies are controlled by a variety of cytokines including IL-4, IL-5, IL-9 and IL-13. Previous studies, from our group, using transgenic mice that constitutively express IL-9 selectively in lung epithelial cells supported an important role for IL-9 in the pathogenesis of asthma, however its contribution in host protection during parasitic infections is still controversial. To definitively determine the role of IL-9, its cellular sources and targets in vivo, we generated two different genetic tools, an IL-9 fluorescent reporter knock-in mice and an IL-9 deficient mice. Using a hookworm infection model, we found that IL-9 expression is induced in T cells (Th9) and innate lymphoid cells (ILC2) in lung and mesenteric lymph nodes of infected mice; importantly, IL-9 expression preceded the expression of other Type 2 cytokines in all organs analyzed. Furthermore, our data suggest that IL-9 amplify Type 2 responses in vivo not only inducing activation of mast cells and goblet cells as previously reported, but also by promoting IL-5 and IL-13 expression as well as basophilia and eosinophilia in mice infected with Nippostrongylus brasiliensis. Interestingly, transfer of Th9 cells but not Th2 cells into Rag deficient mice, promoted basophil recruitment leading to increased worm clearance. In conclusion, innate and adaptive IL-9 orchestrate anti-helminth responses in vivo through activation of different cellular subsets and cytokine secretion that ultimately promote an effective helminth expulsion.

W4.07.05
Mechanisms of the lymphocyte hypo-responsiveness after repeated exposure of the skin to Schistosoma mansoni cercariae

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Schistosomiasis is a debilitating helminth disease currently infecting 200 million people. In areas endemic to Schistosomiasis people are likely to be repeatedly exposed to invasive cercariae. In order to investigate the innate and acquired immune response to repeated infection, we have developed a model in which mice are exposed to repeated doses of S. mansoni cercariae via the pinnae. This allows us to specifically investigate the early immune responses in the skin to larvae, prior to the onset of egg deposition, and to compare the innate immune responses generated by single (1x) versus repeated (4x) infections.
Previously we have shown that 4x exposures of the skin to S. mansoni cercariae results in the development of CD4+ T cell hypo-responsiveness within the skin draining lymph nodes. This project aims to determine how immune cells within the skin are conditioned by repeated exposure, leading to T cell hypo-responsiveness. Key constituent cells in the skin include eosinophils, macrophages and dendritic cells (DCs). A microarray has been performed to determine which key genes are up- or down-regulated in these populations. Relmα, Ym1 and Egr1 are significantly up-regulated in the 4x skin populations indicating a regulatory/alternative activation phenotype which could lead to downstream hypo-responsiveness. In addition IL-10, a known key regulatory cytokine, is produced by these innate populations of the skin. In the absence of IL-10, lymphocyte proliferation is restored, highlighting the importance of IL-10 in inducing the hypo-responsiveness.
The results shown give an insight into the mechanisms which give rise to the lymphocyte hypo-responsiveness.

W4.07.06
IL-10 secreting, type 1 regulatory T cells and naturally occurring regulatory T cells differently modulate IgG secretion by B cells in human hypo-responsive onchocerciasis

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Onchocerciasis is the second leading infectious cause of blindness after trachoma. The disease is caused by the nematode Onchocerca volvulus (O.v) and usually presents two pathological outcomes: the sowda or hyper-reactive form, associated with high levels of IgE and strong local immune reactions and the generalized or hypo-reactive form, characterized by tolerance and elevated amounts of the non cytolytic immunoglobulin IgG4. The molecular mechanisms associated with these polar forms are still not fully clarified. Here, we investigated the role of different regulatory T cell subsets in the human onchocerciasis. We could show that, IgG4 secretion correlated with hypo-responsiveness. We could also demonstrate that, both Tr1 and Treg cells are involved in this IgG4 secretion. However, direct co-cultures of Tr1 and Tregs with B cells reveal differences in the mechanisms of modulation of B cell responses by the two regulatory T cell types. While Tr1 are capable of inducing antibody secretion, purified Foxp3+CD4+CD25+ Tregs inhibit immunoglobulin secretion and only weakly induce IgG4. Remarkable differences were also observable depending on the methods used for Treg-isolation. While CD4+CD25+ Tregs moderately induced IgG4, CD4+CD25+CD127dim and CD4+CD25+CD49d- Tregs inhibited B cells activation and antibody secretion by reducing B cell proliferation, survival and maturation into plasma cells. These findings confirm the implication of both IL-10 secreting regulatory T cells and CD25+ Tregs in IgG4 induction during generalized onchocerciasis. Our results also suggest a direct role for Tr1 cells in IgG4 secretion by B cells, whereas Tregs inhibit IgG secretion and only indirectly promote IgG4 secretion.

W4.08 Immunity to protozoan parasite infection

W4.08.01
Deletion of IL-4 receptor alpha on dendritic cells renders BALB/c mice hypersusceptible to Leishmania major infection

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In BALB/c mice, susceptibility to *Leishmania major* infection is driven by the development of T helper 2 (Th2) responses and the production of interleukin (IL)-4 and IL-13, which share a common receptor subunit, the IL-4 receptor alpha chain (IL-4Rα). While IL-4 is the main inducer of Th2 responses, paradoxical evidence suggests that exogenously administered IL-4 can promote dendritic cell (DC) IL-12 production and enhance Th1 development if given early during infection. To investigate the relevance of this during *in vivo* infection, DC specific IL-4Rα deficient ($CD11c^{cre}IL\text{-}4R\alpha^{-/lox}$) BALB/c mice were generated by gene targeting using the cre/*lox*P system under control of the CD11c promoter. DNA, protein and functional characterization showed abrogated IL-4Rα expression on dendritic cells and alveolar macrophages in $CD11c^{cre}IL\text{-}4R\alpha^{-/lox}$ mice. Following infection with *L. major*, $CD11c^{cre}IL\text{-}4R\alpha^{-/lox}$ mice became hypersusceptible to disease, presenting earlier and increased footpad swelling, necrosis and parasite burdens, upregulated Th2 cytokine and type 2 antibody responses as well as impaired classical activation of macrophages. Hypersusceptibility in $CD11c^{cre}IL\text{-}4R\alpha^{-/lox}$ mice was accompanied by a striking increase in parasite burdens in peripheral organs such as the spleen, liver and even brain. $CD11b^{+}$ inflammatory DCs were the major infected cell population in infected $CD11c^{cre}IL\text{-}4R\alpha^{-/lox}$ mice and showed deficiencies in iNOS production and increased parasite loads. IL-4Rα-deficient DCs produced reduced IL-12 but increased IL-10 due to impaired DC instruction. Together, these data demonstrate that abrogation of IL-4Rα signaling on DCs is severely detrimental to the host, leading to rapid disease progression and increased survival of parasites in infected $CD11b^{+}$ DCs.

W4.08.02
Recognition of Leishmania parasites by the macrophage-inducible C-type lectin Mincle subverts adaptive immunity and promotes susceptibility to infection

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Leishmania utilizes a variety of strategies to escape from an effective immune response. We describe that targeting of the C-type lectin receptor Mincle (Clec4e) by Leishmania results in the inhibition of the adaptive immunity against the parasite. We find that Leishmania parasites encode for a cytosolic ligand for Mincle that is exposed upon infection in vivo. The recognition of this ligand by Mincle promotes the infiltration of neutrophils and monocytes to the infected dermal site, correlating with an enhanced chemokine production early after infection. Mincle-deficient mice are more resistant to L. major intradermal infection in the ear, exhibiting a reduction in the dermal pathology and the parasite burden. Accordingly, Mincle deficiency enhances the adaptive immunity against the parasite, boosting the early priming of CD4+ T cells specific for L. major- derived antigen. This improved priming correlates with an increased activation and migration of the dermal dendritic cells to the draining lymph nodes. These findings suggest that L. major exploits their recognition by Mincle to modulate and subvert the development of acquired resistance.

W4.08.03
Marginal zone B-cells suppress antigen-specific T-cell responses during experimental L. donovani infection

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Various studies have now demonstrated that B-cells play a negative role in several experimental models of leishmaniasis. However, the mechanism by which B-cells exacerbate Leishmania infections is yet unknown. In the present study, we investigate the role of B-cells, in particular marginal zone B-cells (MZB), in the development of adaptive CD8 and CD4 T-cell responses in L. donovani infected mice. Here we show that that marginal zone B-cells contribute to suppress protective T-cell responses during the very early stages of L. donovani infection. MZB were able to capture parasites, which was associated with upregulation of surface IgM and MyD88-dependent IL-10 production. Moreover, MZB presented parasite antigens to CD4 T-cells in vitro. Depletion of MZB also enhanced T-cell responses and led to a decrease in the parasite burden, but did not alter the generation of effector memory T-cells. Thus, MZB appear to suppress protective T-cell responses during the early stages of L. donovani infection.

W4.08.04
Cellular immune polarization to a cell phenotype Th-1 and T-Reg is induced after the treatment of human dendritic cells infected by Leishmania with the compound 11α,19β-dihydroxy-7-acetoxy-7-deoxoichangin

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Several studies for the evaluation of the immune response generated against natural infection and the "required" for the appropriate resolution of the infection caused by Leishmania parasites have been developed in order to design therapies that promote a favorable response and which generate memory cells. It has been proposed that by using bioactive molecules with leishmanicidal and immunomodulatory activity can be achieve that "ideal" response. In the present study we evaluated the ability of 11a, 19b-dihydroxy-7-acetoxy-7-deoxoichangin compound for activate dendritic cells (DCs) and to polarize the effector response, using for this purpose DCs infected in vitro with L. panamensis as well their T cells, for evaluate the expression of surface markers, the production of nitric oxide (NO) and cytokines involved in inflammatory processes by flow cytometry. Here, was observed an increased in the HLA-DR expression as well on NO and proinflammatory cytokine production in DCs infected and treated with the compound. Also, we observed a polarization of effector cell response towards a Th-1 and T-Reg phenotype, which according to reports from literature these phenotypes promotes the development of an adequate response against this intracellular pathogen. These findings are consistent with previous evidence which show the compound's ability to induce the control of the Leishmania infection on in vitro and in vivo assays, showing an antileishmanial activity on antigen presenting cells (APCs) infected without any activity against extracellularly form of the pathogen, as well as the resolution of skin lesions or total cure at least 50% of the animals tested.

W4.08.05
Protective CD8$^+$ T effector memory cells elicited by heterologous prime-boost vaccination expand and re-circulate after an infectious challenge with *Trypanosoma Cruzi*

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INTRODUCTION: Heterologous prime-boost strategy using recombinant plasmid DNA followed by replication-defective human recombinant adenovirus 5 is as a powerful strategy to elicit a stable pool of functional long-lived CD8$^+$ T cells with an effector (TE) or effector memory (TEM) phenotype (CD11a^{High} , CD44^{High} , CD127^{Low}/^{High} and CD62L^{Low}). After a challenge with the human protozoan parasite *Trypanosoma cruzi*, specific CD8$^+$ T cells mediate a strong protective immune response against experimental systemic infection. The present study aimed at characterizing the importance for the protective immunity of the recirculation and proliferation of these specific TE or TEM after an infectious challenge.
RESULTS: To confirm that specific CD8$^+$ T expanded after the infectious challenge were indeed TE(M) induced by vaccination, we used the gzmBCreERT2/ROSA26EYFP transgenic mouse line. After heterologous prime-boost vaccination, specific TE(M) CD8$^+$ T lymphocyte became indelible labeled with enhanced yellow fluorescent protein (EYFP). Following an infectious challenge, these EYFP-labeled TE cells expanded at similar rate as the non-labeled cells and had significantly higher frequency of KLRG1 expressing specific CD8$^+$ T-cells. We also observed that the specific CD8$^+$ T cells capable of producing the anti-parasitic mediators IFN-γ and/or TNF-α re-circulated. This re-circulation was critical for protective immunity as administration of the drug FTYS-720 led to a strong accumulation of these cells in the lymph node and reversed protective immunity induced by vaccination.
CONCLUSION: We concluded that antigen-experienced CD8$^+$ TE(M) cells expanded after experimental infection and that recirculation was critical for the protective immunity they exert.
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W4.08.06
Immunological mechanisms of co-infection of Schistosomiasis and malaria in a primate model

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Co-infections are a common phenomenon in developing countries especially Africa. To determine the effect of co-infections on disease outcome, we conducted a controlled experiment in baboons (papio anubis), to investigate the effect of chronic schistosomiasis on severe malaria, and its influence in the development of acquired immunity to malaria infection. To study the effect of chronic schistosomiasis on severe malaria, we infected three groups of animals with 500 S. mansoni cercarie, and allowed the disease to progress to the chronic phase. To determine the effect of treatment on co-infection, we treated one group with Praziquantel at week 14 and 15 post infection. Four weeks later, the test groups were inoculated with 105 P. knowlesi parasites. In a second experiment designed to explore the development of acquired immunity to malaria, three new groups of animals were subjected to the same experimental procedure as before, except that after the 1st infection with P. knowlesi, animals were treated with anti-malarial drug Coartem® at 2% parasitaemia. Four weeks later, animals were infected two more times with plasmodium parasites, and treatment administered in between the infections. In both experiments animals were monitored daily after malaria infection. Sera and cells were collected at baseline, before and after treatment and at end-point to determine humoral and cellular responses. Results showed that animals infected with P. knowlesi had an early onset of parasitaemia and succumbed to severe malaria, unlike the majority of baboons with co-infection that survived. Comprehensive data on immunological profiles and clinical parameters will be presented.

W4.09 Malaria pathogenesis and vaccines

IL4.09.01
Malaria and its delectable mammalian organ - the liver

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Malaria, caused by *Plasmodium* ssp., often leads to death, particularly amongst young children. It is initiated upon inoculation of sporozoites by a mosquito bite through the skin of a mammalian host.

Sporozoites travel via circulation and/or lymphatics to the liver, and after traversing different cell types, infect a small number of hepatocytes to continue their development, which culminates in blood stage merozoites that infect red blood cells. The morphologically and antigenically distinct phases of parasite development involve different organs: the skin, liver, spleen, lymph nodes and red blood cells. Merozoites can also infiltrate the brain and the lungs, where they cause severe pathologies. The Malaria/Pathogenesis and Vaccines Workshop will highlight immune responses and pathologies associated with the different phases of Plasmodium. Using radiation-attenuated *P.berghei* sporozoite model system of sterile and protracted protection, we demonstrated that induction of protective IFN-γ^+CD8 T cells is mediated by liver, but not splenic, CD8α^+DC in a MHCI- and IL-12-dependent manner; liver CD8 T cells recognize epitopes on Ags expressed by liver-stage parasites. Finally, lasting protection to malaria is achieved by a balance between effector and memory CD8 T cells and survival promoting cytokines, e.g., IL-15. Thus, the mammalian liver not only plays a pivotal role by supporting the life cycle of the *Plasmodium* parasite but also in the induction and maintenance of immune memory responses. Understanding the liver-stage of malaria and the associated liver-stage-specific protective immunity will enhance our knowledge and thus facilitate exploitation of these responses towards vaccine development against this serious infectious disease.

IL4.09.02
Pathogenetic role of hemozoin (malaria pigment) in severe *P. falciparum* malaria

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Severe *P falciparum* malaria, is defined as the occurrence of cerebral symptoms (convulsions, coma), severe anemia or acute respiratory distress (ARDS) with excess inflammatory response in hyperparasitaemic patients. Cytoadherence of infected erythrocytes to the vascular endothelium of different organs, including brain, lungs and bone marrow, is the main pathological feature, and it is associated with elevated cytokine production.
Hemozoin (HZ, malaria pigment), an insoluble crystal made of hematin dimers and the detoxification product of haemoglobin's heme, is tought to contribute to the pathogenesis of severe malaria by interfering with monocytic and endothelial cell functions.
Our recent studies indicated that HZ may contribute to the blood-brain barrier (BBB) damage in cerebral malaria, by inducing *de novo* matrix metalloproteinases-9 (MMP-9) expression and release by human endothelial cells (Prato et al., 2011). Moreover, HZ stimulated the production of CXCL-8 and CCL-5, two chemokines involved in neutrophils/monocytes recruitment. The lipids attached to or generated by HZ, such as 15-HETE, but not 4-HNE, were able to induce both CXCL8 and CCL5, whereas de-lipidated HZ was ineffective. Endothelial cell growth was also impaired.
Monocyte/macrophages respond to the phagocytosis of HZ with the activation of the inflammasome, production of cytokines, oxygen and nitrogen radicals and lipid peroxidation products, which can then impair macrophage functions or cause tissue damage.
It is likely that parasite products and inflammatory mediators contribute to the pathogenesis in the anatomical sites where infected erythrocytes sequester, namely brain, bone marrow or lungs, leading to cerebral symptoms, diserythropoiesis and thus anaemia, or lung pathology.

W4.09.01
Antibodies to *Plasmodium falciparum* merozoite surface protein and neutrophil respiratory burst activity are associated with clinical outcome in severe urban malaria

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Severe malaria are the major complications of *P. falciparum* infection, resulting from a complex cascade of events, possibly including insufficient qualitative and/or quantitative Ab responses against key targets of anti-parasite immunity such as mérozoïte antigens. Immunity against *P. falciparum* blood stage antigens is clearly associated with the type of protective antibodies.
We investigated IgG responses to several recombinant merozoite surface proteins [MSPs] in 110 hospitalised urban patients (13-70 yrs) with confirmed severe malaria (SM), recruited in an urban setting of Dakar. The cohorts included 28 fatal malaria [FM] cases. IgG responses to crude merozoite extracts and to several recombinant MSPs, including MSP1p19, MSP3, MSP4 and MSP5, were evaluated by ELISA and by an antibody dependent polynuclear cell mediated phagocytosis assay.
Collectively, >75% of the sera showed IgG responses to all antigens tested (OD ratios > 2), the levels of those responses were significantly correlated within the antigens (*P<0.01, Rho* from *0.4 to 0.9*) (except for MSP3 and MSP5) but unrelated to age. Interestingly, IgG levels against MSP1p19 were significantly correlated with phagocytic indexes (PI) (*P<0.01, Rho = 0.52*). The correlation was higher in IgG1 subclasses. Comparison between SM and FM showed a significant lower IgG response to MSP1p19 and to MSP4 (*P<0.01*).in FM, measurable for both antigens by the PI. These results are consistent with a role of IgG responses to certain merozoite surface Ags in conferring protection against fatal prognosis of urban SM. Nevertheless, these results suggest that this approach deserves further investigation with more patients.

W4.09.02
Hemozoin induces lung inflammation and correlates with malaria-associated acute respiratory distress syndrome

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Malaria-associated acute respiratory distress syndrome (MA-ARDS) is a deadly complication of malaria infections and its pathophysiology is insufficiently understood. Both in humans and mouse models, MA-ARDS is associated with marked pulmonary inflammation. We investigated the role of hemozoin, a crystal with inflammatory properties produced during intraerythrocytic parasite replication, in MA-ARDS in different murine malaria models. By quantifying hemozoin in the lungs and measuring disease parameters of MA-ARDS, we demonstrate a highly significant correlation between pulmonary hemozoin levels, lung weight and alveolar edema. Histological analysis of the lungs demonstrated that hemozoin is localized in phagocytes and infected erythrocytes, and only occasionally in granulocytes. Species-specific differences in hemozoin production, as measured in individual schizonts, were associated with variations in pulmonary pathogenicity. Furthermore, we found that both pulmonary hemozoin and lung pathology correlated positively with the mRNA expression of IL-6, IL-10, TNF, MCP-1/CCL2, IP-10/CXCL10 and Hmox1, with VEGF protein levels in the bronchoalveolar lavage fluid, and with the number of monocytes/macrophages, inflammatory monocytes, neutrophils, CXCR3$^+$CD4$^+$ T cells and CXCR3$^+$CD8$^+$ T cells in the lungs. The causal relation between hemozoin and inflammation was demonstrated by injection of *P. falciparum*-derived hemozoin intravenously in malaria-free mice. Hemozoin potently induced the pulmonary expression of pro-inflammatory chemokines (IP-10/CXCL10, MCP-1/CCL2, and KC/CXCL1), cytokines (IL-1β, IL-6, IL-10, TNF, and TGF-β) and other inflammatory mediators (iNOS, Hmox1, NOX2, and ICAM-1). Thus, we identified hemozoin as an important parasitic factor in the induction of pulmonary inflammation during MA-ARDS.

W4.09.03
IL-27 receptor signalling restricts the formation of pathogenic, terminally differentiated Th1 cells during malaria infection by repressing IL-12 dependent signals

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The IL-27R, WSX-1, is required to limit IFN-gamma production by effector $CD4^+$ T cells in a number of different inflammatory conditions but the molecular basis of WSX-1-mediated regulation of Th1 responses *in vivo* during infection has not been investigated in detail. In this study we demonstrate that WSX-1 signalling suppresses the development of pathogenic, terminally differentiated ($KLRG\text{-}1^+$) Th1 cells during malaria infection and establishes a restrictive threshold to constrain the emergent Th1 response. Importantly, we show that WSX-1 regulates cell-intrinsic responsiveness to IL-12 and IL-2, but the fate of the effector $CD4^+$ T cell pool during malaria infection is controlled primarily through IL-12 dependent signals. Finally we show that WSX-1 regulates Th1 cell terminal differentiation during malaria infection through IL-10 and Foxp3 independent mechanisms; the kinetics and magnitude of the Th1 response, and the degree of Th1 cell terminal differentiation, were comparable in WT, $IL\text{-}10R1^{-/-}$ and $IL\text{-}10^{-/-}$ mice and the numbers and phenotype of $Foxp3^+$ cells were largely unaltered in $WSX\text{-}1^{-/-}$ mice during infection. As expected, depletion of $Foxp3^+$ cells did not enhance Th1 cell polarisation or terminal differentiation during malaria infection. Our results significantly expand our understanding of how IL-27 regulates Th1 responses *in vivo* during inflammatory conditions and establishes WSX-1 as a critical and non-redundant regulator of the emergent Th1 effector response during malaria infection.

W4.09.04
Apolipoprotein E is critical for the development of cerebral malaria

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Cerebral malaria claims the life of millions of children each year. From our previous work, utilizing proteomic approach and sera from malaria-infected individuals, we have identified unique malaria biomarkers, including Apolipoprotein E (ApoE). Due to its implication in cerebral diseases and as the major apolipoprotein in the brain, we sought to investigate the role of ApoE in cerebral malaria. Herein, we report the first finding that the complete absence of ApoE protects mice from cerebral malaria. While B6 mice die of cerebral malaria within 7-9 days of infection with Plasmodium berghei ANKA, mice lacking ApoE significantly survived up to the third week of infection and then died of anemia due to high parasitemia. Whereas WT mice display the clinical manifestation of cerebral malaria, the surviving ApoE-/- were devoid of these symptoms. Mice lacking the major brain ApoE receptors were used to assess the role of the receptors and none showed a significant survival. Since ApoE-/- mice also exhibit alterations in lipid profile, we utilized the ApoA1-/- mice, which have similar lipid profile to ApoE-/- mice. Nevertheless, our results show that ApoA1-/- mice are susceptible to cerebral malaria. These experiments clearly showed that it is ApoE glycoprotein per se as opposed to the involvement of brain ApoE receptors or the alteration of lipid profiles responsible for the effect. Overall, here we report that absence of ApoE renders resistance to cerebral malaria infections in mice and this could be used as a potential therapeutic target in combating the deadly cerebral malaria disease.

W4.09.05
Validation of novel antigenic targets identified by whole-genome screening for next-generation malaria vaccines

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The identification of protective antigens and development of vaccines against complex pathogens, in particular those for which cell mediated immunity is implicated in protection, has been problematic. For example, despite intense malaria research, only a handful of predicted Plasmodium spp. proteins (representing less than 0.5% of the genome) have been pursued as vaccine targets and there is still no licensed vaccine. Facilitated by the availability of genomic, proteomic and transcriptomic datasets, rational vaccine design strategies can now be employed for malaria and other complex pathogens. We hypothesize that proteins identified from genomic sequence data using biologically relevant criteria, including association with protection and IFN-γ induction, may be excellent vaccine targets. Accordingly, using malaria as a model, we have employed two different immunomics approaches to identify novel proteins targeted by protective immune responses and have evaluated the vaccine potential of the most promising ones. P. yoelii orthologues of these proteins have been characterised for their ability to induce Type I cytokine secreting T cells (especially IFN-γ) and protection against parasite challenge in mice. Most of our newly identified proteins induced partial protection against sporozoite challenge, while immunisation with a combination of the two most promising antigens resulted in sterile protection in 80% of mice. The cytokine profile induced by these protective antigens showed an increase in IL-2, TNF and multi-functional T cells secreting IFN-γ, IL-2 and TNF. These data demonstrate that novel proteins identified by immunomic-based approaches are targets of protective cell-mediated immunity and indicates the potential of multi-antigen malaria vaccines.

W4.09.06
Progress towards a broadly neutralizing vaccine against the asexual blood-stage of Plasmodium falciparum

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There is a need for a more effective vaccine against *P. falciparum* than the current leading candidate, RTS,S. Vaccines against the parasite's asexual blood-stage may reduce mortality, morbidity and transmission of malaria, but face problems of antigenic polymorphism and the apparent requirement for exceptionally high antibody levels to achieve protection.
We recently reported that vaccines based upon the blood-stage merozoite antigen PfRH5 induce antibodies capable of strain-transcending *in vitro* growth inhibition activity (GIA). Here we will describe cross-strain protection achieved by these vaccines in the stringent *Aotus nancymaae* non-human primate - *P. falciparum* challenge model, which correlates strongly with vaccine-induced pre-challenge GIA and total IgG anti-PfRH5 ELISA titres. This is the first example of clinically meaningful cross-strain protection of *Aotus* by a human-compatible blood-stage vaccine regime, and supports the strategy of vaccine selection using *in vitro* GIA.
We will also describe work to characterise the mechanism of parasite neutralisation by anti-PfRH5 antibodies. This includes generation of novel monoclonal antibodies with neutralisation potency among the highest described for any anti-merozoite antibody, and mapping of minimal linear epitopes for two of these. We will present data which suggest that fine epitope specificity and interaction kinetics are more important determinants of neutralisation potency than ability to block the interaction between PfRH5 and its receptor, basigin. These data shed important insight on the mechanism of blood-stage malaria parasite neutralisation, and support the continued clinical development of PfRH5-based vaccines. Phase Ia clinical trials of PfRH5 vaccine candidates are due to commence in Oxford in 2014.

W4.12 Microbiome, gut flora and inflammation

IL4.12.01
Adenoma induced reshaping of the gut microbiome controls host dependent progression of colon cancer

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Inflammation and infection are key promoters of cancer but the molecular interplay between these events is largely unknown. Inflammation mediated by MyD88 dependent signaling promotes intestinal tumor development in mice with a heterozygous mutation in the adenomatous polyposis coli ($Apc^{Min/+}$) gene. Mice deficient in leukotrieneB_4 receptor BLT1 are protected in inflammatory disease models of arthritis, asthma and atherosclerosis. Here, we show that $BLT1^{-/-}$ mice when bred onto $Apc^{Min/+}$ background displayed an increase in the rate of spontaneous colon tumor development and mortality. A paradoxical increase in inflammation in the tumors from the $BLT1^{-/-}Apc^{Min/+}$ mice is coincidental with defective host response to infection. The defective host response in $BLT1^{-/-}$ mice became evident as lethal neonatal infections in the context of MyD88 deficiency**.** Treatment with a broad-spectrum antibiotic eliminated neonatal lethality in $BLT1^{-/-}MyD88^{-/-}$ mice and $BLT1^{-/-}MyD88^{-/-}Apc^{Min+}$ mice are highly protected from colon tumor development. Germ-free $BLT1^{-/-}Apc^{Min/+}$ mice are completely protected from colon tumor development and fecal transplantation led to reappearance of colon tumors in these mice. Analysis of fecal microbiome showed adenoma induced host dependent alterations in bacterial populations suggesting that defective host response in $BLT1^{-/-}$ mice in $Apc^{Min/+}$ background reshapes the gut microbiota to promote colon tumor development. These results identify a novel interplay between the Toll-like receptor mediated microbial sensing mechanisms and BLT1-mediated host response in the control of colon tumor development.

IL4.12.02
Control of mucosal immune responses by vitamin A

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Dendritic cells within the intestine have the ability to drive the differentiation of T regulatory cells (Treg), to induce the production of protective secretory IgA immunoglobulins and to direct the migration of lymphocytes to the intestine, by inducing the expression of gut homing molecules. For these immune controlling functions, $CD103^+$ mucosal dendritic cells express vitamin A converting enzymes, which allows for the generation of retinoic acid. $CD103^+$dendritic cells were furthermore shown to protect against experimental colitis.
We and others have reported that this capacity of dendritic cells to express vitamin A converting enzymes and to act as tolerogenic dendritic cells is induced by intestinal epithelial cells and dependent on the synthesis of retinoic acid within the intestinal environment. $CD103^+$ dendritic cells in BALB/c mice had an increased expression level, when compared to C57BL/6 mice, of vitamin A converting enzymes. Concomitantly, intestinal epithelial cells of BALB/c had an increased ability to convert vitamin A, thus explaining the observed differences in $CD103^+$ dendritic cells. Consequently, BALB/c mice showed higher levels of IgA secretion into the intestinal lumen, as well as increased accumulation of T cells and B cells in the intestinal lamina propria. Furthermore, BALB/c mice were more resistant to Dextran Sulphate Sodium (DSS) induced colitis, while mice that consumed vitamin A deficient diet had a more severe form of DSS induced colitis compared to control mice. Therefore, the level of RA production and consequently the degree of RA-mediated signaling is crucial for the efficiency of the mucosal immune system.

W4.12.01
The molecular characterization of Helicobacter activated regulatory B cells and their role in gastric immunopathology

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Helicobacter infections are acquired during childhood and, despite triggering strong local and systemic immune responses, typically persist for life. Although a majority of infected individuals remain asymptomatic, ~20% develop one or more *Helicobacter*-associated severe gastric and duodenal disease manifestations; these include chronic active gastritis, ulcers, gastric B cell lymphoma, and, rarely, gastric adenocarcinoma. The information about the protective mechanisms that operate in the majority of infected individuals not developing disease symptoms are limited. IL-10 producing regulatory B (Breg) cells can suppress inflammatory responses in autoimmune pathologies and chronic inflammatory conditions. Recently, we have shown that B cells have the ability to negatively regulate adaptive immune responses to bacterial pathogens. Using mouse models of infection with *Helicobacter felis*, a close relative of the human gastrointestinal pathogen *H. pylori*, we found that B cells activated by *Helicobacter* TLR-2 ligands produce IL-10 and induce IL-10–producing $CD4^+CD25^+$ T regulatory-1 (Tr-1)–like cells *in vitro* and *in vivo*. Tr-1 conversion depends on TCR signaling and a direct T-/B-interaction through CD40/CD40L and CD80/CD28. B cell-induced Tr-1 cells acquire suppressive activity *in vitro* and suppress excessive gastric *Helicobacter*-associated immunopathology *in vivo*. Additionally IL-10 producing regulatory B cells express CD1d, CD5, CD21 and CD23 in their surface. Also, $IL\text{-}10^+$ Breg cells do not secrete any antibodies. Our overall findings describe a novel regulatory B cell subset with an important immunomodulatory function during immune responses to persistent bacterial infections.

W4.12.02
Dietary fibers downregulate low-grade inflammation systemically in mice

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Dietary carbohydrates improve growth conditions for distinct populations of bacteria which may affect mucosal and systemic immunity. We have demonstrated that a 10% xylooligosaccharide (XOS) supplemented diet significantly increases *Bifidobacterium* throughout the intestine compared to control fed mice, with the highest proportions found in the ileum after XOS feeding. In the intestinal epithelium, most innate immune-related genes were unaffected by XOS feeding, while expression of *Il1β* and *Ifnγ* was significantly less in the blood from XOS fed mice than from controls. *In vitro* treatment of blood with propionate significantly decreased *Il1β*, *Ifnγ*, and *Il18* expression, supporting our hypothesis that increased production of short chain fatty acids (SCFA) in the gut that are transported across the intestine and into the systemic compartments results in a down-regulation of low-grade inflammatory cytokines. The defensin RegIIIγ was significantly higher expressed in the small intestine in XOS fed mice compared to controls, suggesting only minor contact between bifidobacteria and epithelial cells. A SCFA-induced sodium/hydrogen exchanger isoform 3 expression tended to be greater in the XOS group than in controls, indicating an indirect SCFA mediated anti-inflammatory effect of XOS. In conclusion, XOS feeding decreases systemic inflammation and this effect is most likely caused by higher SCFA concentrations as a result of an increased bifidobacterial saccharolytic fermentation in the entire gut and not only in the large intestine. With the aim of testing the systemic anti-inflammatory effect of dietary fibers in a challenged animal model, we are feeding prebiotics to already established low-grade inflammatory mice.

W4.12.03
Critical IL-23 based cross regulation of colonic DCs by macrophages protects mice from Citrobacter challenge

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Strategically positioned intestinal lamina propria mononuclear phagocytes are considered crucial for maintaining gut homeostasis and immune defense. This includes: monocyte-derived CX3CR1+ CD11c+ cells displaying macrophage (MΦ) features and migratory CD103+ CD11c+ cells that are derived from dedicated dendritic cell (DC) precursors and display DC hallmarks. Understanding differential contributions of these two cell types and their intercellular communication should provide critical insights into the mechanisms that maintain the gut homeostasis or lead to IBD.
Here we focused on the cytokines IL-12 and IL-23 that are considered to play a key role in the development of Th1 and Th17 cell responses. To define the importance of cytokine production by the specific mononuclear phagocyte populations, we used a cell ablation strategy combined with a challenge by the murine Attaching & Effacing (A&E) pathogen Citrobacter rodentium. Chimeras generated with CD11c-DTR and IL-23(p19)-\- BM allowed us to show that DC/MΦ-derived IL-23 is required for the induction of IL-22 and anti-microbial peptides (AMPs). Moreover, also when the IL23 deficiency was restricted to CX3CR1+ cells using newly established CX3CR1Cre:iDTR mice AMP and IL22 production were impaired. Surprisingly, C. rodentium-challenged mice carrying IL23 deficiencies died from the challenge. Our results suggest that in an IL-23 deficient environment, IL-12 secreted by CD103+ DCs drives uncontrolled IFN-γ production by T cells leads causing severe immunopathology. In support of this notion, the immunopathology was prevented by neutralization of IFN-γ that rescued the mice.

W4.12.04
Fungal communities throughout the intestine and their role in intestinal inflammation

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Mucosal fungal infections are relatively common in IBD patients, and antibodies against fungal antigens (ASCA) have been used as a clinical marker for disease. We recently found that fungi are common inhabitants of mammalian intestine. However, how fungi are distributed throughout the gastrointestinal tract and whether immunity to fungi might play a role in inflammatory disease is currently unknown. Fungi are sensed by number of innate immune receptors among which Dectin-1 has emerged as a main innate immune receptor for recognition, phagocytosis, and killing of fungi. We found that mice lacking Dectin-1 are more susceptible to experimental colitis characterized by increased infiltration of Th17 and Th1 cells in the colon. Interestingly this pathology was driven by intestinal fungi, and antifungal therapy ameliorated colitis severity in knockout mice. Deep sequencing analysis of gut mycobiome revealed fungal genera that are overrepresented during experimental colitis. Using this technology we surveyed the mycobiomes of the entire murine gastrointestinal tract, as well as other mucosal sites throughout the body. Our results show specific distribution of certain fungal genera which might be associated with site specific immune responses to fungi and might promote inflammatory conditions at those sites as a result of aberrant immunity to fungi.

W4.12.05
Diet and Gut Microbiota play a key role in Autoimmune Diabetes

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Type 1 Diabetes (T1D) is an autoimmune disease resulting from the specific destruction of insulin-producing beta cells in the pancreas. The incidence of T1D has been growing and this is more pronounced in western countries, where changes in lifestyle and diet differ from developing countries that have relatively low occurrence in T1D. Diet affects the makeup of the gut microbiota and it is now clear that the gut microbiota regulates host immune responses, both locally and in the periphery. Children with T1D have an altered intestinal immune system and increased small intestinal permeability, which suggests T1D may stem from an altered gut homeostasis (leaking gut syndrome). Thus one simple hypothesis is that altered dietary habits in western countries (lack of fibre) modifies intestinal integrity and corrupts the normal processes of immune tolerance. The notion that diet and/or the gut microflora influences immunity and autoimmunity has not been taken that seriously, in part because precise molecular pathways had not been identified. Here we show that a high fiber diet protects NOD mice from diabetes in a process dependent in part on G-protein coupled receptor GPR43. NOD.Gpr43-/- mice developed accelerated diabetes and presented reduced numbers of iNKT cells. The composition of the gut microflora was markedly altered in NOD mice fed a diet deficient in fiber. These results establish the immunoregulatory role of diet and microbiota through the binding between SCFAs and GPR43 as an important pathway, but once disrupted, like with the western diet, may increase the incidence of autoimmune T1D.

W4.12.06
Probiotics as immune-regulators in the rat Experimental Autoimmune Encephalomyelitis and Myasthenia Gravis models

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Probiotics, including Bifidobacteria, mostly present among the human intestinal microbiota, and Lactobacilli, more predominant in the human gastric tract, are viable non-pathogenic bacteria able to modify metabolism and induce immunomodulatory activity. Probiotics can modulate dendritic cell maturation, modify T helper cell balance and induce regulatory response and immunological tolerance, thus representing an innovative therapeutic strategy for autoimmune diseases.
We have selected strains of Lactobacillus and Bifidobacterium based on their immunomodulatory effects on human DCs and leukocytes. In particular, the analysis of maturation (CD80, CD86, MHCII) and regulatory (TGFb, IL10) markers, evaluated by qPCR and FACS, following exposure to probiotics, identified Lactobacillus crispatus (LC) and Bifidobacterium Breve (BB) as candidate immunomodulatory agents. Moreover, biochemical analysis of BB, compared to other bifidi strains, showed its enhanced activity to convert Linoleic Acid in the anti-oxidant and anti-inflammatory fatty acid Conjugated Linoleic Acid (CLA).
Both LC and BB were tested for in vivo gut colonization capability, after three-weeks administration in healthy rats, showing stable persistence of BB leading to TGFb and CCR7 increment in mesenteric LNs and Peyer's Patches.
The selected probiotics BB and LC were tested, in comparison to commercially available lactobacilli and bifidi, in the Lewis rat models of Myasthenia Gravis (EAMG) and Multiple Sclerosis (EAE), showing interesting immunomodulatory effects both locally in GALT and Peyer's Patches and systemically.

W4.13 Immune regulation at barrier sites

W4.13.01
Intestinal epithelium-intrinsic Notch signaling maintains mucosal immune homeostasis by ensuring epithelial barrier functions

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Intestinal epithelial cells (IEC) play a critical role in the first line of defense against diverse microorganisms on the luminal surface. Impaired integrity of IEC has been implicated in increasing the risk of inflammatory bowel disease; however, the underlying pathological mechanism remains to be clarified. Notch signaling plays a critical

role in the maintenance of epithelial integrity by regulating the balance of secretory and absorptive cell lineages, and also by facilitating rapid epithelial turnover. We found that epithelium-intrinsic Notch signaling ensures mucosal barrier functions. Mice harboring IEC-specific deletion of *Rbpj* (RBP-JΔ^{IEC}), a transcription factor responsible for Notch signaling, spontaneously develop T_H17-dominant chronic colitis. Interestingly, regulatory T (Treg) cells accumulated in the inflamed colonic lamina propria (cLP), where nearly half of $CD4^+$ T cells were occupied by Treg cell. Microarray analysis demonstrated that functional molecules for immune regulation were remarkably downregulated in Treg cells of RBP-JΔ^{IEC} mice compared to normal mice. FACS analysis also confirmed the downregulation of functional molecules of Treg cells under inflammatory condition. Reciprocally, genes encoding effector molecules such as IL-2 and IFN-γ increased in these cells. These data suggest that persistent microbial stimulation due to impaired epithelial barrier may lead to the development of functionally defective Treg cells, resulting in T_H17-dominat chronic inflammation. Collectively, epithelial cell-intrinsic Notch signaling ensures the epithelial barrier function and this mechanism is required for the maintenance of appropriate function of colonic Treg cells.

W4.13.02
Oral administration of poly-reactive high-affinity IgA monoclonal antibody against intestinal microbiota improved inflammatory colitis in mice

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Immunoglobulin A (IgA) is the main antibody secreted into the intestinal lumen, and plays a critical role in defense against pathogens and in intestinal microbiota homeostasis. Activation-induced cytidine deaminase (AID) is essential to both class switch recombination and somatic hypermutation (SHM). We have generated SHM-specific deficient mutant mice, AID-G23S mice, which carry a knock-in point mutation (G23S) in AID. They secreted normal amount of IgA in gut but those IgA were less mutated. In these mice, due to low-affinity of IgA against intestinal microbiota, expanded gut microflora caused germinal center B cell hyperplasia as predisposition towards inflammatory colitis in older mice. It indicates SHM in IgA is critical to control intestinal microbiota and to prevent inflammatory colitis. Since microbiota community is huge and easily changed by food, the size of IgA repertoire must be enormous. However, it is unknown how each mutated IgA recognizes its specific bacterial target. We hypothesized that the intestinal IgA may be selected to be cross-reactive to multiple bacteria and to obtain high-affinity against them through SHM. To prove this hypothesis, we generated hybridomas from intestinal IgA-secreting cells and found that several monoclonal IgAs recognized more than ten different intestinal bacteria. Oral administration of the poly-reactive monoclonal IgA with highest affinity into AID-G23S mice normalized germinal center B cell hyperplasia. Oral monoclonal IgA treatment is thus a potential therapeutic approach for inflammatory colitis.

W4.13.03
Caspase-8 is essential to maintain intestinal barrier function in response to mucosal pathogens

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The intestinal epithelium is equipped with numerous Toll like receptors (TLR) that play a pivotal role in pathogen recognition, initiating of immune response and cell death activation of infected cells. Beside apoptosis, we have recently discovered necroptosis as a new form of epithelial cell death and a potential pathogenic mechanism driving the development of ileitis in Caspase-8ΔIEC mice and Crohn´s disease patients. However, the triggering factor for necroptosis is still unknown. Recently, we could identify that additional deletion of Tnf-R1 did not rescue the phenotype of Caspase-8ΔIECmice. To examine the impact of TLR on necroptosis, we injected Poly(I:C) and LPS into mice. TLR-ligands induced a dramatic villous atrophy and severe destruction of the intestine of Caspase-8ΔIECmice as compared to control littermates, leading to the death of the former mice within 6 hours. Immunohistochemistry revealed an excessive number of dying epithelial cells with necrotic morphology after TLR-stimulation in Caspase-8ΔIECmice, but not in Rip3-/-Caspase-8ΔIECmice, indicating that this form of cell death is due to Rip3-mediated necroptosis. Moreover we discovered that Poly(I:C) triggered necroptosis was directly mediated via the TLR3-TRIF pathway, whereas LPS-induced programmed necrosis was prevented in Tnf-R1-/-Caspase-8ΔIECmice, indicating the influence of TNF-α in this setting. Beyond we could demonstrate that TNF-α is produced by intestinal immune cells and not by epithelial cells themselves.
Taken together, our data for the first time demonstrate a host protective function of caspase-8 by maintaining intestinal barrier function in response to mucosal pathogens through controlled shedding of infected epithelial cells and inhibition of necroptosis.

W4.13.04
CD200 receptor signalling facilitates murine cytomegalovirus persistence in mucosal tissue

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Mucosal surfaces are critical ports of entry and/or exit for human cytomegalovirus (HCMV). The salivary glands also represent a significant site of persistent replication of murine cytomegalovirus (MCMV). CD200 receptor (CD200R) is a negative regulator of peripheral and mucosal immunity. Viruses, including certain herpesviruses, have targeted the CD200R signalling pathway through acquisition of functional CD200 homologues. However the role that CD200R signalling plays during persistent herpesvirus infection is unclear. We now report that the CD200-CD200R pathway suppresses innate and adaptive antiviral immunity during MCMV infection. CD200R-/- mice exhibited elevated NK cell accumulation during acute MCMV infection that corresponded with improved control of virus replication in the spleen. During MCMV persistence, CD200R-/- mice exhibited a dramatic increase in CD4 T cell and monocyte/macrophage accumulation, and enhanced NK cell cytotoxicity in the salivary glands that, critically, resulted in decreased virus replication. Concurrent depletion of NK cells and CD4 T cells abrogated improved control of virus replication by CD200R-/- mice. Moreover therapeutic blockade of CD200 during persistent infection of RAG-/- mice also improved control of virus replication. Thus, CD200R inhibits innate immune responses that afford protection from cytomegalovirus persistence independently of adaptive immunity. These results uncover CD200R as a critical regulator of antiviral immunity during cytomegalovirus persistence in mucosal tissue, and highlight the potential importance of promoting innate, as well as adaptive, immune responses during chronic cytomegalovirus infection.

W4.13.05
Endoplasmic reticulum stress aggravates CVB3-induced myocarditis by promoting NF-κB-mediated proinflammatory cytokine production

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Coxsackieviruses B3 (CVB3) is a major pathogen for viral myocarditis with no effective control measures, and the main reason for the lack of effective treatments is the limited understanding of its pathogenic mechanisms. Recently, endoplasmic reticulum (ER) stress response has been proven to participate in CVB3-induced myocarditis by promoting myocardiocyte apoptosis, however its role in myocardial inflammation has not been elucidated. Here, we found that

expression of myocardial GRP78 and GRP94, hallmarks of ER stress, was robustly up-regulated following CVB3 infection and appeared in a time dependent pattern which was highly accordance with the pathogenic process of viral myocarditis. Augment of ER stress by tunicamycin significantly increased myocardial inflammatory cytokine production such as TNF-α, IL-6, IL-12 and MCP-1, and consequently led to aggravated myocardial inflammation and injury. On the contrary, blockade of ER stress by tauroursodeoxycholate substantially reduced the production of myocardial inflammatory cytokines and efficiently relieved viral myocarditis evidenced by the slighter body weight loss, limited inflammation foci and enhanced survival rate. To further explore the underlying mechanism, we found that ER stress response promoted the expression and activation of inflammatory transcriptional factor NF-kB in CVB3-infected heart tissues. Taken together, our results demonstrate that CVB3 infection activated myocardial ER stress, which was involved in the pathogenesis of myocarditis by promoting NF-κB-mediated proinflammatory cytokine production. In addition, ER stress may represent a potential therapeutic target for CVB3-induced myocarditis and other inflammatory heart diseases.

W4.13.06
Impaired maintenance of gut homeostasis in mice lacking GGTase-I in intestinal epithelial cells

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Prenylation is one of the most important posttranslational processes. Although prenylation inhibitors show immunomodulatory and antineoplasic properties, the impact of prenylation on immune response remained undefined. Focusing on intestinal epithelium, main and primary immunological barrier in gastrointestinal immune response, we aim to establish the consequence of a genetic deletion of GGTase-I (geranylgeranyltransferase-I) in Intestinal Epithelial Cells (IEC) for gut homeostasis. Conditional GGTase-I KO mice were generated by crossbreeding Pggt-Iβ$^{flx/flx}$, and VillinCre as well as VillinCre-ERT2 mice. Spontaneous phenotype was reported and gut and IEC were analyzed in order to clarify the underlying mechanism.
Deletion of GGTase-I gene in IEC leads to embryonic lethality in mice. Tamoxifen-induced abrogation of geranylgeranylation in IEC of adult mice caused a lethal enteric disease, more prominent in small intestine (endoscopy), which could be mimicked *in vitro* (organoid culture). Microscopic analysis showed a complete disruption of epithelium architecture and increased cell shedding into the gut lumen (H&E staining). Our data ruled out apoptosis, necroptosis and intestinal microflora as main responsible for intestinal damage. However, GGTase-I deficient epithelium showed a disrupted disposition of actin fibers (confocal microscopy), which pointed out cytoskeleton function as key mediator. IEC lacking geranylageranylation exhibited decreased RhoA activation (pull-down assay), which correlated with its modified subcellular localization (Western blot, immuhistochemistry).Absence of geranylageranylation in IEC implies in the loss of intestinal homeostasis and death. The mechanism underlying this dramatic phenotype might be related to an impaired Rho A activation, which would lead to a defective cytoskeleton function and the breakdown of epithelial barrier.

W5.01 Cytokine regulation in disease

IL5.01.01
Dengue infection triggers platelet-derived IL-1β: Mechanisms and roles to increased vascular permeability

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Dengue is the most frequent hemorrhagic viral disease and re-emergent infection in the world. The severe forms of the disease are characterized by thrombocytopenia and increased vascular permeability that may lead to bleeding and shock. Although thrombocytopenia is characteristically observed in dengue, the role of platelets in dengue pathogenesis has not been fully elucidated. Here we investigate platelet activation and its potential contribution to thrombocytopenia and endothelium activation in dengue. We found that platelets from dengue patients exhibited increased activation, mitochondrial dysfunction and apoptosis. Platelet activation and mitochondrial dysfunction could also be observed when plateles where directly exposed to dengue virus (DENV) in vitro through mechanisms dependent of DC-SIGN. Increased expression of IL-1β in platelets and platelet-derived microparticles (MPs) from patients with dengue or after platelet exposure to DENV in vitro was observed. The mechanisms involved in IL-1β synthesis and processing in platelets was investigated. We demonstrated that DENV infection lead to assembly of NLPR3 inflammasome and caspase-1-dependent IL-1β secretion by platelets. Our findings also indicate that platelet-derived IL-1β is chiefly secreted into MPs through mechanisms dependent of mitochondrial ROS triggered inflammasome. Inflammasome activation and platelet shedding of IL-1β-rich MPs correlate with signs of increased vascular permeability. Moreover, MPs from DENV exposed platelets triggered endothelial cell activation and increased permeability in vitro through IL-1R dependent mechanism. In conclusion, our findings provide new evidence that platelets may contribute to inflammatory response amplification and to the genesis of increased vascular permeability in dengue illness.
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IL5.01.02
Nuclear DNA sensor IFI16 as circulating protein in autoimmune diseases is a signal of damage that impairs endothelial cells through high-affinity membrane binding

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IFI16, a nuclear pathogenic DNA sensor induced by several pro-inflammatory cytokines, is a multifaceted protein with various functions, including suppression of transcription, induction of inflammation, and restriction of virus replication. It is also a target for autoantibodies as specific antibodies have been demonstrated in the sera of patients affected by systemic autoimmune diseases. Various research groups, including ours, have shown that following transfection of virus-derived DNA, or treatment with UVB, IFI16 delocalizes from the nucleus to the cytoplasm and is then eventually released into the extracellular milieu. In this study, using an in-house capture ELISA we demonstrate that significant levels of IFI16 protein can also exist as circulating form in the sera of autoimmune patients. We also show that the recombinant IFI16 protein, when added in vitro to endothelial cells, does not affect cell viability, but severely limits their biological activities. It was further demonstrated that IFI16 released by apoptotic cells bind to neighbouring cells in a co-culture. Immunofluorescence assays revealed the existence of high-affinity binding sites on the plasma membrane of endothelial cells, and radiolabeling experiments allow us to estimate the presence of 250,000 to 450,000 specific binding sites per cell. Corroborating the results from the functional assays, this binding could be completely inhibited using the anti-IFI16 N-terminal antibody. Altogether, these data demonstrate that IFI16 may exist as circulating protein in the sera of autoimmune patients which binds endothelial cells causing damage, suggesting a new pathogenic and alarmin function through which this protein triggers the development of autoimmunity.

W5.01.01
The TNF family member TL1A: a key player in type 2 immunity in both adaptive and innate lymphocytes

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The TNF-family cytokine TL1A costimulates T cells through its receptor DR3. TL1A polymorphisms and increased expression has been linked to Rheumatoid Arthritis and Inflammatory Bowel Disease. TL1A-DR3 interactions are required for diverse mouse models of autoimmune disease. The effects of TL1A on T cell differentiation and its role in innate lymphocyte biology has not been explored. During T cell activation, we have found that TL1A costimulation specifically promotes production of the allergy-promoting cytokine IL-9 through a mechanism dependent on IL-2 and STAT5 signaling, and TL1A promotes pathology in mouse models of asthma and ocular inflammation. Transgenic mice chronically expressing TL1A spontaneously develop small intestinal pathology characterized by high levels of IL-13, muscular and goblet cell hyperplasia, and infiltration with immune cells. Mucosal and goblet cell hyperplasia is dependent on IL-13 but not T cells or commensal flora. We find that the major IL-13 producing cells in TL1A transgenic mice lack T and B cell lineage markers and are phenotypically similar to 'type 2' innate lymphocytes (ILC2), which promote allergic and anti-parasitic responses in mice. ILC2 express surface DR3 and produce IL-13 in response to TL1A ex-vivo. However, DR3 deficient mice mount a vigorous response to the intestinal nematode Nippostrongylus brasiliensis, which depends on ILC2 derived IL-13. Thus, TL1A can directly induce IL-13 production by innate lymphocytes through mechanisms distinct from parasitic infection. TL1A thus coordinately enhances type 2 immunity in both adaptive and innate lymphocytes and may be a good target for therapy in allergic diseases.

W5.01.02
Oxazolone and ethanol induce colitis in NOD-scid IL2R γnull mice engrafted with human peripheral blood mononuclear cells

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Oxazolone induced colitis in mice has become a recognized model to study the mechanism underlying the development of colitis and the efficacy of therapeutics targeting immunological responses. However, this model poorly reflects the pathophysiological mechanisms in the genetically heterogeneous patient population and cannot be used when therapeutics designed to address human targets do not interact with the respective murine counterpart.
In a new model oxazolone mediated colitis was induced in NOD-scid IL2Rγ null mice engrafted with human peripheral blood mononuclear cells (hPBMC) derived from patients suffering from ulcerative colitis (UC). Read outs were the clinical severity- and histological score, hIgG and hIgE levels and colon length. Human lymphocytes were analyzed by immunohistochemistry and FACS analysis.
Mice developed the same symptoms and phenotype as previously observed in immune-competent mice. Challenge with oxazolone resulted in a mixed inflammatory cell infiltrate into the lamina propria consisting of human T- and B-cells and neutrophils and NK T-cells. Colon architecture was characterized by the development of edema, fibrosis and crypt loss. Engraftment alone without further challenge was not sufficient to cause UC, however in contrast to the results obtained in immune-competent mice, ethanol vehicle alone induced UC like symptoms and phenotype. The phenotype was most pronounced in mice engrafted with PBMC derived from a patient suffering with UC. This model has the potential to elucidate molecular mechanisms underlying flares of the disease and to study the efficacy of therapeutics. Drug profiling in this model might deliver results with higher predictive quality for later clinical studies.

W5.01.03
Chronic infection with Toxoplasma gondii aggravates experimental sublethal sepsis induced by CLP and predisposes to septic shock

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Most studies of parasite-host interaction have focused on a single pathogen interaction. However, in natural environment, the host is commonly exposed to multiple pathogens sequentially or simultaneously. Sepsis by Cecal Ligation and Puncture (CLP) is a well characterized model, but has not been correlated with co-infection model. In this context, we hypothesized that chronic infection by Toxoplasma gondii can modulate the host response against CLP. To test this hypothesis, C57BL/6 mice were orally infected with 5 cysts of T. gondii and 40 days post infection, they were subjected to sub lethal CLP (SL-CLP). In our study, when mice were chronically infected with T. gondii, they became more susceptible to SL-CLP, showing improvement of bacterial killing, increased cells recruitment to the site of infection, intestinal damage and increased inflammatory infiltrate in this organ. These mice had also increased pro-inflammatory cytokines (IFN-γ, TNF-α, IL-6 and IL-1β) which induced increased nitric oxide (NO) observed within 24 hours after SL-CLP. We showed that the TCD4+ and TCD8+ cells were responsible to produce IFN-γ and TNF-α. We suggest that early response against SL-CLP after infection with T. gondii happens due to the presence of Th1 memory cells, produced during T. gondii infection. In conclusion, we demonstrate that chronic infection with T. gondii aggravates SL-CLP by Th1 memory generation that induces increased pro-inflammatory cytokines which leads to hypotension and predisposes to septic shock.

W5.01.04
Foxo3 transcription factor controls susceptibility to central nervous system inflammation

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Foxo3 transcription factor regulates cell cycle progression, survival, and DNA repair pathways. Studies have established the crucial role of Foxo3 in the control of antiviral immune response and in the differentiation of induced-regulatory T cells. However, the role of Foxo3 in the physiopathology of autoimmune disease is still unknown. In this context, our goal was to determine the role of Foxo3 in autoimmunity using experimental autoimmune encephalomyelitis (EAE), a well-established animal model of multiple sclerosis.
Here, we show that Foxo3 deficiency is associated with a significant decrease in the severity of an active EAE induced by immunization with myelin-derived peptide MOG35-55. Foxo3-deficient mice display no defect in CD4 T cells migration to the central nervous system. However, the decreased severity of EAE in Foxo3-deficient mice is characterized by an inability of Foxo3-deficient CD4 T cells to differentiate into encephalitogenic T cells. Indeed, CD4 T cell deficient for Foxo3 failed to secrete IFN-γ, IL-17 and GM-CSF. By dissecting the cellular mechanism involved in this phenotype, we have shown that Foxo3 plays crucial roles in both dendritic cells and T cells.
Thus, our results reveal for the first time that Foxo3 plays a role in autoimmune diseases. Identification of Foxo3 as a target gene implicated in the differentiation of CD4 T cells in the context of autoimmunity could give crucial information in the molecular mechanism of autoimmune diseases.

W5.01.05
Factor Xa and thrombin stimulate an inflammatory and fibrotic response by retinal pigment epithelial cells: a role in vitreoretinal disorders?

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Vitreoretinal disorders, including proliferative vitreoretinopathy (PVR), proliferative diabetic retinopathy (PDR) and exudative age-related macular degeneration (AMD) are a major cause of visual impairment worldwide and can lead to blindness. Loss of blood-retinal barrier (BRB) integrity associated with vitreoretinal fibrin deposition, inflammation, fibrosis and neovascularization contribute to the pathophysiology of these disorders. Retinal pigment epithelial (RPE) cells are well recognized to contribute to vitreoretinal inflammation/fibrosis and are likely to encounter contact with coagulation factors upon loss of BRB integrity.
We examined the effect of factor Xa and thrombin on the production of cytokines and growth factors by RPE cells as well as there effects on α-SMA, ZO-1 and collagen type 1 expression. For this purpose we used cytokine arrays that allow simultaneous detection of 120 cytokines and growth factors, ELISA, RQ-PCR and fluorescence microscopy. The involved signaling cascades were examined using specific inhibitors for PAR-1, PAR-2, NF-κB and PDGF-R.
Factor Xa and thrombin regulated the production of many mediators (including GM-CSF, IL-6, IL-8, MCP-3, PDGF-AA, PDGF-BB, TIMP-1 and TGF-α) that fit well in the pathobiology of vitreoretinal disorders. Blocking studies revealed that these effects were mediated via PAR1 induced NF-κB activation. Thrombin enhanced α-SMA and collagen type 1 levels and reduced ZO-1 levels expressed by RPE. The effect of thrombin on α-SMA and collagen type 1 was mediated via PDGF signaling. Our findings suggest that factor Xa and thrombin can drive vitreoretinal inflammation and fibrosis and should be considered as treatment targets in vitreoretinal disorders such as PVR, PDR and AMD.

W5.01.06
Aberrant expression of IL-22RA1 on hematopoietic cells as immunologically signature of primary Sjogren's Syndrome and Sjogren-associated non-Hodgkin lymphomas

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Aim of this study was to better characterize the role of IL-22 and the cognate receptor IL-22R1 in the pathogenesis of primary Sjogren Syndrome (p-SS) and p-SS-associated lymphomas.
Minor salivary gland biopsies were obtained from patients with p-SS and with non-specific chronic sialo-adenitis (n-SS). IL-22, IL-22R1 and IL-22BP were evaluated by RT-PCR, immunohistochemistry and confocal microscopy. IL-22R1 expression was also analyzed by flow cytometry on peripheral blood mononuclear cells (PBMCs). PBMCs were also cultured with recombinant IL-22 and the modulation of the transcripts for pro-inflammatory cytokines was assessed by RT-PCR. Paraffin embedded sections of non-Hodgkin lymphomas from p-SS patients were finally evaluated for the expression of IL-22R1.
Our result showed significantly up-regulated transcript levels for IL-22, STAT3 and IL-22BP but not for IL-22R1 in the inflamed salivary glands of p-SS respect to n-SS. Immunohistochemistry and confocal microscopy analysis confirmed the increased salivary glands expression of IL-22 and p-STAT3 and demonstrated a significant increased protein levels of IL-22R1 in p-SS.
Also $CD14^+$ monocytes aberrantly expressed IL-22R1 in p-SS patients but not controls. The stimulation with recombinant IL-22 of PBMCs from p-SS, significantly up-regulated the expression of IL-17 and IL-22. Non-Hodgkin lymphoma tissues from p-SS patients were also characterized by the aberrant expression of IL-22R1 on macrophages and neoplastic B cells.
The aberrant expression of IL-22R1 on cells of hematopoietic origin seems to be an immunological signature of p-SS and p-SS associated lymphomas. Targeting of IL-22 pathway may represent a successful therapeutic strategy in p-SS patients.

W5.02 Chronic inflammation and fibrosis

IL5.02.01
Regulation of wound healing and fibrosis by macrophages

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Macrophages are found in close proximity with collagen-producing myofibroblasts and play key roles in the mechanisms of wound healing and fibrosis. They produce growth factors and pro-fibrotic mediators that directly activate fibroblasts, including transforming growth factor beta, insulin-like growth factor, vascular endothelial growth factor, and platelet-derived growth factor. They also regulate extracellular matrix turnover by influencing the balance of various matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases. Macrophages also regulate fibrogenesis by secreting chemokines that recruit fibroblasts and other inflammatory cells and by producing various inflammatory and anti-inflammatory cytokines. With their potential to act in both a pro- and anti-fibrotic capacity at distinct stages of the wound healing response, macrophages and the factors they express are integrated into all stages of the fibrotic process. These various and sometimes opposing functions are performed by distinct macrophage subpopulations, the identification of which is a growing focus of fibrosis research. Although collagen-secreting myofibroblasts once were thought of as the master ''mediators" of fibrosis, in this presentation I will illustrate how macrophages function as the master ''regulators'' of fibrosis.

W5.02.01
Th-2 cytokine producing Tregs are present in the skin of patients with systemic sclerosis

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Systemic sclerosis (SSc) is a connective tissue disorder characterized by fibrosis of the skin and internal organs. Immune dysfunction is accepted to play a role in the pathogenesis of SSc, however the exact pathways involved are not understood. Since T regulatory cells (Tregs) are key for preventing the development of autoimmune diseases, a deficiency or dysfunction of this subset may be a key factor in SSc. Deficiencies in Tregs are implicated in several autoimmune disorders but their role in SSc remains controversial. Limitations of previous studies are that in humans, expression of FOXP3 alone is insufficient to define Tregs; Tregs may become unstable and change their cytokine phenotype and/or function and activated effector T cells can also be FOXP3+. Furthermore evaluation of Tregs in the blood may not reflect their status in target organs.
In this study we aimed to determine whether Tregs play a role in the pathogenesis of SSc. Peripheral blood and skin biopsies were from obtained from patients with SSc and healthy controls (HCs). We found no difference in the proportion of Tregs in the blood or skin of patients with SSc compared with HCs. In contrast we found significantly more Th2-cytokine producing Tregs in the skin of patients with SSc. Furthermore, analysis of the TSDR in SSc Tregs revealed a similar pattern of demethylation to that of non-cytokine secreting Tregs from HCs. These data are the first to reveal conversion of Tregs into Th2-cytokine producing cells. Studies are ongoing to elucidate the mechanism underlying the plasticity.

W5.02.02
IL-33 precursor and bleomycin synergize in inducing lymphocyte accumulation and fibrosis in the lungs

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We and others have previously found that precursor IL-33 (PRIL33) protein is biologically active without proteolytic activation. We reported that the levels of PRIL33 are substantially elevated in the lungs of patients with interstitial lung disease (ILD), which is associated with idiopathic pulmonary fibrosis and systemic sclerosis. PRIL33 remains intranuclear and promotes inflammation in a non-Th2 fashion by not engaging the mature IL-33 receptor T1/ST2. To further assess the biological role of the elevated expression of PRIL33 in ILD, intratracheal bleomycin injury to the lung, alone or in combination with recombinant adenovirus delivery of PRIL33, was used to model ILD in mice. Combined PRIL33 gene delivery and bleomycin challenge had a potentiating effect on pulmonary lymphocytosis; accumulation of collagen; expression of HSP70; and the levels of TGF-β, CCL6, MCP-1, and MIP-1α, with these combined effects significantly exceeding the sum of the effects of PRIL33 gene delivery or bleomycin challenge alone ($p < 0.05$). By contrast, the combined effects of PRIL33 expression and bleomycin on bronchoalveolar lavage counts of macrophages and neutrophils in these mice were additive of the effects of each of these factors alone. The Th2 cytokines IL-4, IL-5, and IL-13 were not part of this synergy, and gene deficiency of T1/ST2 did not affect the synergistic nature of the "double-hit" from PRIL33 expression and bleomycin injury. It was concluded that elevated PRIL33 expression in ILD is a likely contributor to inflammatory and fibrotic lung injury, and that this contribution is independent of the T1/ST2 receptor or Th2 cytokines.

W5.02.03
A short-term model of COPD identifies a role for mast cell tryptase

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Chronic obstructive pulmonary disease (COPD) represents a spectrum of airways diseases, and represents a huge social burden. Therapies are largely ineffective with the development of treatments hampered by the lack of an animal model that mimics aspects of human disease in a practical time-frame. We have developed a mouse model of cigarette smoke-induced COPD that develops the major hallmark features of the human disease in a relatively short amount of time. This was achieved by treating mice with tightly controlled amounts of cigarette smoke to the airways of mice. We then assessed the development of the pathological features of COPD. Also, the role of macrophages in the pathogenesis was evaluated in depletion studies, and as tryptases play an important role in tissue homeostasis, we analysed the role of MC protease-6 in COPD by exposing protease-6 deficient mice to cigarette smoke. After 8 weeks of cigarette smoke exposure, wild-type mice developed chronic inflammation, mucus hypersecretion, airway remodeling, emphysema, and reduced lung function. These characteristic features of COPD were glucocorticoid-resistant and did not spontaneously resolve after cigarette smoke sessation. Systemic effects on skeletal muscle and the heart, and increased susceptibility to respiratory infections also were observed. MC tryptase-6 deficient mice had reduced lung pathology, indicating its requirement in COPD development. *In vitro* studies demonstrated that MC tryptase is able to induce TNFα and KC expression from macrophages, indicating a role in enhancing inflammatory responses in the lung. This model provides an opportunity to study the multiple components that result in COPD pathogenesis.

W5.02.04
Suppressive role of neuroimmune semaphorin 4A in allergen-induced and VEGF-regulated lung inflammatory responses

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Asthma-relevant responses can be induced by innate (VEGF, TSLP) as well as adaptive stimuli (allergens). Our studies of VEGF lung overexpressor transgenic (tg) mice highlighted the mechanisms by which innate immune stimuli can predispose the lung to Th2 sensitization (Lee CG et al., Nat Med, 2004, 10:1095-1103; Chapoval SP et al., Clin Immunol, 2009, 132:371-384). Our study on allergen administration to Sema4A$^{-/-}$ mice provided a new insight into the lung immune semaphorin biology and Th2 inflammatory response regulation (Nkyimbeng-Takwi EH et al., Mucosal Immunol, 2012, 5:409-419). We have shown that Sema4A$^{-/-}$ mice displayed a more robust allergic response as compared to WT mice. This included selective increases in eosinophilic airway infiltration, bronchial epithelial cell hyperplasia, AHR, and BAL IL-13 content but lower Treg numbers. We recently generated VEGF tg/Sema4A$^{-/-}$ mice and assessed the effect of Sema4A deficiency on VEGF tg lung tissue phenotype. As we have shown previously, lung bronchial epithelial expression of VEGF transgene lead to an asthma-like phenotype with inflammation, parenchymal remodeling, increased vascularization, edema formation, mucous cell and myocyte hyperplasia and airway hyperreactivity. The observed lung tissue inflammatory response and vascularization in VEGF tg/Sema4A$^{-/-}$ mice were more pronounced than those found in tg mice alone. In addition, similarly to allergen-treated Sema4A$^{-/-}$ mice, we observed higher local levels of IL-13 in VEGF tg mice with Sema4A deficiency. Thus, Sema4A downregulates both innate and adaptive lung allergic responses. The new knowledge obtained from this study will be used for the development of optimal strategies for asthma intervention.

W5.02.05
The condition of host CD4+CD25+ regulatory T cells determines the phenotype of autoimmune-like chronic Graft-versus-Host Disease

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Chronic graft-versus-host disease (cGVHD) is a frequent complication of allogeneic hematopoietic stem cell transplantation and has features similar to various autoimmune diseases such as SLE and scleroderma (SSc). Because cGVHD phenotype varies among patients, we considered that host factors might influence on the decision of cGVHD phenotype. Therefore, we focused on host factors, especially host T cells, and investigated the role of host T cells in the pathogenesis of cGVHD using CD28-deficient mice as a host whose T cells were dysfunctional, because CD28 costimulation is important for T cell activation. We found that the absence of CD28 signaling in host T cells converted cGVHD phenotype from SLE-like to SSc-like. The transfer of allogeneic donor cells into WT host resulted in SLE-like cGVHD with anti-dsDNA autoantibody and glomerulonephritis, whereas CD28-deficient host developed SSc-like cGVHD with fibrotic damage to skin and internal organs. Also, CD28-deficient host manifested no elevation of anti-dsDNA IgG. Furthermore, cytokine pattern of donor CD4$^+$T cells in WT versus CD28-deficient host were qualitatively distinct. Reconstitution experiments demonstrated that cGVHD phenotypic conversion was attributed to the defect of host CD4$^+$T cells, particularly CD25$^+$CD4$^+$T cells, but not CD8$^+$ and NK1.1$^+$T cells. Moreover, SSc-like phenotype of CD28-deficient host was dependent on the disruption of CD28-cytoplasmic C-terminal proline-rich motif, which is the critical motif for regulatory T cells (Treg) development, independently of other two motifs, which contribute to only conventional T cells functions. Therefore, we suggest that the state of host Treg is the determinant of cGVHD phenotype.

W5.02.06
Endogenous and immune cell derived BDNF supports regeneration in human skeletal muscle

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Idiopathic inflammatory myopathies (IIM) are rare skeletal muscle disorders characterized by tissue damage and immune cell infiltration. Despite several evidences point to a pathogenic role of immunity in IIM, some studies provided insights into the cross-talk between inflammatory cells and skeletal muscle after acute damage and in chronic degenerative diseases, suggesting an involvement for immune cells in promoting muscle repair. Recent researches in animal models highlighted a role for the neurotrophin BDNF in muscle regeneration, however its expression, localization and function in human skeletal muscle are unknown. Here we show that BDNF was displayed by muscle satellite cells and mature myofibers in skeletal muscle. Interestingly, in IIM tissue newly-forming myofibers displayed enhanced BDNF immunoreactivity and expressed the neurotrophin receptor p75NTR, suggesting autocrine usage of the neurotrophin supporting in vivo regeneration. Consistently, BDNF and p75NTR were expressed in human myoblasts and myotubes in vitro. Functional experiments demonstrated the role of this neurotrophin in myogenesis, as BDNF gene silencing or protein blockade dampened myocyte differentiation. Importantly, infiltrating immune cells in IIM were not evenly distributed within the tissue, but were preferentially located near p75NTR-positive regenerating fibers and produced BDNF. Both T lymphocytes and macrophages contributed to BDNF synthesis. In conclusion, BDNF is a positive regulator of human myogenesis. Thus, the preferential localization of BDNF-producing immune cells near p75NTR-regenerating fibers supports the hypothesis of protective immune responses in inflamed skeletal muscle. This work was supported by the Association Française contre les Myopathies (AFM) and by the Italian Ministry for Health (Ricerca Corrente)

W5.03.01
Effect of multiple genetic risk factors associated with rheumatoid arthritis on immune cell phenotypes

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The involvement of genetics, environment and autoimmunity in the pathogenesis of rheumatoid arthritis (RA) has been proposed recently. The most important genetic factor is the HLA-DRB gene, followed by more than 30 variants in potentially pathogenic non-MHC genes. The knowledge about their actual effect on immune cell function and related mechanisms is relatively poor. The aim of our study was to obtain a broader picture of relations between circulating immune cell phenotype, cytokine production after stimulation and the genetic background. PBMC were isolated from 35 healthy individuals and 36 RA patients with known RA-associated genotype in the genes: PTPN22, STAT4, CTLA4, PADI4, AFF3, IRF5, TRAF1/C5 and HLA-DRB1. The proportions of selected PBMC subsets were analysed by flow cytometry. TNF-α, IFN-γ and IL-17 production was assessed after stimulation with PMA/ionomycin. Redundancy analysis (RDA) was applied to analyse the data. Our preliminary results suggest an increase in pro-inflammatory cytokines and the proportion of CD14 monocytes in RA patients. Higher proportions of memory B cells were associated with the presence of one risk allele in PTPN22 and two risk alleles in AFF3, PADI4 and TRAF1/C5 genes. The presence of two risk alleles in IRF5, STAT4 and CTLA4 genes or 2 alleles coding the shared epitope sequence was associated with increased percentage of CD8 memory cells and production of TNF-α and IFN-γ by NKT cells regardless of the diagnose. Alterations in immune cell proportions and cytokine secretion are suggested as the possible mechanism behind risk allele association.

W5.03 Genetics of autoimmunity

IL5.03.01
HLA-B27 micropolymorphisms correlate with differences in function and susceptibility to Ankylosing Spondylitis

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More than 100 diseases, many of which with an autoimmune-mediated pathogenic mechanism, have been associated with HLA genes. Very often, HLA region accounts for the greater part of the genetic predisposition of these complex diseases. HLA genes are the most polymorphic in the human genome, and the extensive allelic variation together with the strong linkage disequilibrium among genes primary involved in the regulation of the immune response, have made difficult to identify the exact location of the causative variants. The study of functional differences dictated by micropolymorphisms distinguishing between associated and not associated alleles can help to give some insights. We focused our research on the HLA-B27 family of genes, strongly associated with Ankylosing Spondylitis. In particular, we have reported the existence of an allelic variant, HLA-B*2709, differing from the most frequent HLA-B*2705, for a single amino acid at position 116 (Asp to His substitution) in the pocket F of the groove hosting the C-terminus of the antigenic peptides. Interestingly, the B*2709 allele is virtually absent in patients. The Asp116-His116 variation correlates with differences in binding specificity and epitope presentation to CD8+ T cells. We show here, using theoretical (dynamics simulation and modeling) and experimental (antigen presentation) approaches, how this single substitution influences the structure not only of the F pocket but of the entire peptide-binding groove, thus dictating the binding specificity and TCR recognition.

W5.03.02
Variants in the IRF5-TNPO3 locus are associated with the development of lupus and its clinical presentation and shared with other autoimmune disorders

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Recent improvements in genetic methods allow for more complete identification of candidate causal variants. Exploiting genotyping, DNA sequencing, imputation, and trans-ancestral mapping, we modeled the IRF5-TNPO3 genetic association on chromosome 7, now implicated in two immunotherapies and seven autoimmune diseases. Specifically, in systemic lupus erythematosus (SLE) we resolve separate associations in the IRF5 promoter (all ancestries) and an extended European haplotype. We capture 7,650 IRF5-TNPO3 genetic variants across five ethnicities in 8,395 SLE cases and 7,367 controls. The six plausible causative IRF5 promoter variants are confined to 5.7 kb. The 22 potentially causal variants of an 85.5 kb haplotype of IRF5 and TNPO3. The possible models from this sample virtually eliminate the previously purported IRF5 functional variants as causal. Strikingly, this model also appears to operate in Sjögren's syndrome and systemic sclerosis (both components) and primary biliary cirrhosis (haplotype only), demonstrating the nuances of similarity and difference in autoimmune disease risk mechanisms at IRF5-TNPO3. Finally, we performed case-only subphenotypic analyses to identify variants in the IRF5-TNPO3 region useful in predicting the disease progression of patients with lupus and found strong association with age-of-onset (variants in the haplotype) and the antigen specificity of autoantibodies anti-Ro and anti-dsDNA (variants in the IRF5 promoter). In conclusion, genetic variants in the IRF5-TNPO3 region critically regulate the development of multiple autoimmune diseases and the clinical presentation of lupus. Given the importance of this locus to multiple immune disorders, future studies will be aimed at understanding the immunological mechanisms driving the genetic associations.

W5.03.03
The lupus-associated variant of the ITGAM allele (rs1143679) impairs phagocytosis, but not migration nor TLR7/8 induced cytokine release

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The minor allele of a non-synonymous ITGAM polymorphism (rs1143679, converting arginine to histidine at position 77: R77H) represents one of the strongest genetic risk factors in systemic lupus erythematosus, with heterozygosity (77R/H) being the most common disease associated genotype. ITGAM encodes for an integrin, CD11b, which associates with CD18 to form the complement receptor 3 (CR3) that plays a crucial role in several immunological processes including leukocyte extravasation and phagocytosis. In this study, we assessed the influence of R77H on different CR3-mediated functions in monocytes, neutrophils, macrophages and dendritic cells. The surface expression of CD11b was not altered by R77H in any of these cell types. Nevertheless, phagocytosis of iC3b-coated targets was significantly impaired in heterozygous 77R/H macrophages, monocytes and neutrophils compared to 77R/R cells. We confirmed this using immortalized macrophage cell lines expressing either allele. R77H polymorphism did not affect adhesion and transmigration of neutrophils assessed by *in vivo* and *in vitro* assays. Toll-like receptor 7/8-mediated cytokine release by monocytes or dendritic cells with or without CR3 pre-engagement by iC3b-coated targets was similar between the two alleles. Our findings demonstrate that the reduction in CR3-mediated phagocytosis associated with the 77H CD11b variant is not macrophage-restricted and that susceptibility to systemic lupus erythematosus most likely relates to impaired waste disposal, a key component of lupus pathogenesis.

W5.03.04
Genetic variants in PXK are associated with the development of lupus and critically regulate BCR internalization

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Systemic Lupus Erythematous (SLE) is a systemic autoimmune disease with a strong genetic component. Over 50 genes have been associated with SLE, many with no immediate or obvious biological connection to disease. We previously identified one such gene, *PXK*, as being a candidate gene associated with SLE in women of European descent and these findings have since been replicated. *PXK* has additionally been identified as a risk gene for RA as well, suggesting a broad role in the pathobiology of autoimmune disease. In this work we undertake the fine mapping of the *PXK* genetic locus in an effort to refine the association signal. We identify one independent effect in the region occurring strictly in individuals of European ancestry. In tandem with refinement of the genetic signal, we also attempt to identify the SLE relevant biological import of *PXK* by examining the role it plays in B cells. While PXK has been shown to participate in receptor internalization, nothing is known about its function in the context of SLE. Using ImageStream technology, we find that PXK colocalizes with the B cell receptor (BCR) upon BCR internalization. Finally, we show that the BCR is more rapidly internalized in cells carrying the *PXK* risk allele. These results suggest that PXK may play an important role in the regulation of BCR signaling and B cell differentiation and survival. As B cell regulation is crucial to SLE pathogenesis, understanding the specific changes induced by SLE-associated variants in *PXK* will provide important insight into SLE pathogenesis.

W5.03.05
Enhanced apoptosis by disruption of the STAT3-IκB-ζ signaling pathway in epithelial cells induces Sjögren's syndrome-like autoimmune disease

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Sjögren's syndrome is an autoimmune disease characterized by exocrinopathy that leads to dry eye and dry mouth. Although lymphocyte infiltration into exocrine glands and the generation of autoantibodies have been reported in Sjögren's syndrome, its pathogenic mechanism remains elusive. Here, we show that mice lacking the transcriptional regulator, IκB-ζ, developed Sjögren's syndrome-like inflammation characterized by lymphocyte-infiltrated dacryoadenitis and Sjögren's syndrome-associated autoantibodies. In particular, epithelial cells, but not hematopoietic cells, lacking IκB-ζ were essential for the development of inflammation. IκB-ζ-deficient epithelial cells in the lacrimal glands exhibited enhanced apoptosis even in the absence of lymphocytes. Administration of caspase inhibitors ameliorated the inflammation, indicating the critical role of caspase-mediated apoptosis. Furthermore, epithelial cell-specific STAT3-deficient mice developed Sjögren's syndrome-like inflammation with impaired IκB-ζ expression in the lacrimal glands. Thus, this study reveals a pathogenic mechanism of Sjögren's syndrome in which dysfunction of epithelial cells caused by disruption of STAT3-mediated IκB-ζ induction elicits the activation of self-reactive lymphocytes.
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W5.03.06
Vav1 cooperate with Themis to modulate regulatory T cell suppressive functions

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Natural Foxp3+ regulatory T cells (Treg) are of paramount importance for establishing self-tolerance, controlling inflammatory responses and maintaining immune homeostasis. The identification of new molecules controlling Treg functions is nowadays an important challenge. We previously reported the implication of Themis, a new actor of TCR signaling, in Treg suppressive capacities. Indeed, Treg from Themis deficient BN rats were defective, leading to the development of an inflammatory bowel disease. In the present study, we show that Themis deficiency does not impair Treg suppressive functions and does not induce any pathological manifestations in LEW rats. These findings suggest that an interaction with other(s) gene(s) may contribute to the Treg defect and disease development in Themis deficient BN rats. To test this hypothesis and identify the involved gene(s), we performed a genetic dissection using different congenic lines. We show that the impact of Themis deficiency on Treg suppressive functions requires the expression of a constitutively active Vav1 variant. Together, these studies highlight the importance of Vav1 and Themis signaling hub in regulating the suppressive functions of Treg. Understanding the underlying molecular mechanisms may lead to advances in Treg biology and favor the identification of new pharmacogenetic markers and therapeutic targets in immune mediated diseases.

W5.05 Environmental factors in autoimmunity and allergy

IL5.05.01
'ASIA' - Autoimmune (Auto-inflammatory) syndromes induced by adjuvants

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Four enigmatic medical conditions were described recently, all characterized by hyperactive immune response and similar clinical and laboratory manifestations. These conditions (siliconosis, Gulf War Syndrome, macrophagic myofasciitis syndrome and post-vaccination phenomena) imply that environmental factors may play a role in inducing or aggravating autoimmunity and auto-inflammation.
In rare occasions vaccines may induce autoimmune or auto-inflammatory conditions both in animals and in humans. These conditions - defined diseases (Gullian-Barre syndrome) or enigmatic ones - have been reported following vaccines and vaccination protocols. The susceptibility factors and the temporal association between vaccines and these immune mediated reactions remain to be defined; however, the similarities between vaccines and infections and the addition of adjuvants to almost every vaccine are considered major contributors to such adverse events. In MMF a cause was clearly delineated. MMF is a rare condition caused by deposition of alum, an adjuvant in different vaccines, which cause an immune mediated muscles disease. In genetically prone patients, alum may induce this syndrome.
Another similar phenomenon is exposure to silicones. In a study, a group of patients with silicone breast implants had a statistically significant increase in 16 of 28 investigated symptoms consistent with fibromyalgia and chronic fatigue syndrome criteria, compared to a group of women who underwent reduction mammoplasties, congruent with the FDA's establishment of a link between fibromyalgia and ruptured silicone implants.
A common denominator to these syndromes is the trigger entailing adjuvant activity.
We suggest including these four conditions in one syndrome, the "Autoimmune Syndrome Induced by adjuvants" (ASIA).

IL5.05.02
Early-life microbial exposures and allergy development - exploring the role of herpesviruses

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There is increasing evidence to support a role for early-life microbial exposures in influencing immune- and allergy development in children. Epstein-Barr virus (EBV), a human herpesvirus, is often acquired during childhood and persists latently in B cells. We have found that EBV sero-positivity connects to immune-modulatory effects in children such as altered T- and NK-cell functional responses in vitro as well as protection against early IgE-sensitization. This potential "allergy-protection" was further dependent on time-point for infection. Therefore we have continued to study whether age at infection and if co-infection with another common herpesvirus, cytomegalovirus (CMV), influence immune characteristics following EBV infection in vitro. This has been investigated in cells from a well-characterized cohort of children with known EBV and CMV serostatus. Upon in vitro EBV infection of peripheral blood cells from children of different ages we found that age, and the capacity to produce IFN-γ in particular, clearly had an impact on the degree of EBV-induced B-cell activation and expansion. Further CMV serostatus significantly influenced EBV-infection in vitro, as we observed a reduced enrichment of (switched memory) IgD-CD27+ B cells associated with high levels of IFN-γ and frequencies of highly mature CD8+CD57+ T cells in CMV+ children following EBV-infection. Our results demonstrate that both a child's age and serostatus to CMV will have an impact on EBV-induced B-cell activation and expansion, and points to the ability of persistent viruses with immune-modulatory functions to impact on immune responses within the host.

W5.05.01
Ectopic lymphoid structures support Epstein-Barr virus persistence and autoreactive plasma cell infection in Sjogren's Syndrome salivary glands

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Background: Epstein-Barr virus (EBV) infects B-cells and modifies their differentiation programme leading to B-cell activation and immortalization. EBV has been suggested to rescue autoreactive B-cells and promote autoimmunity. Although different evidences support a link between EBV infection and Sjogren's syndrome (SS), the role of EBV in SS pathogenesis remains elusive.
Objectives: To investigate the role of EBV in SS by analysing EBV infection in the salivary glands and its relationship with ectopic lymphoid structures (ELS), in situ autoreactive plasma cell differentiation, autoantibody production and cytotoxic immune response.
Methods: Latent and lytic EBV infection was investigated in SS salivary glands characterised for the presence/absence of ELS by RT-PCR, in situ hybridization and immunohistochemistry/ immunofluorescence. Local production of anti-Ro52 antibodies and anti-EBV antibodies was investigated in situ and/or in vivo in the SCID/SS chimeric model.
Results: EBV dysregulation was observed exclusively in ELS+ SS salivary glands as revealed by EBER transcripts and EBER+ cells and immunoreactivity for EBV latent (LMP1/LMP2A) and lytic (BFRF1) antigens in B-cells and plasma cells, respectively. A subset of EBV+ plasma cells produced anti-Ro52 antibodies and ELS-containing SS salivary glands transplanted into SCID mice supported the production of anti-Ro52, anti-La and anti-EBV antibodies. Analysis of CD4/CD8 T-cell localization and granzyme-B expression suggested that EBV persistence is favoured by impaired cytotoxicity and exclusion of CD8+ T-cells from B-cell follicles.
Conclusions: We demonstrated latent and active EBV infection within ELS in SS salivary glands that appears to contribute to local growth and differentiation of autoreactive B cells producing SS-associated autoantibodies.

W5.05.02
A single non-synonymous polymorphism of TLR2 is sufficient to recapitulate the variability of Experimental Multiple Sclerosis

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In the pathogenesis of Multiple Sclerosis a growing body of evidence is directing the attention also to bacteria. It is known that TLRs are involved in maintaining tolerance and inducing inflammation against the pathogens. We showed that M tb-derived motives play an essential role in the induction of the experimental model of Multiple Sclerosis (EAE). To examine TLR2 (the main receptor for M tb) in the development of EAE we crossed different strains of mice, SJL and B6, in both of which is possible to induce EAE after immunization with myelin derived proteins. These two strains display a non-synonymous SNP of TLR2 that condition mobility of encephalitogenic T cells, with the allele of B6 dominant over that of SJL. Using of a strain lacking for TLR2 expression ($B6^{tlr2-}$) we observed that a single NSP of TLR2 has a complex effect on clinical and histology of EAE. Although TLR2 haplotype does not influence incidence of disease, it is crucial for the course of EAE (more severe in F1 SJL x $B6^{tlr2-}$ than in F1 SJL x $B6^{wt}$ mice), significant for remission of EAE symptoms and involved in localization of infiltrates and demyelination in frontal lobes. The polymorphic site of TLR2 influences polarization of T cells by the expression of cytokines modifying the bias towards Th1/Th17, while no effect was seen in FoxP3 expression.

W5.05.03
High salt diet exacerbates autoimmune disease by induction of pathogenic Th17 cells

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Over the last half-century there has been a marked increase of autoimmune diseases in the Western world. This increase could not be explained by genetic predisposition but must be triggered by changes in the environment. It has been shown that pathogenic IL-23 dependent Th17 cells play a critical role for the development of autoimmune diseases. Genetic risk factors associated with multiple sclerosis (MS) are related to the IL23/Th17 axis and Th17 cell are crucial for the induction of experimental autoimmune encephalomyelitis (EAE), an animal model for MS. However, how changes in the environment could directly influence Th17 cells is not well established. With this study we show that high-salt (sodium chloride; NaCl) concentrations boost the induction of murine and human Th17 cells in vitro. High-salt concentrations activate the p38/MAPK pathway involving nuclear factor of activated T cells 5 (NFAT5) and serum/glucocorticoid-regulated kinase 1 (SGK1) during cytokine-induced Th17 polarization. In line with increased IL-17 secretion, high-salt induced Th17 cells up regulate the pro-inflammatory cytokines GM-CSF, TNFα and IL-2, characteristic for a highly pathogenic phenotype. Finally, mice fed a high-salt diet develop a severe form of EAE, in line with augmented central nervous system infiltrating and peripherally induced Th17 cells. Thus, increased dietary salt intake might represent an environmental risk factor for the development of autoimmune diseases through the induction of pathogenic Th17 cells.

W5.05.04
Impact of infectious agents on trafficking of effector T cells is mediated by a polymorphic site of TLR2 and CD44 isoforms expression

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The nature of triggers activating auto-aggressive lymphocytes in Multiple Sclerosis is poorly understood, but both environmental and genetic factors are relevant. The role of infectious agents in the regulation of antigen-specific T cell trafficking is currently unknown. We showed that the amount of *M tuberculosis* in the adjuvant modulates rapid relocation of antigen-specific T cells from draining lymph nodes (LN) to spleen, in the SJL mouse. We observed that the modulation of T cell mobilization was strain dependent and linked to a NSP of TLR2 responsible for early/late relocation phenotype. To clarify the mechanisms controlling early and late mobilization of T cells we examined the expression of activation markers and adhesion molecules involved in T cell trafficking. We show that early relocation associated with intermediate expression of CD44, a molecule controlling T cell migration under inflammatory conditions. TLR2 directly regulates the distribution of CD44 and the expression of its isoforms. Our results show that pathogens engaging TLR2 on activated T cells through a polymorphic site modulate expression of CD44 and regulate effector T cells trafficking in vivo.

W5.05.05
Aryl hydrocarbon receptor modulates psoriatic skin inflammation

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Psoriasis is a chronic inflammatory skin disease resulting from the interaction of genetic and environmental factors. Cross-talk between innate, adaptive and epithelial/stromal cells, such as keratinocytes and fibroblasts, underpins the pathological response in this disease. More than 40 disease-associated loci have been identified to contribute to psoriasis. Environmental risk factors on the other hand remain less well-defined on a mechanistic basis.
The Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor responsive to environmental stimuli and it is essential for the maintenance of intestinal barrier integrity. The wide expression of AhR in several skin cell types suggests a role for AhR signalling at this barrier organ. Here, we combine the imiquimod-induced mouse model of psoriasiform skin inflammation with the analysis of psoriasis patient skin biopsies, to investigate the role of AhR in psoriasis.
AhR deficiency in the skin resulted in a more severe psoriasiform skin inflammation with increased epidermal thickness, neutrophils infiltration and expression of pro-inflammatory mediators. Conversely, AhR ligation in wild-type mice ameliorated the skin phenotype. Finally, exposure of psoriasis skin biopsies to either AhR agonist or antagonist modulated psoriasis-related genes with AhR agonist reducing their expression in lesional skin, and AhR antagonist up-regulating these genes in non-lesional skin.Taken together, our results suggest that AhR has a critical role in dampening skin immune responses and that lack of AhR in the skin exacerbates dysregulated cross-talk between innate and adaptive cells in psoriasis.

W5.05.06
IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation

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How the innate and adaptive immune systems cooperate in the natural history of allergic diseases has been largely unknown. Plant-derived allergen, papain, and mite allergens, Der f 1 and Der p 1, belong to the same family of cysteine proteases. We examined the role of protease allergens in the induction of antibody production and airway inflammation after repeated intranasal administration without adjuvants and that in basophil/mast cell stimulation *in vitro*. Papain induced papain-specific IgE/IgG1 and lung eosinophilia. Der f 1 induced Der f 1-specific IgG1 and eosinophilia. Although papain-, Der f 1-, and Der p 1-stimulated basophils expressed allergy-inducing cytokines including IL-4 *in vitro*, basophil-depleting antibody and mast cell deficiency did not suppress the papain-induced *in vivo* responses. Protease inhibitor-treated allergens and a catalytic site-mutant did not induce the responses. These results indicate that the protease activity is essential to antibody production and eosinophilia *in vivo* and basophil activation *in vitro*. IL-33-deficient mice lacked eosinophilia and had reduced papain-specific IgE/IgG1. Coadministration of ovalbumin with papain induced ovalbumin-specific IgE/IgG1, which was reduced in IL-33-deficient mice. We demonstrated IL-33 release, subsequent IL-33-dependent IL-5/IL-13 release, and activation of T1/ST2-expressing lineage$^-$CD25$^+$CD44$^+$ innate lymphoid cells in the lung after the papain inhalation, suggesting the contribution of the IL-33_type 2 innate lymphoid cell_IL-5/IL-13 axis to the papain-induced airway eosinophilia. Rag2-deficient mice, which lack adaptive immune cells, showed significant, but less severe eosinophilia. Collectively, these results suggest cooperation of adaptive immune cells and IL-

33-responsive innate cells in protease-dependent allergic airway inflammation.

W5.06 Regulatory T cells in autoimmunity and allergy

IL5.06.01
The CD28 superagonist TGN1412: After the storm

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During a first-in-human trial in 2006 in London, application of the CD28 superagonist TGN1412 resulted in a life-threatening cytokine release syndrome. This is in contrast to rodent models, where Treg cells are preferentially activated, allowing interference in multiple models of autoimmunity and inflammation. Using a novel cell culture system in which the response to soluble TGN1412 is readily detected, we have found that a) the dose of TGN1412 applied during the FIH study was in functional saturation, maximally activating both pro-inflammatory CD4EM T-cells as well as Treg cells; b) just as in rodents, reduction of CD28 SA concentration leads to a loss of the pro-inflammatory response while partially maintaining the regulatory T-cell response, and c) that using this protocol, effective Treg activation is readily observed in vitro with PBMC from patients with various autoimmune/inflammatory disorders such as RA, MS, SLE, and Psoriasis. Finally, results from a new, low-dose study with human volunteers will be reported.

IL5.06.02
Molecular Control of Treg Stability and Function

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Regulatory T cells (T_{reg}) represent a critical component of the immune system that prevents autoimmunity and inflammatory diseases, and maintains immune homeostasis, but also limits effective anti-tumor immunity. The signals that maintain T_{reg} stability and potentiate their function remain obscure. We have shown that the immune cell surface ligand semaphorin-4a (Sema4a) on conventional T cells and the T_{reg}-restricted receptor neuropilin-1 (Nrp1) interact to potentiate T_{reg} function. Mice with a T_{reg}-restricted deletion of Nrp1 exhibit limited tumor-induced tolerance and thus substantial resistance to certain tumors, yet do not develop any autoimmune manifestations. Nrp1-deficient T_{reg} are also unable to control colitis. Increased Nrp1 signaling represses Akt activity, facilitates Foxo nuclear translocation and induces a transcriptional program that promotes T_{reg} stability and function while repressing the induction of lineage-specific transcription factors. Nrp1 is also expressed on activated human T_{regs} and promotes their suppressive function. Thus, Nrp1 ligation maintains T_{reg} stability and function in highly inflammatory sites but is dispensable for the maintenance of immune homeostasis, highlighting Nrp1 as a potential immunotherapeutic target in cancer while induction of this pathway may present novel treatments for inflammatory and autoimmune diseases. The utilization of this pathway in autoimmune diseases will be discussed.

W5.06.01
TRAF6 is essential for maintenance of regulatory T cells and suppression of Th2 type inflammation

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Regulatory T cells (Tregs) maintain immune homeostasis by limiting inflammatory responses.
Tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) has a key role in the regulation of innate and adaptive immune responses by mediating signals from various receptors including T-cell receptor. Previously, T cell-specific deletion of TRAF6 has been shown to induce multiorgan inflammatory disease, however, the role of TRAF6 in Tregs remained to be investigated. Therefore, we generated Treg-specific TRAF6-deficient mice by using Foxp3-Cre and TRAF6-flox mice. Treg-specific TRAF6-deficeint mice developed fulminant multiorgan inflammatory diseases including dermatitis, multiple lymphadenopathy, arthritis, and hyper IgE phenotypes. Although TRAF6-deficeint Tregs possesses similar in vitro suppression activity compared to wild-type Tregs, TRAF6-deficeint Tregs could not suppress autoimmune colitis induced by naïve T cells in Rag2-deficeint mice due to low proliferation capacity under lymphopenic conditions. Consistently, the fraction of TRAF6-deficeint Tregs was reduced in number compared with wild-type Tregs in TRAF6 flox/flox, Foxp3YFP -Cre/+ mice, which suggests the in vivo instability of TRAF6-deficeint Tregs without inflammation. Moreover fate-mapping analysis of Tregs suggested the number of exFoxp3 cells increased, and IL-4 was mostly produced from exFoxp3 cells under Th2 type inflammation. These data indicate that TRAF6 play important roles in stable maintenance of Tregs against Th2 type inflammation.

W5.06.02
Breakdown of immune tolerance by quantitative and qualitative alterations of ZAP-70

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ZAP-70 protein is an essential mediator of TCR signaling and its alteration can drastically change the course of thymic development and immune tolerance. Here, we show that a series of qualitative and quantitative alterations of ZAP-70 result in spontaneous developments of arthritis and other chronic autoimmune diseases. Qualitative alterations of ZAP-70 by knock-in mutagenesis resulting in 10- or 20-fold reduction in affinity to phosphorylated ITAMs of CD3ζ lead to developments of arthritis and other autoimmune diseases. Quantitative alterations in a tetracycline-inducible ZAP-70 reconstitution system in ZAP-70 KO mice showed that approximately 4-fold reduction resulted in chronic arthritis and a variety of autoimmune diseases. All the alterations of ZAP-70 that caused CD4+ T cell dependent arthritis showed selection of few CD4 SP cells with skewed TCR towards self-reactive repertoire and defective thymic development of natural regulatory T cells in the thymus. Furthermore, modulation of ZAP-70 levels distinctly in the thymus and the periphery demonstrated that those T cells developed in the thymus with a quantitatively low level of ZAP-70 lead to the development of autoimmune diseases irrespective of their ZAP-70 levels in the periphery. Our results suggest that a range of ZAP-70 reduction low enough to impair Treg selection and promote the selection of self-reactive TCR, but sufficiently high to maintain their TCR responsiveness confer spontaneous autoimmunity.

W5.06.03
KLRG1 expression in ICOS+Foxp3+ Treg cells delineates a functionally fatigued Treg subset during T1D progression in NOD mice

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A progressive waning in Treg cell function is thought to provoke autoimmunity in the non-obese diabetic (NOD) model of type 1 diabetes (T1D). We have shown that the abrogation of the inducible T cell co-stimulator (ICOS) pathway in NOD-BDC2.5 mice exacerbates T1D. More specifically, we demonstrated that the majority of islet-infiltrating Treg cells express ICOS in pre-diabetic mice, and that ICOS+ Treg cells display improved fitness and suppressive function. We also showed that T1D progression is associated with a decline in ICOS expression, in expansion and in suppression in these cells. Strikingly, as much as 50% of ICOS+ Treg cells express KLRG1, a

marker of terminal differentiation. We thus hypothesized that KLRG1 expression designates a subpopulation of dysfunctional ICOS+ Treg cells in islets and might therefore provoke T1D progression. We find that ICOS+ Treg cells expressing KLRG1 have decreased proliferative capacity and CD25 expression in vitro and in vivo. In addition, loss of fitness in KLRG1+ICOS+ Treg cells correlates with a poorer suppressive capacity, both in vitro and in vivo. We also observe an increased inflammatory cytokine production by KLRG1+ICOS+ Treg cells which correlates with a loss of Foxp3 expression in these cells. Overall, we demonstrate that KLRG1 expression delineates a subpopulation of dysfunctional ICOS+ Treg cells during the progression of T1D. Finally, the KLRG1 ligand E-cadherin is expressed by the beta cells of the pancreas, suggesting a crosstalk between these cells and the autoimmune target organ, and highlighting the potential relevance of KLRG1+ICOS+ Treg cells in T1D physiopathology.

W5.06.04
B cell derived IFN-γ contributes to the negative regulation of T-regulatory cell differentiation in arthritis

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Depletion of mature B cells using Rituximab has proven efficacious in patients with rheumatoid arthritis (RA). We have previously shown in our mouse model of RA, proteoglycan induced arthritis (PGIA), that depletion of B cells leads to enhanced T regulatory cells (Tregs) numbers and activity essential in the suppression of arthritis. This increase in Treg numbers and function correlated with a decrease in T cell IFN-γ production. In this study, we investigate the effects of B cells on the generation of Tregs and how IFN-γ contributes to this process. We show that in vitro IFN-γ suppresses the differentiation of $CD4^+CD25^-CD62L^+$ T cells into $Foxp3^+$ Tregs. $CD4^+CD25^-CD62L^-$ T cells were resistant to Treg differentiation and this resistance correlates with copious IFN-γ production. Through an in vivo transfer model of transgenic T cells into immunized and B cell depleted mice, we find that $CD4^+CD62L^+Foxp3^-$ T cells are converted into Tregs more effectively than $CD4^+CD62L^-Foxp3^-$ T cells. To determine if IFN-γ is responsible for the suppression of Treg generation, we setup in vitro Treg differentiation using wild type and IFN-$\gamma^{-/-}$ $CD4^+CD25^-CD62L^+$ T cells in the presence of TGF-β and wild type or IFN-$\gamma^{-/-}$ B cells. We find that IFN-$\gamma^{-/-}$ B cells resulted in an increase in Treg differentiation in comparison to WT B cells. Chimeras with a specific deficiency in IFN-γ in B cells also show an increase in the percentage of Tregs. Our findings demonstrate that B cell derived IFN-γ can negatively regulate the generation of Tregs in arthritis.

W5.06.05
The *in vivo* impact of CD25 blockade on human regulatory T cells and evidence for compensatory maintenance by IL2Rβγ and IL7R signaling

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The biology of IL-2 is pleiotropic and complex. As a growth factor for effector T cells and regulatory T cells (Treg), IL-2 can both potentiate and limit the adaptive immune response. In mouse models, the role of IL-2 in mediating immune tolerance through the support of Treg homeostasis appears dominant. Mice deficient in IL-2, IL2Rβ (CD122), and IL2Rα (CD25) all succumb to lymphoproliferative autoimmune pathology. Monoclonal antibodies against CD25 exacerbate EAE. In contrast, data from two recent Phase II clinical trials demonstrate that therapy using a humanized monoclonal antibody against CD25 (Daclizumab HYP) is efficacious in the treatment of relapsing-remitting multiple sclerosis (RRMS) despite reducing Treg numbers in the peripheral blood.
To better understand the role of CD25 in human Treg biology and to reconcile seemingly contradictory observations from mouse and human biology, we analyzed Treg subsets using refined immunophenotyping and epigenetic analysis of the *FOXP3* promoter of peripheral blood lymphocytes. Together this data provides the largest and most comprehensive analysis of human Tregs in a single study. A comparison of placebo- and Daclizumab-treated RRMS patients from the SELECT phase II clinical trial reveals differential effects of Daclizumab on specific Treg subsets. The observed changes in Treg subsets did not appear to be associated with treatment response or adverse events. Additionally, we find evidence that intermediate-affinity IL-2 receptor and IL-7 receptor signaling maintain the Treg compartment in the face of CD25 blockade.

W5.06.06
Restoration of normoglycemia in diabetic NOD mice by oral L.lactis secreting human pro-insulin plus IL-10 combined with short-term low-dose anti-CD3 is dependent on FOXP3+ Tregs

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INTRODUCTION/GOALS: In Type 1 Diabetes (T1D) re-establishing tolerance to beta-cell antigens is an ultimate goal. We have previously shown that combination therapy (CT) of L.lactis secreting human pro-insulin plus IL10 and short-term low-dose of anti-CD3 could stably revert diabetes in new-onset diabetic NOD mice and induce an increase in CD4+CD25+FOXP3+Tregs. The AIM of this study was to characterize and investigate dependence of the clinical effect on the presence of these induced Tregs. METHODS: New-onset diabetic NOD or transgenic NOD-FOXP3-DTR mice received anti-CD3 (145-2C11 clone) i.v for 5 consecutive days and were gavaged during 6 weeks with genetically-modified L.lactis. At 6 weeks, CD4+CD25+Tregs from spleen and pancreatic draining lymph nodes of CT-cured mice were tested in polyclonal suppression assays using transwell plates or blocking antibodies against TGFβ, CTLA-4 or IL10. In cured male NOD-FOXP3-DTR mice FOXP3+Tregs were specifically depleted by difteria toxin ((DT) injections i.p.) RESULTS: In vitro, we demonstrated that Treg suppression was cell contact-dependent as use of transwell abrogated suppression completely. Addition of anti-TGFβ or anti-CTLA4 decreased suppression by 74% and 75% respectively ($0.001<p<0.0001$), while blocking IL10 had no effect. In NOD-FOXP3-DTR mice, CT induced disease reversal in 66% of mice. In cured male NOD-FOXP3-DTR mice, DT injections eliminated >90% of FOXP3+Tregs followed by return to hyperglycemia, indicating break of tolerance in 3/3 mice. CONCLUSION: The combined therapy of low-dose anti-CD3 and L.lactis secreting pro-insulin plus IL-10 induces diabetes remission in newly diagnosed diabetic NOD mice and maintenance of tolerance is dependent on the presence of FOXP3+Tregs.

W5.07 Animal models of autoimmunity

IL5.07.01
Experimental model of autoimmune prostatitis

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Animal models have long been used to study human disease in order to identify etiologies, pathogenic mechanisms and development of therapies. Ideally, they must mimic, if not all, most of the features observed in human disease. Despite continuing debate about ethics on using animal models in biomedical research, the field has experienced considerable progress during last decades. Several animal models, particularly mouse models, have been developed. Diseases can be spontaneous (naturally occurring), or be induced by physical, chemical or biological means.
The prostate is the target of several diseases, such as infection, chronic inflammation, benign hyperplasia and cancer. Autoimmune prostatitis (AP) has become to be recognized as a disease occurring in patients, and animal models of Experimental Autoimmune Prostatitis (EAP) are available. Certainly, NOD mice develop AP spontaneously with age and immunization of young males with prostate antigens induces an exacerbated disease mimicking most features observed in human pathology. Florid infiltrates circumscribed

to dorsolateral prostatic lobes are observed, accompanied of humoral and T cell-mediated responses. Prostate-specific IFN-γ secreting cells are detected in periphery and Th1-related cytokines in the target organ. Increased IFN-γ and IL-12 levels are observed in prostate tissue from immunized animals, while IL-10 and IL-4 levels are decreased. Moreover, mice deficient in IFN-γ, IL-12, or in factors involved in IFN-γ signaling cascade (IRF-1 or STAT-1) are resistant to EAP induction. On the contrary, IL-4-deficient animals develop a more exacerbated disease.
After reviewing all the experimental evidence, we propose EAP models as valid for the study of human disease.

W5.07.01
Effector Memory Exhausted Tissue Resident CD8 T Cells Accumulate During Chronic Experimental Autoimmune Uveitis (EAU)

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Using CD4 T cell mediated Experimental Autoimmune Uveitis (EAU) as a model for human intraocular inflammation we have demonstrated that different infiltrating leukocyte populations accumulate during disease. CD8 T cells increase in the secondary progressive phase of EAU and it remains unknown whether they contribute to the persistence of disease. Because CD8 T cells of an effector memory (EM) phenotype take up residence in the tissue following infection we determined whether the retinal CD8 T cell population fulfilled a similar function. C57BL/6J mice were immunised with RBP-3 (IRBP) 1-20 peptide to induce EAU. Disease progression was measured by Topical Endoscopic Fundal Imaging (TEFI) and CD8 T cell retinal infiltrate and phenotype assessed by flow cytometry. Cytokine production and cytotoxic potential was measured by intracellular cytokine staining. Treatment with FTY720 was used to interrogate the tissue half-life of retinal CD8 T cells. Retinal CD8 T cells expanded significantly late in disease. These cells lacked effector function and upregulated PD-1 and CD69 expression but not CD103. Treatment with FTY720 decreased circulating levels of CD4 and CD8 T cells in the blood; while analysis of retinal cell content revealed a significant decline in the numbers of CD4 T cells but not CD8 T cells. In conclusion, effector memory CD8 T cells migrate to the retina during peak inflammation and upregulate markers associated with recent antigen stimulation. Over time the CD8 T cells expand while chronic antigen stimulation leads to changes in phenotype associated with T cell exhaustion.

W5.07.02
Small intestine inflammation in roquin-mutant and roquin-deficient mice

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Roquin is an E3 ubiquitin ligase that localizes to cytosolic RNA granules that is involved in regulating mRNA stability and translation. Mice that have a M199R mutation in the Roquin protein (sanroque, *Roquin*$^{san/san}$ mice) develop autoimmune pathologies, although the extent to which these occur in the intestine has not been assessed. *Roquin*$^{san/san}$ mice were found to reproducibly develop intestinal inflammation in the small intestine but not the colon. Similar findings were observed in mice generated in our laboratory in which the *Roquin* gene was disrupted by insertion of a gene trap cassette (*Roquin*$^{gt/gt}$ mice). MLN cells in *Roquin*$^{san/san}$ mice consisted of activated proliferating T cells that included CD44^{hi} CD62L^{lo} KLRG1^{+} short-lived effector cells (SLECs). More small intestinal intraepithelial lymphocytes in *Roquin*$^{san/san}$ mice expressed the ICOS T cell activation marker compared to normal mice. Importantly, small intestinal lamina propria lymphocytes in *Roquin*$^{san/san}$ mice consisted of a high proportion of Gr-1^{+} T cells that included IL-17A^{+} cells and CD8^{+} IFN-γ^{+} cells. There was extensive cytokine dysregulation resulting in both over-expression and under-expression of chemotactic cytokines in the ileum of *Roquin*$^{san/san}$ mice, the intestinal region most prone to inflammation. These findings demonstrate that chronic inflammation ensues in the intestine following Roquin protein mutation or gene disruption, and they have implications for understanding how chronic small intestinal inflammation is perpetuated. Because the primary gene/protein defects in these Roquin animal systems are known, it will be possible to elucidate the underlying genetic and molecular mechanisms that drive the disease process.

W5.07.03
Tolerance induction by hair-specific keratins in murine alopecia areata

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Alopecia areata (AA) is a T cell-mediated autoimmune disease of the skin with non-scarring hair loss by destruction of anagen stage hair follicles. AA is characterized by perifollicular infiltrates of CD4+ and CD8+ T cells and aberrant MHC expression on hair follicle epithelium, the loss of immune privilege being suggested to allow for autoreactive T cell activation. The autoantigen(s) have not been defined. Hair- and nail-specific keratins are potential candidates, which we controlled in C3H/HeJ mice that develop AA spontaneously or after skin transplantation. From 9 keratins, K6irs and Ha1 peptides supported T cell activation when presented by dendritic cell (DC) to syngeneic naive T cells and young C3H/HeJ mice receiving subcutaneous injections of peptide-loaded DC developed AA. The frequency of K6irs- and Ha1-specific CD4+ and CD8+ T cells increased 4-5-fold by vaccination, which corresponds with the frequency seen in skin transplantation-induced AA mice. Also, accessory molecule expression, the cytokine profile with a dominance of IFNγ, the proliferative response against AA lysate or peptide-loaded DC as well as peptide-specific cytotoxic T cells were similar in keratin peptide- and skin transplantation-induced AA. Instead, vaccination with soluble K6irs or Ha1 peptides significantly retarded AA induction and prevented progression. Soluble peptide vaccination did not provoke immunosuppression, but induced long-lasting T cell anergy with unresponsiveness to DC-presented K6irs and Ha1 peptides.
Thus, keratins (K6irs and Ha1) are an AA autoantigen, our data suggesting a contribution of additional self antigens. Importantly, keratin peptide vaccination prevents AA induction and progression in about 50% of mice.

W5.07.04
Inducible tertiary lymphoid structures, autoimmunity, and exocrine dysfunction in a novel model of sialoadenitis in wild-type mice

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Salivary glands (SG) of patients with Sjögren's syndrome (SS) develop ectopic lymphoid structures (ELS) characterized by B/T cell compartmentalization, the formation of high endothelial venules (HEV), follicular dendritic cell networks (FDCs), functional B cell activation and local differentiation of autoreactive plasma cells. The mechanisms triggering these phenomena in SS are largely unknown. Here we present a novel model of inducible ELS formation, breach of self-tolerance and salivary hypofunction following delivery of a replication-deficient adenovirus-5 (AdV5) in SGs of C57BL/6 mice. Luciferase- or LacZ-encoding Ad5 were delivered in mice SGs through retrograde cannulation. SGs were collected at various time-points 1, 2 and 3 weeks post-cannulation (pc) and sections were graded for infiltration and stained for T/B cell segregation, FDCs and HEV markers. Submandibular salivary flow was induced by pilocarpine stimulation. Expression of ELS-related genes was investigated by TaqMan-PCR. Anti-viral antibodies and autoantibodies were detected by IF and western blot. In this model, inflammation rapidly and consistently evolves from diffuse infiltration towards the development of SS-like periductal lymphoid aggregates

within 2 weeks pc. These infiltrates progressively acquire ELS features and support functional GL7+/AID+ germinal centers. Formation of ELS is preceded by ectopic expression of lymphoid chemokines CXCL13, CCL21, CCL19 and lymphotoxin-β and is associated with development of anti-nuclear antibodies in 75% of the mice. Reduction in salivary flow was observed over 3 weeks pc. This novel model has the potential to unravel the cellular and molecular mechanisms regulating ELS formation and their role in exocrine dysfunction and autoimmunity in SS.

W5.07.05
Phenotype conversion from rheumatoid arthritis to systemic lupus erythematosus by introduction of Yaa mutation into FcγRIIB-deficient C57BL/6 mice

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We previously established an IgG Fc receptor IIB (FcγRIIB)-deficient C57BL/6 (B6)-congenic mouse strain (KO1), which spontaneously develops rheumatoid arthritis (RA), but not systemic lupus erythematosus (SLE). Here we show that when Y chromosome-linked autoimmune acceleration (Yaa) mutation was introduced in KO1 strain (KO1.Yaa), the majority of KO1.Yaa mice did not develop RA, but instead did develop SLE. This phenotype conversion did not depend on autoantibody specificity, since KO1.Yaa mice, compared with KO1, showed a marked increase in serum levels of both lupus-related and RA-related autoantibodies. The increase in frequencies of CD69+ activated B cells and T cells, and the spontaneous splenic GC formation with T follicular helper (TFH) cell generation were manifest early in life of KO1.Yaa, but not KO1 and B6.Yaa, mice. Activated CD4+ T cells from KO1.Yaa mice showed up-regulated production of IL-21 and IL-10, compared with the finding in KO1 mice, indicating the possibility that this aberrant cytokine milieu relates to the disease phenotype conversion. Thus, our model is useful to clarify the shared and the disease-specific mechanisms underlying the clinically distinct systemic autoimmune diseases RA and SLE.

W5.07.06
SPARC deficiency promotes lupus like autoimmunity by defective clearance of dead PMN

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Increased apoptotic neutrophils and impaired clearance by macrophages have been associated to pathogenesis of systemic lupus erythematosus (SLE). Accumulation of apoptotic bodies leads to availability of autoantigens promoting autoimmunity.
SPARC (Secreted Protein, Acidic and Rich in Cysteine) is a matricellular protein with known functions in wound healing, inflammation and cancer. In such conditions, SPARC is upregulated to modulate cell survival, adhesion, migration and matrix deposition.
To test whether SPARC deficiency impact on the outcome of an autoimmune disease, we compared Sparc-/- and wt mice for the evolution of a lupus-like model induced by injection of Pristane which causes chronic peritonitis followed by SLE-like manifestations (auto-antibodies, arthritis, glomerulonephritis and lung vasculitis).
Sparc deficiency accelerated the onset of autoimmune symptoms. Sparc-/- produced detectable titers of autoantibodies against small nuclear ribonucleoproteins (snRNP), single- and double-stranded DNA (ssDNA, dsDNA) 120 days after treatment, while Sparc+/+ mice took 170-200 days to show similar titers. Also, Sparc-/- mice developed accelerated arthritis with augmented frequency of Th17 cells in popliteal lymph nodes.
We excluded that SPARC deficiency had a direct effect on B cell activation, since vaccination of wt and Sparc-/- mice with the T-dependent and -independent model antigens produced similar antibody titers.
Rather, accelerated autoimmunity depends on augmented availability of auto-antigens, according to the increased fraction of necrotic neutrophils found in the peritoneal cavity and circulation of Sparc-/- than wt mice.
Collectively, our data uncover a new activity for the matricellular protein SPARC in homeostasis and clearance of apoptotic PMN that, if lost, might promote autoimmunity.

W5.08 Atherosclerosis and cardiovascular diseases

IL5.08.01
T cells regulate lipid metabolism and vascular stability. Role in atherosclerosis

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Atherosclerosis is an inflammatory disease. When infiltrating the artery wall, cholesterol-laden plasma lipoproteins trigger activation of innate immune responses that lead to recruitment of professional immune cells to the forming lesion. By activating antigen-specific T cells, oligopeptides of plasma lipoproteins elicit adaptive immune responses, largely of the Th1 type, which aggravate vascular inflammation and promote atherosclerosis. Depending on signals provided by the dendritic cell during antigen presentation of lipoprotein components, CD4+ T cells develop into alternative Th pathways that have entirely different consequences for disease. We have characterized the role of Treg and Th17 cells in atherosclerosis by eradicating Foxp3+ regulatory T cells or targeting Smad7 in the TGF-beta signal transduction cascade in a mouse model of human disease. In the absence of Treg, disease development is accelerated, not only because of increased vascular inflammation but also since hyperlipidemia is aggravated in the absence of Treg. This is likely due loss of cytokine regulation of sortilin-1, a hepatocyte receptor for very low density lipoproteins. These findings highlight the interplay between the immune and metabolic systems and its importance in atherosclerosis.

IL5.08.02
Anti-VEGF therapy normalizes pulmonary vasculature in Endoglin and Alk1 heterozygous mice

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Mutations in endoglin (*ENG*) and *ALK1* genes lead to Hereditary Hemorrhagic Telangiectasia (HHT), a vascular dysplasia affecting 1 in 5000 people worldwide and associated with arteriovenous malformations in multiple organs. Haploinsufficiency is the underlying mechanism of disease and heterozygous (*Eng*$^{+/-}$ and *Alk1*$^{+/-}$) mice serve as experimental models. We characterized their phenotype and angiogenic profile and tested the effects of anti-VEGF therapy. As we demonstrated previously, these mice develop with age a rarefied peripheral pulmonary vasculature, leading to increased resistance and right ventricular hypertrophy (RVH). We now assessed peripheral microvascular density, RVH and several angiogenic factors in these mice after treatment with an antibody to mouse VEGF. Administering 4 weekly i.p. injections (5mg/kg, G6-31 monoclonal antibody) to 15 week-old mice led to high levels of circulating antibody-bound VEGF and to reduced VEGF levels in *Eng*$^{+/-}$ and *Alk1*$^{+/-}$ lungs. The treatment paradoxically increased the number of peripheral lung vessels and consequently attenuated RVH. Basal pulmonary levels of the angiostatic factor TSP-1 were higher in *Eng*$^{+/-}$ than control mice and were normalized by anti-VEGF treatment. In *Alk1*$^{+/-}$ mice, the vessel destabilizing factor, angiopoietin-2, was increased under basal conditions and normalized by treatment. Our findings suggest that the factors responsible for the rarefied pulmonary vasculature may differ between *Eng*$^{+/-}$ and *Alk1*$^{+/-}$ mice, possibly explaining some of the disease heterogeneity. Our data also indicate that anti-VEGF therapy can reverse the angiogenic/angiostatic imbalance in lungs of *Eng*$^{+/-}$

and *Alk1*$^{+/-}$ mice, and could account for its emerging beneficial effects in patients with HHT.

W5.08.01
Circulating CD40$^+$ and CD80$^+$ B cell subsets are opposingly associated with risk of stroke

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Background: Accumulating evidence show that immune cells play an important role in atherosclerosis. Most attention has focused on the role of different T cell subsets, while the possible involvement of B cells has been less studied. In this study we assessed the association of two different B cell subsets, CD19$^+$CD40$^+$ and CD19$^+$CD86$^+$ B cells, with risk for development of acute cardiovascular events.
Methods and Results: The prospective study included 700 subjects randomly selected from the cardiovascular cohort of the Malmö Diet and Cancer study. Mononuclear leukocytes, stored at -140°C at the baseline investigation in 1991-94, were thawed and B cell subsets analyzed by flow cytometry. Cytokine release from stimulated mononuclear leukocytes was measured with multiplex ELISA. Baseline carotid intima-media thickness and stenosis were assessed by ultrasonography and clinical events were monitored through validated national registers during a median/mean follow-up time of 15 years. The CD19$^+$CD40$^+$ B cell subset correlated with release of an anti-inflammatory cytokine profile and subjects in the highest tertile of CD19$^+$CD40$^+$ B cells had a significantly lower risk of incident stroke after adjustment for other risk factors. In contrast, CD19$^+$CD86$^+$ B cells correlated with increased release of pro-inflammatory cytokines and risk for development of a stroke event.
Conclusions: These observations provide evidence for an involvement of B cells in the incidence of stroke and suggest that both pathogenic and protective B cell subsets exist.

W5.08.02
Genetically controlled myocardial expression of CXCL9 may be responsible for the selective migration of Th1 T cells and magnitude of myocarditis in Chagas disease cardiomyopathy

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Chronic Chagas cardiomyopathy (CCC) affects 30% of those infected by the protozoan *Trypanosoma cruzi*. Even though the Th1 T cell-rich myocarditis plays a pivotal role in CCC pathogenesis, little is known about migration and dynamics of T cell subsets in CCC myocardium. We first analyzed expression of hallmark transcription factors and cytokines of the Th1, Th2, and Treg populations, using Real time qPCR in myocardial samples from end-stage CCC patients and controls. CCC myocardial samples displayed robust mRNA expression of IFNγ and T-bet, with absent effector Th2 (IL-4, IL5, IL13) or limited expression of Th17 (IL17; no RorγT) or Treg-associated cytokines and molecules (CTLA4, FoxP3; no IL10 or TGFβ). We also assessed mRNA expression of chemokines chemotactic for different T cell subsets using qPCR and confocal microscopy. Expression of CXCL9 and CCL5, chemoattractants for Th1 cells, was robustly upregulated in CCC myocardium; other CCR5/CXCR3 ligands were also upregulated. CXCL9 expression positively correlated with the intensity of myocarditis. Analyzing single-nucleotide polymorphisms from the chemokines in a cohort of CCC patients with different degrees of severity, we found that the *CXCL9* rs10336CC genotype was associated with less severe CCC. Significantly, myocardial samples from end-stage CCC carrying this genotype displayed lower CXCL9 expression and intensity of myocarditis. Results suggest that the robust Th1 response in CCC myocardium is apparently unopposed by local Th2 and Treg cells. Genetically controlled myocardial expression of CXCL9 may be responsible for the selective migration of Th1 T cells to CCC myocardium, and thus modulate the magnitude of myocarditis.

W5.08.03
Transforming growth factor-beta signaling in T cells promotes stabilization of atherosclerotic plaques through an interleukin-17 dependent pathway

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Adaptive immunity has a major impact on atherosclerosis, with pro- and anti-atherosclerotic effects exerted by different subpopulations of T cells. Transforming growth factor- β (TGF-β) may promote development either of regulatory T cells or Th17 cells, depending on factors in the local milieu. In the present study, we have addressed the effect on atherosclerosis of enhanced TGF-β signaling in T cells. Bone marrow from mice with a T-cell specific deletion of *Smad7*, a potent inhibitor of TGF-β signaling, was transplanted into hypercholesterolemic *Ldlr*$^{-/-}$ mice. *Smad7*-deficient mice had significantly larger lesions that contained a large collagen-rich smooth muscle cap, consistent with a more stable phenotype. The inflammatory cytokine interleukin-6 was expressed in the atherosclerotic aorta and increased mRNA for IL-17A and the Th17 specific transcription factor RORγt were detected in draining lymph nodes. Treating *Smad7*-deficient chimeras with neutralizing IL-17A antibodies reversed the stable cap formation. IL-17A stimulated collagen production by human vascular smooth muscle cells and RORγt mRNA correlated positively with collagen type-1 and alpha-smooth muscle actin mRNA in a biobank of human atherosclerotic plaques. These data demonstrate that TGF-β modulates atherosclerosis in a context dependent manner and links IL-17A to induction of a stable plaque phenotype.

W5.08.04
A Novel Role for Rip2 in T cell regulation; Rip2 Deficiency leads to increased Th17 Skewing

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Background: Receptor-interacting serine/threonine protein kinase 2 (Rip2) is the critical adapter molecule for both Nod1 and Nod2 signaling complexes in the innate and adaptive immune pathways in myeloid, epithelial, and endothelial cells. However, the role of Rip2 in T cells is unknown.
Methods and Results: We observed that Rip2-/- CD4 T cells are skewed towards increased Th17 skewing. To investigate the mechanism of this observation, we adoptively transferred naïve CD4 T cells from Rip2-/- mice into Rag1-/- mice, and infected them with Chlamydia pneumoniae (CP). Rag1-/- mice receiving Rip2-/- CD4 T cells displayed significantly more IL-17A but less IFN-γ production during infection when compared to Rag1-/- mice receiving wt CD4 T cells despite similar bacterial burdens, indicating that T cell-derived Rip2 deficiency is that factor that leads to increased Th17 skewing. To investigate the functional impact of this observation, we used two different experimental models. We found that in Rip2-/- mice had a severe and chronic lung inflammation following CP infection, which was not observed in the Rip2-/-Il17a-/- mice. When irradiated Ldlr-/- mice (on high fat diet) received Rip2-/- bone marrow, we found a significant increase in atherosclerotic lesion development, which was not observed if recipient mice were given Rip2-/-Il17a-/- BM .
Conclusion: We demonstrate in multiple models that increased inflammation in Rip2-/- mice is due to increased Th17 skewing observed in Rip2-/- T-cells. Our study demonstrates a novel role for Rip2 in T cell regulation and suggests that Rip2 in CD4 T cells negatively regulates Th17 mediated chronic inflammation.

W5.08.05
TIPE2 prevents atherosclerosis by negatively regulating macrophage responses to oxLDL

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Atherosclerosis has been widely recognized as an inflammatory disease of the arterial wall in which macrophages play a major role. TIPE2, the tumor necrosis factor (TNF)-alpha-induced protein 8-like 2 (TNFAIP8L2), is highly expressed in resting macrophages and can negatively regulate inflammation through inhibiting immune receptor signaling. In this study, we first report that TIPE2-deficient macrophages treated with ox-LDL produced more oxidative stress and proinflammatory cytokines, were resistant to activation-induced cell death, and exhibited heightened activation of the JNK, NF-κB, and p38 signaling pathways. As a consequence, TIPE2 deficiency in bone marrow-derived cells exacerbated atherosclerosis development in *Ldlr*$^{-/-}$ mice fed a high-fat diet. Interestingly, TIPE2 deficiency doesn't affect lipoprotein metabolism or blood cell counts of *Ldlr*$^{-/-}$ recipients mice, while the sera levels of some cytokines were significant higher than WT controls. These results indicate that TIPE2 plays a crucial atheroprotective role by negatively regulating macrophage responses to ox-LDL and may be a potential new drug target for treating the disease.

W5.08.06
Co-stimulatory signaling through lymphocyte CD40 limits adipose tissue inflammation and protects from de novo and pre-established metabolic disease in mice

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Background: Immune cell functioning drives adipose tissue inflammation during the metabolic syndrome. Co-stimulatory cascades such as the CD40L-CD40 dyad enhance immune cell activation during chronic inflammation. Here, we tested the hypothesis that CD40 modulates diet-induced obesity in vivo.
Methods and Results: To induce the metabolic syndrome, WT or CD40-/- mice consumed a high fat diet (HFD) for 20 weeks. CD40-/- mice exhibited an aggravated metabolic phenotype with increased weight gain, enhanced fat depositions, aggravated insulin resistance, and increased accumulation of inflammatory cells in adipose tissue. Accordingly, multiple pro-inflammatory gene sets were up-regulated in adipose tissue. This phenotype could be transplanted in lethally irradiated WT mice by reconstitution with bone marrow lacking CD40 on lymphocytes, indicating that lymphocyte CD40 accounts for its phenotype. In a gain-of-function approach, mice were fed HFD for 6 weeks and treated with the activating CD40 antibody FGK45 for 6 weeks. Activation of CD40 signaling with FGK45 abolished further weight gain, lowered glucose levels and improved insulin resistance. Correspondingly, immune cell accumulation in adipose tissue was substantially reduced. Mechanistically, expression of the TH1-cytokine IFN-γ in T-helper cells decreased after CD40 activation. In a cohort of 183 patients with a high prevalence of the metabolic syndrome levels of soluble CD40 were elevated in obese patients, indicating clinical relevance of our findings.
Conclusion: We present that CD40 deficiency aggravates, while activation of CD40 signaling improves adipose tissue inflammation and its metabolic complications in mice. Positive modulation of co-stimulatory pathways might describe a novel therapeutic concept against cardio-metabolic disease.

W5.09 Neuroimmunology

IL5.09.01
EBV specific CD8+ T cells in Multiple Sclerosis

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Epstein-Barr virus (EBV) has been associated with multiple sclerosis (MS).
We used a highly standardized flow cytometric analysis with EBV-specific pentamers, that unequivocally identifies antigen-specific CD8+ T cells, on freshly isolated PBMCs from HD and MS patients. We find that differences in the prevalence and magnitude of the CD8+ T cell response to EBV latent and lytic proteins between MS patients and HD and within the MS cohort emerge only when patients are stratified according to disease activity.
Fewer inactive MS patients have a detectable CD8+ T cell response against EBV lytic antigens compared with HD and active MS patients, while the frequency of lytic antigen-specific CD8+ T cells is higher in active MS patients. On the other hand, responses to the latent antigen EBNA-3A are higher in inactive MS patients. Thus, changes in the immune control of EBV replication are associated with MS disease activity.
PD-1 was upregulated only in EBV-specific CD8 T cells of inactive MS patients, whereas PD-1 expression levels in total CD8 were comparable to controls. We speculate that in inactive MS patients there is an attempt by CD8+ T cells to control abnormal expansion of a latently infected B-cell pool.
Based on the present findings, we propose that failure to fully control EBV latent infection in the CNS could lead to recrudescence of EBV reactivation. Exposure to newly synthesized viral antigens would promote expansion of lytic antigen-specific CD8+ T-cells targeting intracerebral viral deposits and hence induce the active phase of MS.

W5.09.01
Regulatory T cells together with resolving macrophages orchestrated by Th1 cells are non-redundant pivotal players in CNS repair

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The perception of the central nervous system (CNS) as an immunologically inert tissue, where the presence of immune components was considered an imminent sign of pathology, is now obsolete. Over the last few years, the understanding of the role of monocyte-derived macrophages (mo-MΦ) in CNS repair has undergone a major revolution, and the beneficial contribution of these cells to repair is now well appreciated. In parallel, several studies highlighted the neuroprotective role of T cells, but as T cell activity in the CNS can be potentially hazardous, understanding the mechanisms that ensure their beneficial rather than destructive effect is of critical importance.
Using a paradigm of spinal cord injury, we found that T helper-1 (Th1) cells activated the remote blood-cerebrospinal fluid barrier, facilitating the recruitment of IL-10-expressing mo-MΦ to the injury site. Subsequently, the mo-MΦ controlled the local levels of Foxp3+ regulatory T cells (Tregs); with late ablation of the Tregs impairing the extent of myelination. The IL-10 mo-MΦ and Tregs were non-redundant in resolving the innate and adaptive inflammatory responses, respectively. As a corollary to the need for effector T cells at the acute stage, reducing Treg levels at the time of injury is beneficial, allowing a risk-free effector immune response, and highlighting the need for a delicate balance between Th1 and Tregs for proper repair.
Overall, recovery from CNS injury requires a spatially and temporally orchestrated response involving Th1 cells, mo-MΦ, and Foxp3+ Tregs; all of these cells are needed, none of them are only harmful or harmless.

W5.09.02
Triggers of anti-CNS autoimmunity in mouse model of Multiple sclerosis

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Multiple sclerosis (MS) is an inflammatory and demyelinating central nervous system disorder of unknown etiology. Recent data, from induced and spontaneous mouse models (experimental autoimmune encephalomyelitis, EAE) of the disease implicate a pivotal role for gut microbiota not only in disease progression but also in protection from it. Toll-like receptors (TLRs), key components of innate system are the prominent players in recognising microbial products. This recognition culminates in induction of inflammatory signalling cascades. Currently, confusion is reigning over the role of TLRs in EAE. We have performed EAE experiments in mice quintuple-deficient for TLRs 2, 3, 4, 7, and 9 to gain better understanding of the role of TLR activation in anti-CNS autoimmunity. Surprisingly, we could not observe a pronounced influence of simultaneous absence of these five TLRs on EAE disease course. We also observed that quintuple deficient mice displayed a delayed but rapidly progressing disease suggesting less prominent role for these TLRs in EAE. This observation was confirmed in histological analysis. Interestingly, the immunization against the myelin peptide resulted only in a delayed T cell response.

W5.09.03
IL-33/ST2 axis mediates resistance to EAE by promoting regulatory B and tolerogenic dendritic cells

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BALB/c mice are resistant to EAE induction with MOG35-55. ST2 molecule is expressed on immune cells and its signaling is accompanied by accentuated ST2 response. We recently published (Milovanovic et al. PlosOne 2012) that deletion of ST2 molecule abrogates resistance to EAE, as evaluated by clinical and histological findings. Adoptive transfer of ST2 KO lymphocytes restimulated with MOG35-55 induced clinical signs of the disease in ST2 KO and BALB/c mice. MOG35-55 specific CD4+ lymphocytes of ST KO mice had higher expression of transcriptional factor T-bet, inflammatory cytokines IFNγ, IL-17, GM-CSF, TNFα and chemokine receptors important for migration to CNS (CCR6, CXCR5 and CXCR3). ST2 KO mice had higher incidence of activated antigen presenting cells, myeloid/regulatory dendritic cells (DC) ratio and percentages of myeloid cells containing inflammatory cytokines, IL-1, IL-12 and IL-6. ST KO DC induced higher in vitro proliferation of CD4+ cells and stimulated in vitro with TLR1/2 agonist produced more cytokines involved in Th1 and Th17 differentiation compared with DC isolated from BALB/c mice. Interestingly we also observed the increase of regulatory CD11b+CD5+ B lymphocytes, IL-10 producing B1 lymphocytes and decrease of IL-6 producing B cells in BALB/c mice after immunization with MOG35-55 peptide. The opposite results were found in ST2 KO mice. Results indicate that ST2 signaling influences the activity of regulatory B1 cells and differentiation of proinflammatory antigen presenting cells, consecutively affecting the differentiation of encephalytogenic T cells in the periphery.

W5.09.04
N-methyl-D-aspartate receptor (NMDAR) Antibodies in Post Herpes Simplex Virus Encephalitis (HSVE) Neurological Relapse

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Background: Herpes Simplex Virus encephalitis (HSVE) is a devastating condition that relapses, often with a chorea in children, despite adequate antiviral treatment. At relapse, evidence of viral replication is frequently absent, suggesting that the relapse may be immune-mediated.
Methods: Seven children who had a neurological relapse following their initial encephalitis, identified from 20 cases of HSVE, were studied. Serum and/or CSF were tested for N-methyl-D-aspartate receptor (NMDAR) and other antibodies previously reported in central nervous system autoimmunity.
Results: The children initially presented with clinical, laboratory and radiological findings consistent with HSVE and had made a significant clinical improvement with intravenous acyclovir. Viral reactivation was identified only in one patient (14%). For the remaining 6 patients with no evidence of viral reactivation, a range of infective and alternative inflammatory causes were sought but not identified at the time. Three out of six (50%) children who had relapsed with choreoathetosis, seizures and/or cognitive regression following HSVE, had NMDAR antibodies. In two of the NMDAR antibody positive patients who were treated at relapse, a beneficial response was observed.
Conclusion: Neurological relapses after HSVE may frequently be immune-mediated, particularly (but not exclusively) in children with chorea. NMDAR antibodies are common, and immunotherapy should be considered.

W5.09.05
Neuroimmunological changes in the Cstb-/- mouse: a model for progressive myoclonus epilepsy

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Loss-of-function mutations in the *cystatin B* gene encoding cystatin B (CSTB) an inhibitor of cysteine proteases cathepsins cause neurodegenerative disorder called progressive myoclonus epilepsy of Unverricht-Lundborg type (EPM1). Neuropathological change in *Cstb-/-* mouse include early microglial activation (P14) that precedes astrocytosis and neurodegeneration and, presumably, is central in initiating of the neuronal loss. Our aim is to characterize in detail cystatin B-deficient microglia.
Resting and activated (lipopolysaccharide) cultured *Cstb-/-* and control microglia were analysed for cytokine, NO production, chemotaxis and phagocytosis. Microglia extracted directly from the brains was studied for expression of MHC II and M1-M2 polarization using flow cytometry. Myeloid cell population in bone marrow and spleen, cytokines' level in blood serum were analysed in P14 animals. Our results show increased secretion of proinflammatory chemokines by *Cstb-/-* microglia, which is chemotactically more active whereas their phagocytic activity is decreased. *Cstb-/-* microglia show decreased expression of MHC II on the cell surface. Activated *Cstb-/-* microglia directly extracted from the brain has predominantly proinflammatory M1 phenotype. We have registered high concentrations of proinflammatory chemokines and cytokines in blood serum of *Cstb-/-* pups. Amount of granulocytes is enhanced in *Cstb-/-* bone marrow and spleen as well as level of GM-CSF in their blood serum and microglial conditioned medium.
Results suggest a role for cystatin B in regulation of immune response. CSTB-deficiency is associated with early inflammatory processes both in the brain and the peripheral tissues. We consider that EPM1 should be treated as a disorder combining neuropathological and immunological features.

W5.09.06
Elucidation of pathogenic mechanism of myasthenia gravis with MuSK antibodies using a novel murine model

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Myasthenia gravis (MG) is caused by autoantibodies against postsynaptic membranes at neuromuscular junctions (NMJs). Antibodies against muscle-specific kinase (MuSK) are found in a

variable proportion of MG patients who lack antibodies against acetylcholine receptors. MuSK antibodies predominantly belong to the IgG4 subclass, which has distinct features compared with other human IgG subclasses. (1) IgG4 does not activate the classical complement pathway. (2) The production is promoted by T-helper 2 (Th2) cytokines. These unique properties are directly associated with the pathogenic mechanisms of MG with MuSK antibodies (MuSK-MG). Although several models of experimental autoimmune MG (EAMG) with MuSK antibodies have been produced, it has been doubt on the similarity in the pathogenic mechanism of MuSK-EAMG caused by complement-fixing antibodies. Here we generated a new EAMG model by injecting MuSK protein into complement-deficient mouse strains. Nearly all of the mice tested manifested MG with morphological and functional changes in NMJs observed in MuSK-MG patients, providing the evidence that complement activation is dispensable for the onset of MuSK-MG. In addition, MuSK antibodies produced by all strains were predominantly IgG1 subclass, which has functional similarities with human IgG4 in terms of Th2 dependency for production and complement-binding capacity, suggesting that these EAMG mice share a common immune regulatory pathway with MuSK-MG patients. Taken together, our novel EAMG model provides a platform for research of MuSK-MG in humans not only to develop appropriate medication, but also to reveal the immune mechanisms.

W5.10 Multiple sclerosis

IL5.10.01
CNS tissue damage by T cells

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The CNS is confronted to a double challenge regarding its interactions with the immune system. On the one hand it should allow the immune system to fight invading pathogens and on the other it should prevent inflammatory damage given its vital functions and poorly regenerative capacity. A series of mechanisms, collectively referred to as 'immune privilege', ensures that immune reactions are kept minimal and are rapidly controlled within the CNS. However, accumulating evidence show that T cells readily penetrate the brain and spinal cord parenchyma in numerous inflammatory neurological diseases such as Multiple Sclerosis. The consequence for CNS resident cells, and more specifically for neurons, of their encounter with activated T cells is a question that we have addressed recently using experimental rodent models.
Shedding light on the mechanisms by which T cells promote CNS tissue damage may allow the design of more refined therapeutic strategies for immune-mediated neurological diseases, including multiple sclerosis.

IL5.10.02
Immunological knowledge integration in MS

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The immunology of multiple sclerosis is providing an ever growing picture of potential mechanisms whose expansion parallels the progress of our understanding of basic immunology. During the last two decades this knowledge has led to great advances in the therapy of this disease. The challenge ahead is now to exploit this pathogenetic information to infer an etiologic understanding that may lead to definitive cures. To do so we should ameliorate our ability to intepret these data across different knowledge domains. In particular, we should strive to constantly put immunological data into the context of what we are learning about heritable and nonheritable causative factors and their interactions. A constant process of "cross-validation" between these domains may lead to a better characterization of genome to phenome correlation and bring about substantial progress for the cure, diagnosis and prevention of this disease. Examples of how this could be done, in a pragmatic way, for existing knowledge will be provided.

W5.10.01
Identification, isolation and characterization of brain-infiltrating, in situ clonally expanded T cell clones in multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). MS is considered an autoimmune disease mediated by T cells. However, the spectrum of autoantigens targeted by pathogenic T cells in MS is until now poorly understood. Only few myelin antigens are considered well defined autoantigens. There is also limited knowledge about the possible environmental triggers that could activate the autoreactive T cells in the periphery. Our hypothesis is that the spectrum of autoantigens in MS is broader than previously assumed and that new autoantigens and molecular triggers will be identified by using unbiased methods to test the antigen specificity pathogenic T cell populations in MS.
Most T cell specificity studies in MS are based on peripheral blood T cells, and for these it is difficult to determine if they are related to the disease. Our purpose is to test the specificity of brain-infiltrating, in situ clonally expanded T cell clones (TCC) from a MS patient, who died due to aggressive MS. We have first identified by T cell receptor sequencing T cells that are clonally expanded in brain lesions (frozen tissue). Currently we are isolating the same TCC from the cerebrospinal fluid T cells of the patient, expand them *in vitro* and characterize them phenotypically. Our purpose is to test the antigen specificity of these disease-relevant brain-infiltrating clonally expanded CD4+ and CD8+ TCC combining synthethic combinatorial peptide libraries and a brain-derived cDNA library screening system with cDNAs from affected brain tissue from the patient.

W5.10.02
T cells specific for both a myelin and a neuronal antigen are frequent in C57BL6 mice and contribute to CNS inflammation during EAE

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Pathogenic T cells in autoimmunity can recognise multiple self-antigens simultaneously. We have shown that in T cell receptor (TcR)-transgenic mice on a C57BL/6 background (H-2b), the 2D2 TcR co-recognises the myelin oligodendrocyte glycoprotein epitope MOG35-55 and an epitope within the axonal protein Neurofilament medium (NF-M15-35). Here we assessed whether this is a common feature of the pathogenic MOG35-55 specific T cell response. To this end, we analysed the CD4 T cell response of MOG35-55-immunised C57BL/6 mice for cross-reactivity with NF-M15-35. Using antigen recall responses and ELISpot assays we established that purified CD4+ T cells from all MOG35-55-immunised mice tested revealed cross-reactivity to NF-M15-35. To study the clonality of this response we analysed twenty-four MOG35-55-specific T-cell hybridomas expressing distinct T cell receptors. Eight hybridomas were found to cross-react to NF-M15-35, while sixteen responded only to MOG35-55. Using an alanine-scan of NF-M18-30, we established that the aminoacids E19 and R21 are I-Ab anchoring residues, while F24, R26 and V27 proved important TCR contact residues. Strikingly, the three identified TCR contact residues are conserved within the MOG38-50 epitope. Our data indicates that part of the MOG35-55-specific T cell repertoire of C57BL/6 mice co-recognises NF-M15-35 due to linear sequence homology. Using approaches to silence these bi-specific cells or trace their frequency over time in the central nervous system of C57BL/6 mice with experimental autoimmune encephalomyelitis (EAE), we subsequently defined the functional

contribution of these distinct T cell pools during MOG35-55-induced EAE. These observations provide insight into the mechanisms underlying chronic organ-specific autoimmune diseases.

W5.10.03
Cell-intrinsic estrogen receptor α activation in CD4+ T cells control Th1/Th17 differentiation in trans and protect from CNS autoimmunity

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Estrogens influence many physiological processes in mammals. In autoimmune diseases, estrogens can display either beneficial or deleterious effects. Clinical remissions in patients with multiple sclerosis (MS) during pregnancy have suggested that sex hormones, particularly estrogens, could modulate CNS-autoimmunity and inflammation. Indeed, 17ß-estradiol (E2) treatment has been shown to inhibit the development of experimental autoimmune encephalomyelitis (EAE), the animal model of (MS). Two molecular targets essentially mediate E2 actions: estrogen receptor alpha (ERα) and beta (ERβ). We and others have shown that the protective effect of E2 on EAE was strictly dependent on ERα. However, the underlying mechanisms responsible of anti-inflammatory effect of E2 in EAE protection are still ill defined. Using tissue-specific ERαKO mouse models, we demonstrated that ERα-signaling in T lymphocytes, but not myeloid cells (monocytes/macrophages or dendritic cells), was required for sustained EAE protection by E2 (Lélu et al., *J. Immunol.* 2011 187:2386). Using adoptive transfer model of MOG-specific 2D2 CD4 T cells, we now show that ERα-signaling in endogenous host CD4+ T lymphocytes rather than responding 2D2 CD4+ T cells orchestrates the inhibition of MOG-specific Th1/Th17 cell priming. Coadministration of naive 2D2 $ER\alpha^{-/-}$ T cells with E2-responsive $ER\alpha^{+/+}$, but not $ER\alpha^{-/-}$, CD4 T cells strongly delayed EAE development in $Rag2^{-/-}$ $ER\alpha^{-/-}$ hosts. Thus, these results identify a suppressive activity of E2-primed CD4+ T cells, by which they can restrict differentiation of Th17 cells in *trans* and protect from CNS autoimmunity.

W5.10.04
A role for TNF signalling in cortical grey matter pathology in multiple sclerosis

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Neuroimaging and neuropathology studies indicate that accumulating grey matter pathology is the main correlate of clinical progression in multiple sclerosis (MS). Recent studies of cortical pathology in secondary progressive MS (SPMS) have shown that a more severe clinical course and the presence of extended subpial grey matter lesions (GMLs) with significant neuronal/glia loss and microglia activation are associated with diffuse meningeal inflammation and presence of lymphoid-like structures in the subarachnoid space. We investigated the hypothesis that pro-inflammatory and cytotoxic molecules diffusing from the meninges are the main determinant of GM pathology.
We used 20 post-mortem MS brains with and without substantial meningeal inflammation and 10 non-neurological controls to determine differentially expressed genes and pathways in subpial GMLs and normal appearing GM (NAGM) of the motor cortex by using Illumina HumanRef8 Beadchip arrays.
Gene expression profiling of GMLs and NAGM not only confirmed the substantial GM pathological cell changes, but also demonstrated the upregulation of multiple genes/pathways associated with the inflammatory response. In particular, genes involved in TNF mediated apoptosis/survival signalling have been found significantly deregulated in MS cases compared to controls, and increased meningeal inflammation was found associated with a shift in the balance of TNF signalling from the TNFR2 and NFkB anti-apoptotic pathway towards TNFR1 and RIP3 pro-apoptotic signalling.
These results support the hypothesis that the inflammatory milieu in the subarachnoid space of the meninges has a fundamental role in subpial grey matter pathology in MS and that TNF is a key mediator of brain tissue damage.

W5.10.05
Characterization of the impact of CD25 blockade on the NK and T cell populations of relapsing-remitting multiple sclerosis patients

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Multiple sclerosis (MS) is an immune-mediated neuroinflammatory disorder believed to be mediated by autoreactive T cells. Therapies that target T cell activation and effector function have shown promise in patients with relapsing-remitting MS (RRMS). Daclizumab high yield process (DAC HYP), a humanized monoclonal antibody that binds the high-affinity IL-2R (IL-2Rα or CD25) and prevents its association with IL-2, has demonstrated efficacy in two phase II clinical trials. Despite evidence of changes to immune cell populations based on altered IL-2 signaling, including an increase in CD56bright NK cells and a decrease in regulatory T cells, the mechanisms by which DAC HYP mediates its therapeutic benefit are poorly understood.
The biology of IL-2 is complex. As a growth factor for natural killer (NK) cells, conventional T cells and regulatory T cells, IL-2 is able to both potentiate and limit innate and adaptive immune responses. Adding to this complexity, IL-2 can signal through both high-affinity (IL-2Rαβγ) and intermediate-affinity (IL-2Rβγ) receptors. Thus, the response to IL-2 at the single cell level is dependent on both receptor expression and local IL-2 concentration.
Here we present data characterizing the impact of Daclizumab therapy on NK and T cell populations of RRMS patients. Using multi-color flow cytometric analysis of PBMCs from DAC HYP- and placebo-treated patients at baseline and week 24, we provide evidence of dynamic changes to immune cell subsets based on requirements for IL-2 signaling and expression of IL-2R components.

W5.10.06
Confirmation of Human Endogenous Retrovirus HERV-Fc1 role in multiple sclerosis risk

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Background: Multiple sclerosis (MS) is a chronic demyelinating disease with an aetiology not completely ascertained, albeit the prevailing hypothesis contemplates the triggering effect of environmental factors in genetically susceptible individuals. The genetics of MS has undergone a spectacular advance propelled by the genome wide association studies (GWAS) recently performed. However, this robust approach accounts only for part of the estimated genetic load, and where the so called missing heritability lies is intriguing. Provided GWAS do not scrutinize repetitive regions of the genome, their role in MS predisposition gains interest. Part of these repetitive sequences corresponds to human endogenous retroviruses (HERVs), which are proviral loci inherited under a Mendelian pattern. We aimed to validate the previously reported association of HERV-Fc1 with MS susceptibility.
Methods: The rs391745 polymorphism located upstream of the HERV-Fc1 locus in chromosome X was genotyped by TaqMan assays in independent cohorts from Central (714 patients and 692 controls), South (880 patients and 1359 controls) and North (368 patients and 980 controls) Spain.

Results: North and Central Spanish cohorts including non primary progressive MS patients and showing association results without heterogeneity were combined to achieve a higher statistical power. A significant association with MS risk was evidenced [pM-H=0.02; OR (95% CI)=1.27 (1.03-1.55)], replicating previously reported Danish and Norwegian results. Meta-analysis of all available data yielded a strong effect [p=0.0003; OR (95% CI)=1.32 (1.32-1.53)].
Conclusion: Our work supports the association of HERV-Fc1 in MS pathogenesis, identifying a chromosome-X genetic marker that could lay behind genetic differences in MS clinical forms.

W5.12 Rheumatoid arthritis and other inflammatory joint diseases

IL5.12.01
Necrotizing autoimmune myopathy: emergence of a new autoimmune disease

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Necrotizing autoimmune myopathy (NAM) is a new group of acquired myopathies. Patients present with severe progressive muscle weakness, high serum creatine kinase (CK) levels and prominent myofiber necrosis with little muscle inflammation. Hence, NAM patients may be misdiagnosed as having muscular dystrophy, which prevents the initiation of an appropriate therapy. NAM is commonly associated with deposit of C5b-9 in muscle and presence of autoantibodies (aAbs) against signal-recognition particle (SRP) or 3-hydroxy-3-methylglutaryl-coenzymeA reductase (HMGCR) in serum.
We developed Luminex assays to quantify these aAbs. Anti-SRP aAbs stained the endoplasmic reticulum (ER) on muscle sections (electronic microscopy) and their titers (Luminex) correlated to CK levels. Anti-SRP$^+$ plasma was toxic for myoblast and myotubes in culture. It induced muscle weakness (grip test) after injection to mice, with *in vivo* binding to myofibers and C5b-9 deposits. This suggests that anti-SRP aAbs are directly pathogenic.
NAM may also be a complication of treatment with statins, an anticholesterol drug targeting HMGCR. In a series of 150 patients with suspicion of NAM, 37 were anti-HMGCR$^+$. These patients presented with proximal and bilateral muscle weakness and had very elevated (mean > 6,000 IU/L) CK levels. Sixty percent had not taken statins. Histologically, muscle biopsies always showed regenerating and necrotic muscle fibers.Detection of anti-SRP/HMGCR aAbs allows to ascribe patients with necrotizing myopathy to an autoimmune form, rendering them eligible to immunosuppression. These aAbs are likely to be directly pathogenic. Yet, more than 20% of NAM patients are seronegative, suggesting that other aAbs remain to be discovered.

IL5.12.02
Role of soluble CD30/CD30L and of CD30/CD30L+ T cells in the modulation of inflammatory response in rheumatoid synovitis

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CD30 and CD30 ligand (CD30L) are members of TNF-Receptor and TNF superfamilies, respectively. CD30+ Tcells are increased in several human diseases and interaction of CD30L with CD30+ cells induces signals that cause cell proliferation or apoptosis. A percentage of Tcells from synovial fluid (SF) from patients affected by Rheumatoid Arthritis (RA) express surface CD30 and high levels of soluble CD30 (sCD30) have been reported in sera and SF of RA patients. The increase of sCD30 levels seems to reflect the recruitment of CD30+ T cells into the inflamed joints and is predictive of a positive response to immunosuppressive therapy. Recently a soluble form of CD30L has been described in RA patients' sera. Our aim was to investigate the role of sCD30L and CD30L+ Tcells in RA.
sCD30L was not detectable in PB of healthy controls whereas it was present in PB and SF of patients with RA showing a correlation with the disease activity and with response TNFα blockade therapy. sCD30L molecule is shedded upon interaction between CD30+ and CD30L+ cells, binds to surface CD30 and is functionally active, since it induces a decrease in CD30+ Tcell proliferation due to apoptosis. Therefore sCD30L may favour chronic inflammation by inducing apoptosis of CD30+ Tcells. Moreover a great number of Treg cells are CD30+. Finally, synovial CD30L+ Tcells stimulated with CD30-chimera produce high levels of the proinflammatory cyotkines IFN-g and IL-17.
In conclusion, sCD30L and CD30L+ Tcells may play a pivotal role in RA synovitis and are correlated with response to therapy.

W5.12.01
DERAA directed T-cells: a link between HLA class II association and Rheumatoid Arthritis

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The HLA system represents the most prominent genetic risk factor for rheumatoid arthritis (RA), but the biological mechanism is poorly understood.
Recently, we showed that HLA-DRB1*13:01 protects against RA characterized by the presence of RA-specific anti-citrullinated protein antibodies (ACPA). These antibodies recognize citrullinated proteins, often found among structural proteins.
A dominant peptide source presented by HLA class II molecules are HLA derived peptides. Natural processing of the protective HLA-DRB1*13:01 yields a peptide containing the amino acid sequence DERAA that is found in few self-proteins but many pathogens. We hypothesized that cross-reactive DERAA-directed T-cells against "DERAA-containing" pathogens "help" B-cells recognizing the "DERAA-containing" structural protein vinculin which is recently identified as a citrullinated self-protein present in synovial fluid of RA-patients. Such T-cells would be tolerized in HLA-DRB1*13:01 donors.
Intriguingly, we detected autoreactive T-cells, restricted by RA predisposing HLA molecules HLA-DQ7 and HLA-DQ8, specifically directed to the DERAA containing epitope of vinculin. Moreover, T-cell responses to pathogen-derived DERAA epitopes were readily detectable. Importantly, these T-cell responses were absent in HLA-DRB1*13:01-carriers. Likewise, we showed cross-reactivity of a DERAA-directed T-cell receptor with both pathogen- and self-derived DERAA-sequences. Moreover, we showed that ACPA recognize citrullinated vinculin.
Together, our data indicate the presence of pathogen-induced "DERAA"-directed T-cells that cross-react to human vinculin. These T-cells are absent in HLA-DRB1*13:01 carriers and recognize DERAA-antigens presented by RA-predisposing HLA-molecules, possibly explaining the long known HLA-RA connection. These data point to a role for DERAA-directed T-cells in the pathogenesis of ACPA-positive RA by helping ACPA-producing B-cells recognizing citrullinated vinculin.

W5.12.02
Impaired TNFα production by dendritic cells from rheumatoid arthritis patients upon contact with Porphyromonas gingivalis

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The prevalence of periodontitis is increased in patients with rheumatoid arthritis (RA) and the severity of periodontitis can affect the level of arthritis. Porphyromonas gingivalis is one of the main bacteria causing periodontitis and our aim was to determine if there are differences in the immune response against P. gingivalis between healthy controls and RA patients. Therefore we compared the response of dendritic cells (DCs) from RA patients with healthy donors for their response towards P. gingivalis. We isolated monocytes from healthy controls, RA patients and psoriatic arthritis (PsA) patients and cultured them into monocyte-derived dendritic cells. DCs were stimulated with a set of bacteria possibly involved in RA including the periodontal bacteria P. gingivalis and Prevotella intermedia. TNFα production was significantly decreased in RA patients as compared to healthy controls upon stimulation with P. gingivalis, but not with any of the other bacteria or single TLR agonists. PsA patients were included as a diseased control group and the cytokine production by PsA DCs was not different from healthy controls, suggesting a RA specific deregulated response to P. gingivalis. The decreased response towards P. gingivalis is present in all medication groups. The response to P. gingivalis was not correlated with the presence of auto-antibodies, disease activity or erosions. In conclusion, DCs from RA patients produce less pro-inflammatory cytokines upon P. gingivalis stimulation. This could result in prolonged survival of P. gingivalis resulting in an increased bacterial burden in RA patients.

W5.12.03
Antibodies From Rheumatoid Arthritis Patients Target Citrullinated Histone 4 Contained in Neutrophils Extracellular Traps

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Background: Histone deimination is a crucial event in cell biology, regulating gene function and contributing to antimicrobial response, through the formation of neutrophil extracellular traps (NETs). Deiminated proteins are target of disease-specific antibodies (ACPA) in rheumatoid arthritis (RA).
Objective: Aim of the present study is to investigate the presence in RA sera of a new subset of ACPA, antibodies reactive with deiminated histones.
Methods: Neutrophils from peripheral blood were stimulated with calcium ionophore; NETosis was induced by phorbol myristate acetate and NET proteins were isolated. Sera were tested by immunoblot on acid extracted proteins from neutrophils and from NETs and by ELISA on deiminated histone H4 or H4-derived peptides. Bands reactive with RA sera were digested with trypsin and subjected to MALDI-TOF analysis, before and after derivatization to detect citrullinated peptides.
Results: RA sera reacted with an antigen of 11 KDa expressed in nuclei of activated neutrophils, identified as H4. MALDI TOF analysis indicated that this antigen, present also in NETs, is citrullinated. By ELISA, RA sera bound in vitro citrullinated H4. Citrullinated H4 14-34 and 31-50 AA peptides detected antibodies in 67% and 63% of RA sera and in less than 5% of controls; preincubation with peptides inhibited binding to citrullinated H4.
Conclusion: Citrullinated H4 from activated neutrophils and NETs is a target of antibodies in RA. As NETosis can generate antigens for ACPA, these data suggest a novel connection between innate and adaptive immunity in RA.

W5.12.04
DNAse IgG activity in patients with early arthritis

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The aim of our study was to study the levels of DNAse IgG activity in patients with early arthritis (EA).
We studied 190 persons: 64 patients with early rheumatoid arthritis (ERA), 55 patients with acute reactive arthritis (AReA), 36 patients with unclassified arthritis (UA) and 39 healthy controls.
The samples of purified from the sera by combined method polyclonal IgG isolated from patients and healthy persons were investigated. The experiments, confirming that DNAse activity is the essential quality of IgG were performed. DNAse IgG activity was determined by rivanol clot prevention test and agarose electrophoresis.
The levels of DNAse IgG activity in patients with ERA (Me 4.00, 95%DI 3.50-4.50), AReA (Me 2.50, 95%DI 2.00-3.00), UA (Me 3.50, 95%DI 2.50-4.00) were higher ($p<0.01$) than in controls (Me 0.00, 95%DI 0.00-0.50). The levels of DNAse activity in ERA were higher ($p<0.05$) than in AReA and UA.
There were correlation between DNAse IgG activity and ESR ($r=0.42$) in ERA, T-lymphocytes number ($r=0.40$), immune complexes number ($r=0.42$) in AReA, CRP ($r=0.37$), CD4+lymphocytes number ($r=-0.49$), CD8+lymphocytes number ($r=-0.50$) in UA, $p<0.05$ respectively.
The determination DNAse IgG activity elevated levels might be applied for discrimination of ERA from AReA and UA (sensitivity 82.81%, specificity 71.43%, PLR 2.90, NLR 0.24).
For the first time we confirmed the presence of elevated levels of DNAse IgG activity in EA in comparison with controls and prevalence of this activity in ERA. We proposed original test for differentiation of ERA from AReA and UA.

W5.12.05
Endogenous SLPI controls B cell survival factor expression in TLR3-stimulated rheumatoid synovial fibroblasts

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Background and aims: Secretory leukocyte protease inhibitor (SLPI) is known as a serine protease inhibitor mainly synthesized by epithelial cells which also exerts anti-inflammatory and microbicidal activities. Rheumatoid arthritis (RA) is characterized by the presence of synovial niches of autoreactive B cells. Importantly, autocrine mechanisms such as production of B cell survival factors BAFF and APRIL by RA synovial fibroblasts, support ongoing B cell activation within the RA synovium. Here we investigated whether SLPI is produced by RASF and regulates BAFF expression in response to Toll-like receptors (TLRs).
Methods: mRNA and protein expression of SLPI in RASF/RADF stimulated with/without TLR2/TLR3/TLR4 ligands was assessed by QT-PCR and ELISA, respectively. We also study the effect on BAFF mRNA/protein expression by RASF in the presence/absence of recombinant SLPI and blocking anti-SLPI antibodies.
Results: In vitro stimulation of TLR3, but not TLR2/TLR4 on RASF led to an about 15-fold induction of SLPI mRNA expression. In response to TLR3, SLPI was time-dependently released in the supernatant of RASF but not RADF. SLPI restrained BAFF production released by TLR3-stimulated RASF. In turn, TLR3-induced BAFF production was upregulated by endogenous SLPI blockade.
Conclusion: We demonstrated that RASF are able to release high levels of SLPI constitutively and upon TLR3 stimulation. The release of SLPI can directly modulate BAFF released by TLR3-stimulated RASF, highlighting the existence of an endogenous negative feedback loop centred on SLPI which regulates the release of B cell survival factors by RASF.

W5.12.06
In-vivo rnai mediated silencing of HNRNP-A2 decreases chronic inflammation by inhibiting proliferative and secretory capacity of monocytic cells

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Background: HnRNP-A2 belongs to a heterogenous family of nuclear proteins, importantly involved in mRNA processes. Recent evidence let suggest that hnRNPs posttranscriptionally modulate inflammatory mediators such as COX-2, TNF-α, IL-1β and iNOS by affecting mRNA stability.
Upregulation of hnRNP-A2 at sites of inflammation, and the generation of autoreactive T-cells against hnRNP-A2 in different arthritis models points towards an involvement in arthritis pathogenesis.
Objective: To gain more insight in the role of hnRNP-A2 in inflammation we have investigated the impact of hnRNP-A2 silencing in-vivo in mice suffering from collagen-induced arthritis (CIA) and in the KRN serum transfer arthritis model.
Animals and methods: In both arthritis models hnRNP-A2 was targeted using siRNA containing lipoplexes injected intravenously once a week. Control animals were treated with unspecific siRNA/lipoplexes or PBS. Arthritis was measured by a clinical scoring system. Inflammation and bone erosions were analyzed by histomorphometry.
Results: HnRNP-A2 was exclusively expressed in lymphoid organs. Cells of the mononuclear phagocyte system (MPS) showed the strongest expression of hnRNP-A2. Incidence of arthritis in hnRNPA2 silenced mice was significantly lower. Arthritis scores and weight loss differed significantly from control animals. Histological analysis of paws confirmed that both inflammation and erosion was significantly diminished in animals treated with hnRNP-A2 specific siRNA. Cytokines typically produced by cells of the MPS such as TNF-α, IL-23 and IL-1 were strongly reduced.
Conclusion: In-vivo silencing of hnRNP-A2 in two inflammatory arthritis models largely prevents induction of disease presumably by affecting the mononuclear phagocyte system.

W5.14 Psoriasis and skin diseases

W5.14.01
Innate immune activation promotes autoimmune response in generalized pustular psoriasis

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Generalized pustular psoriasis (GPP) is a rare, life-threatening disease characterized by recurrent episodes of systemic inflammation and pustulosis. It has been thought to belong to the spectrum of psoriasis, which is an HLA-class-I associated autoimmune disease. However, the recent identification of genetic defects in the IL-36 receptor antagonist (IL-36RN), a physiological antagonist of IL-36 signaling, suggests an autoinflammatory pathomechanism with excessive activation of the IL-1 pathway in GPP (Marrakchi *et al* 2011, Onoufriadis *et al* 2011). In this study, we investigated how autoinflammation and autoimmunity contribute to GPP pathogenesis. We identified two novel mutations of IL-36RN and particular HLA-molecules in GPP patients, which may correspond with a genetic predisposition for autoinflammation and autoimmunity, respectively. Real-time PCR for cytokine expressions and immunohistochemical studies confirmed local and systemic inflammation in GPP patients. Meanwhile, increased susceptibility to proinflammatory signals promoted auto-antigen driving T-cell activation in T-cell stimulation assays using lymphocytes from patients. T-cell receptor (TCR)-beta chain fragment length spectratyping and single cell TCR analysis were performed, and strong clonal T-cell expansions were detected in the skin and circulation of GPP patients. These results provide a comprehensive picture of GPP pathogenesis; whereby aberrant innate immune reactions enhance adaptive autoimmune responses, and have important therapeutic implications.

W5.14.02
PD-1 and other new molecules in the psoriatic infiltrate: an immunohistochemical and confocal laser microscopic study

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Basic and translational research is recently focusing on immunopathogenesis of psoriasis for the development of new therapeutic strategies. Few studies have recently demonstrated the role of PD-1, PD1-L inhibitory molecules in the control of T-cell activation and in favouring T-reg proliferation. The aim of our study was to evaluate the psoriatic infiltrate of 10 patients at baseline conditions. An immunohistochemical method and confocal laser microscopy with a large panel of monoclonal antibodies was used to better characterize the different cell subtypes. Our results showed many CD11c+ cells, several BDCA2+ plasmacytoid dendritic cells in a perivascular distribution and isolated Langerin+, CD103+ DCs. Many macrophages were also present, particularly CD163+ (M2) cells. Few CTLA-4+ Tcells and T-reg (Fox-P3+) were demonstrated. Monoclonal antibodies against classic cytokines, such as IFN, TNFα and IL17 labelled macrophages, lymphocytes or endothelial cells. PD1 expression was clearly detected on CD8+, TIA1+ cytotoxic T cells in the epidermis, on CD4+, T-Bet+, TIA-1- (Th1) lymphocytes in the papillary and superficial dermis and on macrophages, but not on CD11c+ dendritic cells. High expression of PD1-L was observed on stromal cells and keratinocytes in lesional skin. PD-1:PD-L interactions exert a wide range of immunoregulatory actions in T cell activation, tolerance, and immune-mediated tissue damage. Thus, further studies are needed to better understand the role of PD-1:PD-L pathway in the regulation of the immune response, in both physiological and pathological conditions, paving the way to new drugs that act by regulating PD1 expression in immune-mediated disorders.

W5.14.03
Inhibition of keratinocyte differentiation by the synergistic effect of pro-inflammatory cytokines mimics psoriasis

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Epidermal integrity is based on tight regulation of the communication between keratinocytes and leucocytes, particularly provided by balanced cytokine production. An imbalance network leads to skin inflammatory diseases such as psoriasis. Our attempt to model skin inflammation showed that combination of IL1a-IL17A-IL22-OSM-TNFa synergistically increases expression of chemokines and antimicrobial-peptides, leading to neutrophil infiltration, recapitulating some features of psoriasis.
Our goal was to characterize in vitro and in vivo the activity of cytokine combinations on keratinocyte differentiation and to compare with psoriatic skin lesions.
If IL1a, IL17A, IL22, OSM or TNFa were able to decrease cytokeratin-10, loricrin, filaggrin and cadherin-1 expressions, IL22, OSM and TNFa were the most powerful. Combination of these 5 cytokines (M5) generated a synergistic effect on inhibition of differentiation markers expression. This was confirmed on reconstructed epidermis. IL22 and OSM significantly decreased expressions of cytokeratin-10, filaggrin and cadherin-1, and induced epidermal hyperplasia. Injection of M5 in vivo in mice caused a thickening of the epidermis associated with decreased expression of cytokeratin-10, filaggrin and cadherin-1. Similarly we demonstrated a decreased expression of these markers in psoriatic skin lesions, as obtained in vitro and in vivo.
Our results showed that synergistic effect of cytokines was responsible for the production of antimicrobial-peptides, chemokines and inhibition of keratinocyte differentiation. If IL1a, IL17A and TNFa

were important for the production of antimicrobial-peptides and chemokines, IL22, OSM and TNFa seemed essential to the differentiation inhibition. Establishment of these models should clarify the role of cytokines in the establishment of the inflammatory response.

W5.14.04
In vivo induction of cutaneous inflammation results in the accumulation extracellular trap forming neutrophils expressing RORγt and IL-17, while IL-17+RORγ+ T cells remain absent

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Successful clinical trials using antibodies targeting IL-17 in psoriasis support the importance of IL-17 in the pathophysiology of psoriasis. However, there is debate concerning the cell source of IL-17 in the inflamed skin. In this study were to characterized IL-17-producing innate and adaptive cell populations in time by utilizing two established models for the induction of human skin inflammation *in vivo* that share many histological features with psoriasis: application of leukotriene B4 (LTB4) and tape stripping. Immunohistochemical studies showed that the application of LTB4 first led to an influx of neutrophils followed by an influx of T cells. Tape stripping caused an early influx of both neutrophils and T cells. Characterization of IL-17 associated cells, indicated that IL-17 was expressed by neutrophils and mast cells in both models. Only neutrophils co-expressed the IL-17 associated transcription factor RORyt. While the presence of mast cells remained steady during the skin inflammatory process, presence of neutrophils was clearly dynamic in time. Therefore, it is attractive to hypothesize that IL-17+/RORyt+ neutrophils contribute to the development of psoriasis. Surprisingly, none of the T cell subsets expressed RORγt or IL-17. These observations challenge the classical opinion that IL-17 is predominantly associated with T cells in psoriasis.

W5.14.05
Skin inflammation in Act1-deficient mice is T-cell intrinsic and due to exaggerated Th17 derived IL-22

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Psoriasis is a chronic inflammatory skin disease mediated in part by T helper 17 (Th17) cells and their cytokines (IL-17, IL-21, and IL-22). Act1 is the essential adaptor molecule for IL-17 receptor signaling. Here we report that Act1 is a 'client' protein of the molecular chaperone, Hsp90. Treatment of MEFs with Hsp90 inhibitors led to proteasomal-dependent Act1 degradation. We identified a highly conserved region in the N-terminus of Act1 that is required for the interaction with Hsp90. Recent genome-wide association studies linked a variant of Act1 (Act1(D10N)) to psoriasis susceptibility. As the variance occurred at the N-terminus, we found that Act1 (D10N) was indeed unable to interact with Hsp90, which resulted in an impaired interaction with all of the IL-17-signaling components (IL-17R, TRAF2, TRAF5, TRAF6, and IKKi) and thus failed to mediate IL-17-dependent gene induction. Given that IL-17 is linked to psoriasis pathogenesis, these findings are potential conundrums for the field. Using the Act1-deficient mice to model the mechanistic link between loss of Act1 function and susceptibility to psoriasis, we found that Act1-deficient mice had exaggerated Th17 cell generation and developed spontaneous skin inflammation that was resolved with anti-IL-22 treatment. Moreover, ex vivo Th17 polarization resulted in enhanced IL-17 and IL-22 production, indicating a T cell-intrinsic regulatory role for Act1. Taken together, in the absence of IL-17-signaling, IL-22 appeared to be the main contributor to skin inflammation, providing a molecular mechanism for the association of Act1(D10N) with psoriasis susceptibility.

W5.14.06
Autoreactive T cells in chronic idiopathic urticaria target the high-affinity IgE receptor Iα subunit (FcεRIα)

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Background. Chronic idiopathic urticaria (CIU) is the recurrence of "hives" without an apparent trigger. 50% of CIU patients have IgG autoantibodies to the FcεRIα on dermal mast cells and basophils, which upon activation release mediators responsible for urticaria. T cells are present in the skin lesions but autoantigen-specific T cells have not been identified in CIU.
Aims. We aimed to identify autoreactive T cells to FcεRIα in CIU, to help elucidate the pathogenesis and to improve the diagnosis of this disease.
Methods. Peripheral blood T-cell responses to FcεRIα were assayed in 30 CIU and 18 control subjects. Cell proliferation was measured by CFSE dye dilution. IFN-γ, IL-5 and IL-13 secretion were measured by ELISpot. Serum autoantibodies to FcεRIα were measured by immunoprecipitation of ^{125}I-labelled FcεRIα.
Results. We detected significant $CD4^+$ T-cell proliferation to FcεRIα in 30% of CIU and 0% of control subjects. IFN-γ responses were detected in 58% of CIU subjects, while IL-5 and IL-13 responses were detected in 29% and 37% of CIU subjects respectively. We detected autoantibodies to FcεRIα in 45% of CIU and 0% of control subjects. Overall, at least one FcεRIα-specific response was detected in 97% of CIU and only 5% of control subjects.
Conclusion. Autoreactive $CD4^+$ T cells specific for FcεRIα were detected in a proportion of subjects with CIU and, in combination with the assay for autoantibodies to FcεRIα, may improve the diagnosis of CIU.

W5.16 Liver immunology

IL5.16.01
Progress in the genetics and epigenetics of primary biliary cirrhosis

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Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease that primarily affects middle-aged women. PBC has strong autoimmune features, including highly specific loss of tolerance to a ubiquitous mitochondrial antigen. Both environmental and genetic factors are considered important in the pathogenesis of disease. Prior to the advent of genome wide association studies, only HLA class II loci (HLA-DRB1*08, *11, and *13) had been reproducibly shown to be associated with disease. With application of genome wide technology HLA was confirmed as the strongest association and many other risk loci have been identified, including IL12A, IL12RB2, STAT4, IRF5-TNPO3, and CTLA-4. These collectively support an important role for innate and adaptive immunity in development of disease. A second wave of genome wide association studies is now in progress based on dense arrays, such as the immunochip assay, larger cohorts and different ethnicity. Disease sub-phenotypes such as response to therapy, clinical progression and symptoms remain additional areas for further dedicated studies, and in which different genetic risk factors may be relevant. In addition to genetics, also epigenetic factors seem to play a role in PBC; in particular epigenetic defects were found in immune-related genes located on the X chromosome, such as the CD40L gene. Finally, due to a loss of X and Y chromosomes in female and male patients respectively, a progressive aploinsufficiency of sex chromosome genes can be hypothesized.

IL5.16.02
Constitutive IKK Activation Promotes Hepatocellular Carcinoma via Formation of Inflammatory Micro-niches

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Hepatocellular carcinoma (HCC) is a common fatal malignancy developing in chronically inflamed livers. Chronic viral hepatitides commonly show NF-κB activation and are the most frequent underlying cause of HCC worldwide. Yet, the role of NF-κB signaling in the pathogenesis of HCC is controversial. We dissected the role of IKK activation in a new mouse model of HCC. Constitutive activation of IKKβ in mouse hepatocytes suffices to induce HCC in old age. Moreover, it dramatically accelerates carcinogen induced HCC. In both cases, HCC is preceded by discrete inflammatory follicles, forming micro-niches in which progenitor malignant hepatocytes are nursed until they are ready to exit micro-niches to form bona-fide HCCs. These follicles are characterized by a complex cellular and cytokine milieu, particularly lymphotoxins, and their downstream effectors. When outmigrating from micro-niches, the malignant hepatocytes acquire autocrine properties, previously provided non-autonomously in the niche. Hepatitis C infected human livers, previously shown to induce chronic NF-κB activation, harbor similar inflammatory follicles. Pharmacological blockade of all lymphotoxin family cytokines dramatically attenuates mouse HCC, despite persistent NF-κB activation. Our findings indicate that liver NF-κB promotes early carcinogenesis non-cell-autonomously, by stimulating distinctive focal inflammatory micro-niches that foster tumor progenitor cell growth.

W5.16.01
IL-17A Produced by γδT Cells Promotes Tumor Growth in Hepatocellular Carcinoma

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IL-17A has been found in both mouse and human tumors, and shown to be critical involved in tumor development. However, the role of endogenous IL-17A in anti-tumor immunity remains controversial. Here we showed that IL-17A-deficient mice exhibited a significant reduction of tumor growth, while systemic administration of rmIL-17A promoted tumor growth in hepatocellular carcinoma (HCC), indicating a promoting role of IL-17A in HCC development. Further analysis showed that IL-17A promoted tumor growth through suppression of the antitumor responses, especially CTL responses. Furthermore, we demonstrated that, in the tumor microenvironment, IL-17A was predominantly produced by Vγ4 γδT cells. Moreover, depletion of Vγ4 γδ T cells reduced tumor growth while adoptive transfer of Vγ4 γδ T cells promoted tumor growth. Further studies showed that IL-17A inhibited antitumor immune responses though enhancing the migration and suppressive activity of MDSCs in tumor microenvironment. Thus, our results demonstrated that IL-17A produced by Vγ4 γδ T cells promoted tumor growth and suppressed anti-tumor immune responses in HCC through enhancing the migration and suppressive activity of the MDSCs in tumor microenvironment. These findings provided new insights into the role of IL-17A in tumor immunity and may have important implications in tumor immunotherapies.

W5.16.02
A second generation therapeutic cellular vaccine for hepatitis C virus

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We reported the results of a Phase I clinical trial using a cellular vaccine to treat HCV patients who had previously failed therapy. This trial fulfilled a major criterion of a Phase I trial viz. safety, the HCV-specific immune responses were transient. We have now designed a second generation vaccine based on preclinical data in mice and conducted a new Phase I trial.
A novel mouse dendritic cell line expressing the HCV NS3 was manipulated to ensure maximum immunogenicity, by inducing cell necrosis. Mice were vaccinated and ten days after the second dose, NS3 ELIspots were performed. Mice vaccinated with necrotic cells generated levels which were approximately 80-100 fold greater than viable cells. This protocol was used to vaccinate HCV patients.
Twelve chronically infected patients (HCV g1b) received interferon-alpha therapy for 4 weeks prior to vaccination. One week before the first vaccination, autologous monocyte derived dendritic cells (MoDc) were generated and transduced with a recombinant human adenovirus (rAd5), encoding HCV E1, E2 and NS3. Necrosis was induced in rAd5 transduced MoDc and patients received escalating dose of vaccine and vaccine boosts. Safety, viral load and responses to HCV peptides were assayed at various time points post final boost and compared to baseline levels for each patient. Here we report the preliminary data of this trial.
It will be expensive and time consuming to prove the efficacy of an HCV prophylactic vaccine. Consequently, we formed the hypothesis that an effective therapeutic vaccine will also represent an effective prophylactic vaccine.

W5.16.03
Gender predominance is mediated via testosterone in a mouse model of autoimmune cholangitis

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Background and aims: Many autoimmune liver diseases, including autoimmune liver diseases predominantly affect women. A deeper understanding of gender effects exerts on disease pathogenesis was limited by the lack of suitable animal models. We investigated how gender affects the immune pathogenesis in a novel mouse model of autoimmune cholangitis.
Methods and results: Adoptive cell transfer of ovalbumin (OVA)-specific CD8+ T cells into mice, which express OVA on cholangiocytes (K14-OVAp) resulted in portal inflammation and cholangitis in female, but not in male recipient mice. Gender differences seemed to be mediated by significant increased recruitment of endogenous CD4+ T cells in female as compared to male livers. Furthermore the levels of IL-17, RORc and the chemokines CXCL-9 and -10 were markedly elevated in female cholangitis. In castration and hormone replacement studies we could demonstrate, that the male insusceptibility to liver inflammation could be attributed to the protective effects of testosterone; estrogen levels had no effect on the severity of inflammation
Conclusion: We here demonstrate that the protection from autoimmune cholangitis which was observed in male mice was mediated by testosterone. Indeed, testosterone supplementation induced resistance to disease in otherwise susceptible female mice. The increased inflammation observed in female mice was associated with increased IL-17 expression by endogenous CD4 positive T cells which seems to be recruited to the liver via an increased expression of chemokines that recruit T cells to the liver. These results may stimulate future investigations into the mechanisms involved in gender differences in autoimmune diseases.

W5.16.04
CD49a$^+$DX5$^-$ NK cells are liver-resident and confer adaptive immunity in skin-contact inflammation

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Liver natural killer (NK) cells were recently reported to possess memory-like properties in contact hypersensitivity (CHS) models. However, the phenotype and origin of these “memory” NK cells cannot be distinguished from other NK cell subpopulations. Here, we defined the transcriptional, phenotypic and functional features of liver NK cell subsets and their roles in mediating CHS. Liver NK cells can be divided into two distinct subsets, CD49a$^+$DX5$^-$ and CD49a$^-$DX5$^+$. Substantial transcriptional and phenotypic differences existed between liver CD49a$^+$DX5$^-$ NK cells and other NK cell subsets; the former possessed memory potential and conferred hapten-specific CHS responses upon hapten challenge. Importantly, CD49a$^+$DX5$^-$ NK cells were liver-resident, present in the liver sinusoid blood but not the afferent and efferent blood of the liver. Moreover, they appeared to originate from hepatic hematopoietic progenitor/stem cells (HPC/HSC) but not from the bone marrow (BM), and maintained their phenotypes in the steady-state. Our findings of liver-resident NK cells shed new light on acquisition of memory-like properties of NK cells.

W5.16.05
Phenotypic evaluation of CD28 negative T cells in primary sclerosing cholangitis

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Background/Aims: Genetic, functional and epidemiologic studies implicate CD28 and vitamin D (vitD) as relevant to autoimmune diseases including primary sclerosing cholangitis (PSC). Here we studied the role of CD28-ve T cells in PSC and the effects of vitD on their phenotype. Methods: Liver-infiltrating (LIMCs) and peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors or patients with PSC. The frequency and phenotype of CD28-ve cells [expression of activation (CD69, CD25) and exhaustion (PD-1) markers, perforin and granzyme B] were studied by flow cytometry. PSC LIMCs and PBMCs were activated and the release of IFNγ was examined. CD28-ve and CD28+ve cells from PSC PBMCs were stimulated with anti-CD3, autologous CD14+monocytes (5:1 ratio) with/without vitD for 4 days. Results: CD4+CD28-ve and CD8+CD28-ve T cells were highly expanded in PSC peripheral blood (3.9- and 2.1-fold increase respectively versus normal blood) and in PSC liver (17.8- and 2.45-fold increase respectively versus normal liver). The frequency of CD4+CD28-ve T cells in PSC liver was significantly higher than PSC peripheral blood (*p<0.01).* Liver-infiltrating CD4+CD28-ve T cells showed an activated CD25+CD69+PD-1- and cytotoxic phenotype granzymeB+perforin+. *In-vitro* stimulation of PSC liver-infiltrating CD4+CD28-ve cells induced the production of IFNγ (41%), which was much higher compared to their CD28+ve counterparts (12%). Stimulation of PSC peripheral blood CD28-ve and CD28+ve cells with vitD induced CD28 expression on CD28-ve (+52%) and CD28+ve (+65%) cells and suppressed IFNγ production (-43% and 67%, respectively). Conclusion: In PSC, CD28 negative T cells are pro-inflammatory, an effect that can be reduced by vitamin D supplementation.

W5.16.06
Tumor-infiltrating IL-10 producing T cells are potent suppressors of the local anti-tumor immunity in patients with liver cancer

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Hepatocellular carcinoma (HCC) and liver metastases from colorectal cancer (LM-CRC) are the main malignancies affecting the liver and are among the most common cancers and leading causes of cancer mortality. Curative treatment options are limited to very early stages, and they cannot prevent the recurrence of the disease. Several attempts to design alternative immunotherapeutic strategies have only shown limited efficacy in clinical trials. The failure of immunotherapy may be related to suppressive mechanisms in the tumor environment. In support of this, we recently described the accumulation of highly activated FoxP3+ regulatory T cells (Tregs) that are potent suppressors of anti-tumor immunity at the tumor site of patients with liver cancer. Now, we observed that in addition to Treg, tumor-infiltrating lymphocytes (TILs) contain a subset of CD4+ T cells that produces IL-10, and does not express FoxP3. These cells do not produce the Th2-like cytokine IL-13, but the majority can produce IFNg. Tumor-derived IL-10 producing CD4+ T cells have a potent capacity to suppress proliferation and cytokine production by autologous and allogenic effector T cells in an IL-10-dependent manner. These results indicate for the first time to our knowledge that besides CD4+FoxP3+ Tregs, liver tumors contain another subset of CD4+ T cells that is able to suppress the local immune response. It's phenotype and function resemble that of type 1 regulatory T (Tr1) cells, and it may also interfere with immunotherapeutic efforts designed to treat patients with liver cancer.

W5.17 Autoinflammatory diseases

IL5.17.01
Epigenetic regulation following AhR activation leads to suppression of inflammatory and autoimmune diseases

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Aryl hydrocarbon receptor (AhR) plays a central role in mediating detoxification of xenobiotics. Interestingly, recent studies have demonstrated an exciting new role for AhR in the regulation of T cells leading to suppression of inflammation and autoimmune diseases. Chronic inflammation is considered to be the underlying cause of most clinical disorders including autoimmune, cardiovascular, neurodegenerative diseases, obesity, as well as certain types of cancer. Thus, identifying novel pathways to regulate inflammation could lead to new drug discovery with global impact. Numerous endogenous and plant derived products act as AhR ligands. In the current study, we investigated the effect of compounds such as Resveratrol (RES; 3,5,4’-trihydroxystilbene) and other dietary AhR ligands, on T cell differentiation and suppression of inflammation. We found that AhR ligands could suppress experimental autoimmune encephalomyelitis (EAE) as well as colitis, We noted that treatment of mice with AhR ligands resulted in a dramatic decrease in infiltrating T-cells at sites of inflammtion. Based on miRNA array screening, AhR ligands induced the expression of certain microRNA that targeted cytokine genes such as IL-17 and transcriptional regulator, FoxP3. Moreover, AhR ligands also altered the DNA methylation, specifically upregulating the methylation of IL-17 gene while down-regulating the methylation of FoxP3. Together, these studies demonstrated that dietary AhR ligands may promote Th17 to Treg switch through epigenetic pathways involving DNA methylation and micro-RNA regulation, to suppress inflammation and autoimmune diseases (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357).

IL5.17.02
Autoinflammatory diseases

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"Autoinflammatory" diseases represent and expanding spectrum of genetic and non-genetic inflammatory diseases characterized by recurrent episodes of fever and systemic inflammation affecting the eyes, joints, skin, and serosal surfaces. Autoinflammatory differ from autoimmune diseases by several features, including the periodicity whereas autoimmune diseases are progressive, and the lack of signs of involvement of adaptive immunity such as association with HLA aplotypes, high-titer autoantibodies or antigen-specific T cells. Autoimmune diseases are responsive to biologic agents that targets T- and B-cell functions, including anti-TNFα, anti-IL-6 receptor, anti-IL-12/IL-23 antibodies etc. However, these therapeutics have no sustained effects in treating autoinflammatory diseases that, in contrast, are uniquely responsive to IL-1β blockade. Thus, autoinflammatory syndromes are recognized as disorders of innate immunity, where the monocyte-macrophage rather than the T-cell is the culprit and the defect is dysregulated secretion of active IL-1β. Although many autoinflammatory diseases have a genetic cause, increasing evidence indicates that the degree of cell stress concurs to the severity of the disease phenotype. In this workshop, we will discuss the recent advances on pathogenesis, pathophysiology and therapeutic approaches in autoinflammatory syndromes compared with autoimmune diseases.

W5.17.01
The molecular link between PSTPIP1 and S100A8/S100A9 in autoinflammatory diseases

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Hypercalprotectinaemia & hyperzincaemia is a rare autoinflammatory syndrome that is characterized by excessively high S100A8/A9 complex (calprotectin) serum concentrations. S100A8 and S100A9 are Damage Associated Molecular Pattern (DAMP) proteins that can be released by microtubuli (MT)-dependent alternative secretory pathways to promote inflammatory processes via TLR-4 dependent signalling pathways. By analyzing eleven patients we could identify a novel mutation in the proline serine threonine phosphatase-interacting protein 1 (PSTPIP1) gene. Ten patients were carrier of an E250K substitution encoded by the PSTPIP1 gene. Other PSTPIP1 mutations are responsible for the classical, clinically different Pyogenic sterile Arthritis, Pyoderma gangrenosum, and Acne (PAPA)-syndrome which presents with elevated, albeit significantly lower S100A8/A9 levels compared to patients with E250K mutation. Whether there is a molecular link between PSTPIP1 mutations and elevated S100A8/A9 concentrations is currently unknown. This project focuses therefore on the identification of molecular links between PSTPIP1 and S100A8/A9.
The double-staining immunofluorescence, proximity ligation assays (DuoLink II®), and immunoprecipitation results show an interaction between PSTPIP1 and S100A8/A9 after activation of monocytes by phorbolester. In biochemical assays we could specify a direct interaction of these proteins in a strictly calcium-dependent manner. Deletion constructs of PSTPIP1 indicate that the S100A8/A9 binding motif is located in the same region where the mutation of patients is located. MT co-sedimentation experiments indicate a mutual interference of PSTPIP1 and S100A8/A9 on their interaction with MT which is altered by E250K mutation. This in turn might be of important relevance for the uncontrolled, MT-dependent secretion of S100A8/A9 promoting inflammation in these patients.

W5.17.02
Increased Nlrp3-dependent Interleukin (IL) 1B secretion in patients with Familial Mediterranean Fever (FMF): correlation with Mefv genotype

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Objectives To define in patients affected by Familial Mediterranean Fever (FMF), whether or not interleukin (IL)-1β secretion (1) is enhanced, (2) correlates with the type of MEFV mutation and (3) is mediated by NLRP3. **Methods** Freshly isolated monocytes from 20 FMF patients (12 homozygous and 8 heterozygous), 14 MEFV healthy carriers (HC) and 30 healthy donors (HD), unstimulated or after LPS-induced activation, were analyzed for redox state (reactive oxygen species (ROS) production and antioxidant responses), and for IL-1β and IL-1Receptor antagonist (IL-1Ra) secretion. NLRP3 down-modulation was induced by NLRP3 *in vitro* silencing. **Results** LPS-stimulated monocytes from FMF patients displayed enhanced IL-1β secretion which correlated with the number and penetrance of MEFV mutations. Silencing of NLRP3 consistently inhibited IL-1β secretion. As in other autoinflammatory diseases, MEFV mutated monocytes produced more ROS than genetically negative controls. However, contrary to CAPS, they were featured by a conserved and sustained antioxidant response. Consistent with this finding, MEFV mutated monocytes did not exhibit the functional indicators of oxidative stress observed in CAPS, including accelerated IL-1β secretion and deficient IL-1Ra production. **Conclusion** MEFV mutated monocytes display enhanced IL-1β secretion which correlates with the number of high-penetrance mutations and level of endogenous ROS. Unlike NLRP3 mutated cells, monocytes carrying MEFV mutations withstand oxidative stress and preserve IL-1Ra production, thereby limiting inflammation. Finally, in contrast to what found in the animal model, the increased secretion of IL-1β by LPS-stimulated FMF monocytes is NLRP3-dependent.

W5.17.03
Defective IFN-γ-induced indoleamine 2,3-dioxygenase production in patients with macrophage activation syndrome

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Macrophage activation syndrome (MAS) is a rare but severe immune-inflammatory condition seen in hemophagocytic syndromes, namely hemophagocytic lymphohistiocytosis (HLH), and as a complication of systemic juvenile idiopathic arthritis (sJIA). The syndrome is associated with increased production of inflammatory cytokines. Although increased IFN-γ serum levels have been reported in MAS and sJIA patients, gene expression studies on peripheral blood mononuclear cells (PBMCs) of these patients revealed a conspicuous absence of IFN-γ-upregulated genes, suggesting perturbed IFN-γ signaling. This study aimed to investigate IFN-γ responses and the regulatory role of IFN-γ in PBMCs of patients with MAS and sJIA. Hereto, freshly isolated PBMCs were stimulated with IFN-γ and induction of genes and proteins was measured by qPCR, ELISA, western blot, flow cytometry and HPLC. IFN-γ responses were studied in MAS (n=7), inactive and active sJIA (n=19) patients and healthy age-matched controls (n=18). In PBMCs of all patients and controls, IFN-γ equally induced IFN-γ-associated genes and proteins such as phosphorylated STAT-1 and interferon-inducible protein 10 (IP-10). In all study groups, IFN-γ also down-regulated autocrine induction of IL1β as well as IL-1β-induced IL-8. In contrast, upon analysis of IFN-γ-induced indoleamine 2,3-dioxygenase (IDO) protein, a >50% decrease in IDO production was observed in MAS patients compared to sJIA patients and healthy controls. In conclusion, MAS patients have no general defect in the IFN-γ response but display a

selective defect in IFN-γ-induced IDO production. Since IDO possesses potent immunosuppressive activity, this defect may contribute to uncontrolled immune activation and inflammation in MAS.

W5.17.04
Study MHC class II presentation of Multiple Sclerosis associated autoantigen using antibodies with T-cell receptor specificity

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Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination, and axonal damage triggered by auto reactive T cells specific for myelin antigens, including Myelin Oligodendrocyte Glycoprotein (MOG). Antigen-presenting cells (APC) -associated MHC class-II molecules are key for activating these specific CD4+ auto-reactive T cells. Susceptibility to MS is strongly linked to the HLA-DR2 allele that confers the highest MS risk.
The main goal of this study was to generate MS-specific, recombinant antibody with MHC-restricted, peptide-specific reactivity, termed T-Cell Receptor-Like (TCRL). These antibodies will be used as antigen-specific immunotherapeutic approach. Such approaches could promote tolerance to MS antigens without generally weakening the immune system.
We have cloned and purified a soluble recombinant HLA-DR2 molecule and loaded it with the MOG35-55 peptide. Then, we isolated the desired antibodies by introducing the DR2/ MOG35-55 molecule to a large, naïve library using Phage Display approaches. The different antibody clones isolated from the library screening were characterized with some of them exhibiting TCR like specificity, thus, their binding was dependent on both DR2 and MOG peptide recognition. They were also able to bind naturally presented DR2/ MOG35-55 complexes. Most significant, the DR2/MOG-specific TCRL antibody was capable to block the recognition between MOG35-55 pulsed DR2 positive APCs and their cognate T cell hybridoma as measured by inhibition of IL-2 secretion.
In the future, the DR2/MOG-specific TCRL antibody will be used for two major research directions; to induce antigen-specific tolerance in vivo, in the EAE mouse model, and to analyze MS epitope presentation.

W5.17.05
Identification of commensal flora associated antigen,as a pathogenic factor of autoimmune pancreatitis

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Background: Autoimmune pancreatitis (AIP) is a nosological entity of IgG4-related systemic sclerosing diseases. Although autoantibody and the MHC genotypes are suspected to have role in the etiology, pathogenic factors of AIP have not fully been elucidated. We previously reported a mouse model of AIP by repeatedly inoculating Escherichia coli in C57BL/6 mice. Methods: To determine pathogenic antigen of E. coli, 2D-DIGE was carried out followed by TOF/MS. Recombinant proteins of identified antigens were generated and inoculated into C57BL/6 mice as the same protocol as previously described (ref. Lab Invest 2010; 90(12):1757), to examine for the induction of AIP-like disorder in C57BL/6 mice. Results: (i) The immune-reactive spot against AIP-mice sera was determined as FliC of E. coli. (ii) Marked cellular infiltration, which was predominantly consistent with CD3-positive cell, was observed in the exocrine pancreas of rFliC-inoculated mice. (iii) Anti-lactoferrin and anti-carbonic anhydrase II titers were significantly higher in sera of rFliC-inoculated mice. Conclusions: FliC of E. coli could induce AIP-like pancreatitis in C57BL/6 mice. Bacterial protein itself would be directly pathogenic or molecularly mimic to unknown autoantigen to generate the status of AIP-like inflammation, perhaps, being influenced by additional factors such as MHC. To reconsider the importance of commensal bacteria as an environmental factor of the pathogenesis of autoimmune disease would give us many hints to overcome AIP. (Collaboration; Kyoko Shimizu[3], Toru Furukawa[4], [4]Integrated Medical Sciences, TWMU)

W5.17.06
Soluble CD91/LRP1 is elevated in the sera of a subset of children with different inflammatory diseases

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CD91 also known as LRP1 is a large 2-chain receptor that binds many ligands including heat shock proteins, lipoproteins, myelin basic protein, and alpha-2 macroglobulin. Proteolytic cleavage results in its shedding. Little is known about the half-life of soluble CD91 (sCD91) in circulation or other fluids or even its normal levels. Using an ELISA established in the laboratory, levels of sCD91 were measured in sera from children with juvenile idiopathic arthritis (JIA), children who had had a demyelinating event one year prior or in normal children. We also tested synovial fluid from 12 Rheumatoid Arthritis patients for sCD91. Elevated levels of sCD91 were detected in the sera of 14 and 27% of poly or oligo JIA patients respectively but were not detected in those with systemic disease. Numbers were too small to determine any correlations with disease activity. There was a trend for elevated levels of sCD91 in those diagnosed with JIA prior to 6-years of age compared to those with later onset. SCD91 was detected in 5 of 12 synovial fluid samples from RA patients. In patients who had experienced a demyelinating event, those with elevated levels of sCD91 appeared to have an increased risk of being diagnosed with multiple sclerosis. A 50 unit increase in sCD91 was associated with a 50% increased risk. These data suggest that CD91 can be shed as a component of different inflammatory responses, and that further characterization of sCD91 could reveal novel insight into disease pathogenesis.

W5.18 Inflammatory bowel diseases

W5.18.01
Depletion of regulatory T cells improves anti-tumour immunity in colitis-associated colon cancer

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Chronic inflammation severely increases the risk for cancer development as seen in patients with inflammatory bowel diseases. Although the exact mechanism of inflammation-associated tumor development remains to be shown, a role for the adaptive immune system has been implicated in colitis-associated cancer (CAC). In this context, the role of regulatory T cells (Tregs) is controversial as they act as potent suppressor of inflammation and thus can have protective effects in CAC, but simultaneously can prevent antitumor immunity. Therefore, we investigated the role of $CD4^+Foxp3^+$ Tregs in a mouse model of CAC. Blood and tissue specific $CD4^+Foxp3^+$ Tregs from CAC mice were compared to Tregs from healthy mice and mice with chronic colitis. We found a strong increase of $CD4^+Foxp3^+$ Tregs in the colonic tumors and the mesenteric lymph nodes of mice suffering from CAC. Tumor-infiltrating $CD4^+Foxp3^+$ Tregs were highly activated, suggested by the increased expression of CD25, CTLA-4 and IL-10. Moreover, they exhibited an increased suppressive effect on $CD4^+CD25^-$ responder T cell proliferation and TH1-cytokine production *ex vivo*. Mice expressing a diphtheria toxin (DT) receptor-enhanced fluorescent fusion protein under the control of the foxp3 gene locus (DEREG mice) allow the depletion of $Foxp3^+$ Tregs by DT injection. *In vivo* depletion of $CD4^+Foxp3^+$ Tregs during carcinogenesis in DEREG mice suppressed the tumor progression accompanied by an increase of $CD8^+$ INF-γ producing effector cells. Our data suggest that selective, transient depletion of Tregs has the potential to evoke an efficient antitumor response and might have implications for therapeutic interventions in CAC patients.

W5.18.02
RIP3 promotes injury-induced cytokine expression and tissue repair

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Receptor interacting protein kinase 3 (RIPK3/RIP3) is critical for programmed necrosis/necroptosis driven by tumor necrosis factor-like death cytokines, toll-like receptors, and antigen receptors. RIP3 was thought to promote inflammation through the release of danger-associated molecular patterns from necrotic cells when FADD or caspase-8 was inhibited. However, it is unclear if RIP3 can facilitate inflammation independent of necrosis when apoptosis pathway is intact. Here we present evidence that RIP3 has a unique role in promoting injury-induced inflammation and tissue repair that is independent of its pro-necrotic function. $RIP3^{-/-}$ mice were highly susceptible to colitis induced by dextran sodium sulfate (DSS). Bone marrow chimera experiments show that RIP3 expression in the hematopoietic compartment was critical for tissue repair upon DSS treatment. Induction of IL-22, a key cytokine for repair of intestinal epithelium, was significantly impaired in $RIP3^{-/-}$ mice. The blunted IL-22 expression was not due to intrinsic defects of innate lymphoid cells, the main cellular source of IL-22 in the gut. Rather, dendritic cells (DCs) expression of IL-23, a strong inducer of IL-22, was significantly suppressed in $RIP3^{-/-}$ mice. We found that LPS-induced activation of the NFκB pathway was severely dampened in $RIP3^{-/-}$ bone marrow derived DCs. These deficiencies led to sustained injury, inflammation, and higher incidence of colitis-associated colon adenomas in $RIP3^{-/-}$ mice. Our results reveal a previously unappreciated function of RIP3 in NFκB activation, innate inflammatory cytokine expression, intestinal tissue repair and inflammation-induced tumorigenesis.

W5.18.03
Induction of IL-33 is dependent on the gut microflora and is essential for EOS activation/migration and Th2 immune responses in the pathogenesis of experimental IBD

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IL-33 is a member of the IL-1 cytokine family known to promote Th2 immunity and has been linked to several inflammatory disorders, including inflammatory bowel disease (IBD). SAMP1/YitFc (SAMP) mice, a spontaneous model of IBD, raised under germ-free (GF) conditions develop a delayed onset of ileitis, with lower penetrance and severity, compared to age-matched specific pathogen free (SPF)-reared SAMP. Interestingly, mucosal-derived CD4+ T-cells from GF-SAMP display a vigorous Th1, but not Th2, immune response in the absence of bacterial flora that can be reversed upon fecal antigen exposure. The aim of this study was to determine the effects of the gut microflora on IL-33 expression and downstream mucosal Th2 immune responses, leading to chronic intestinal inflammation in SAMP mice. Our results showed that 20-26-wk-old GF-SAMP displayed a 5.2-fold decrease in ileal IL-33 mRNA levels compared to age-matched SPF-SAMP with comparable inflammatory scores (6.25±1.60 vs. 8.33±0.53, n.s.). In the absence of gut microflora, a dramatic decrease of infiltrating eosinophils (EOS) (44.10±7.15 vs. 22.67±4.65 EOS/HPF), and IL-4, IL-5, and IL-13 mRNA levels (12.7-, 2- and 3.5-fold, respectively) was observed in ilea of GF- vs. SPF-SAMP. Importantly, EOS depletion in SPF-SAMP did not affect IL-33 mRNA expression, suggesting that upregulation of IL-33 likely occurs before EOS activation and homing to the gut. Together, these data indicate that the gut microbiota induces IL-33 production that can promote EOS infiltration into the intestinal mucosa and confirm that the commensal flora is essential to mount potent Th2 immune responses, leading to chronic ileitis in SAMP mice.

W5.18.04
Targeting IL-23/T_H17 axis to suppress intestinal inflammation in murine spontaneous colitis induced by an epithelial defect

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Whilst the IL-23/T_H17 axis is activated in inflammatory bowel disease, its contribution to pathology and the best way to target it therapeutically remain unclear. Previously we found that genetic deficiency of IL-17A suppressed colitis in *Winnie* mice which carry a *Muc2* mutation, and develop goblet cell endoplasmic reticulum stress and progressive T_H17-dominant colitis. In an attempt to mimic biological therapy with antibodies, we assessed the efficacy of anti-IL-17A antibodies in *Winnie* with emerging and established colitis. Neutralizing IL-17A in young *Winnie* mice failed to alleviate colonic inflammation, while blocking IL-17A in older *Winnie* mice resulted in decreased histological colitis in the proximal colon, suggesting neutralizing IL-17A may have differential effects depending on the nature of colitis as it progresses. Neutralizing IL-17A together with IL-17F or blocking IL-17Ra did not further suppress intestinal inflammation suggesting that targeting T_H17 effector cytokines or IL-17 receptor signalling is not sufficient to alleviate *Winnie* colitis even in the context of strong T_H17 inflammation.
However, targeting IL-23 by monoclonal antibodies against the p19 subunit greatly reduced colon weight of *Winnie* mice with established colitis compared to a control group ($P < 0.01$). Anti-p19-treated *Winnie* mice had significantly decreased distal histological colitis scores ($P < 0.05$), with less neutrophil infiltration, limited crypt hyperplasia and reduced epithelial damage. Anti-p19 treatment was more efficacious than anti-p40, which is shared with IL-12, indicating that the IL-23/T_H17 axis, not IL-12/T_H1, is responsible for pathology in *Winnie* colitis. Clinical trials also suggest that targeting IL-23 is more efficacious than targeting IL-17.

W5.18.05
The p50 NF-κB subunit is a key orchestrator of cancer-related intestinal inflammation

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Colorectal cancer (CRC) is one of the best example of pathological association between chronic inflammation and cancer development. Tumor Associated Macrophages (TAM) represent the major leukocyte population present in tumors. During tumor-development, TAM undergo to phenotypic switch promoting an M2 polarized phenotype with tumor promoting properties. We previously demonstrated that nuclear accumulation of p50 NF-κB promotes an M2-polarized pro-tumoral phenotype, a condition shared also by endotoxin tolerant macrophages. This may be relevant in the gut, where macrophages are central regulators of intestinal homeostasis and orchestrate the balance between immune response and tolerance to intestinal microflora. Hence, we investigated the role of p50-driven polarized inflammation in CRC development, by using two distinct models of genetic- (ApcMin mice) and colitis-associated cancer (CAC). In the CAC model, we observed that p50-/- mice exhibit a dramatic intestinal inflammation (addressed by weight loss, colon shortening and histological analysis of colon tissues), paralleled by reduced incidence of tumor development. In agreement, analysis of mice survival, tumor incidence, progression, in ApcMin versus ApcMin-p50-/- mice, demonstrated that p50 NF-κB is required to support cancer growth at different stages of the neoplastic process, including early and late stages of tumor development. Finally, biochemical studies identified the p50 NF-κB subunit as a key player tuning the balance between two differentially expressed clusters of inflammatory genes, respectively involved in tumor inhibition and development. Overall our results indicate that, irrespective of the etiological events triggering CRC development, the p50 NF-κB subunit is required to promote cancer-related inflammation supporting CRC development.

W5.18.06
Novel HLA-DR transgenic mice that spontaneously develop colitis with serious defects in lymphoid organs

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HLA-DR4 (HLA-DRA*01:01/HLA-DRB1*04:05) is one of the frequent HLA-DR molecules found in 26% of the Japanese population. We have established C57BL/6-background HLA-DR4-transgenic mice (DR4-tgm) and the peptide-specific HLA-DR4-restricted Th-cell responses were successfully observed in the DR4-tgm immunized with the peptide. Unexpectedly, the mice with homozygous transgenic alleles (homo-DR4-tgm) spontaneously developed serious colitis similar to human ulcerative colitis without any further genetic modification, adoptive cell transfer, bacterial infection or chemical treatment reported in previous experimental colitis model mice. The majority of the homo-DR4-tgm showed severe wasting and rectal prolapse within 4-5 months and died within 5-6 months. The histological analyses revealed that many neutrophils infiltrated in the lamina propria of colon but not in small intestine. Interestingly, the thymus of homo-DR4-tgm shrank and morphologically collapsed up to one month of age and disappeared in adult (after 2-3 months of age). Moreover, other lymphoid organs such as white pulp in the spleen and Payer's patches in the colon were hardly observed. Flow-cytometry showed that in adult homo-DR4-tgm, lymphocytes in the peripheral blood and spleen were markedly reduced while Gr-1+CD11b+ granulocytes were accumulated. The genome-wide sequencing and FISH analyses revealed that the HLA-DR transgenes were inserted in the telomeric region of chromosome 3 with deletion of 39 kb genomic fragment, which is close to but definitely separated from the colitis-susceptible Cdcs1 region reported in PNAS, 98: 13820, 2001. Hence, this novel animal model may contribute to our understanding of the pathogenesis of the inflammatory bowel disease and the immune manipulation against autoimmunity.

W5.19 Reproductive immunology

IL5.19.01
Galectins in pregnancy: the bitter side of sweet

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Members of the galectin family are expressed within the female reproductive tract and have been shown to be involved in multiple biological functions that support the progression of mammalian gestation, a physiological process that sustains life in this universe. Specific expression of several galectins has been identified at the maternal and placental compartments, where in some cases dysregulated functions have been associated with adverse pregnancy outcomes. Recent studies on galectins have shown that some members of this lectin family seem to be important for pregnancy maintenance, emphasizing their role as major regulators of the maternal immune adaptation to pregnancy (e.g. induction of tolerogenic dendritic cells, IL-10 expressing regulatory T cells, IFN-γ expression by NK cells and apoptosis of maternal T cells) and placental development (such as immuno-modulatory function exhibited by trophoblast cells and regulation of trophoblast invasion and fusion properties). This lecture will summarize current knowledge on galectins as regulators of pregnancy and discuss their implication in preeclampsia, a life threatening pregnancy specific disorder.

W5.19.01
Fetal loss induced by depletion of innate 33D1+DC subset in mice

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Dendritic cells (DCs) play a crucial role in providing an appropriate fetal/maternal balance between Th1 and Th2 during pregnancy. The Th1/Th2 balance is thought to be regulated mainly by two distinct DC subsets, DEC-205+ DCs having the capacity to establish Th1 polarization and 33D1+ DCs to induce Th2 dominance. In addition, maternal hormones, such as progesterone and estrogen, have been thought to be important for the maintenance of pregnancy. We found that the balance of DC subtypes was affected mainly by progesterone, which induced a dose-dependent reduction of the DEC-205/33D1 ratio together with/without a stable amount of estrogen (Negishi, et al., Immunobiol. 217:951, 2012). The DEC-205/33D1 ratio decreased gradually with the progress of pregnancy and rapid augmentation of this ratio was seen around delivery period in vivo. Here, we demonstrate that the fetal loss was induced by the depletion of 33D1+DCs during perinatal period mediated through Th1 up-regulation via transient IL-12 secretion, and pre-administration of progesterone could rescue this fetal loss. Similar miscarriages were also observed when pregnant mice were intraperitoneally (i.p.) injected twice with IL-12 on Gd 9.5 and 10.5. Moreover, prior inoculation of progesterone suppressed the enhanced serum IL-12 production in mice treated with 33D1 antibody, indicating that progesterone might inhibit temporal IL-12 secretion around Gd 10.5 and miscarriage was prevented. These findings suggest that the balance of DC subsets is crucial for maintaining pregnancy and we can prevent miscarriage by manipulating the activity of the DC subpopulation of pregnant individuals by progesterone administration.

W5.19.02
HLA-G expressing DC-10 and CD4$^+$ T cells accumulate in human decidua in the first trimester of pregnancy

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During human pregnancy, HLA-G molecules specifically expressed in the trophoblast have a role in reprogramming the local maternal immune response towards tolerance. DC-10 are tolerogenic dendritic cells (DCs) that secrete high amounts of IL-10 and express membrane-bound HLA-G and the immunoglobulin like transcript (ILT)4. DC-10 are present in the peripheral blood and promote the induction of adaptive regulatory T cells (Tr1) *in vitro*.
We investigated the presence of DC-10 and of a subset of regulatory CD4$^+$ T cells constitutively expressing HLA-G in the human decidua during the first trimester of pregnancy. Results showed a significantly higher percentage of DC-10 in the decidua (dDC-10) as compared to those present in the peripheral blood. Interestingly, dDC-10 expressed similar levels of HLA-G but significantly lower levels of ILT4 compared to circulating DC-10. We also observed a significantly higher frequency of HLA-G-expressing CD4$^+$ T cells in the peripheral

blood and in the decidua of pregnant women compared to that observed in peripheral blood of healthy donors. Notably, no significant differences in the frequency of DC-10 and HLA-G-expressing $CD4^+$ T cells were observed when decidua from women with spontaneous abortions and with elective abortions were compared.
This is the first report describing the presence of DC-10 and $CD4^+HLA\text{-}G^+$ T cells in the human decidua, indicating that these regulatory cells may contribute to control tolerance at the fetal-maternal interface.

W5.19.03
Placental cells promote a tolerant fetal environment by inducing homeostatic macrophages and regulatory T cells

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Proper adaptation of the maternal immune system is essential to avoid rejection of the semi-allogeneic fetus. Fetally derived trophoblast cells have immune modulatory properties and thus the placenta itself might promote a tolerant immune environment at the fetal-maternal interface. We investigated the ability of human placental tissue and trophoblast cells to induce macrophages and Th cells with regulatory properties. Peripheral blood $CD14^+$ monocytes and $CD4^+$ Th cells from non-pregnant women were obtained by MACS. Macrophages were differentiated from monocytes in the presence of M-CSF or GM-CSF. The effect of conditioned medium (CM) from first trimester placental explants or the trophoblast cell line HTR8 on macrophages and Th cells was analyzed with flow cytometry. CM induced high expression of the homeostatic scavenger receptor (CD163) and the M2 marker DC-SIGN (CD209) in macrophages, consistent with the phenotype of decidual (uterine) macrophages. M-CSF, which promotes regulatory decidual macrophages *in vitro*, was produced at high levels by both HTR8 trophoblasts and placental tissue. The expression of CD163 was reversed by anti-M-CSF blocking antibodies, confirming the involvement of trophoblast-derived M-CSF in the polarization of decidual macrophages. Placental explant CM also reduced the expression of HLA-DR on Th cells and induced $CD25^{bright}Foxp3^+$ Treg cells, while transcription factors for Th1, Th2 and Th17 cells (Tbet, GATA-3 and Rorc) were not consistently affected. Altogether, our data shows that placental cells promote the polarization of homeostatic macrophages, limit T cell activation and contribute to the induction of Treg cells, supporting the tolerant environment required for normal fetal development.

W5.19.04
Demonstration of the deleterious impact of foeto-maternal MHC identity on the success of pregnancy in a macaque model

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The impact of foeto-maternal histocompatibility on reproduction has inspired long-lasting debates. However, after the review of numerous articles, the impact of HLA allele sharing within couples on fecundity remains questionable. We decided to explore the impact of MHC foeto-maternal compatibility on reproduction in a cynomolgus macaque breeding unit composed of animals originating from Mauritius Island. The Mauritian macaque population presents a very restricted MHC polymorphism (only seven founding haplotypes) due to a strong founding bottleneck. The MHC polymorphism was investigated in 237 trios (male, female and offspring) using 17 microsatellite markers distributed across the MHC. Haplotypes were confirmed by segregation analysis. We evaluated the relative frequencies of offspring MHC-compatible and MHC-semicompatible with the mothers. Among the 237 trios, 106 offsprings were semi-compatible and 47 offsprings were fully compatible with their mother for all MHC markers. This repartition is incompatible with a random distribution with an interval of confidence of 99,9 %. In a second step, we selected 42 trios for which the identity of the father is certain with a theoretical equal probabilities of full compatible and semi-compatible offspring. We found 11 offsprings fully compatible and 31 offsprings semi-compatible with their respective mother. Again, the observed repartition was clearly outside the interval of confidence of 99 %, and therefore most probably resulted from a selection of the semi-compatible foetuses during pregnancy. We concluded that MHC fully compatible cynomolgus macaque foetuses have a selective survival disadvantage in comparison with foetuses inheriting a paternal MHC haplotype differing from maternal haplotypes.

W5.19.05
The atypical chemokine receptor D6 is expressed by trophoblasts and plays roles in placental formation and chemokine scavenging

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Placental dysfunction contributes to many important obstetric problems, including preeclampsia and fetal growth restriction. The crosstalk between maternal and fetal cells is critically important for effective placental function. Chemokines have been implicated in both the regulation of trophoblast migration into the uterus, and the recruitment of maternal immune cells into gestational tissues. The focus of this study is the atypical chemokine receptor D6, which has been hypothesized to be involved in reducing inflammation at the fetomaternal interface. Chemokine receptor expression by primary human trophoblasts did not vary with length of time in culture, however D6 mRNA was more abundant than other receptors (CCR1, CCR2, CCR3, CCR5, CCRL1, CXCR4) examined. To confirm expression of D6 protein in these cells, Western blotting and immunofluorescent staining were performed. The ability of trophoblasts to internalise CCL2 from the culture medium was found to be D6-dependent, thus demonstrating the chemokine scavenging ability of D6. D6-deficient mice exhibit higher rates of stillbirth and neonatal death than their wild-type counterparts (stillbirth, KO 21.3%, WT 10.2%; neonatal death, KO 37.0%, WT 16.7%, both $p<0.0001$). Placental stereology revealed that at E14 of gestation, D6-deficient mice have a larger junctional zone than wild type mice, and a smaller labyrinthine zone. However, by E18 of gestation, these differences are diminished. This is the first study to examine D6 in primary human cells, demonstrating a role for this receptor in immune regulation at the fetomaternal interface. In addition, our animal studies also suggest a role for D6 in regulating placenta formation.

W5.19.06
Genital Chlamydia trachomatis Infection Induced MicroRNA Profiles May Contribute to Associated Inflammation and Reproductive Sequelae

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Chlamydia trachomatis (CT) is the leading cause of human bacterial sexually transmitted infections and reproductive pathological sequelae. Given the involvement of microRNAs (miRs) as immune regulators and that CD4+ and CD8+ T cells and cytokines IFN-γ and IL-17A are associated with anti-chlamydial immunity, we analyzed modulation of miRs in genital tracts (GT) following CT challenge of wild type (WT), CD4+ deficient (CD4-/-), CD8+ deficient (CD8-/-), IFNγR deficient (IFNγR-/-) and IL17A deficient (IL17A-/-) C57BL/6 mice. CT elementary bodies (5X104 IFU) were inoculated intravaginally for analysis of GTs at days 6 (early infection) and 12 (established infection). Within WT LGT (vagina & cervix) at day 6, miRs significantly down-regulated were miR-125b-5p (-17.34 fold change), miR-16(-8.87), miR-214(-4.30), miR-182(-19.29) and miR-30c (-19.15) while miR-135a (+13.89) was significantly upregulated, profiles not exhibited by day 12 in LGT. We found significant differences in regulation of miR-125b-5p (+4.9), miR-135a (+4.7), miR-183 (+7.8) and miR-182 (+3.23) in CD4-/- GT and miR-125-5p (+5.7), miR-183 (+5.6) and miR-214 (+3.3) in IL17A-/- GT compared to WT mice. Antigen specific CD8+ T cells or IFNγ did not contribute to changes in these miRs. Moreover, these miRs were differentially modulated upon increasing CT challenge doses and in vitro knockdown analyses with specific inhibitors of these miRs resulted in 30-45% increase in CT infectivity. Since antigen specific CD4+ T cells and IL17A have been associated with CT clearance and GT pathology, we are currently investigating the contribution of these down selected miRs in modulating immunity in cohorts of CT-infected women with inflammation and reproductive pathology.

W5.20 Tumor immunity and immunosurveillance

IL5.20.01
IL-1β is a major inflammatory cytokine that mediates the angiogenic switch in tumor invasiveness

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Previously, we have demonstrated the crucial involvement of IL-1β in tumor-mediated angiogenesis, through mechanisms not yet elucidated. Here, we have assessed the role IL-1β in the early angiogenic response of tumors using Matrigel plugs supplemented with B16 melanoma cells. We described a new cross-talk between bone marrow-derived $VEGFR1^+/IL\text{-}1R1^+$ immature myeloid cells and tissue-resident endothelial cells in the early angiogenic response of tumors. Myeloid cells produce IL-1β and other pro-inflammatory molecules, while endothelial cells, activated by myeloid cell-derived cytokines, produce in turn VEGF and other direct angiogenic factors. Thus, endothelial cells are initially activated by IL-1β and subsequently they support in an autocrine manner blood vessel formation. This mechanism was also observed in inflammation-induced angiogenesis in Matrigel plugs supplemented with recombinant IL-1β or VEGF, as well as in Matrigel plugs containing supernatants of hypoxic macrophages, representing non-tumor scenarios of inflammation-induced angiogenesis. Furthermore, IL-1β inhibition stably reduces tumor progression, through limiting the local inflammatory response, but also by induction of maturation of $VEGFR1^+/IL\text{-}1R1^+$ cells into M1 macrophages with anti-tumor features. In sharp contrast, VEGF neutralization induced only a transient reduction in progression of tumor, followed by rebound angiogenesis and recurrence. Rebound angiogenesis occurs via re-programming $VEGFR1^+/IL\text{-}1R1^+$ cells that start to express HIF-1α and subsequently secrete VEGF and other direct angiogenic factors, which support in a paracrine manner endothelial cells for blood vessel formation. We suggest IL-1β inhibition as an efficacious anti-tumor therapy and its further optimization may leads to its application in the clinic.

IL5.20.02
Notch regulates anti tumor functions of gamma delta T cells in oral cancer

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Gamma delta (γδ) T cells exhibit anti microbial and anti tumor functions and are activated by phosphoantigens produced as intermediates of the mevalonate pathway in eukaryotic cells. The aim of the present study was to investigate the role of Notch signaling in regulating anti tumor responses of γδ T cells isolated from patients with oral cancer. γδ T cells from oral cancer patients showed impaired proliferative responses and IFN-γ release in response to stimulation with phospho antigens. mRNA expression of Notch1 and Notch2 genes were predominantly observed in peripheral γδ T cells. The presence of active Notch intracellular domain in γδ T cells was confirmed by confocal microscopy and western blotting. Proliferation of γδ T cells and IFN-γ release in response to phosphoantigens was inhibited in the presence of gamma secretase inhibitor (GSI) confirming involvement of Notch signal. Delta like ligands 1 and 4 activated γδ T cells while Jagged1 inhibited the γδ T cell response. Decreased expression of CD107a, and diminished lysis of oral tumors in the presence of GSI confirmed that Notch was involved in cytotoxic effector functions of γδ T cells. mRNA and protein expression of Jagged1 was observed on oral tumors. Blocking of Jagged1 ligand on regulatory T cells rescued the Treg induced suppression of γδ T cells. Thus, our data demonstrates that Notch signal plays an indispensable role in anti tumor responses of γδ T cells. Future immunotherapeutic strategies can exploit Notch signaling in γδ T cells to achieve therapeutic benefits in oral cancer patients.

W5.20.01
CD70 reverse signaling on NK cells enhances anti-tumoral immune response

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CD70 is the unique ligand for the TNF-receptor superfamily member CD27. CD70 is not expressed in healthy individuals. Upon immune activation CD70 is transiently induced on B- and T lymphocytes, NK cells and subsets of dendritic cells. The CD27-CD70 interaction provides a positive costimulatory signal through various downstream pathways resulting in proliferation,differentiation and survival of lymphocytes. As for other TNF family members, CD70 reverse signaling has been reported on normal and malignant B cells, T cells and NK cells. However, the physiological relevance of CD70 reverse signaling in vivo is poorly understood. In the present study, we analysed the role of CD70 reverse signaling in the immunological control of solid tumors. Tumor fragments expressing a truncated CD27 receptor consisting of the extracellular domain were transplanted subcutanteously on both flanks of BL/6 mice and tumor growth was monitored. Growth of tumors expressing the truncated form of CD27 was significantly reduced when compared to growth of parental tumor cells. Furthermore, tumor growth of cells expressing truncated CD27 was restored to growth of non-transfected control cells in mice treated with a monoclonal antibody blocking the CD27-CD70 interaction. In contrast, tumor growth of cells expressing truncated CD27 was significantly slower in Rag2-/- mice, indicating that inhibition of tumor development was independent of adaptive immunity. In contrast, depletion of NK cells in Rag2-/- restored growth of tumors expressing truncated CD27 to growth kinetics of control cells. Taken together, CD70 reverse signaling on NK cells contributes to tumor growth control.

W5.20.02
Braf inhibitor impacts on melanoma cell immunogenicity

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Melanoma incidence is increasing for several decades. Malignant melanoma displays a high metastatic potential. Conventional therapies induce rare sustained clinical responses in metastatic patients. Recently, targeted therapies directed towards signaling pathways altered in melanoma are evaluated in clinical trials.
BRAF mutations (V600E/K) are present in 50% of melanoma metastases. When the tumor harbors one of these mutations, treatment with BRAF inhibitors induces clinical responses in high percentages of patients. However, patients often experience a relapse of the disease, after 6-9 months of treatment. Thereby a better understanding of the mechanisms responsible for the resistance is required to design more active molecules or combined treatments. Furthermore MAPK inhibitors can induce off-target effects and thus may affect the regulation of immune cells.
We have recently showed that melanoma cell lines express stress molecules and are efficiently lysed in vitro by Natural Killer (NK) cells. In that context, we investigate the interest of combining immunotherapy with targeted therapy.
Seven melanoma cell lines displaying different mutational status were phenotyped for the expression of NK ligands. NK mediated cytolysis of melanoma cell lines was assessed by dynamic measure of adherent target cell index (xCELLigence system). We have studied how BRAF inhibitor interferes with melanoma/NK cell interactions. We showed that treatment with BRAF inhibitor modulates the expression of NK ligands on metastatic melanoma cell lines with different BRAF mutated status. It also interferes with the lysis of target cell by NK cells. Our data support the therapeutic potential of combining BRAF inhibitors with immunotherapy.

W5.20.03
Predictive clinical significance of tumor infiltrating neutrophils in patients with colorectal cancer

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Background: Infiltrating myeloid cells, mainly macrophages, have been involved in tumor growth and progression. Neutrophils have emerged as candidate cells that may modulate the tumorigenic process and the antitumor immunity. Epidemiological studies and animal models suggest that tumor-infiltrating neutrophils may be associated with poor clinical outcome.
Objective: To investigate the clinical significance of tumor-infiltrating neutrophils in 128 primary Stage I-Stage IV colorectal cancer (CRC) patients.
Method: A previous comparative analysis showed that CD66b is a more specific neutrophil marker compared to myeloperoxidase (MPO), which is also expressed by monocytes/macrophages. CRC histological sections were immunohistochemically treated with monoclonal antibodies against CD66b. For each section, intratumoral and peritumoral neutrophils densities were expressed as immunoreactive area ($CD66b^+$ IRA) in three randomly selected and non-contiguous fields using a computer-aided image analysis system.
Results: None of the clinico-pathological aspects affected values of CD66b+IRA, assessed both at the invasive margin (IM) and in the intratumoral compartment. Higher percentage of $CD66b^+$ IRA (based on the median value) both at the intratumoral compartment and IM were associated with better Disease Specific Survival (DSS) (p=0.001 and p=0.01, respectively) and Disease Free Survival (DFS) (p=0.01 and p=0.03, respectively). Multivariate analysis demonstrated that high intratumoral $CD66b^+$ IRA is an independent risk factor for better patient DSS (HR 0.48; 95% CI: 0.25-0.92; p= 0.03) and DFS (HR 0.58; 95% CI: 0.33-1.02; p= 0.0579).
Conclusions: Our findings suggest that intratumoral neutrophils are associated with a positive clinical outcome and might be considered as a novel independent prognostic factor in CRC patients.

W5.20.04
MicroRNA-9 regulates maturation and function of myeloid-derived suppressor cells by modulating Runx1

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Myeloid-derived suppressor cells (MDSCs) accumulate in tumor-bearing hosts and play a major role in tumor-induced immunosuppression, which hampers effective immunotherapeutic approaches. Our previous study showed that β-glucans could promote monocytic MDSCs (M-MDSCs) differentiate into more maturate myeloid cells and down-regulate the suppressive capacity of MDSCs. However, the molecular networks regulating maturation and function of MDSCs are largely unknown. MicroRNAs (miRNAs) are involved in the regulation of immunity, including the immune cell development and differentiation. In this study, we identified that microRNA-9 (miR-9), whose expression was dramatically reduced after β-glucan stimulation, was considered an essential player in regulating the maturation and function of MDSCs. Inhibition of miR-9 remarkably promoted the maturation of MDSCs and down-regulated the suppressive effect of the cells by targeting runt related transcription factor 1 (Runx1), whereas overexpression of miR-9 significantly enhanced the activity of MDSCs and reversed β-glucan induced maturation. In addition, down-regulation of Runx1 resulted in increased suppressive effect of MDSCs and inhibited the maturation process. Furthermore, knockdown of miR-9 significantly impaired the activity of MDSCs and inhibited the tumor growth of Lewis lung carcinoma in vivo. Collectively, our findings reveal that miR-9 expression in MDSCs plays a critical role in the molecular events governing the maturation and function of MDSCs and might be identified as a potential target in cancer therapy.
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W5.20.05
Neuropilin-1 guides regulatory T cells into tumor tissues resulting in impaired anti-tumor immunity

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Based on their immunosuppressive activity CD4+CD25+Foxp3+ regulatory T cells (Tregs) interfere with different immune responses including anti-tumor immunity. Elevated numbers of Tregs are found in the peripheral blood and tumor mass of cancer patients and are proposed to correlate with a poor prognosis. However, by which mechanism Tregs infiltrate the tumor tissue to locally suppress an effective anti-tumor immune response remains elusive. Here, we demonstrate that Neuropilin-1 (Nrp-1) highly expressed by Foxp3+ Tregs, mediates Treg infiltration into melanoma in dependency on tumor-derived Vascular Endothelial Growth Factor (VEGF). Mice deficient for T cell-expressed Nrp-1 exhibited a tremendously reduced tumor growth in a tumor transplantation model as well as in a spontaneously-driven melanoma model accompanied by a more pronounced CD8+ T cell response and significantly decreased numbers of Foxp3+ Tregs within the tumor section. Importantly, this phenotype was reversed by application of Nrp-1+ Tregs from wildtype mice. Due to the known function of Nrp-1 as a co-receptor for VEGF, we propose that Nrp-1 driven Treg tumor infiltration is dependent on tumor-produced VEGF. Indeed, we detected less Treg numbers in VEGF deficient tumors associated with a more effective CD8+ T cell response and clearly reduced tumor growth. From this we conclude that Nrp-1 guides Tregs into VEGF-producing tumor sections to locally suppress anti-tumor immunity.

W5.20.06
A novel self-lipid antigen targets human T cells against CD1c+ leukemias

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T cells that recognize self-lipid antigens presented by CD1c molecules are frequent in the peripheral blood of healthy individuals and are capable of killing transformed hematopoietic cells, but little is known about the antigen specificity and the potential anti-leukemia effects of this intriguing T cell population. We find that CD1 molecules are frequently present on primary acute myeloid leukemia and B-cell acute lymphoblastic leukemia cells and exhibit disease-specific patterns of expression, with CD1c being the most prevalent isoform. CD1c self-reactive T-cells recognize the novel class of self-lipid antigen methyl-lysophosphatidic acids (mLPAs), which are expressed in primary leukemia cell lines and blasts. CD1c self-reactive T-cells efficiently recognized CD1c+ leukemia cells and were poorly responsive to non-transformed CD1c-expressing cells. Methyl-lysophosphatidic acid-specific T-cells protected NOD/scid mice against grafted CD1c+ human leukemia cells in vivo. The identification of a novel self-lipid antigen expressed in leukemia cells and the observed control of malignant cell growth by lipid-specific T-cells in vivo provide a new conceptual framework for leukemia immune surveillance and immunotherapy.

W5.20.07
Complement dependent immunosurveillance in Her-2/neu autochtonous carcinomas arising in BALB-neuT transgenic mice

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Complement is a plethora of plasma and membrane-bound proteins acting at the interface of innate and adaptive immunity. Beside its role against infections, complement is an important component of tumor microenvironment. To evade complement-mediated inhibition cancer cells express membrane-bound and fluid-phase complement regulators. However, the importance of complement as microenvironmental regulator of tumor growth is not yet elucidated.
With a 100% penetrance all inbred BALB/c female mice transgenic for the rat ErbB-2 (Her-2/neu) oncogene (BALB-neuT mice) develop a carcinoma in all their mammary glands. The step-wise progression of these autochthonous tumors recapitulates several molecular and genetic features of human cancer, among which a slow progression, the natural occurrence of invasion and metastasis, and the presence of a long-lasting interaction between the evolving lesion and its microenvironment. By crossing BALB-neuT with BALB/c mice deficient for C3, we observed that the onset of the first palpable tumor is accelerated with a dramatic increase both in the number of mammary glands with tumor and in the tumor growth rate. Whole mounts of the mammary gland show that at the same week of age a higher number of focal lesions is spread all over mammary glands in BALB-neuT/C3KO compared to BALB-neuT mice. These data show, probably for the first time, that complement proteins can hamper even the onset of a mammary cancer aggressively driven by the overexpression of the ErbB2 oncogene that results markedly accelerated by the absence C3 in the tumor microenvironment.

W5.20.08
Active STAT5 imprints a T-Bet-dependent Tc-1 program in CD8 T cells inducing efficient regression of autochthonous melanoma

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We have developed a genetically engineered TiRP mouse model which recapitulates key aspects of human melanoma,through the association of a melanocyte-specific deletion of both p16 and p19/Arf with a gain-of-function mutation of Ras. Development of melanoma in the inducible TiRP mouse strain is associated with systemic chronic inflammation and non functional endogenous CD8 T cells (TCs). A main limitation of TCs-based immunotherapy is the poor ability of transferred TCs to infiltrate the tumor mass and to resist the suppressive microenvironment. This includes abrogation by TGFβ1 of effectors molecules in CD8 TCs and promotion of aberrant Tc-17 polarization in conjunction with IL-6.
We engineered expression of activated STAT5 transcription factors (STAT5CA) in CD8 effector TCs (eTCs). When adoptively transferred in melanoma bearing TiRP mice, STAT5CA-transduced eTCs produced superior anti-tumor effects compared to non-transduced eTCs (Grange, Cancer Research 2012-72:p76). STAT5CA expression highly increased Granzyme B expression in intra-tumor CD8 eTCs implying that it can render eTCs resistant to the immunosuppressive environment.
At the molecular level, STAT5CA induced sustained expression of genes controlling tissue homing, cytolytic granule composition, Tc-1-associated cytokines and the potential for secondary responses. ChIP-Seq analyses showed that genes encoding T-Bet and Eomes transcription factors are
direct STAT5 target genes. Additionally, STAT5CA-expressing CD8 eTCs demonstrated reduced IL-6R/TGFbRII expression and dampened IL-6 and TGFβ1 signaling. The absence of T-Bet restored IL-2 secretion and IL-6R/TGFbRII expression and signaling, as illustrated by IL-17 induction. Therefore concerted STAT5/T-Bet/Eomes regulation controls homing, recall responses and resistance to Tc-17 polarization in CD8 eTCs.

W5.20.09
T cells from DNAM-1 deficient mice display polarity defects that impair anti-tumour immunity

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DNAX accessory molecule 1 (DNAM-1/CD226) is an adhesion molecule expressed on all CD8+ cytotoxic T cells (CTLs) that acts as an accessory molecule to promote CTL functions such as activation, differentiation, migration and effector function. DNAM-1 interacts with LFA-1, a critical molecule for immunological synapse formation between T cells and antigen presenting cells (APC), and for cytotoxic killing of target/tumour cells. Indeed, mice lacking DNAM-1 display abnormal T cell responses and anti-tumour activity; however, the mechanism involved is not well understood. Here, we investigate the immunological synapse between DNAM-1 deficient T cells and APC, and show that DNAM-1 deficiency results in reduced proliferation and expansion of T cells in response to antigen presentation, which correlates with a decrease in cytokine production. Furthermore, we demonstrate that activated DNAM-1 deficient T cells show reduced stable conjugations with tumour cells and decreased recruitment of LFA-1 and lipid rafts to the immunological synapse, which correlates with reduced killing in vitro. This polarity defect may explain why DNAM-1 deficient mice cannot clear tumours in vivo and highlights the importance of the immunological synapse in T cell mediated anti-tumour immunity.

W5.20.10
Density of Tertiary lymphoid structures predicts T cell infiltration with coordinated Th1 polarization and cytotoxic effectors functions in human lung cancer

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Purpose/Objective: Numerous studies have demonstrated a strong correlation between the density of tumor-infiltrating T cells (TIL) and clinical outcome in human solid cancers. Nevertheless, the mechanisms governing T cells recruitment and activation into tumors remain poorly characterized. In lung cancer patients, we demonstrated that density of tertiary lymphoid structures (TLS) is associated with a favorable clinical outcome. We hypothesized that TLS are implicated in the shaping of a protective anti-tumor immune response and investigated their impact on the infiltration, differentiation and functionality of TIL in lung tumors.
Materials and Methods: The expression of relevant molecules was assessed by multicolor flow cytometry on fresh tumor samples, by immunohistochemistry on tissue sections and by low density array analysis on frozen tumors.
Results: A specific chemoattractants gene expression signature associated with high endothelial venules and T cells presence was identified in TLS. Most TLS T cells had a naive and early-memory T cells phenotype, as observed in canonical secondary lymphoid organs, whereas T cells located outside TLS were mainly composed of experienced T cells. We demonstrated that TLS density predicts a strong infiltration of activated effector-memory T cells. Finally, T-helper 1, activation and cytotoxic-related genes were overexpressed and coordinated with TLS density in human lung tumors.
Conclusions: Altogether, these data suggest that TLS represent a privileged area for T cell recruitment and activation in the primary site of the tumor. These results provide strong evidence supporting a major role of these structures in the shaping of a local anti-tumor immune response.

W5.20.11
Galectin-3 is involved in the immunosuppressive activity of prostate cancer stem cells

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As cancer stem/initiating cells (CSC) are the major culprits of tumor development, it's reasonable to believe that they can adopt mechanisms of immune evasion to maintain their niche. Here we provide evidence that prostate CSC (PCSC) obtained from autochthonous tumors developed in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice exert immunosuppressive activities on T lymphocytes, by inhibiting their proliferation and activation in vitro. PCSC-conditioned T cells showed reduced activation of TCR and IL-2 pathways, characteristics of anergic T cells. Immune suppression is mediated by several molecules, including Galectin-3. Indeed, Galectin-3 was found overexpressed on PCSC and in TRAMP and human prostate cancer tissues. Furthermore, silencing of Galectin-3 in PCSC reduced their immunosuppressive activity on T cells. While an immunosuppressive role of PCSC in vivo still needs to be demonstrated, our data suggest that targeting Galectin-3 in vivo might render the PCSC more susceptible to the attack by T cells.

W5.20.12
The BCR regulates Burkitt lymphoma survival and proliferation through the modulation of GSK3β activity

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Mature B-lymphocytes rely on expression of a functional B-cell-antigen-receptor (BCR) for their survival(1,2,3). Non-Hodgkin B-cell lymphomas (NHL) originate from mature B cells recruited into the germinal center reaction during a T-cell-dependent immune response. Most NHL retains expression of a functional BCR, suggesting a role for the BCR in the proliferation and/or survival of lymphoma cells. Using a Burkitt's lymphoma (BL) mouse model, in which conditional ablation of the BCR can be induced in tumor B cells through the Cre/loxP recombination system, we provide genetic proof that the BCR plays an essential role in BL maintenance. In response to BCR loss, tumor cells succumbed to their BCR+ counterparts both in vitro and in vivo. In BL cells, the BCR regulated survival and cell-cycle progression. Pharmacological inhibition of GSK3β prevented the competitive loss of BCR- tumors, whereas interference with the mTOR pathway accelerated their disappearance. The BCR controlled over 400 genes in BL cells most of which through modulation of GSK3β activity. Experiments are underway to determine which gene(s) critically contributes to the competitive advantage provided by the BCR to BL cells. Taken together, our study shows that the BCR supports the growth and survival of c-Myc-transformed B cells through the modulation of the PI3K/GSK3β pathway. Importantly, our data provide the rationale for the combination of small molecule inhibitors targeting BCR signaling (through PI3K/GSK3β) and the mTOR pathway for the treatment of BL and possibly other NHL.
1)Lam et al, Cell 1997
2)Kraus et al, Cell 2004
3)Srinivasan et al, Cell 2009

W5.20.13
Inhibiting the development of immunosuppressive Gr-1+ Dendritic Cells in Tumors by blocking TLR2 enhances immunotherapy

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Cancer cells secrete tumor-derived factors that activate immune cells to modulate the tumor inflammatory microenvironment. Recently, we found that tumor-derived TLR2 ligands promote differentiation of DC precursors (pre-cDC) towards an immunosuppressive Gr-1+ subpopulation (Gr-1+ cDC). Here, we attempt to enhance adoptive transfer immunotherapy in B16 melanoma models by inhibiting Gr-1+ cDC development. Compared to wildtype mice, we found that tumors from TLR2-/- mice contained much lower frequency of Gr-1+cDC. In addition, wildtype mice treated with monoclonal TLR2 antibody also contained a lower frequency of Gr-1+ DC in tumors compared to mice treated with isotype control antibody. To assess the effects of blocking TLR2 in adoptive transfer immunotherapy, wildtype and TLR2-/- mice bearing B16 or B16-OVA tumors were treated with CTL derived from OT-I mice. OT-I CTL treatment of TLR2-/- mice bearing B16-OVA tumors showed delayed tumor growth and improved survival compared to their wildtype counterparts. Furthermore, adoptively transferred OT-I CTLs produced more IFNγ after OVA peptide stimulation in vitro. Using the same immunotherapy model, we showed that inhibition of Gr-1+cDC in TLR2-/- mice enhanced proliferation and expansion of cytotoxic CD8+ cells. Compared to wildtype mice bearing B16-OVA, adoptively transferred CD8+ T cells in TLR2-/- tumor bearing mice showed higher expression of T cell activation markers, CD25 and CD69. Together, these results demonstrate that inhibiting the development of intratumor Gr-1+cDC by blocking TLR2 improves antitumor CTL responses and suggest a possible target for tumor immunotherapy.

W5.20.14
Evaluating parameters that influence T cell responses to tumor antigens: A role for B7-H4

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The B7 family plays a critical role in regulating immune responses by engaging a variety of receptors on lymphocytes. Importantly, monoclonal antibodies against co-inhibitory molecules CTLA-4 and PD-1 have been shown to have an impact on promoting tumor immunity in patients. It is critical to understand the role of different family members as potential therapeutic targets. B7-H4 is another member with inhibitory function that is expressed on antigen presenting cells and also up-regulated on a variety of tumors. Here we show that B7-H4 expression by the tumor inhibits tumor growth in mouse models as well as in breast cancer patients. Furthermore, using a mouse model of breast cancer, we show that B7-H4 is critical for anti-tumor immunity. Importantly, in both mice and human samples, B7-H4 has an impact on class I expression via the polycomb repressor complex 2, which dampens IFN-γ signals. Consequently, the reduced class I expression impairs vaccine induced CD8+ immunity to tumors. Significance: The data presented are novel and timely appropriate for a broad range of audience. Notably, innovative monoclonal antibody treatments (i.e. ipilumumab) have been proposed to block negative signals, which improve antitumor immunity. Our data suggests that targeting B7-H4 in a similar fashion may not be beneficial for immune therapy. These findings are significant in that they present a slight paradigm shift, alternative to previous results. Additionally, our data provide insight into understanding factors critical for the induction of anti-tumor immunity, which can potentially predict patient populations that may respond best to immune therapy.

W5.21 Myelo- and lympho-proliferative disorders

IL5.21.01
Cytokines and B cell lymphoproliferative disorders

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We have studied for a long time the relationships between cytokines and malignant B cells. These cells produce cytokines which may act as autocrine/paracrine growth factors or modulate cells in the tumor microenvironment towards a cancer-promoting phenotype. Conversely, cytokines produced by the latter cells can bind receptors expressed by neoplastic B cells and interfere with their functions. IL-12 is a heterodimeric cytokine binding a heterodimeric receptor (R) composed of the beta 1 and beta 2 chains and belonging to a superfamily that includes IL-23, IL-27 and IL-35. In the last decade, we have investigated the expression and function of the IL-12R in human malignant B cells spanning all stages of B cell differentiation, i.e. from acute lymphoblastic leukaemia (B-ALL) to multiple myeloma (MM) and identified three different paradigms. In paradigm 1, referred to B-ALL, the IL-12R beta 2 chain is not expressed as happens also in progenitor/precursor B cells; in paradigm 2, referred to chronic B cell malignancies, the beta 2 chain is not expressed due to methylation of a CpG island in exon 1 of the gene; in paradigm 3, referred to MM, the beta 2 chain is expressed at lower levels than in normal plasma cells. In paradigm 2, the IL-12R beta 2 chain gene has been formally identified as tumor suppressor. Additional studies carried out by the Airoldi's group with IL-23 and IL-27 will be discussed. Taken together, these results have translational relevance and may lead to the development of novel therapeutic strategies.

W5.21.01
Variation in B cell receptor signaling between stereotypic and heterogeneous chronic lymphocytic leukemia

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The existence of stereotyped B cell receptors (BCR) in different CLL patients is interpreted as an indication for specific antigenic stimulation driving CLL pathogenesis. These stereotypic BCRs can be found in 30% of CLL patients, while the other 70% express a heterogeneous receptor. So far, it has not been fully clarified which antigens are recognized by the CLL BCRs. In a recent paper by Dühren-von Minden (Nature 2012), CLL was shown to be driven by (higher basal) autonomous signaling based on Ca^{2+} influx assays rather than antigen-dependent signals. This autonomous signal is induced by the heavy chain complementarity determining region (HCDR3) of the CLL BCRs, which recognize an internal epitope within framework 2 of the BCR. However, the observed autonomous signaling does not explain the selection for stereotypic BCRs. Therefore, we aimed to see if there is variation in autonomous BCR signaling between stereotypic versus heterogeneous BCRs in CLL. From our cohort of well-defined CLL samples consisting of cases belonging to specific stereotypic CLL subsets and heterogeneous CLL, we selected representative samples for all major stereotypic subsets. In these samples, we investigated BCR signaling by use of Ca^{2+} influx assays.
Our results show variation in BCR signaling between stereotypic and heterogeneous CLL cells, with frequently a higher basal Ca^{2+} influx in heterogeneous CLL cells compared with CLL cells belonging to stereotypic subsets. These results would imply that heterogeneous CLL cells generally show autonomous BCR signaling, while stereotypic CLL cells might need their specific antigen to effectively induce BCR signaling.

W5.21.02
The bone-targeting of IL-27 is mediated by the poly-glutamic acid motif of its p28 subunit

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Interleukin 27 (IL-27) is a cytokine known to play an important role in the regulation of T, B or NK cells functions. Its potent antitumoral properties have made it attractive for the treatment of certain types of cancer.
The heterodimeric cytokine IL-27 is composed of the p28 subunit associated to the soluble receptor EBI3 (EBV-induced gene 3). Like the other members of the IL-6/IL-12 family, the p28 subunit is composed of four α helixes (A, B, C, D). Unlike other cytokines, p28 comprises a unique, negatively charged motif, in the loop connecting helixes C and D. The high conservation of this poly glutamic acids (polyE) motif, suggests a physiological role. A homologous polyE motif in the bone sialoprotein mediate binding to hydroxyapatite, the main bone mineral component.
We analyzed whether the p28 polyE motif allows : 1) IL-27 binding to hydroxyapatite and bone matrix ; 2) can be used as a tag for protein targeting to bone. Using a mutated form of IL-27, we demonstrated that IL-27 binds to hydroxyapatite and bone matrix through the polyE domain *in vitro*. Furthermore, our results demonstrate that IL-27 immobilized on bone remains active. Finally, we observed that *in vivo*, the p28 polyE motif targets IL-27 to the bone and is important for the activation of bone marrow cells.
In conclusion, the acidic motif specific to p28 might confer to IL-27 benefical pharmacological properties for therapy targeting immune cells located in endosteal niches.
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W5.21.03
MIP-1α/CCL3-mediated maintenance of leukemia initiating cells in the initiation process of chronic myeloid leukemia

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In the initiation process of chronic myeloid leukemia (CML), a small number of transformed leukemia initiating cells (LICs) co-exist with a large number of normal hematopoietic cells, gradually increasing thereafter and eventually predominate in the hematopoietic space. However, the interaction between LICs and normal hematopoietic cells at the early phase has not been clearly delineated due to the lack of a suitable experimental model. In this study, we succeeded in causing a marked leukocytosis resembling CML from restricted foci of LICs in the normal hematopoietic system by direct transplantation of *BCR-ABL* gene-transduced LICs into the BM cavity of non-irradiated mice. Herein, we observed that BCR-ABL^{+}lineage^{-}ckit^{-} immature leukemia cells produced high levels of an inflammatory chemokine, MIP-1α/CCL3, which promoted the development of CML. Conversely, the ablation of *CCL3* gene in LICs dramatically inhibited the development of CML. Furthermore, normal hematopoietic stem/progenitor cells (HSPCs) can directly impede the maintenance of LICs in BM in the absence of CCL3 signal.

W5.21.05
Early growth response gene 2 and 3 are essential for the regulation of tumour suppressor genes, Ikaros, Aiolos and FOXO3

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Non-Hodgkin lymphoma is the fifth most common cancer in the UK, accounting for 4% of all new cases and is associated with inflammatory autoimmunity. The understanding of lymphomagenesis still remains a challenge. We have discovered that early growth response gene (Egr) -2 and -3 are essential for the control of inflammatory responses of lymphocytes and also involve in the regulation of antigen-mediated proliferation. In addition to the control of inflammatory responses, here we report a novel role of Egr- 2 and/or -3, in controlling the development of lymphoma. The deletion of Egr-2 and Egr-3 in lymphocytes results in spontaneous B or T lymphoma. Despite poor responses to antigen stimulation, Egr-2 and -3 deficient B and T cells are resistant to apoptosis and survival longer in vitro in the absence of overt stimulation. We have discovered that Egr-2 and/or -3 directly bind to the promoter regions on Ikaros, Aiolos and FOXO3. The deletion of Egr-2 and -3 down regulates Ikaros, Aiolos and FOXO3 in T and B cells. The impaired expression associates with proliferative disorder and the development of T and B cell lymphoma. Thus, Egr-2 and -3 are suppressors for lymphomagenesis through the regulation of tumour suppressor genes Ikaros, Aiolos and FOXO3.

W5.21.04
Detection of *de novo IGHV* mutations by ultra-deep sequencing from *in vitro* activated B-cell chronic lymphocytic leukemia cells: Evidence for activation-induced deaminase function

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Human B-cell chronic lymphocytic leukemia (CLL) is a clonal CD5^{+}CD19^{+} B-lymphocyte whose B-cell receptor may be classified as unmutated (U-CLL) or mutated (M-CLL) depending on the level of *IGHV* mutation. Aggressive CLL associates with acquisition of new gene mutations and cytogenetic aberrations, perhaps caused by activation-induced deaminase (AID). To test if CLL cells can produce functional AID, CLL cells were activated by *in vitro* culture with CD32-transfected murine L cells, anti-CD40 and interleukin-4 (7 and 14 days, U-CLL1278 0.0% mutated *IGHV3-30*)(14 days M-CLL1299, 4.9% mutated *IGHV3-23*), plus irradiated T lymphocytes (10 or 14 days M-CLL1299). CLL cells in these cultures produced detetable AID protein. To evaluate mutational activity, CLL *IGHV* cDNA was ultra-deep sequenced using the 454 FLX system (Roche) prior to (day 0) or after activation. The resulting 458,124 sequence reads were processed to generate fixed sequence length datasets. Individual subclone sequences occuring at least twice were extracted and unique *de novo* subclones not shared between day 0 and activation were analyzed for new mutations. All culture conditions showed increases in *IGHV* mutation frequency relative to the *IGHM* constant region. U-CLL1278 showed increased mutation at AID hotspots, but an overall increase in transversion frequency. M-CLL1299 showed an overall high frequency of transitions, but an increase in mutation at AID hotspots only in the 10 day culture. Thus, *de novo* mutations consistent with AID activity were found, with some differences between U-CLL and M-CLL. Mutational active AID in CLL could lead to adverse consequences.

W5.21.06
CD27 signaling promotes the proliferation of human acute myeloid leukemia cells

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The TNF receptor superfamily member CD27 is a costimulatory molecule mainly expressed on lymphocytes. Interaction of CD27 with CD70, the unique CD27-ligand which is only expressed on activated immune cells, leads to lymphocyte expansion and differentiation towards effector cells. Recently, it was shown that CD27 is also expressed on hematopoietic stem cells. We now demonstrate that CD27 and CD70 are both expressed on cell lines of acute myeloid leukemia (AML). The CD70-CD27-interaction promoted proliferation of AML cells by enhancing the Wnt signaling pathway through nuclear translocation of β-catenin. Similarly, CD27 was expressed on immature myeloid blasts in the blood and bone marrow (BM) of AML patients. Blocking CD27 on primary AML blasts reduced myeloid colony formation in vitro. In addition, serum soluble CD27, a marker for the CD70-CD27-interaction in vivo, was increased in AML patients and correlated with BM infiltration by immature blasts. Our results show for the first time that CD27 signaling plays a role in the pathogenesis of AML, revealing CD27 as a potential new therapeutic target in leukemia.

W5.22 Primary immunodeficiencies

W5.22.01
Mucosal immune dysregulation in Omenn Syndrome

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Omenn syndrome (OS) is a rare monogenic disorder associating immunodeficiency and autoimmune-like manifestations that can present in the form of inflammatory bowel disease (IBD)-illness. We studied the intestinal homeostasis of the Rag2R229Q/R229Q murine model, closely recapitulating the human disease. Constant signs of inflammation, in both small and large intestines of mutant mice, were associated to different degree of spontaneous colitis, wasting diarrhea, and rectal prolapse, leading sometimes to early death. Increased cellularity of CD4+T cells and, intriguingly, of Foxp3+ Treg cells in the Rag2R229Q/R229Q mice was a unique feature of the lamina propria compartment, sharply contrasting with the overall

lymphoid depletion observed in the other peripheral lymphoid organs. Abnormal leukocyte trafficking to the intestinal mucosa was confirmed by the significantly higher percentages of CCR9 and a4b7-expressing splenic memory T cells in the mutants compared to WT mice. Interestingly, intestinal inflammation was associated to a Th1/Th17 cytokine predominance, strongly associated to the development of IBD in humans. In contrast to T cells, B cells are poorly present in the gut of Rag2R229Q/R229Q mice, as shown by the lack of Peyer's Patches and markedly reduced mucosal IgA levels. Studies are underway to investigate whether gut-blood barrier defects and break of tolerance to commensal microbiota play a pathogenic role in the immune dysregulation associated with hypomorphic RAG defects.
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W5.22.02
A new form of global leucopenia associated with mutation in the moesin gene

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In 5 young patients (1.3- 15 years) from 4 differents families, we observed a profound lymphopenia (181-660 CD3+/ul;7-14 CD19+/ul) and low neutrophils count (0.2-1.4. 10-3/ul). Among the T lymphocytes, the naive compartment was particularly low and T cell proliferation in vitro is decreased as compared to the controls. All patients have a hypogammglobulinemia and a poor response to vaccinal Ag. During childhood, most of them developed severe varicella, pneumopathies and recurrent pulmonary infections.
Using exome sequencing analysis, we have identified in all patients a unique missense mutation in the moesin gene (Xq11.1), introducing an amino acid change into the highly conserved FERM domain of the protein (p.R171W). Moesin is a member of the ERM protein family which link plasma membrane proteins with actin-based cytoskeletons and are implicated in various cellular functions such as survival, adhesion, migration and activation. This mutation is associated with a low protein expression especially in T cells. Upon in vitro activation, proliferation (as measured by CFSE analysis and cell cycle checkpoints analysis) and survival capacity of patient's T cells were impaired despite expression of activation markers like CD25 and CD69. In addition, we observed enlarged synapses in patient's blasts. We reported here, for the first time, the association of a defect in an ERM protein and a primary immune deficiency.

W5.22.03
Critical role of DOCK8 in dendritic cell trafficking during T cell immune responses

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DOCK8 is a member of the evolutionarily conserved DOCK family proteins that function as guanine nucleotide exchange factors (GEFs) for the Rho family of GTPases. Mutations of DOCK8 cause combined immunodeficiency syndrome in humans, yet the mechanism underlying T-cell dysfunctions remains unknown. In this study, we show that in the absence of DOCK8, dendritic cells (DCs) fail to accumulate in the lymph node parenchyma for T-cell priming. We found that DOCK8 KO mice exhibited a severe defect in antigen-specific T cell proliferation when antigens were injected into mouse footpads. Adoptive transfer experiments revealed that antigen presenting cell functions are primarily affected in the absence of DOCK8. DOCK8 deficiency affected neither antigen uptake nor presentation by DCs. However, when mice were painted on the flank skin with 1% FITC solution, accumulation of FITC+ DCs was severely impaired in the case of DOCK8 KO mice. Indeed, the migration efficiency of DOCK8-deficient DCs was reduced to less than 25% of the wild-type level. Collectively, these results indicate that DOCK8 plays a key role in DC trafficking during T cell immune responses.

W5.22.04
Investigating T cell subsets in DOCK8 immunodeficiency

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DOCK8 immunodeficiency is a rare human immunodeficiency that gives rise to susceptibility to a limited range of viral infections (human papilloma virus, herpes virus and molluscum contagiosum) as well as frequent sino-pulmonary infections. The patients also have allergic disease, increased levels of IgE and a significant rate of hematological and non-hematological malignancy[1,2]. Patients have a complete resolution of their infections with PBSC transplant.
Previous work carried out in ENU-mutant mouse models of DOCK8 immune deficiency have shown abnormal immune synapse formation in both B and T cells[3,4] but no clear mechanism for the specificities of the infections, nor the presence of allergic disease, have been found. Mouse models show some but not all features of DOCK8 disease, in particular no increase in IgE has been found in the DOCK8$^{pri/pri}$ mouse model on a C57Bl/6 background.
To further investigate the T cell polarization of CD4 T cells isolated from the DOCK8$^{pri/pri}$ mouse model, we have analyzed the number of splenic TH1, TH2 and TH17 cells. Analysis has been carried out using both polarizing and non-polarizing conditions, and for total splenic cells and FACS-sorted naïve T cells. No increase in TH2 cells were seen with either TH2 polarizing or non-polarizing conditions. In addition, no defect in TH17 cells was noted.
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W5.22.05
Resolving the heterogeneity of common variable immune deficiency by exome sequencing and deep phenotyping

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Considerable progress has been made elucidating the cellular and genetic basis of congenital human primary antibody deficiency. More than 80% of cases are accounted for by mutations in BTK. Recently, additional autosomal recessive genetic defects have been identified by whole exome sequencing of affected offspring of consanguineous parents. In contrast, most cases of non-congenital primary antibody deficiency (including common variable immune deficiency) remain unexplained. This group is clinically and serologically heterogeneous, and slow progress in elucidating the genetic cause has been attributed to assumed genetic heterogeneity. We have assembled a large CVID cohort from centres across Australia and are investigating causal pathways by combining whole exome sequencing with deep phenotyping. The results of the first 50 whole exome sequences obtained from a subgroup within the cohort confirm the genetic heterogeneity of the disorder. Furthermore, recessive or biallelic damaging mutations appear to be uncommon. Consequently, illumination of causal pathways demands specific analysis of candidate damaging mutations guided by comprehensive analysis of both B and T cell phenotypes. Our results have revealed novel gene defects within known CVID-associated signaling pathways, as well as defects in new pathways that combine to help explain the complexity of the disorder.

W5.22.06
Altered B and T lymphocyte homeostasis in the bone marrow and the periphery correlates with clinical findings in Common Variable Immunodeficiency (CVID)

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Introduction. Common Variable Immunodeficiency (CVID) is characterized by hypogammaglobulinemia due to defective B cell maturation. In almost 10% of cases, affected patients may present a reduction in the number and/or percentage of peripheral B cells; T cell alterations may also be present.
Objective. Evaluate B cell maturation in the bone marrow of CVID patients and correlate the results with peripheral B and T lymphocyte homeostasis and clinical findings.
Methods. Lymphocyte homeostasis (bone marrow, periphery) was studied by means of four-color flow cytometry.
Results. One third of the patients presented a developmental defect in the early phases of B cell maturation (group A). This defect was associated with B cell lymphopenia in the periphery in the presence of normal T cell development. Peripheral B cell lymphopenia was observed in a second group of CVID patients (group B) where bone marrow development was undisturbed. In this group however, CD4+ T cells and RTE cells were severely reduced. Finally, 50% of CVID patients presented normal B cell development in the bone marrow, normal percentages/numbers of B and T cells in the periphery (group C). Group C presented only URTI, group B was characterized by URTI and LRTI, important splenomegaly and gastrointestinal infections (during 10 year follow-up). Finally, group A was characterized by URTI and LRTI, bronchiectasis (already at diagnosis), invasive infections (at diagnosis) and autoimmune phaenomena.
Conclusions. CVID patients may be subdivided in three groups based on B and T lymphocyte homeostasis with significant differences in the severity of clinical manifestations.

W6.01 Immunomodulation by nutrients and vitamins

IL6.01.01
Stopping T cells in their tracks, vitamin D suppresses naïve CD8 T cell proliferation

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Diseases where T cell derived IFN-γ and IL-17 is pathologic like inflammatory bowel disease are suppressed by active vitamin D (1,25(OH)2D3). Paradoxically, infectious diseases that require these same responses for protection are unaffected by 1,25(OH)2D3 treatments. Vitamin D receptor (VDR) knockout (KO) CD8+ T cells, but not wildtype (WT) CD8+ T cells, induced colitis in Rag KO recipients. In addition, co-transfer of VDR KO CD8+ T cells with naïve CD4+ T cells accelerated colitis development. The more severe colitis was associated with rapidly proliferating VDR KO CD8+ cells and increased IFN-γ and IL-17 in the gut. Naïve CD8+ VDR KO T cells proliferated more rapidly than WT CD8+ T cells in vivo and in vitro. The increased proliferation of VDR KO CD8+ cells was due in part to the higher production and response of the VDR KO cells to IL-2. Vitamin D is critical in the control of CD8+ T cell proliferation. T cells express low levels of the vitamin D receptor until 48h post-stimulation. In addition, CD8+ T cells produce the 1-alpha hydroxylase that converts 25(OH)D3 into 1,25(OH)2D3 but the enzyme is not induced before 48h of stimulation. T cell regulation by vitamin D is a late event. Therefore the data support a new model where vitamin D is required to shut off the T cell response. The inability to signal through the VDR results in the generation of pathogenic CD8+ T cells from rapidly proliferating cells that contribute to the development of inflammation in the gut.

IL6.01.02
Vitamin D regulates complement receptor immunoglobulin (CRIg) expression in human macrophages

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An entirely new facet of vitamin D effects distinct from those on homeostasis and bone metabolism has been recognised, which includes the regulation of macrophage function in immunity to infection and in chronic inflammatory diseases. Macrophages have all the requirements to synthesise and respond to 1,25-dihydroxy vitamin D3 (1,25D). This active form of vitamin D stimulates innate immunity and the anti-bacterial activity of macrophages. Since CRIg, a complement receptor distinct structurally and functionally from the classical complement receptors CR3/4, is expressed only by a subpopulation of macrophages and is involved in the rapid phagocytosis of complement (C3b/iC3b)-opsonised bacteria and immunity to *Listeria monocytogenes* and *Staphylococcus aureus*, the relationship between CRIg expression and vitamin D metabolism was studied in human macrophages. Monocytes derived macrophages cultured in the presence of 1,25D showed a marked increase in CRIg mRNA and protein expression. This effect was reflected in increased phagocytosis of complement-opsonised particles and microbial killing by the macrophages. In contrast, 1,25D had no effect on the expression of CR3 and CR4. These results were conducive with the effects of the steroidal anti-inflammatory agent dexamethasone, which also selectively increased CRIg expression and increased the anti-microbial activity of macrophages. Interestingly, the pro-hormone 25 hydroxy vitamin D had no effect on CRIg expression on macrophages unless the cells were stimulated with a Toll-like receptor 2/1 agonist. The results suggest a role for an intracrine action of vitamin D in innate immunity and underscore the need to maintain adequate levels of vitamin D in the community.

W6.01.01
Immunomodulatory role of vitamin D in allergic airway inflammation and airway hyperresponsiveness in bronchial asthma

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Rationale: Allergic airway inflammation and airway hyperresponsiveness (AHR) are hallmarks of asthma. NF-κB is a potent transcriptional factor involved in the generation of many inflammatory cytokines and chemokines. Active subunits of NF-κB are transported from cytosol to the nucleus by importin-α3 and importin-α4. Vitamin D is a potent immunomodulator regulating immune response. However, role of vitamin D in allergic asthma is still unclear. Here, we examined the effect of vitamin D status on allergic immune response in a mouse model.
Methods: Female pups from vitamin D-deficient BALB/c parents were continued on vitamin D-deficient mice. In parallel, mice were fed with vitamin D-sufficient (2,000IU/kg) or vitamin D-supplemented (10,000IU/kg) diet for 13wks, then sensitized and challenged with ovalbumin (OVA). Effect of vitamin D status was examined on AHR, airway inflammation, and the expression of importin-α3 and NF-κB in the lungs.
Results: Three groups of mice had serum 25(OH)D levels (ng/ml) of 5.0+0.2, 31.0+0.7, and 67.1+0.5, respectively at 13wks following OVA challenge. Vitamin D deficiency exaggerated AHR, collagen deposition, mucus gland hyperplasia, BAL eosinophilia, levels of BALF pro-inflammatory cytokines, and reduced blood T-regulatory cells and BALF IL-10 levels. In the lungs of vitamin D-deficient mice, there was increased expression of importin-α3 and NF-κB and decreased expression of VDR. Vitamin D supplementation did not completely reverse the features of allergic airway inflammation and AHR, but increased circulating T-regulatory cells and reduced pro-inflammatory response.
Conclusion: Vitamin D could be beneficial as an adjunct therapy in the treatment of allergic asthma.

W6.01.02
Effects of w3 fatty acid in experimental model of mucositis induced by 5-FU

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Mucositis is caused by chemotherapy and radiotherapy cytotoxic in cancer patients and is important to investigate new strategies for control mucositis. The administration of immunomodulators such as ω3 fatty acid could be an alternative to this. So, the aim of this study was to evaluate the effects of ω3 fatty acids in an experimental model of mucositis.
Thirty two male BALB/c were divided into four groups: Control (CTL), Mucositis (M), Control+ω3 (CTL+ω3) and Mucositis+ω3 (M+ ω3). The animals of ω3 group received a diet prepared with ω3 (50% of total lipid content) during ten days. On day tenth, mice received an intraperitoneal injection containing 200mg/kg of 5-FU. Three days after, the small intestines were collected for histological, oxidative stress and cytokine analyzes.
Animals subjected to mucositis showed microscopic lesions in jejunum and ileum. There were increased amount of lamina propria cells, altered villus/crypt ratio, necrosis, hypersecretion of Paneth cells and inflammatory infiltration. However, ω3 pretreatment was able to reduce the intensity of those effects. There was also a decrease in the levels of GSH in M compared to CTL. In mice treated with ω3, there is a decrease in IL-6 in ileum. There was no difference between the levels of IFN, IL-4 and IL-10 with ω3 treatment, although 5-FU seems to reduce cytokine secretion in jejunum and ileum.
The 5-FU leads to alterations in intestinal mucosal architecture and decrease levels of GHS and cytokines in BALB/c mice. The pre-treatment with ω3 appears to alleviate the damage of small intestine mucosal.

W6.01.03
The *Agaricus blazed*-based mushroom extract, Andosan™, has immuno- suppressive and anti-inflammatory effects when used as supplement to multiple myeloma patients receiving high-dose chemotherapy and autologous bone marrow transplantation

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Agaricus blazei Murill (AbM) is an edible Brazilian *Basidiomycetes* mushroom used in traditional medicine against cancer that has antitumor effects against myeloma and other cancers in mouse models. Ahn et al 2004, reported increased NK cell activity and life quality in patients with gynecological cancer who received AbM extract as supplement to high-dose chemotherapy. We found anti-inflammatory effects in inflammatory bowel disease patients of the AbM-based extract, Andosan™, also containing related medicinal mushrooms; *Hericium erinaceus* (15%) and *Grifola frondosa* (3%). In vitro Andosan™ activated human MDDC and increased proliferation of normal CD56+ hematopoietic stem cells. A placebo-controlled, double-blinded study in 33 multiple myeloma patients who drank 60 ml/day of Andosan™ for 7 weeks as supplement to high dose chemotherapy with autologous stem cell support, showed these immunological findings:
In the Andosan™ relative to placebo group there were increased Treg cells (CD4+, CD127d + CD25+) in the apheresis product harvested for bone marrow transplantation after 2 weeks treatment, and increased plasma levels after 7 weeks in IL-1 receptor antagonist, IL-5 and IL-7 cytokines and up-regulation of immune function-related genes in the bone marrow. This shows that the AbM-based mushroom extract, Andosan™, may have clinically significant immuno-suppressive and anti-inflammatory effects in multiple myeloma patients when used as supplement to high-dose chemotherapy with autologous bone marrow transplantation. The T reg cell increase is probably influenced by enhanced IL-7, a modulator of T cell development. There was no difference in circulating lymphocytes between the patient groups. Clinical status and survival rates are being supervised.

W6.01.04
Hepatocyte nuclear factor 4 alpha regulates immune cell activation and autoimmunity

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Hepatocyte nuclear factor (HNF) 4α, a member of the nuclear receptor superfamily, plays an important role in regulating metabolic processes such as glucose and lipid homeostasis. Long chain fatty acyl-CoA (FA-CoA) thioesters are endogenous, high affinity ligands for HNF4α and, depending on their chain length and degree of saturation, differentially affect HNF4a transcriptional activity. In fact, saturated FA-CoA activate HNF4α while polyunsaturated FA-CoA decrease HNF4α function. HNF4a is essential for liver development and function, but it has been also implicated in a number of diseases, including diabetes, inflammatory bowel disease and cancer, so that specific antagonists of this nuclear receptor have been developed for therapeutic intervention. Here we provide evidences for a role of HNF4α in immunity. In fact, HNF4a is expressed in human immune cells and its blockade by the antagonist BIM5078 impairs activation of and cytokine release from human monocytes and T-cells. Similarly, in animal models BIM5078 inhibits splenic T cell responses to the self antigen MOG35-55. Finally, in vivo administration of BIM5078 after induction of experimental autoimmune encephalitis (EAE) ameliorates clinical expression of EAE. In conclusion, HNF4a-dependent gene transcription sustains immune cell activation and autoimmunity, and therapeutic targeting of this signaling pathway is beneficial in experimental neuroinflammation. This work was financially supported by Merck Serono S.A., Geneva, Switzerland. Merck Serono is the biopharmaceutical division of Merck KGaA, Darmstadt, Germany

W6.01.05
Mast cell protease expression is reduced in vitro and in vivo in response to cinnamon treatment

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Mast cells (MC) contain large amounts of proteases like chymase, tryptase and carboxypeptidase A (MC-CPA) in their secretory granules. MC proteases being released play a critical role in inflammatory processes. Cinnamon extract is known to affect diverse immunological cells like macrophages and shows anti-inflammatory capacities. Here, we analyzed the effect of cinnamon extract on the expression of pro-inflammatory MC proteases in vitro and in vivo. Expression of tryptase and MC-CPA was analyzed in vivo in the intestine of wild type mice as well as of IL-10 knockout mice - as a model of inflammatory bowel disease - after oral treatment with cinnamon extract in comparison to controls. In addition, human MC isolated from intestinal tissue were incubated with cinnamon extract prior to IgE mediated stimulation. Expression of MC proteases was examined by real time RT-PCR and Western blotting. Following oral treatment with cinnamon extract, expression of MC-specific proteases tryptase and MC-CPA was significantly reduced in wild type and IL-10 knockout mice. Moreover, severity of disease (weight loss, consistency of stool, rectal inflammation) was reduced in IL-10 knockout mice. In human MC from intestinal mucosa, cinnamon extract also caused a significantly decreased expression of tryptase and chymase. In summary, cinnamon extract mediates anti-inflammatory activities by reducing expression of MC-specific proteases in vitro and in vivo, and thus is a new plant-originated candidate for diverse MC associated therapeutical applications.

W6.01.06
Immunomodulatory effects and anti-tumor activities of conjugated linolenic acids on the murine macrophage-like leukemia cells

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Naturally-occurring conjugated linolenic acids (CLN) derived from various plant seed oils have shown to possess various biological and pharmacological activities, including antioxidative, hypolipidemic, chemopreventive and anti-tumor properties. Although CLN are known to inhibit the growth of several cancer cell lines *in vitro*, however, their modulatory effects on macrophages and their action mechanisms on macrophage-like leukemia have not been investigated. In the present study, various CLN isomers were found to exhibit potent anti-proliferative effect on the murine macrophage-like leukemia PU5-1.8 cells in a time- and dose-dependent manner. The most potent CLN isomer, jacaric acid, also exerted significant growth-inhibitory effects on other murine macrophage-like leukemia cell lines *in vitro*. Mechanistic studies indicated that jacaric acid could lead to cell cycle arrest of PU5-1.8 cells at the G0/G1 phase, and this was associated with a decrease in cyclin E and CDK2 proteins, and an increase in p21, p27 and p53 proteins. Moreover, jacaric acid could induce apoptosis in PU5-1.8 cells as revealed by DNA fragmentation, phosphatidylserine externalization, and down-regulation of anti-apoptotic Bcl-2 and Bcl-xL proteins expression. Furthermore, pre-treatment of PU5-1.8 cells with jacaric acid significantly reduced the *in vivo* tumorigenicity of the leukemia cells in syngeneic BALB/c mice. Interestingly, jacaric acid exhibited no significant cytotoxicity on the thioglycollate-induced peritoneal macrophages but stimulated their cytostatic activity towards tumor cells and production of nitric oxide *in vitro*. Collectively, our results indicate that CLN can stimulate macrophages and exert their anti-tumor effects on the macrophage-like leukemia cells through cell cycle arrest and apoptosis induction.

W6.02 Metabolism and immunity interfaces

IL6.02.01
Polyphenols from red grape and modulation of the immune Response

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Polyphenols, largely present in the vegetal kingdom, are endowed with a plethora of biological activities. Over recent years, we have been working with different extractions of polyphenols derived from red grape. In our initial studies, polyphenols extracted from red wine Negroamaro (N) were shown to *in vitro* release nitric oxide from human circulating monocytes as well as an array of cytokines from lymphomonocytes. In this last regard, a balance between production of inflammatory and anti-inflammatory cytokines was demonstrated. In addition, N polyphenols were able to inhibit activation of NF-KB pathway by LPS. In a second model, using fermented grape marc (FGM) from Koshu (K) and N *Vitis Vinifera*, especially K-FGM was able to *in vivo* attenuate murine experimental colitis with a dramatic reduction of TNF-α and IL-1β in colon homogenates. Both, N-FGM and K-FGM when *in vitro* incubated with peripheral human granulocytes and lymphomonocytes, led to the following results:
1. Increased expression of FoxP3 in CD4 lymphocytes;
2. Increased production of IL-10;
3. Decreased release of granzyme B from CD8+ lymphocytes;
4. Decreased release of oxygen radicals from granulocytes and monocytes;
5. Reduction of basophil burst.
Finally, epigallocathechin gallate extracted from grape seeds and contained in the Leucoselect® Phytosome® preparation, was able, when administered to frail elderly subjects, to recover the depressed Th1 response in these subjects. Taken together, these results emphasize the immunological properties of polyphenols and their potential application in inflammatory conditions.

IL6.02.02
Intracellular metabolic pathways control immune tolerance

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The field that links immunity and metabolism is rapidly expanding. Apparently non-immunological disorders such as obesity and type 2 diabetes have been linked to immune dysregulation suggesting that metabolic alterations can be induced by or be consequence of an altered self-immune tolerance. In this context, a key role is played by signalling systems acting as metabolic "sensors" linking energy/nutritional status to regulatory T (Treg) cell functions such s the adipose tissue derived hormone, leptin. We propose that a dynamic/oscillatory activity of intracellular metabolism, particularly through the mammalian target of rapamycin (mTOR) complex, might represent a shift in understanding the molecular mechanisms governing Treg cell tolerance. In particular, the decision between Treg cell proliferation and hyporesponsiveness arises from their ability to probe the extracellular milieu and, modulating the metabolic intracellular signalling, to determine different qualitative and quantitative functional outcomes. The possibility to interfere with these pathways should lead to novel approaches for treatment of chronic-inflammatory and autoimmune disorders.

W6.02.01
Insulin impairs regulatory T cell function: implications for obesity

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Chronic inflammation is known to drive metabolic dysregulation in obesity and type 2 diabetes. Although the precise origin of the unchecked inflammatory responses in obesity is unclear, it is known that over-production of pro-inflammatory cytokines such as TNF-α by innate immune cells has a key role in the development of metabolic dysregulation. One key hallmark of obesity is high levels of the pancreatic hormone insulin, and we hypothesized that there may be an unknown link between hyperinsulinemia and chronic inflammation. Here we show that high levels of insulin impair the ability of regulatory T cells (Tregs) to suppress inflammatory responses via effects on the AKT/mTOR signaling pathway. Insulin strongly activates AKT/mTOR signalling in Tregs, leading to specific inhibition of the production of the anti-inflammatory cytokine IL-10. As a result, insulin hinders the ability of Tregs to suppress the production of TNF-α by macrophages. Tregs from the visceral adipose tissue of hyperinsulinemic, obese mice also have a decrease in IL-10 production and a parallel increase in production of IFN-γ. These data suggest that the hyperinsulinemia associated with obesity may contribute to the development of obesity-associated inflammation via a previously unknown effect on Treg function.

W6.02.02
Investigating the anti-inflammatory effects of High Density Lipoprotein in macrophages

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Elevated plasma levels of High Density Lipoprotein (HDL) are associated with decreased risk of cardiovascular disease (CVD). The protective role of HDL in atherosclerosis has been attributed primarily to its ability to remove excess cholesterol from lipid-laden macrophages (foam cells) within the arterial walls. Clinical trials that simply raise HDL cholesterol levels have failed to show a therapeutic

benefit, casting doubts on our basic understanding of HDL function. Atherosclerosis is a chronic inflammatory condition underlying CVD and driven in part by the recognition of metabolic danger signals by innate immune receptors on macrophages. A potential feature that could contribute to its protective effects in CVD could be HDL's anti-inflammatory nature, such as its ability to reduce endothelial cell activation. However, the molecular mechanisms by which HDL reduces inflammatory macrophage responses remain poorly understood. Here we show that HDL protects against Toll-like receptor (TLR)-induced inflammation both in vivo and in vitro under normocholesteremic conditions by suppressing the transcription of inflammatory cytokines. We identify Activating Transcription Factor 3 (ATF3), a transcriptional repressor of the CREB family of basic leucine zipper transcription factors, as a HDL-inducible regulator of macrophage activation. HDL's ability to down modulate TLR responses was severely compromised in ATF3-deficient cells demonstrating that ATF3 mediates HDL's anti-inflammatory effects in macrophages and may explain the broad anti-inflammatory functions of HDL.

W6.02.03
Obesity-associated autoantibody production requires AIM to retain IgM immune complex on follicular dendritic cells

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Natural IgM is polyreactive not only to foreign antigens but also to autoantigens, and thus, believed to be important for progression of autoimmunity. In blood, pentameric IgM loaded with antigens forms a large immune-complex containing various elements including apoptosis inhibitor of macrophage (AIM). Here we demonstrate that this IgM-AIM association critically contributes to the development of autoantibody producing plasma cells under obese condition. In mice fed a high-fat diet (HFD), natural IgM increased through B cell toll-like receptor-4 stimulation. Blood AIM associated with IgM pentamer at the Fc region. This association protected AIM from renal excretion, increasing blood AIM levels along with the obesity-induced natural IgM augmentation. Meanwhile, the AIM association inhibited IgM binding to the Fcα/μ receptor on splenic follicular dendritic cells (FDCs), thereby protecting the IgM immune complex from Fcα/μ receptor-mediated internalization. This supported IgM-dependent autoantigen presentation to B cells, stimulating IgG autoantibody production. In obese AIM-deficient (*AIM*$^{-/-}$) mice, the increase of multiple IgG-autoantibodies, observed in obese wild-type mice, was abrogated. Accordingly, the increase in high-affinity IgG producing plasma cells in response to HFD was diminished in the *AIM*$^{-/-}$ bone marrow. Thus, the AIM-IgM association plays a critical role in the obesity-associated autoimmune process.

W6.02.04
Novel factors regulating chemerin synthesis and function in fat tissue

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Chemerin is a chemoattractant protein with adipokine properties. First identified as tazarotene- (retinoic acid-analog) induced gene 2 (TIG2) in tazarotene treated psoriatic skin, it has gained more attention over the past few years due to its multilevel impact on metabolism and immune responses. Pleiotropic actions of chemerin include chemotaxis of dendritic cell, macrophage and NK subsets, bactericidal activity, regulation of adipogenesis and glucose metabolism. The mechanisms underlying systemic and/or local chemerin expression remain obscure. Given the high levels of chemerin message in the liver and adipocyte tissue, as well as nanomolar levels of circulating protein, we hypothesize that, like certain other plasma proteins, chemerin expression and secretion by liver and adipocytes might be regulated by factors released upon inflammation. In these studies we demonstrate that oncostatin M (OSM), a cytokine of the IL-6 family, regulates chemerin expression and/or synthesis in mouse visceral white adipose tissue (vWAT) as well as mouse adipocytes differentiated from vWAT derived preadipocytes. Moreover, we show that OSM acts synergistically with IL-1b, leading to strong up-regulation of chemerin levels in vWAT and adipocyte cultures. In contrast to adipocytes, OSM or OSM+IL1b exerted weak if any response in liver or primary cultures of mouse hepatocytes, suggesting that the effect of the cytokines on chemerin expression is specific to fat tissue. Up-regulation of chemerin expression in vWAT by OSM and IL-1b correlates with changes in differentitation of adipocytes and the influx of natural killer cells into vWAT, suggesting that increases in chemerin levels might contribute to fat tissue remodeling.

W6.02.05
Amino acid sensing by mTORC1 is the vital prerequisite for alternative activation of macrophages

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Mechanistic target of rapamycin complex 1 (mTORC1) is an integrative sensor for nutrition and growth signals. Although the role of mTORC1 in several organs and some immune cells, such as T lymphocytes and dendritic cells, have been elucidated, little is known about its implications for macrophages. Gene expression microarrays comparing classically activated macrophages (CAM) with alternatively activated macrophages (AAM) revealed that a molecule of amino acid sensing pathway, namely p18/LAMTOR1, was predominantly expressed in AAM. Macrophage-specific p18 conditional knockout mice (p18 cKO) showed massive accumulation of macrophages in peripheral organs, and 50 to 200 fold greater serum levels of pro-inflammatory cytokines after the challenge with endotoxin. Surprisingly, macrophages derived from p18 cKO mice showed defective polarization of AAM with little expression of its signature genes such as arginase-1, resistin-like molecule alpha, and IL-10. Consistently, this defective polarization was reproduced by interfering amino acid sensing pathways: amino acid starvation, v-ATPase inhibition, and mTOR inhibition. These data indicated that mTORC1 pathway is the vital prerequisite for the polarization of AAM. Collectively, our present study shows first evidence that the amino acid sensing by mTORC1 is essentially required for AAM polarization.

W6.02.06
Gene profile of myeloid-derived suppressive cells from the bone marrow of lysosomal acid lipase knock-out mice

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Lysosomal acid lipase (LAL) controls development and homeostasis of myeloid lineage cells. Loss of the lysosomal acid lipase (LAL) function leads to expansion of myeloid-derived suppressive cells (MDSCs) in LAL knock-out (lal-/-) mice. Ly6G^{+} positive MDSCs actively participate in inflammation-induced pathogenic processes in lal-/- mice by suppressing T lymphocytes. Affymetrix GeneChip microarray analysis identified detailed intrinsic defects in Ly6G^{+} myeloid lineage cells of *lal-/-* mice. Ingenuity Pathway Analysis revealed activation of the mammalian target of rapamycin (mTOR) signaling, which functions as a nutrient/energy/redox sensor, and controls cell growth, cell cycle entry, cell survival, and cell motility. Loss of the LAL function led to major alteration of large GTPase and small GTPase signal transduction pathways. *lal-/-* Ly6G^{+} myeloid cells in the bone marrow showed substantial increase of cell proliferation in association with up-regulation of cyclin and cyclin-dependent kinase (cdk) genes. The epigenetic microenvironment was significantly changed due to the increased expression of multiple

histone cluster genes, centromere protein genes and chromosome modification genes. Gene expression of bioenergetic pathways, including glycolysis, aerobic glycolysis, mitochondrial oxidative phosphorylation, and respiratory chain proteins, was also increased, while the mitochondrial function was impaired in *lal-/-* Ly6G^{+} myeloid cells. The concentration of reactive oxygen species (ROS) was significantly increased accompanied by up-regulation of nitric oxide/ROS production genes in these cells. This comprehensive gene profile study for the first time identifies and defines important gene pathways involved in the myeloid lineage cells towards MDSCs using *lal-/-* mouse model.

W6.03 Pathogenesis and immunointervention in diabetes

IL6.03.01
Soluble CD52 released by activated T cells interacts with Siglec-10 to suppress T cell activation and prevent autoimmune disease

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Functionally diverse T-cell populations interact to maintain immune homeostasis. In testing the hypothesis that paracrine mechanisms limit proliferation of T cells following their activation we found that a sub-population of antigen-activated T cells characterized by high expression of the GPI-anchored glycoprotein CD52 suppresses other T cells. CD52hi CD4+ T cells are distinct from CD4+CD25+FOXP3+ regulatory T cells by several criteria, including their methylation at the FOXP3 locus and their mode of action. Suppression by CD52hi CD4+ T cells is mediated by soluble CD52, released by the action of phospholipase C. Soluble CD52 then binds to the inhibitory Siglec-10 receptor on T cells, leading to impaired phosphorylation of the T-cell receptor-associated tyrosine kinases Lck and ZAP-70 and impaired T-cell activation. This homeostatic mechanism may be important for the prevention of autoimmune disease. Thus, humans with, and at risk for, type 1 diabetes displayed reduced frequency and function of CD52hi CD4+ T cells generated in response to the pancreatic islet autoantigen GAD65 but not tetanus toxoid. Furthermore, in an autoimmune mouse adoptive transfer model, CD52hi-depleted splenic T cells from young, pre-diabetic mice markedly accelerated the onset of diabetes in syngeneic recipients. Our studies identify a previously undiscovered ligand and receptor mechanism of T-cell regulation that protects against autoimmune disease.

W6.03.01
Detection of Chromogranin A-reactive CD4^{+} T cells in type 1 diabetes in the nonobese diabetic (NOD) mouse

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We have identified the peptide WE14 as chromogranin A (ChgA) antigen for highly diabetogenic CD4+ T cell clones. Peptide truncation and extension analysis shows that WE14 bound to major histocompatibility complex class II (MHCII) molecule I-A(g7) in an atypical manner, occupying only the carboxy-terminal half of the I-A(g7) peptide-binding groove. We constructed modified fluorescent WE14 peptide-MHCII tetramer, in which WE14 was only bind to p5-p9 position of the I-A(g7) peptide-binding grove. In the nonobese diabetic (NOD) mouse, an animal model of type 1 diabetes (T1D), we used this peptide-MHCII tetramer to detect ChgA reactive CD4+ T cell in the pancreas, pancreatic lymph node and spleen, both in prediabetic and diabetic NOD mice. The results showed that ChgA reactive CD4+ cells accumulated into the pancreas at prediabetic stage and were disappearing from pancreas upon the onset of the disease. Further study of ChgA reactive CD4+ T cell hybridmas from NOD mice showed those T cells express a variety of T cell repertoire.

W6.03.02
IL21 is a critical regulator of chronic/persistent but not acute/accelerated auto-immune and allo-immune responses

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IL21 is critical for T1D development in NOD mice, but its role in sustaining T cell responses against islets during T1D progression remains unexplored. Our preliminary studies show that NOD mice produce high levels of serum IL21 during T1D onset. Thus, we hypothesize that IL21 produced by activated CD4 T cells in spontaneously diabetic NOD mice sustains T cell responses to islets. We show that IL21 is required for maintaining the function of auto-reactive immune cells during T1D progression as transient therapy with IL21 receptor fusion protein (IL21RFc) is effective in reversing T1D onset in NOD mice (n=11) and inducing long-term T1D remission for up to 30 weeks. Pancreatic infiltrates of IL21RFc-treated NOD mice contain mostly CD4 T cells, that have lost surface ICOS expression, and few CD8 T cells, suggesting that IL21RFc inhibits CD4 T cell help to CD8 T cells. In a parallel study, we also show that IL21 deficient mice are protected from chronic allograft vasculopathy in a MHC class-II mismatched cardiac allograft model. However, IL21 deficient mice acutely reject fully mismatched cardiac allografts, similar to wild type. Thus, we demonstrate a novel and critical role for IL21 signaling in driving chronic immune responses against auto and allo-antigens. Based on our findings, IL21 has the potential to act as a serum biomarker for early T1D detection and a therapeutic target to abrogate persistent T cell responses in T1D and chronic allograft rejection. We continue to investigate the molecular mechanisms governing IL21 mediated chronic immune responses.

W6.03.03
Postnatal events in intestinal gene expression and splenic cell composition is altered in NOD mice

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Evidence suggests that colonisation pattern of the gut in the early postnatal period is highly correlated with the risk of developing type 1 diabetes (T1D). We have recently shown that colonization in SPF mice accelerates gut maturation and that at postnatal day (PND) 1, in comparison with germ free mice, certain chemokines, including *Cxcl2* encoding macrophage inflammatory protein (MIP)-2 and involved in attraction of neutrophils was downregulated in the gut epithelium. The non-obese diabetes (NOD) mouse is widely used as a model for studying the pathogenesis of T1D, as it spontaneously develops insulitis at three to five weeks of age. The neonatal gut microbiota seems to play an important role in the development and control of T1D. We hypothesized that NOD mice in the perinatal period respond differently than mice not prone to develop T1D (C57/Bl6), and we investigated the differences in postnatal expression of genes in gut, spleen, liver and pancreas, and in cellular composition in spleen.
At PND1 and 2, the number of Ly-6G and CD11b positive cells in NOD mice was significantly higher as compared to C57/bl6. Furthermore, gene expression analyses of liver, spleen and intestine showed difference between the two mouse strains in the early postnatal expression of *Cxcl2* and the antibacterial lectin encoding *RegIII* gene. Our findings suggest that very early postnatal microbiota dependent events contribute to the development of T1D in NOD mice.

W6.03.04
Antigen-specific protection against highly aggressive cytotoxic T cells in diabetes by intradermal injection of heme-oxygenase-1 inducer

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Diabetes is the result of chronic inflammation created by CD4 or CD8 autoreactive T-cells attack of beta-cells in the pancreas. Once autoreactive CD4 or CD8 T-cells are activated, hijacking these cells from their initial target is challenging.
Heme oxygenase-1 (HO-1) is a cellular induced enzyme in response to inflammation that degrades free heme and results in the production of carbon monoxide (CO). We have recently obtained experimental data showing that anti-inflammatory properties of HO-1/CO can be used to in vitro induce tolerogenic dendritic cells that can decrease diabetes (Simon et al.Eur. J. Immunol. 2013).
The main objective of this research is to efficiently induce tolerance by manipulating HO-1 pathway directly in vivo that can be applicable to clinical practice. Autoimmune diabetes can be induced in Rat Insulin Promotor-Ovalbumin (RIP-OVA) transgenic mice by transfer of highly aggressive in vitro activated anti-OVA OT-I cells. Once transferred, these cells are very difficult to stop. However if an HO-1 inducer is intradermally injected along with OVA antigen, insulitis is reduced and diabetes can be prevented by more than 65%. This protection necessitates both the injection of the antigen and of HO-1 inducer. Inhibition of HO-1 activity is sufficient to abrogate the protection. Interestingly, we have been able to identify a tolerogenic MHC-class II population expressing high level of HO-1 induced in protected animals that can abolish the pathogenic capabilities of cytotoxic T cells. We are currently investigating the mechanism underlying the tolerogenic effect of these cells on cytotoxic T-cells.

W6.03.05
Inhibiting Th17 Cells and Reducing Insulitis in Diabetic NOD Mice by GW9508, a GPR40 and GRP120 Dual Agonist

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G-protein coupled receptor 40 (GPR40) and G-protein coupled receptor 120 (GPR120) are expressed on monocytes, macrophages, and intestinal L cells. We investigated the effect of GW9508 (GW), a GRP40 and GPR120 dual agonist, on modulating autoimmunity in NOD mice. New-onset diabetic NOD mice were treated with GW for up to 8 weeks. Nonfasting blood glucose was monitored. After the treatment, $CD4^+CD25^+FoxP3^+$ T (Treg) cells in thymus, spleen and pancreatic lymph nodes were measured. To detect $IL\text{-}17^+CD4^+$ T (Th17) cells, lymphocytes from these mice were cultured for 5 days. To determine in vitro effect of GW on Th17 cells, lymphocytes from NOD mice were cultured with or without GW. Cytokines and chemokines in supernatants were determined. Diabetes was reversed in 13% of vehicle (DMSO) treated mice, in 54% of mice treated with GW at 10mg/kg/day and in 69% of mice treated with GW at 20 mg/kg/day ($P<0.01$). The insulitis score was significantly lower in GW-treated mice ($P<0.05$). Although the percentage of Treg cells was not significant difference, the percentage of Th17 cells and the ratio of Th17 cells to Treg cells in GW-treated mice were significantly lower ($P<0.01$). Th17 cells in GW-treated lymphocytes in culture were significantly reduced ($P<0.05$). IL-17, IFN-γ, IP-10, RANTES, and MIP-1α in supernatants of GW-treated lymphocytes were also significantly reduced ($P<0.05$). The percentage of $insulin^+/Ki67^+$ β cells in islets was significantly higher in GW-treated mice than in prediabetic mice ($P<0.01$). Our data indicate that targeting GPR40 /GPR120 is a new therapeutic approach for treating type 1 diabetes.

W6.03.06
New insights into Type 1 diabetes development and therapy

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Type 1 diabetes (T1D) is an autoimmune disease in which the insulin-producing beta cells in the pancreas are destroyed by the immune system, although the molecular basis of this autoimmune destruction is unclear. We have recently reported that mouse islets express extraordinarily high levels of the glycosaminoglycan heparan sulfate (HS) intracellularly and are also surrounded by a basement membrane (BM) rich in HS. In fact, beta cells are exquisitely dependent on intracellular HS for their survival, with in vitro addition of HS or related molecules maintaining beta cell viability and rendering the cells highly resistant to damage by reactive oxygen species. Furthermore, we have found in vivo that destructive insulitis is associated with high level expression by leukocytes of enzymatically active heparanase, an endoglycosidase that degrades HS. In addition, in vivo treatment with several heparanase inhibitors significantly protected NOD mice from clinical diabetes. Based on these data we propose that initially leukocyte-derived heparanase degrades HS in the islet BM, allowing leukocyte entry into islets. Thereafter, heparanase-mediated degradation of beta cell-associated HS results in a novel mechanism of beta cell death. Recent studies using an acute T1D model in heparanase knock out mice has confirmed the importance of heparanase in T1D development, with heparanase expression by several different cell types being important. Thus heparanase inhibition, possibly combined with HS replacement in beta cells, has great therapeutic promise for treating T1D patients at an early stage of the disease and, potentially, for the prevention of T1D in “at risk” individuals.

W6.04 Treatment of rheumatoid arthritis and other inflammatory joint diseases

W6.04.01
Human cathelicidin-derived peptide IG-19 regulates cytokine IL-32-induced inflammation in human blood mononuclear cells, and provides protection in a murine model of arthritis

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Human cathelicidin host defence peptide LL-37 can suppress pathogen-induced inflammatory responses, while maintaining the ability to resolve infections. The objective of this study was to investigate the effects of LL-37 and its derivatives in immune-mediated ‘sterile’ inflammation. We demonstrated that LL-37 and its derivative peptide IG-19 significantly suppressed inflammatory cytokine IL-32γ-induced TNF-α and IL-1β production, induced anti-inflammatory cytokine IL-1RA, without neutralizing chemokine responses in human peripheral blood derived mononuclear cells. Mechanistic studies revealed that IL-32γ-induced phosphorylations of Akt-1 (T308) and MKP-1 (S359), and the activation of p44/42 MAPK was not suppressed by either LL-37 or IG-19. The peptides induced the phosphorylation of MKP-1 (S359), a known negative regulator of inflammation. In contrast, the peptides abrogated IL-32γ-induced phosphorylation of Fyn (Y420), an inducer of inflammation. The peptides exhibited an overall anti-inflammatory effect in the presence of IL-32, by abrogating the Fyn protein tyrosine kinase phosphorylation, and in contrast inducing the activity of the negative regulator MKP-1 dual phosphatase. As IL-32γ is directly linked to the severity of chronic inflammatory diseases including rheumatoid arthritis, we further investigated the effect of the peptide IG-19 *in-vivo* using a murine model of collagen-induced arthritis (CIA). We demonstrated that the administration of the peptide IG-19 decreased disease severity, infiltration of immune cells into the joints, and serum levels of anti-collagen antibodies and pro-inflammatory cytokines TNF-α and IFN-γ, in the CIA mice. This study demonstrates the potential of human cathelicidin-derived peptides to regulate immune-mediated inflammation and provide a rationale for examining their use for chronic inflammatory diseases.

W6.04.02
The pro-inflammatory activities of Interleukin-6 are mediated by the soluble Interleukin-6 receptor via trans-signaling

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Cytokines receptors exist in membrane bound and soluble form. The IL-6/soluble IL-6R complex stimulates target cells not stimulated by IL-6 alone, since they do not express the membrane bound IL-6R. We have named this process 'trans-signaling'. Soluble gp130 is the natural inhibitor of IL-6/soluble IL-6R complex responses. Recombinant soluble gp130 protein is a molecular tool to discriminate between gp130 responses via membrane bound and soluble IL-6R responses. We used neutralizing monoclonal antibodies for global blockade of IL-6 signaling and the sgp130Fc protein for selective blockade of IL-6 trans-signaling in several animal models of human diseases. Inhibition of IL-6 trans-signaling was beneficial in a sepsis model. Defense against bacterial infections rely on the membrane bound IL-6R. The extent of inflammation is controlled by trans-signaling via the soluble IL-6R. Using the sgp130Fc protein or sgp130Fc transgenic mice we demonstrate in animal models of inflammatory bowel disease, peritonitis, rheumatoid arthritis, atherosclerosis pancreatitis, colon cancer, ovarian cancer and pancreatic cancer, that IL-6 trans-signaling via the soluble IL-6R is the crucial step in the development and the progression of the disease. Therefore, sgp130Fc is a novel therapeutic agent for the treatment of chronic inflammatory diseases and cancer and it will undergo phase I clinical trials as an anti-inflammatory drug later in 2013.

1. Jones SA, Scheller J, Rose-John S (2011) Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. J Clin Invest 121: 3375-3383
2. Zhang H et al (2013) IL-6 trans-signaling promotes pancreatitis-associated lung injury and lethality. J Clin Invest, in press

W6.04.03
Survivin as a target for therapeutic RNA interference in experimental arthritis

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Survivin is a proto-oncogene with a major function in cell division and regulation of apoptosis. It has an emerging role in autoimmune diseases. In patients with rheumatoid arthritis, survivin is an independent predictor of joint damage and resistance to the conventional and biological anti-rheumatic treatment.
We study whether in vivo silencing of survivin is sufficient to reduce inflammation and joint damage in experimental arthritis.
Survivin targeting shRNA (shSurv) in a lentiviral delivery system was provided by a single injection (10^6-10^7 particles /mouse) on first immunization day 0 (mBSA arthritis) or at the onset of arthritis (collagenII arthritis, CIA). Control groups received non-targeting shRNA.
shSurv-treated mice had significant inhibition of survivin in spleen (12-51%) and bone marrow (7-29%). shSurv significantly alleviates clinical and histological signs of mBSA-arthritis and CIA, joint damage (microCT) and MMP13 expression in synovial tissue.
shSurv mice had low MHCII expression on dendritic and B cells, suggesting impaired antigen presentation. Additionally, shSurv increases Treg (Foxp3+CD4+) subset in spleen and lymph nodes, and increases Foxp3 mRNA and GATA3 mRNA and production of IL 10 and IL-4 in splenocytes. shSurv reduces serum levels of antigen-specific and autoantibodies (anti-Fc-gamma), both in CIA and mBSA-arthritis. Low antibody levels correlates to the reduction of survivin and to the severity of arthritis.
We show that in vivo silencing of survivin expression is sufficient to alleviate arthritis by promoting anti-inflammatory immune responses and by reduction of antigen- specific antibodies.

W6.04.04
Critical role of all-trans retinoic acid in stabilizing human nTregs under inflammatory conditions

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Objective Recent studies have demonstrated that thymus-derived naturally-occurring CD4+Foxp3+ regulatory T cells (nTregs) in both human and mouse are unstable and dysfunctional in the presence of pro-inflammatory cytokines. All-trans Retinoic Acid (atRA), the active derivative of vitamin A, has been shown to regulate Treg and T effector cell differentiation. We hypothesize atRA stabilizes human nTregs in the inflammation condition.
Methods Human nTregs pre-treated with atRA or control (DMSO) were expanded and re-stimulated with IL-1 and IL-6. Foxp3 expression and suppressive activities were analyzed in vitro and in vivo using a standard assay and a humanized animal model. The epigenetic regulation in the Foxp3 locus has been analyzed by DNA methylation and histone acetylation.
Results atRA prevents human nTregs from converting to Th1 and/or Th17 cells and sustains their Foxp3 expression and suppressive function in vitro or in vivo following encountering with IL-1 and IL-6. Interestingly, adoptive transfer of human nTregs pre-treated with atRA significantly enhanced their suppressive effect on xeno-graft versus host diseases (xGVHD) and only these cells after stimulating with IL-1/IL-6 sustained the functional activity against xGVHD in a humanized animal model. atRA suppresses IL-1R upregulation and accelerates IL-6R downregulation and diminishes their signaling events, as well as increases histone acetylation on FOXP3 gene promoter and CpG demethylation in the region of Foxp3 locus (TSDR).
Conclusions These results strongly implicate that nTregs primed with atRA may represent a novel treatment strategy to control established chronic immune-mediated autoimmune and inflammatory diseases.

W6.04.05
Blockade of CCR6 and CCL20 interaction ameliorates arthritis and contact hypersensitivity reaction in mice

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The immune response depends on the migration of leukocyte subsets, which track secreted chemokines via the activation of surface GPCRs. CCR6 is expressed to varying extents on effector memory T cells, mature B cells and immature dendritic cells and Th17 cells. It was reported that Th17 play an important role in arthritis models. So, to clarify the role of the CCR6-CCL20 pathway in experimental arthritis, the effects of an anti-CCL20 monoclonal antibody (mAb) on the collagen-induced arthritis model was investigated in mice. Anti-CCL20 mAb significantly inhibited the development of arthritis symptoms. We observed same result with CCR6 knockout mice. In this study, the infiltration of CD3+ T cells and F4/80+ macrophages was evaluated by immunohistochemical analysis. These infiltrating cells were decreased in CCR6 KO mice compared with WT mice. We also observed the suppressive effect of anti-CCL20 mAb in a contact hypersensitivity model, type IV allergic reaction. To evaluate migration of leukocytes, fluorescently labeled T cells from WT and CCR6 KO mice were injected into WT mice and then infiltrated cells were evaluated by imaging technology. There was no difference in edema formation between WT and KO mice, but infiltrating cells were decreased in the mice which CCR6 KO mice derived cells were injected. CCR6 KO mouse and application of anti-CCL20 antibody showed retardation of mouse arthritis development, suggesting that anti-CCL20 antibody could be a benefit to RA patients.

W6.04.06
The effect of CXCL10 blockade in C protein-induced myositis

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Background: CXCL10 (also called interferon-γ-inducible protein 10 [IP-10]) is a chemokine that plays a critical role in the infiltration of T cell in autoimmune diseases. CXCL10 is reported to be expressed in muscle tissue of polymyositis, thus we investigated the role of CXCL10 and the effect of CXCL10 blockade in C protein-induced myositis, an animal model of polymyositis.
Methods: C protein-induced myositis model was induced with human skeletal C protein fragment in 8-week-old female C57BL/6 mice. Immunohistochemistry was performed to detect CXCL10 and CXCR3, its receptor. CXCR3 in mouse splenocyte was investigated by flow cytometry. Migration assay of mouse splenocyte was performed with 5 μm pore transwell system. Mice with C protein-induced myositis were treated with anti-CXCL10 antibody or control IgG 8 days after the induction of myositis and the muscle inflammation was assessed 3 week after the induction.
Results: Immunohistochemistry showed the expression of CXCL10 and CXCR3 in the muscle of C protein-induced myositis. Flow cytometry demonstrated IFN-γ+ cells were increased among CXCR3+CD8+ T cells compared to CXCR3-CD8+ T cells (CXCR3+CD8+ T cell, 28.0 ± 4.2% vs. CXCR3-CD8+ T cell, 9.5 ± 1.5%, p = 0.016). Migration of splenocyte was increased in response to CXCL10 (chemotactic index=1.91±0.45). Treatment with anti-CXCL10 antibody (n=10) showed less inflammation score in muscles than treatment with control IgG (n=10; median [range], anti IP-10, 0.75 [0.25-2.00] vs. control IgG, 1.43 [1.125-4.25], p=0.045).
Conclusion: CXCL10 was expressed in the inflammation of C protein-induced myositis model and its blockade suppressed inflammation in muscle.

W6.05.01
Application of HLA classII peptide tetramers vs. allergen-induced proliferation for identification of allergen-specific CD4 T cells in the model of mugwort pollen allergy

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MHC-classII/peptide tetramer complexes are praised as tool to monitor antigen-specific CD4 T cells. The downsides of this costly technique are HLA-classII-polymorphism, the knowledge of immunodominant epitopes and scarcity of cells. Therefore, we investigated whether allergen-reactive T cells would identify a T cell subset comparable to tetramer-stained cells.
We used the HLA-DRB1*01:01/Artv1$_{19-36}$ tetramer, as Artv1, the major mugwort pollen-allergen, has only one single HLA-DR1-restricted epitope (Artv1$_{25-36}$). PBMC were obtained from mugwort-allergic patients. Artv1-reactive, dividing (CFSElo) T cells were studied at day 8 and compared to Artv1$_{25-36}$-specific T cell lines (TCL) established by Artv1-stimulation and expansion for 3-4 weeks.
Ex vivo characterization of tetramer$^+$ T cells was not possible due to low frequencies. The phenotype of tetramer+ and CFSElo CD4+ T cells was analyzed for cytokines and differentiation markers. Both, tetramer$^+$ cells in TCL and CFSElo T cells showed higher levels of Th2 cells duringallergen-specific treatment. The levels of Th1 cells tended to be higher in CFSElo compared to tetramer+T cells. Tetramer$^+$ cells in TCL showed an intermediate to highly differentiated phenotype compared to CFSElow T cells.
In summary, *ex vivo* analysis of allergen-specific CD4$^+$ T cells with MHC-classII tetramers for was hampered by their low frequency. Tetramer$^+$ T cells in long-term TCL maintained the original cytokine responses, but exhibited an altered, late differentiation phenotype. Although the CFSE-dilution method may also detect by-stander Th1 cells, earlier differentiation stages can be analysed. Therefore, it may be a more cost-/time-effective method to study allergen specific T cells when tetramer-staining *ex vivo* is not possible.

W6.05 Prevention and intervention in allergy

IL6.05.01
Allergy in the CIS-countries

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To summarize the results of the CIS-countries (CIS-C) research on the prevalence, structure and peculiarities of the course of allergic diseases (AD) for the last 5-10 years. in children(Ch), teenagers(Tg) and adults(Ad) living in industrial cities and rural areas. It was found out that the structure of AD of CIS-C is dominated by: bronchial asthma (BA), allergic rhinitis (AR), atopic dermatitis (AD), pollinosis (P). The average incidence of AD ranges from 15% to 40%. It was stated that 7% of 48.3% of the Ad and from 4% to 21% of Ch+Tg suffer from BA. The highest incidence of BA is reported in Tajikistan and Armenia. AR is diagnosed in 8%-34% of Ad and 4%-9% of Ch+Tg. Prevalence of symptoms of AR is particularly high in Armenia and Azerbaijan. The average incidence of AD in Ad is 5%-19%, in Ch+Tg- 5.3%. The high prevalence of AD is characteristic of Ukraine, Tajikistan and Kyrgyzstan. In Armenia and Tajikistan the level of AD diseases among Ch+Tg noted within 2.7%-4.7%. In Tajikistan and Armenia a significant number of patients is revealed with P, more often Ad are ill (15.7%-29.3%), Ch-4.3%-5.2%.In Armenia a significant incidence of allergic conjunctivitis is established among Ch+Tg (39.1%-62.2%), Ad-13.6%-32.9%. The structure of AD recorded by uptake, dominated the disease with medium-severe course. Monitoring of the true prevalence of AD shows hypodiagnostics of all forms of AD in the CIS-C, related to the underestimation of mild forms of the disease.

W6.05.02
Antibodies specific for the junction of CH4 domain and CεmX in membrane-bound IgE are potentially useful for down-regulating mIgE-B lymphocytes

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It has been shown that membrane-bound IgE (mIgE) on B lymphocytes is essential for generating isotype-specific IgE response. The successful development of omalizumab, which neutralizes free IgE and binds to mIgE on B cells, as a treatment for allergic asthma has validated the IgE-mediated pathway as an effective therapeutic target for allergic diseases. The two mAbs, 4B12 and 47H4, which recognize their respective epitopes in the 52 a. a. CεmX, a discrete domain located between the CH4 and the C-terminal membrane-anchor peptide of the heavy chain of mIgE, are now in active development for the treatment of allergic diseases. Herein, we employed virus-like particles formed by hepatitis B virus core antigen that harbors a peptide segment encompassing a C-terminal segment of CH4 and an N-terminal segment of CεmX as an immunogen and generated mouse mAbs that are specific for linear epitopes representing the junction of CH4 and CεmX. One such mAb, 5A8, binds to SVNPGLAGGSAQSQRAP, of which SVNP is from the CH4 and GLAGGSAQSQRAP from CεmX. The mAb 5A8 cannot bind to secreted IgE and hence does not activate and degranulate IgE-pulsed human FcεRI-expressing rat basophilic leukemic cell line RBL SX-38 cells. Furthermore, 5A8 can mediate apoptosis and antibody-dependent cellular cytotoxicity of human mIgE-expressing B cell transfectoma Ramos and A20 cells. These results suggest that mAbs specific for the CH4-CεmX junction of mIgE can provide additional therapeutic candidates for the down-regulation of mIgE-B cells in allergic patients.

W6.05.03
Mast cell-derived prostaglandin D_2 suppresses intestinal mastocytosis in food allergy

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Objective:
Prostaglandin D_2 (PGD_2) is a major prostanoid secreted by mast cells. There have been controversial reports showing a pro- or anti-inflammatory role of PGD_2 in diverse allergy models. Its precise contribution in allergic inflammation remains unclear. We here investigated the role of PGD_2 in ovalbumin (OVA)-induced intestinal allergy using hematopoietic PGD synthase deficient (H-$PGDS^{-/-}$) mice.
Results: Oral administration of OVA induced allergic responses characterized by diarrhea, scratching, immovability, eye puffiness and piloerection in OVA-sensitized Wild-type (WT) mice. H-PGDS deficiency exacerbated all the OVA-induced allergic responses without changing in serum OVA-specific IgE level. Quantitative RT-PCR showed that H-PGDS deficiency did not influence the Th2-cytokine expressions, but it elevated TNF-α expression in the inflamed intestine. Morphological studies showed that c-kit/FcεRI-positive mast cells strongly expressed both H-PGDS and TNF-α in WT intestine. Of interest, H-PGDS deficiency greatly increased the mast cell-infiltration into intestine especially colonic mucosa. Administration of neutralizing antibody against TNF-α prevented the mastocytosis and exacerbation of allergic responses seen in H-$PGDS^{-/-}$.
Conclusion: We identified mast cell-derived PGD_2 as a negative regulator of mastocytosis and allergic responses in OVA-induced food allergy model. The PGD_2-mediated inhibition of TNF-α may be responsible for these phenomena.
Footnote: This work is supported by Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

W6.05.04
Robust long-term tolerance in IgE-mediated allergy through transfer of allergen-expressing bone marrow cells

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Objective: Robust and durable tolerance was established in IgE-mediated allergy towards the major grass pollen allergen Phlp5 by molecular chimerism. This strategy requires myelosuppressive conditioning of the recipient (i.e. irradiation). Here we investigated, if a tolerance protocol can be developed obviating the need for recipient conditioning.
Material and Methods: To this end we generated a novel transgenic mouse (on the Balb/c background) expressing Phlp5 ubiquitously on the surface. This transgenic mouse served as cell donor. Balb/c recipients were treated with a short-course of rapamycin (d-1,0,2) and costimulation blockade (CTLA4-Ig, d2) and transplanted with $15x10^6$ bone marrow (BM) cells isolated from Phlp5-transgenic mice. Subsequently, mice were challenged several times with splenocytes (+AlOH3) of transgenic mice and control allergen (Betv1, weeks 4,7,10 after BM transplantation [BMT]). Tolerance was analyzed by ELISA for allergen-specific isotypes and T-cell proliferation assays. Airway-hyperresponsiveness was determined by whole-body-plethysmography.
Results: Phlp5-specific IgE remained absent inrecipients of the full treatment protocol (e.g. w20 post BMT, 7/10), whereas controls transplanted with Phlp5-positive BM only developed high levels of anti-Phlp5 IgE. High levels of Betv1-specific IgE developed in both groups. Furthermore Phl p 5-specific T-cell proliferation was not detectable at the end of follow up (w35 post BMT). Additionally preliminary data reveal no airway hyperresponsiveness in BMT recipients in response to Phlp5 (6/6) compared to untreated sensitized controls (n=3).Conclusions: Transfer of allergen-bearing BM cells with costimulation blockade and short course rapamycin leads to robust tolerance without recipient conditioning. These data suggest that preventive cell-therapy is feasible with clinically acceptable toxicity.

W6.05.05
Sublingual immunotherapy induces regulatory T cells in cervical lymph node of murine allergic rhinitis model

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Sublingual immunotherapy has been considered to be a painless and efficacious therapeutic treatment of allergic rhinitis which is known as type-I allergy of nasal mucosa. Nevertheless, its mechanisms need to be further investigated. In this study, we constructed an effective murine model of sublingual immunotherapy (SLIT) in allergic rhinitis, in which mice were sublingually administered with ovalbumin (OVA) followed by an intraperitoneal sensitization and nasal challenge of OVA. Sublingually treated mice showed significantly decreased allergic responses as well as suppressed Th2 immune responses. Sublingual administration of OVA did not alter the frequency of $CD4^+CD25^+$ regulatory T cells (Tregs), but led to up-regulation of Foxp3- and IL-10-specific mRNAs in the Tregs of cervical lymph nodes (CLN), which strongly suppressed Th2 cytokine production from $CD4^+CD25^-$ effector T cells *in vitro*. Furthermore, sublingual administration of plasmids encoding the lymphoid chemokines CCL19 and CCL21-Ser DNA together with OVA suppressed allergic responses. These results suggest that IL-10 expressing $CD4^+CD25^+Foxp3^+$ Tregs in CLN are involved in the suppression of allergic responses and that CCL19/CCL21 may contribute to it in mice received SLIT.

W6.05.06
Effective suppression of grass-pollen induced asthma manifestations by subcutaneous and sublingual immunotherapy in a mouse model

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Allergic asthma is characterized by high levels of IgE, eosinophilic airway inflammation and airway hyperresponsiveness (AHR). Although specific immunotherapy is the only treatment providing long-term protection against allergic diseases, it needs further improvement to increase its efficacy. Previously, we developed a preclinical grasspollen (GP) model of asthma. Here, we aim to perform dose-finding experiments for subcutaneous- (SCIT) and for sublingual (SLIT) immunotherapy.
All Balb/c mice were sensitized with a crude extract of GP, after which we performed a dose-finding experiment (30, 100, and 300kSQ or placebo) for SCIT and SLIT. SCIT was given by three subcutaneous injections and SLIT by 40 sublingual administrations. Subsequently, mice were challenged intranasal three times on alternate days. Two days after the last challenge, we measured AHR to methacholine using FlexiVent, GP-specific IgE, IgG1, and IgG2a in serum, ear-swelling responses (ESR), eosinophils in broncho-alveolar lavage fluid (BALF) and T-cell cytokine release after re-stimulation of lung cells (IL-5, -10, and -13). In asthmatic mice, GP-challenge induced AHR, BALF eosinophilia, up-regulation of serum IgE levels and increased levels of Th2-type cytokines upon lung T-cell restimulation as compared to control mice. The highest dose of SCIT significantly decreased AHR and eosinophils in BALF, whereas ESR showed a trend for suppression. High dose-SLIT significantly suppressed AHR and ERS, and there was a trend for the suppression of BALF eosinophils. Interestingly, SCIT and SLIT have differential effects on

Th2-type cytokine production by lung cells. In conclusion, high dose-SLIT and SCIT effectively suppress asthma manifestations in an asthma mouse model.

W6.06 Cancer immunotherapy and anti-tumor vaccines

IL6.06.01

Cell therapy of cancer with alloreactive intentionally mismatched IL-2 activated donor lymphocytes targeting anti-cancer killer NK & T cells using monoclonal and bispecific antibodies

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Graft-vs.-leukemia or solid tumors effects following allogeneic stem cell transplantation (SCT) are well established, however, use of SCT may be hazardous and associated with unavoidble graft-vs-host disease (GVHD). We investigated whether short-term immunotherapy could be induced by intentionally mismatched alloreactive killer NK and T cells (IMAK), maximally activated with interleukin 2 with no SCT. Monoclonal and trifunctional bispecific antibodies (BSA) were used for targeting IMAK against antigens over-expressed on malignant cells confirmed efficacy of eradication of otherwise lethal inoculum of B16 melanoma while avoiding GVHD in mice. Successfully treated mice could also resist fresh tumor challenge, suggesting development of anti-cancer immunity by Fc binding of malignant cells to host dendritic cells and presentation to T cells cells. Commercially available monoclonal and BSA against target antigens (EGFR, VEGF, Her-2/neu, CD20, EpCAM) using Erbitux, Avastin, Herceptin, MabThera & Catumaxomab, respectively were used in a pilot clinical trial. We have confirmed that treatment of patients with hematologic malignancies and solid tumors with IMAK with (n=16) or without (n=40) targetting antibody is safe and potentially effective with no GVHD in patients with hematological malignancies and metastatic solid tumors. We are now investigating whether targeting of IMAK may be further improved using low energy acoustic shockwave therapy (AST) that may increase the perfusion of malignant tissue as well as increase binding of killer lymphocytes to cancer cells. Based on our cumulative experience we conclude that treatment with short-lived targeted IMAK may represent an effective future approach for personalized targeted cancer immunotherapy.

W6.06.01

Examination of cancer testis antibodies and Th1/Th2 cytokines as prognosis markers in glioma patients treated with Delta-24-RGD oncolytic adenovirus

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In a Phase I clinical trial of patients with recurrent gliomas treated with the intratumoral injection of Delta-24-RGD oncolytic adenovirus, we showed that a subset of patients had partial or complete responses as assessed by decrease of tumor volume or complete regression of the tumor mass in MRI images. These findings correlated with extended survival. Tissue samples and sera were examined to discover prognosis markers. Examination of the tumor tissue after the treatment showed that Delta-24-RGD replicates in gliomas. Importantly, spread of the virus caused tumor necrosis and recruitment of CD8 lymphocytes. We speculate that the trigger of an immune response after the injection of Delta-24-RGD, could be the cause for the tumor regression in some patients. Supporting this hypothesis, we showed a skew to Th1 response in patients with positive outcome. Thus, examination of cancer autoantibodies and Th1/Th2 cytokines in the serum of the patients enrolled in the clinical trial, showed that predominance of a humoral response (high titer of autoantibodies against cancer testis antigens) and lack of cytokine immune response, were associated with suboptimum outcome. On the contrary, high levels of Th1-related cytokines and lack of autoantibodies, seemed to potentially correlate with positive clinical outcome. This is the first study of the expression of anti-cancer related antibodies and Th1 cytokines in glioma patients treated with oncolytic viruses and suggest that it is possible to elucidate molecular markers to predict the clinical response to Delta-24-RGD. Our data could be relevant for any form of immunotherapy for solid tumors.

W6.06.02

Synergistic 4-1BB and CD28 costimulation enables CD19-targeted T cells to eliminate aggressive leukemia, in an IRF7-dependent manner

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Adoptive T-cell therapy using chimeric antigen receptor (CAR) engineered T cells is a promising cancer treatment. CARs include an scFv targeting a surface antigen and the CD3ζ chain to direct tumor cell killing. CAR- transduced T cells however need appropriate costimulation to proliferate and persist.
Here we assessed in a B-cell leukemia xenograft model the anti-tumor activities of human T cells transduced with an anti-CD19 CAR harboring either the zeta chain alone (19z1+) or CD3z fused to the CD28 or 4-1BB cytoplasmic domain (19-28z+, 19-BBz+), or co-expressed with 4-1BBL (19z1+4-1BBL+), or with 19-28z co-expressed with 4-1BBL (19-28z+4-1BBL+). At low T cell doses, we observed complete NALM-6 tumor regression in mice treated with 19-28z+4-1BBL+ T cells and no or partial regression in the other T-cell groups, as measured by bioluminescence imaging.19-28z+4-1BBL+ T cells showed the highest and longest bone marrow accumulation. Gene expression signatures of CD28 and/or 4-1BB costimulated T cells notably contained the transcription factors KLF2 and IRF7, solely overexpressed in T cells receiving dual costimulation. CD62L, described as directly regulated by KLF2, was overexpressed in 19-28z+4-1BBL+ T cells compared to 19-28z+ T cells. IRF7 and associated type I interferon-stimulated genes (ISG) were strongly expressed in 19-28z+4-1BBL+ CD4 and CD8 T cells. Using RNA interference, IRF7 down-regulation in 19-28z+4-1BBL+ T cells abolished their therapeutic activity due to decreased long-term survival.
Thus, concomitant CD28 and 4-1BB costimulation in human T cells induces the IRF7 pathway which is associated with the generation of long-lived tumor-reactive T cells capable of eradicating aggressive leukemia.

W6.06.03

Development of a tumor stem cell specific T-cell engaging antibody for the treatment of Glioblastoma multiforme

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Glioblastoma multiforme is the most malignant form of primary brain tumor and is resistant to chemo- and radiotherapy. CD133 is a marker for glioblastoma stem cells which are thought to be responsible for tumor propagation and treatment resistance. We therefore developed a novel bispecific T-cell engaging antibody targeting CD133+ tumor stem cells in the format of a bispecific single chain variable fragment (bs-scFv). The recombinant antibody shows high binding to both CD133+ tumor cells and CD3+ human T cells. It also shows high cytotoxicity against CD133-overexpressing U251 glioma cells and primary patient-derived tumor stem cells *in vitro*. In immunodeficient mice, the bs-scFv antibody efficiently prevented the outgrowth of both subcutaneously implanted CD133-overexpressing glioma cells and primary CD133+ tumor stem cells, when co-implanted with human T cells. Tumor growth could also be efficiently prevented when CD133+ glioma cells were orthotopically co-implanted with human T cells into the brain of immunodeficient mice. In these experiments, the bs-scFv antibody was applied by continuous pressure-backed local infusion. In conclusion, tumor stem cell-directed T-cell recruiting antibodies might enlarge the armamentarium of therapeutics against highly aggressive, tumor stem cell-driven malignancies.

W6.06.04
Regeneration of Human Melanoma Antigen-Specific T cells from iPSCs Derived from Mature CD8+ T cells

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Antigen-specific T cells represent a potential therapeutic avenue for a variety of conditions, but current approaches for generating such cells for therapeutic purposes are limited. In this study, we established human induced pluripotent stem cells (hiPSCs) from mature cytotoxic T cells specific for the melanoma epitope MART-1. When cocultured with OP9/DLL1 cells, these hiPSCs efficiently generated TCRb+CD4+CD8+ double positive (DP) cells expressing a T cell receptor (TCR) specific for the MART-1 epitope. Stimulation of these DP cells with anti-CD3 antibody generated a large number of CD8+ T cells, and more than 90% of the resulting cells were specific for the original MART-1 epitope. Stimulation of the CD8+ T cells with MART-1 antigen-presenting cells led to the secretion of considerable ammount of IFNg, demonstrating their specific reactivity. The present study therefore illustrates an approach for cloning and expanding functional antigen-specific CD8+ T cells that might be applicable in cell-based therapy of cancer.

W6.06.05
Isolation of antigen-specific T cell receptor (TCR) genes and analysis of disease-associated TCR repertoires by TCR gene capture.

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The transfer of T-cell receptor (TCR) genes into patient T-cells is a promising approach for the treatment of both viral infections and cancer. However, efficient strategies for TCRs gene isolation have thus far been lacking. Likewise, the study of TCR-repertoires related to different disease states has been limited to description of TCR Variable-gene usage and did not include assessing the properties of disease-associated TCRαβ pairs. To address these issues we have developed a high-throughput DNA-based TCR gene capture approach for the identification of TCR gene sequences of interest.
The approach involves the capture and sequencing of genomic DNA fragments encoding the TCRαβ gene-loci. The quantitative nature of the resulting next-generation-sequencing data reveals relative frequencies of all TCRα- and β-sequences and, because of this, TCRαβ pairs can be directly determined within oligoclonal T-cell populations, through 'frequency-based matching'.
We have exemplified the potential of TCR gene capture in two different applications. First, we demonstrate the possibility to rapidly assemble TCRαβ gene libraries against antigens of interest, in our case the Cancer/Germline (C/G) antigens that are frequently expressed in human tumors. Second, we demonstrate the ability to analyze TCR-repertoires of intratumoral T-cell subsets without knowledge of their antigen-specificity, and show that TCR gene capture can be used to identify dominant tumor-reactive TCRαβ pairs within such populations.
Taken together, our data shows that TCR gene capture can be used to describe the TCR-repertoire in diverse types of biological samples and to create large collections of TCR genes for genetic engineering of T-cell immunity.

W6.06.06
Glycoengineered defucosylated anti-CD20 antibodies activate neutrophils mediated phagocytosis more efficiently than rituximab

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GA101 (obinutuzumab) is a glycoengineered, defucosylated anti-CD20 antibody with higher affinity for CD16A (FcγRIIIa) and stronger NK mediated antibody dependent cellular cytotoxicity activity (ADCC) than fully glycosylated rituximab (RTX) antibody. Polymorphonuclear leukocytes (PMN) express the homologous GPI-anchored CD16B (FcγRIIIb) molecule, which also shows enhanced affinity for glycoengineered antibodies, in addition to the activating CD32A (FcγRIIa) receptor. We have investigated the functional activity of human PMN in response to GA101 or defucosylated rituximab (G2), compared to the parent RTX antibody. We show that GA101 activated purified PMN more efficiently than RTX, leading to 50% increased CD11b expression and 70% down modulation of CD62L. This activation was not accompanied by generation of reactive oxygen species or by ADCC. In contrast, it was accompanied by up to 45% phagocytosis of opsonised targets and then followed by PMN death. Furthermore, significant phagocytosis (15% above background) could be observed in whole blood assays only in presence of defucosylated antibodies but not with unmodified antibodies, similarly to what was previously described for NK cell activation and ADCC. Finally, we demonstrated using blocking $F(ab)_2$ antibodies that the CD16B and CD32A FcγRs cooperate in PMN activation and phagocytosis of opsonised targets.
We conclude that phagocytosis of targets by PMN is an additional mechanism of action for anti-CD20 antibodies, which is more efficient with defucosylated compared to fully glycosylated parent molecules. Furthermore, these data may offer an explanation for the increased neutropenia observed in patients treated with GA101, therefore an effect likely to be related to its anti-lymphoma activity.

W6.06.07
FOXO3 regulates tumor-associated DC tolerogenicity by binding and sequestering NF-κB

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Tumor-associated dendritic cells (TADC) are a major contributor of immune tolerance in the tumor microenvironment. We previously reported that the transcription factor FOXO3 is a critical mediator of TADC tolerogenicity and that silencing *Foxo3* expression promoted anti-tumor immunity. In the current study, we sought to identify the mechanism by which FOXO3 regulates TADC function. It was previously reported that FOXO3 to inhibits NF-kB activity in T cells through the transcriptional regulation of IkB proteins. In contrast, our data demonstrate that unlike in non-DC populations, FOXO3 was principally detected in the cytoplasm of TADC. Further analysis revealed that despite extensive phosphorylation, cytoplasmic FOXO3 was stable. These findings suggested that FOXO3 may not solely function as a transcriptional regulator. Instead, our findings show for the first time that FOXO3 binds both the RelA and p50 NF-kB subunits and sequesters them to the cytoplasm. The formation of this protein complex prevents nuclear translocation and downstream pro-inflammatory signaling by NF-kB. In the absence of FOXO3, NF-kB re-localizes to the nucleus and turns on expression of pro-inflammatory genes. We further demonstrate that NF-kB enhances cytoplasmic FOXO3 stability. These new insights into the regulation of DC tolerogenicity provide new potential targets for the enhancement of immune-based therapies for cancer, where FOXO3 may reduce pro-inflammatory activities of TADC.

W6.06.08
Host immunity contributes to the anti-melanoma activity of BRAF inhibitors

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The BRAF mutant, $BRAF^{V600E}$, is expressed in nearly half of melanomas, and oral BRAF inhibitors induce substantial tumor regression in patients with *$BRAF^{V600E}$* metastatic melanoma. The inhibitors are believed to work primarily by inhibiting $BRAF^{V600E}$-induced oncogenic MAPK signaling; however, some patients treated with BRAF inhibitors exhibit increased tumor immune infiltration, suggesting that a combination of BRAF inhibitors and immunotherapy may be beneficial. We used two relatively resistant variants of *BrafV600E* driven mouse melanoma (SM1 and SM1WT1) and melanoma-prone mice to determine the role of host immunity in type I BRAF inhibitor PLX4720 antitumor activity. We found that PLX4720 treatment downregulated tumor *Ccl2* gene expression and decreased tumor CCL2 expression in both transplanted *BrafV600E* mouse melanomas and in *de novo* melanomas, coincident with reduced tumor growth. While PLX4720 did not directly increase tumor immunogenicity, analysis of SM1 tumor-infiltrating leukocytes in PLX4720-treated mice demonstrated a robust increase in $CD8^+$ $T/Foxp3^+CD4^+$ T cell ratio and NK cells. Combination therapy with PLX4720 and anti-CCL2 or agonistic anti-CD137 antibodies demonstrated significant antitumor activity in mouse transplant and *de novo* tumorigenesis models. These data elucidate a role for host CCR2 in the mechanism of action of type I BRAF inhibitors and support the therapeutic potential of combining BRAF inhibitors with immunotherapy.

W6.06.09
Exploiting the good and blocking the bad: new strategies to convert the tumor into an in situ personalized vaccine

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Ionizing radiation therapy (RT) has the potential to convert the tumor into an in situ, personalized vaccine, however this potential is rarely realized by RT alone. We have previously shown in mice models of poorly immunogenic tumors that the pro-immunogenic effects of RT can be exploited to improve the response to targeted immunotherapy strategies. For instance, effector T cell recruitment to the tumor by RT-induced CXCL16 chemokine and stabilization of interactions between CD8 T cells and tumor cells by RT-induced NKG2D ligands were critical for the therapeutic synergy of RT with anti-CTLA-4 antibody treatment (J Clin Invest 2012).
To determine if TGFβ, an immunosuppressive cytokine that is activated by RT, hinders the ability of RT to promote anti-tumor immunity we employed a mouse model of established metastatic carcinoma. Antibody-mediated neutralization of TGFβ did not have any effect by itself but improved significantly control of the irradiated tumor. CD8 T cells producing IFNγ in response to a tumor-specific antigen and inhibition of non-irradiated lung metastases were seen only in mice treated with RT+TGFβ blockade, but not each modality used alone. Depletion of $CD4^+$ or $CD8^+$ T cells abrogated the therapeutic benefit of the combination treatment. Notably, whole genome transcriptional analysis demonstrated the selective upregulation of genes associated with immune-mediated rejection only in tumors of mice treated with RT+TGFβ blockade. Data identify TGFβ as a master regulator of RT ability to generate an in situ tumor vaccine, and support testing inhibition of TGFβ during radiotherapy to promote therapeutically effective anti-tumor immunity.

W6.06.10
A new class of iNKT cell agonist to harness tumor immunity

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Invariant (or type I) NKT (iNKT) cells are CD1d restricted T cells recognizing lipid antigens. iNKT cells enhance tumor immunity in mouse tumor models, especially when activated with the agonistic antigen α-GalCer. It also has been shown that IFN-γ is necessary for protection induced by α-GalCer. It was believed that that alpha-linked sugar moiety in α-GalCer's structure is essential for its anti-tumor activity. Here, we report a new class of iNKT cell tumor-protective agonistic antigen with a beta-linked sugar moiety, exemplified by β-ManCer. In both CT26 colon carcinoma and B16F10 melanoma lung metastasis models, we observed significant reduction of tumor burden in the lungs of β-ManCer treated mice compared to vehicle treated controls. In contrast to α-GalCer, β-ManCer still induced significant protection in IFN-γ-/- mice, whereas its protection was abrogated by blockade of TNF-α or NOS. Further, suboptimal doses of β-ManCer and α-GalCer synergized to induce protection. We conclude that β-ManCer protects against tumors through a pathway different from that induced by α-GalCer, defining β-ManCer as a new class of NKT cell agonist. We further observed that β-ManCer does not induce long lasting unresponsiveness or anergy of activated iNKT cells, which is known to be induced by α-GalCer. While prior treatment with α-GalCer completely abrogated the protective effect of either α-GalCer or β-ManCer against the CT26 lung metastasis, β-ManCer pretreatment did not affect the protection. Since β-ManCer can activate human NKT cells, this new NKT cell agonist may provide a novel opportunity for NKT cell-targeted immunotherapy in cancer.

W6.06.11
Naive-derived memory stem T cells: a novel promising platform for cancer immune-gene therapy

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Memory stem T cells (TSCM) with ability to self renew and plasticity to differentiate into potent effector cells represent ideal weapons to treat cancer. Nonetheless, clinical-grade procedures to target this T-cell population remain elusive. Here we report that it is possible and feasible to differentiate in vitro, vigorously expand and genetically engineer TSCM lymphocytes starting from naïve precursors. Requirements for the generation of this T-cell subset, defined as CD62L+CCR7+CD45RA+CD45R0+IL-7Rα+CD95+, are CD3/CD28 engagement and culture with low-dose IL-7/IL-15. The gene expression profile and functional phenotype validate this population as a distinct memory T lymphocyte subset, hierarchically superior to central memory (TCM) and effector memory (TEM) lymphocytes. To define naive-derived TSCM function and potential in a clinically relevant context, we evaluated their ability to mediate xenogeneic GvHD in vivo. Naïve-derived TSCM prove superior in vivo expansion and persistence than TCM counterparts, and are able to differentiate into effector cells, mediating a lethal xenogeneic GvHD with the same kinetics and intensity of unmanipulated lymphocytes. TSCM are the only T-cell subset able to engraft and mediate GvHD upon serial transplantation, thus demonstrating self-renewal abilities. Furthermore, TSCM lymphocytes are less sensitive to CD95-mediated apoptosis compared to memory counterparts, and do not express the inhibitory molecule PD-1. Finally, we identified and functionally validated the natural counterpart of our gene-modified TSCM population in healthy donors as a rare fraction of naive T cells, recapitulating the CD62L+CCR7+CD45RA+CD45R0+IL-7Rα+CD95+ phenotype. Our findings pave the way for a rapid clinical translation of

cancer immunotherapy approaches based on ex-vivo generated TSCM lymphocytes.

W6.06.12
Conditioning tumor microenvironments for effective entry and function of effector T cells

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The desirable effector cells of antitumor immunity (CTLs, Th1 and NK cells) express high levels of "type-1" chemokine receptors CXCR3 and CCR5 and depend on their chemokine ligands (CXCL9, CXCL10 and CCL5) for effective entry into tumors. We observed that prostaglandin E_2 (PGE_2), a ubiquitous mediator of cancer-associated inflammation, suppresses the induction of immune effector cells and enhances local development and function of myeloid-derived suppressor cells (MDSCs). In addition, PGE_2 selectively inhibits the production of the effector cell-attracting chemokines within ovarian and colorectal cancers and promotes the MDC/CCL22- and SDF1/CXCL12-mediated accumulation of Tregs and myeloid-derived suppressor cells (MDSCs) in tumor tissues, orchestrating local inhibition of CTL function.
Blockers COX2 activity and selective antagonists of PGE_2 receptors (EP2 and EP4) antagonized the migration and immunosuppressive function of MDSCs and were necessary (although not sufficient) to restore local immune surveillance. While TLR-ligands used as stand-alone adjuvants were effective only in selected tumor samples and, in some cases, amplified the PGE_2-driven suppressive events, their combination with COX2 inhibitors and IFNα allowed to selectively induce effector cell-attracting chemokines within tumors (rather than marginal tissues) and suppressed the local production of the MDSC- and Treg-attracting CXCL12 and CCL22. Tumor-selectivity of the chemokine modulation by the combination of TLR3 ligands, IFNα and Celecoxib resulted from the preferential activation of NF-κB in tumor-associated fibroblasts and myeloid cells, the main chemokine sources in tumor tissues.
The safety and effectiveness of tumor-selective conditioning of tumor microenvironments is being currently tested in our phase I/II clinical trials.

W6.06.13
A bi-institutional pilot study of peptide-based vaccines in combination with poly ICLC in patients with WHO grade 2 low-grade glioma

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Adults with WHO grade 2 low-grade glioma (LGG) have a significant risk of tumor progression, especially via transformation to high-grade glioma (HGG), and most patients eventually die of the disease. We conducted a pilot study of subcutaneous vaccinations with synthetic peptides for glioma-associated antigen (GAA) epitopes emulsified in Montanide-ISA-51 and intramuscular administration of poly-ICLC in HLA-$A2^+$ patients with: newly diagnosed high-risk LGG without prior radiation therapy (RT) (Cohort 1); newly diagnosed high-risk LGG with prior RT (Cohort 2); or recurrent LGG (Cohort 3). Primary endpoints were safety and $CD8^+$ T-cell responses against vaccine-targeted GAAs: IL-13Rα2, EphA2, WT1, and Survivin. Cohorts 1, 2, and 3 have enrolled 12, 1, and 10 patients, respectively. No regimen-limiting toxicity has been encountered except for one case with Grade 3 fever (Cohort 1). ELISPOT assays, completed in 7, 1 and 7 patients in Cohorts 1, 2 and 3 respectively, demonstrated robust and sustained IFN-γ responses against at least 3 of the 4 GAAs in 6 and 3 cases of Cohorts 1 and 3, respectively. Cohort 1 patients demonstrated significantly higher magnitude of IFN-γ responses than Cohort 3 patients for all 4 GAA epitopes, suggesting that newly diagnosed patients may have better vaccine-responsiveness than recurrent patients. Median progression-free survival periods are 21 months (Cohort 1; since diagnosis; range 10-38) and 12 months (Cohort 3; since the 1st vaccine; range 3-14). The only patient in Cohort 2 with large astrocytoma has been progression-free for over 48 months since diagnosis. These data warrant further evaluation of the regimen.

W6.06.14
Entire requirement of endogenous type I Interferon for the efficacious cancer treatment with viral vector-encoded IL-12

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To date little is known about how the innate Interferon (IFN)-α/β response affects the efficacy of cancer treatments based in viral vector delivery of therapeutic genes. Using Semliki Forest virus encoding IL-12 (SFV/IL-12) we have explored this. Intratumoral injection of SFV/IL-12 induces production of IFNα/β that is readily detected in serum. Such production is abolished in interferon-beta promoter stimulator 1 (IPS1)ko mice and to some extent in TIR-domain-containing adapter-inducing IFN-β (TRIF)ko mice. Using bone-marrow chimeric mice we show that both hematopoietic and stromal cells are involved in the IFNα/β response. Macrophages, plasmacytoid and conventional dendritic cells are all implicated in the IFNα/β production, as was revealed by depleting experiments. The therapeutic activity of SFV/IL-12 against MC38 tumor is absolutely lost in mice deficient for the IFNα/β receptor (IFNAR) and when IFNAR signaling is blocked in vivo with anti-IFNAR mAb. This is also true for TC1 tumor. The lack of efficacy is not related to an impaired expression of IL-12 because IFNARko mice even express higher levels of the transgene. IFNAR absolute requirement is mainly operational at hematopoietic-derived cells. Interestingly, tumor-specific CTLs are outstandingly expanded upon SFV/IL-12 intratumoral injection to WT mice, but this does not happen when tumor-bearing mice are IFNARko, or have been treated with anti-IFNAR mAb. All in all, our data show that the efficacy of tumor immunotherapy with SFV/IL-12 is totally dependent on IFNs-I and unravels an unexpected mechanism of action of a therapy that is being translated to patients.

W6.07 Cell-based therapy

IL6.07.01
Cell therapy to induce tolerance: which cell type should we use?

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Different mechanisms are active in the periphery to guarantee that tolerance to self-antigens is maintained. The most important mechanism is regulation in which specialized T cells, called regulatory T cells (Tregs), control the immune responses to autoantigens.
Tregs can be divided into two groups: naturally occurring (thymus-derived Tregs-nTregs) and induced or adaptive (iTregs) each with different functions.
In recent years many laboratories worldwide have started to develop strategies to tilt the balance between effector T cells and Tregs in favor of the latter to achieve tolerance. Two different approaches have been used to increase the number of Tregs. The first is the injection of different agents or cells that are able to induce/expand Tregs *in vivo*. Among these, dendritic cells (DCs) manipulated *in vitro* to become "tolerogenic" and mesenchymal stem cells (MSC).
The second approach involves the injection of either freshly isolated or *in vitro* expanded nTregs or iTregs. Three clinical trials have been already completed with nTregs to prevent GvHD showing that Treg

therapy is safe. Our laboratory has been involved in the polyclonal expansion of clinical grade nTregs and we are ready for the first clinical trials with nTregs in kidney and liver transplant patients. In the last few years other populations of cells with regulatory properties have emerged with the potential to be used in cell therapy (e.g. B cells and macrophages). Whether any of these cell types will dominate the scenes of cell therapy in the future is still an open question.

W6.07.01
Human CD8+ regulatory T cells inhibit GVHD and preserve general immunity in humanized mice

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Graft-versus-host disease (GVHD) is a lethal complication of allogeneic bone marrow transplantation (BMT). Immunosuppressive agents are currently used to control GVHD but may cause general immune suppression and limit the effectiveness of BMT. Adoptive transfer of regulatory T cells (Tregs) can prevent GVHD in rodents, suggesting a therapeutic potential of Tregs for GVHD in humans. However, the clinical application of Treg-based therapy is hampered by the low frequency of human Tregs and the lack of a reliable model to test their therapeutic effects in vivo. Recently, we successfully generated human alloantigen-specific $CD8^{hi}$ Tregs in a large scale from antigenically naïve precursors ex vivo using allogeneic CD40-activated B cells as stimulators. We report a human allogeneic GVHD model established in humanized mice to mimic GVHD after BMT in humans. We demonstrate that ex vivo-induced $CD8^{hi}$ Tregs controlled GVHD in an allospecific manner by reducing alloreactive T cell proliferation as well as decreasing inflammatory cytokine and chemokine secretion within target organs through a CTLA-4-dependent mechanism in humanized mice. These $CD8^{hi}$ Tregs induced long-term tolerance effectively without compromising general immunity and graft-versus-tumor activity. Our results support testing of human $CD8^{hi}$ Tregs in GVHD in clinical trials.

W6.07.02
Therapeutic efficacy of human Mesenchymal Stromal Cells in a humanised mouse model of acute Graft versus Host Disease

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Mesenchymal stromal cells (MSC) are a heterogenous population of cells first identified in the bone marrow. MSC have the capacity to modulate the immune response both in vitro and in vivo. Based on a large body of data, autologous and allogeneic *ex vivo* expanded human MSC have been used to treat a number of inflammatory diseases including acute graft versus host disease (aGvHD) with promising results. However, the exact mechanisms of therapeutic action are unclear. Acute graft versus host disease (aGvHD) is a life threatening complication following allogeneic haematopoietic stem cell transplantation (HSCT), occurring in up to 50% of patients receiving HLA-matched sibling transplants. Current therapies focus on corticosteroids, however in cases of steroid refractory aGvHD, there are limited therapeutic options leading to poor patient prognosis. This study utilised a robust humanised mouse model of aGvHD based on delivery of human peripheral blood mononuclear cells (PBMC) to NOD-scid IL-2rγ^{null} (NSG) mice. This novel model allowed the investigation of the role of MSC in cell therapy. MSC therapy significantly increased survival and reduced liver and gut pathology associated with aGvHD. MSC therapeutic efficacy was dependent on the timing of MSC administration with un-stimulated MSC proving effective only after delayed administration. In contrast, IFNγ stimulated MSC were effective when delivered with PBMC on day 0. Interestingly, MSC did not inhibit donor PBMC chimerism, as $CD45^{+}$ and T cells successfully engrafted in this model. However, in this model MSC inhibited donor $CD4^{+}$ T cell proliferation and reduced human TNFα in the serum.

W6.07.03
Induction of donor-specific tolerance in organ transplantation using in vitro-generated immunosuppressive myeloid blood cells

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Immunosuppressive treatment in organ transplantation improves graft survival but causes serious side effects. Cell therapeutic approaches for induction of antigen-specific tolerance are on the rise.
Monocytes are one source of dendritic cells (DCs). We observed that when DCs are generated from monocytes preincubated with the chemotherapeutic drug Mitomycin C, myeloid cells (M-MCs), which suppress allogeneic T cell responses, are resulting. T lymphocytes which had encountered M-MCs could not be restimulated. Interestingly, M-MCs resemble morphologically and phenotypically myeloid-derived suppressor cells which occur in cancer patients. Analyses of M-MCs revealed downregulation of immunostimulatory cell surface molecules and upregulated transcription of immunosuppressive and pro-apoptotic genes.
We further analyzed the immunosuppressive property of M-MCs *in vivo*. For practical reasons MMC-treated peripheral blood mononuclear cells (PBMCs), containing a substantial proportion of monocytes, were used. In a rat heart transplant model a single treatment of recipients with MMC-treated donor PBMCs one week before transplantation resulted in a significant prolongation of allograft survival, 50% of recipients becoming tolerant. Importantly, rejection of third-party allografts revealed donor-specific tolerance. Depletion of monocytes from PBMCs abrogated the immunosuppressive effect, proving the essential role of these cells. Similar results were obtained in a kidney transplant model in pigs. Furthermore, in a compassionate treatment, a 6-year-old girl with acute lymphatic leukemia and therapy-resistant rejection of a haploidentical stem cell transplant received MMC-treated donor monocytes, after which the signs of rejection disappeared.
In conclusion, we have developed a therapeutic approach to specifically inhibit organ rejection episodes. A transfer into the clinic is possible.

W6.07.04
Gene Therapy mediated by lentiviral vector transduced CD34+ cells for the treatment of Wiskott-Aldrich Syndrome

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Wiskott-Aldrich Syndrome (WAS) is an X-linked immunodeficiency characterized by thrombocytopenia, infections, autoimmunity and lymphomas. Gene therapy with ex vivo transduced hematopoietic stem cells could represent a valid therapeutic option to transplantation. We developed an approach based on a lentiviral vector (LV) encoding for WAS under the control of the homologous 1.6 kb WAS promoter. We designed a phase I/II clinical trial based on infusion of autologous transduced CD34+ cells derived from bone marrow (BM) or mobilized peripheral blood (PB) and reduced intensity conditioning. Transduction of clonogenic progenitors was

highly efficient (94.4 ± 4.2%), with a mean vector copy number (VCN)/genome in bulk CD34+ cells of 2.7 ± 0.98. In the first 3 treated patients, at the latest follow up, robust multilineage engraftment was observed in BM (VCN in myeloid lineages: 0.36-0.78) including clonogenic progenitors (25.8-48.2%), and in PB cells (VCN range of 0.30-1.30 in myeloid cells). As expected, a selective advantage was observed in lymphoid lineages (VCN: 0.93-2.04 and WASP+ cells: 70.9 ± 4.8%). TCR repertoire, proliferative responses to anti-CD3, and NK cytotoxic ability were improved. WASP expression was also observed in the majority of platelets. All patients are currently clinically well and independent from platelet transfusions.
In conclusion, the unprecedented level of gene transfer obtained with LV-WAS resulted in robust engraftment of transduced HSC even when combined to reduced intensity conditioning. The ongoing follow up will allow to establish the long-term safety and clinical efficacy of LV gene therapy for WAS.

W6.07.05
Multifunctional CD26hi Th17 cells eradicate large human tumors

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Human $CD4^+$ T cells differentiate into multiple effector subsets, but their distinct roles in anti-tumor immunity remain elusive. $CD4^+$ T cell subsets enriched from bulk human $CD4^+$ T cells [Th1 ($CXCR3^+$), Th2 ($CCR4^+$), and Th17 ($CCR6^+$ or $CD26^{hi}$)] were stimulated with anti-CD3 beads bearing agonists to either CD28 or ICOS and were then engineered with a chimeric antigen receptor that recognizes human mesothelioma. *In vitro*, ICOS costimulation proved superior to CD28 for augmenting the function of human Th1, Th2, $CCR6^+$ Th17 and $CD26^{hi}$ Th17 cells, as indicated by elevated production of IFN-γ, IL-4 and IL-17A, respectively. Moreover, $CD26^{hi}$ Th17 cells possessed strikingly enhanced polyfunctionality compared to Th1, Th2 or $CCR6^+$ Th17 cells, as demonstrated by their heightened capacity to co-secrete IL-17A, IFN-γ, IL-22, IL-2, and TNF-α simultaneously. Compared to other enriched T cell subsets, a greater percentage of $CD26^{hi}$ Th17 cells exhibit an effector memory phenotype. Additionally, $CD26^{hi}$ Th17 cells expressed higher levels of ICOS, but lower levels of the regulatory-associated molecule CD39 on their cell surface than the other subsets. *In vivo,* $CD26^{hi}$ Th17 cells more efficiently reconstituted immunodeficient hosts and persisted long-term. Furthermore, $CD26^{hi}$ Th17 cells possessed a superior ability to kill large human tumors (>150mm^2) when infused into mice compared to Th1, Th2 or $CCR6^+$ Th17 cells. These results suggest that the generation of multifunctional, long-lived human Th17 populations could be instrumental to the design of novel, effective T cell-based cancer therapies.

W6.07.06
Identification of an alternative specificity for engineered T cells expressing an enhanced affinity MAGE-A3 TCR

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MAGE-A3 belongs to the family of cancer testis antigens and is considered an ideal target for adoptive therapy given its re-activation in various tumours and limited normal tissue expression. We developed an affinity optimised T cell receptor (TCR) specific for the HLA-A1 MAGE-A3 antigen for use in adoptive therapy. Pre-clinical studies indicated no off-target concerns and a clinical study in melanoma and multiple myeloma was initiated. Four days following administration the first patient died. The resulting autopsy revealed ASCVD with two prior silent MIs. Death was attributed to hemorrhagic MI precipitated by demand ischemia and review by the FDA allowed the study to be reopened. Infusion of the second patient resulted in cardiogenic shock and death at day 5. Immunohistochemical evaluation of the heart revealed T cell infiltrates; however, MAGE-A3 was not detected in cardiomyocytes or heart autopsy tissue. Post-mortem investigations did not reveal cross-reactivity against an array of cardiac cells. However, beating cardiomyocytes derived from induced pluripotent stem (iPS) cells triggered T cell killing. Using amino acid scanning, a peptide from the protein Titin was identified as an alternative target for the MAGE A3 TCR. Titin expression in iPs derived cardiomyocytes was confirmed and the displayed peptide was identified by mass spectrometry indicating targeting to Titin as the likely cause of *in vivo* toxicity. The results indicate recognition of similar but unrelated peptides by TCRs presents a safety risk for T cell-based therapies and highlights the requirement for preclinical studies to mitigate these risks in future clinical studies.

W6.08 Stem cells in immunity

IL6.08.01
Harnessing stem-cell like memory T cells for adoptive cell transfer therapy of cancer

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Stem cells are defined by the ability to self-renew and to generate differentiated progeny, qualities that are maintained by evolutionarily conserved pathways that can lead to cancer when deregulated. There is now evidence that these stem cell-like attributes and signaling pathways are also active in subsets of antigen experienced memory T lymphocytes, especially in T memory stem cells and IL-17-producing CD4+ and CD8+ T cells. Here, we will discuss how using stem cell-like T cells can overcome the limitations of current adoptive T cell therapies, including inefficient T cell engraftment, persistence and ability to mediate prolonged immune attack. Conferring stemness to naturally occurring or genetically engineered antitumor T cells by activation of pathways governing stem cell behavior or reprogramming terminally differentiated T cells with stem cell-associated transcription factors and microRNAs could unleash the full potential of cellular therapies and enable cancer immunotherapists to fight 'fire with fire' with ever increasing effectiveness.

W6.08.01
Potential roles for adipose-derived mesenchymal stem cells in the regulation of the mucosal immune system in aging

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Various clinical trials have shown the regenerative capacity of adipose-derived stem cells to differentiate into blood cells, epithelial cells, adipocytes, chondrocytes, and osteoblasts. Age-associated dysregulation of the gastrointestinal (GI) immune system has also been well documented. It has been shown that antigen (Ag)-specific secretory IgA (SIgA) antibody (Ab) responses in the GI tract are impaired in aged mice. In this study, we have examined the potential of adipose-derived mesenchymal stem cells (AMSCs) to restore the GI mucosal immune system in aged mice. One year-old mice were adoptively transferred with AMSCs (2 x 10^6 /mouse). Eight to ten months later, mice were orally immunized with ovalbumin (OVA, 1 mg) plus cholera toxin (10 μg) as mucosal adjuvant three times at weekly intervals. When fecal extracts and plasma were subjected to OVA-specific ELISA, elevated levels of mucosal SIgA and plasma IgG Ab responses were noted in aged recipients. When OVA-stimulated $CD4^+$ T cell cytokine production was examined, Peyer's patch $CD4^+$ T cells produced increased levels of IL-4. Further, $CD4^+$ T cells from GI tract lamina propria revealed elevated levels of IFN-γ and IL-4 production. In contrast, aged mice without AMSC transfer

showed essentially no OVA-specific Ab or $CD4^+$ T cell cytokine responses. Interestingly, total numbers of thymocytes and the frequency of double negative cells were increased in aged mice with AMSC transfer when compared with aged mice without AMSC transfer. These results show major roles for AMSCs in restoring impaired mucosal immunity and the thymus in aged mice.

W6.08.02
Clinical effects of intraperitoneal Mesenchymal stem cells and Mesenchmal stem cells - derived Neural Progenitor Cells in a chronic experimental model of multiple sclerosis

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Mesenchymal stem cells (MSCs) and neural stem cells have been shown to have immunoregulatory and regenerative potential. In this study we investigated the therapeutic effects of injection of mice Bone Marrow-derived MSCs and MSCs-derived neural progenitor cells (NPCs) in chronic experimental autoimmune encephalomyelitis (EAE), a well known animal model for multiple sclerosis. In this study, 1x106 MSCs or NPCs or a combination of MSCs and NPCs were injected intraperitoneally at the peak of disease (on day 22) into three EAE groups of mice and clinical scores, antigen-specific ex vivo proliferation of T cells as well as IFN-g, IL-10 and IL-17 secretion by splenocytes were assessed to evaluate the in vivo immunomodulatory effects of MSCs and NPCs. Clinical scores were significantly decreased in MSCs (1.6±0.17), NPCs (1.1±0.29) and MSCs+NPCs (1.1±0.36) treated groups compared to those of EAE control group (2.4±0.26) received PBS injection (p=0.001). Interestingly, in NPCs and MSCs+NPCs-treated groups continued clinical score improvement were observed without relapsing and worsening of EAE. In addition, in comparison to EAE control mice, injection of NPCs has resulted a significant decrease in proliferation of MOG-specific CD4+ T cells (p=0.001), reduced splenocytes-derived IFN-γ (1600 ±31 pg/mL and 200± 15 pg/mL, respectively; p=0.009) and IL-17 (108±506 for EAE control mice and 27±5.6 for NPCs, respectively; p=0.01) production as well as augmentation of IL-10 production (p=0.03). In conclusion, our study demonstrated that MSCs-derived NPCs are therapeutically more efficient than MSCs in the treatment of chronic EAE and their immunomodulatory potentials might be involved in this phenomenon.

W6.08.03
Interferon-gamma impairs the self-renewal of hematopoietic stem cells during viral infection

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Balancing the processes of hematopoietic stem cell (HSC) differentiation and self-renewal is critical in maintaining life-long supply of blood cells. The bone marrow (BM) produces a stable output of newly generated cells, but immunological stress conditions inducing leukopenia increase the demand for peripheral blood cell supply. An important mediator in this respect is the pro-inflammatory cytokine Interferon-gamma (IFN-γ), which is produced in the bone marrow by activated T cells during the course of a viral infection. Here we demonstrate that IFN-γ impairs the maintenance of HSCs by directly reducing their proliferative capacity both in vitro and in vivo, and that IFN-γ impairs restoration of HSC numbers upon viral infection. We show that IFN-γ reduces thrombopoietin (TPO)-mediated phosphorylation of STAT5, which is an important positive regulator of HSC self-renewal. Furthermore, IFN-γ deregulates the expression of the STAT5-mediated cell cycle genes CyclinD1 and p57. Interestingly, on more committed progenitors IFN-γ rather induced rapid differentiation towards the myeloid lineage. Based on these experiments we conclude that IFN-γ reduces HSC self renewal by modifying cytokine responses and expression of genes involved in HSC proliferation, whereas it boosts myelopoiesis in downstream hematopoietic progenitor cells. Our findings thereby provide challenging new insight regarding the impact of immune activation on hematopoiesis, in particular during viral infection. We postulate that the occurrence of BM failure in chronic inflammatory conditions, such as HIV, aplastic anemia and graft-versus-host disease is related to a sustained impairment of HSC self-renewal caused by chronic IFN-γ-signaling in these disorders.

W6.08.04
Mouse Compact Bone Derived Mesenchymal Stem Cells Supress Airway Inflammation in Both Chronic and Acute Murine Asthma Model

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Aim: The aim of the present study was to investigate the efficacy of mouse compact bone (mCB) derived MSCs on lung histopathology and lymphocyte proliferation.
Methods: mCB-MCS were isolated from BALB/c mice, characterized and marked by GFP. To generate murine models of chronic and acute asthma, mice were i.p. sensitized with OVA and exposed to aerolized OVA. mCB-MSCs (2.5x105 cells) were administrated i.v. after last nebulization. Mice were sacrified, and splenocytes and lung lymphocytes were isolated and marked with CFSE. Cells stimulated with OVA (40µg/ml) were cultured under suitable conditions for 5 days. Flow cytometric analysis and histopathological examination of lungs were evaluated. In histopathological analysis, the measurements were performed from minimum 5 points of each airway and mean values were calculated. Goblet cells stained with PAS enumarated in 2500 cells.
Results: In sections stained with H&E, the distal [without MCS chronic:29,9µm acute:32,03µm; with MSC chronic:13,3µm acute:12,25µm] and proximal [without MCS chronic:42,6µm acute:28,97µm; with MSC chronic:17,4µm acute:18,9µm] airway epithelial thicknesses were observed to decrease in both mouse models. Likewise, in sections stained with PAS, a significant reduction in number of hyperplasic goblet cells in the proximal [without MSC chronic:140 acute:1200; with MSC chronic:4 acute:211] and distal [without MSC chronic:55 acute:118; with MSC chronic:0 acute:0] airways was observed. Morever, in the CFSE staining experiment, CB-MSCs inhibited lymphocyte proliferation in both asthma model.
Conclusion: The results reported here provide that mCB-MSCs may provide powerful alternative therapeutic for the treatment of chronic and acute asthma.

W6.08.05
Allogenicity of human cardiac stem/progenitor cells orchestrated by programmed death ligand 1 (PD-L1)

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Rationale: Transplantation of allogeneic cardiac stem/progenitor cells (CPC) in experimental myocardial infarction promoted cardiac regeneration and improved heart function. While this has enhanced prospects of using allogeneic CPC for cardiac repair, the mechanisms regulating the behavior of these allogeneic cells, which are central to clinical applications, remain poorly understood.
Objective: T cells orchestrate the allogeneic adaptive immune response. Therefore, to provide insight into the mechanisms regulating the immunologic behavior of human CPC (hCPC), we investigated the allogeneic T-cell response elicited by cryopreserved c-kit-selected hCPC.
Methods and Results: By using an experimental model of allogeneic stimulation, we demonstrate that, whether under inflammatory conditions or not, hCPC do not trigger conventional allogeneic Th1 or Th2 type responses but instead induce proliferation and selective expansion of suppressive CD25highCD127lowHLA-DR+FoxP3high effector regulatory T cells (Treg). The Treg proliferation and amplification was dependent on the interaction with the B7 family

member programmed death-ligand 1 (PD-L1) which is substantially expressed on hCPC and increased under inflammatory conditions. Thus, hCPC in allogeneic settings acquire the capacity to down-regulate an ongoing immune response, which was dependent on PD-L1. Conclusions: Collectively these data reveal that hCPC in allogeneic settings have a "tolerogenic" immune behavior, promoting a contact-PD-L1-dependent regulatory response and a PD-L1-dependent allogeneic-driven immunomodulation. Our study attributes an important role for PD-L1 in the immune behavior of allogeneic hCPC and raises the possibility of using PD-L1 expression as a marker to identify and select low-risk high-benefit allogeneic cardiac repair cells.

W6.08.06
The acquisition of immunomodulating properties by mouse mesenchymal stem cells in presence of interferon-gamma is associated with a reduced expression of Nanog and alteration of their differentiation potential

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Bone marrow-derived adult stromal stem cells, referred to as mesenchymal stem cells (MSC), display a therapeutic plasticity, which has been exploited for the treatment of diseases where both immune and degenerative components are present. Indeed, MSC have the ability to modulate immunity and foster tissue repair, and to differentiate into cells of mesodermal lineage. The role of interferon-gamma (IFN-g) in the physiology of MSC has been demonstrated: IFN-g is necessary for the immunomodulatory activity of MSC and affects their differentiation potential. Here, we investigated the changes in gene expression profile of MSC following exposure to IFN-g. We observed a reduced expression of genes involved in WNT and TGF-beta pathway and of the transcription factor Nanog, which is essential for the maintenance of pluripotency, together with an increased expression of genes involved in MSC immunomodulatory function; the increased immunomodulatory effect of IFN-g-treated MSC was associated with a concomitant reversible alteration of pluripotency and differentiation potential. We have analyzed the dynamics of molecular pathways targeted by IFN-g in MSC. We show that IFN-g induces a time-dependent switch of mTOR activation. Incubation of MSC with the mTOR inhibitor Rapamycin increased the IFN-g-dependent induction of genes involved in the immunomodulatory effect of MSC and impaired differentiation towards adipocytes and osteocytes. In contrast, inhibition of STAT3 and ERK1/2 affected self-renewal and promoted differentiation, respectively. These data support the pivotal role of inflammation in inducing an immunomodulatory trait in MSC and suggest a relationship between the acquisition of strong immunomodulating activity and pluripotency in MSC.

W6.09 Vaccine adjuvants

IL6.09.01
Vaccine adjuvants for mucosal immunity: recent progress and future challenges

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Most human pathogens take their portal of entry or establish infection in the mucosal tissues. There is an urgent need to develop new vaccines or improve the existing human vaccines to counter mucosally transmitted pathogens. Development of safe and potent vaccine adjuvants able to mount broad, long-lasting protective immune responses in the mucosal tissues represents a top priority. By employment of transcriptomics and metabolomics combined with systems biology, we could identify immunological and metabolical biomarkers predicting adjuvanticity in mice. A number of genes involved in immunological and metabolism process and pathways were found to be correlating with adjuvanticity in mice. Recent developments in the field of vaccine adjuvants as pertain to understanding the mode of action of the existing human vaccine adjuvants along with recent data from our lab on discovery of novel biomarkers of experimental mucosal adjuvants will be presented. The underlying premise is that understanding the mode of action of potent and safe immunomodulatory agents/adjuvants could inform rational development of novel adjuvants able to engender protective immune responses in the mucosal tissues.

IL6.09.02
Perspectives in vaccine adjuvants

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The design of more powerful adjuvants is a tool of crucial interest to ameliorate vaccination strategies to reduce injections and/or dose of antigen, induce local immunity and obtain better protection. Effective vaccines against pathogens should elicit protective T_H1 responses, cytotoxic CD8+ cells and antibody-forming cells. Type and persistence of the antigen, nature and differentiation state of antigen-presenting cells, cytokine microenvironment are equally key points for the development of appropriate adaptive immune responses in naive recipients and prophylactic or therapeutic vaccinations. There is a general consensus about the optimal cytokine milieu for protective immunization as IL-12 and type I IFN are necessary for adequateT_H1-polarization. Efficient adjuvants and delivery to appropriate DC subsets would be highly appreciated in order to properly boost innate immune cells especially in targeted therapeutic vaccinations when interference with an already-existing inappropriate immunity is required. Aluminium hydroxide used since almost one century, although safe, has been classically associated with the induction of a TH2 response to co-formulated antigens, at least in the mouse. Therefore, the use of appropriately conditioning as safe as alum, and potentially orally available, adjuvants, would be highly hopeful. In this perspective, MF-59 and detoxified LPS (MPL-A) actually represent improvements in vaccine strategies. However, large experiences both from in vivo as well as from in vitro cultured human cells come from several low molecular weight synthetic compounds, such as CpG-ODNs, imidazoquinolines and adenine derivatives, which could provide the optimal cytokine milieu to induce T_H1 differentiation and interfere with pathogenic T cells via innate sensors.

W6.09.01
Novel adjuvants for DNA vaccines

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The failure of protein-based vaccines to prevent HIV/HCV infection highlights the need for novel vaccine strategies. We provide evidence that induction of necrosis or co-expression of antigen with heat shock protein 70 (Hsp70) in DNA vaccine-targeted cells provides a potent adjuvant effect. Necrotic, antigen-positive cells release a range of intracellular factors that signal via receptors on dendritic cells (DC) and lead to enhanced cross-presentation of antigen. We report that a DNA vaccine that induces cytolytic, rather than apoptotic, cell death enhances CD11c+ CD8α+ DC activation, broad and multifunctional CD8 T cell responses and increases protection in mice challenged with a chimeric virus, EcoHIV. This was dependent on both the mechanism and timing of cell death after antigen expression.
Hsp70, as damage associated molecular pattern, can bind to dendritic cells and up-regulate the expression of co-stimulatory molecules, making Hsp70 a potential DNA vaccine adjuvant. A DNA vaccine encoding HIV gag and Hsp70 with antigen expression controlled by separate promoters was constructed. Genetic modifications resulted in cytoplasmic, secreted and membrane-bound forms of Hsp70. Membrane bound and secreted Hsp70 significantly increased the numbers of IFN-γ secreting T cells, $CD4^+$ and $CD8^+$ triple cytokine-producing T cells and T cell proliferation. Thus, indicating that modifying HSP70 to translocate to the plasma

membrane or to the extracellular milieu may greatly improve the potency of DNA vaccines.
These novel strategies have the potential to increase the efficacy of DNA vaccines, either as a DNA vaccine *per se* or as a prime in a DNA prime/virus boost regimen.

W6.09.02
Using immunopotentiating capacity of flagellin for sublingual immunotherapy

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Mucosal vaccination is an attractive strategy for preventing infectious diseases. Intranasal vaccination is particularly effective for prophylaxis of respiratory infections, but poses safety issues associated with brain toxicity. Instead, sublingual (s.l.) immunotherapy may offer a safer alternative, although due to the pro-tolerogenic environment of the s.l. mucosa strong adjuvants are often required to potentiate immunogenicity of non-living antigens.
We evaluated adjuvanticity of flagellin (FliC) from Salmonella enterica serovar Typhimurium, via s.l. route. Furthermore, we tested FliC as s.l. immunostimulant by assessing its therapeutic value against invasive pneumococcal pneumonia.To address FliC adjuvanticity by s.l. route, we used OVA as model antigen, and evaluated the response in mice vaccinated with OVA alone or OVA+FliC. Sublingual vaccination with OVA+FliC augmented serum levels of OVA-specific antibodies and induced isotype-switching. Using flagellin mutants deficient in TLR5 or NLRC4 activation, we confirmed that engagement of a single pattern-recognition receptor is sufficient for adjuvant effect. Adoptive transfer of OT-II lymphocytes to wild-type counterparts, demonstrated that OVA+FliC vaccination promotes priming of naïve CD4+-T cells in submaxilar lymph nodes. Subsets of dendritic cells involved in antigen processing were determined using self-quenched OVA, emitting fluorescence after lisosomal degradation.
S.l. FliC administration was also effective as a therapeutic agent to prevent mortality in a mouse model of pneumococcal pneumonia. FliC-treated animals had increased levels of Il6 and Cxcl1/2 mRNA in lungs compared to infected controls and protection depended on neutrophil recruitment into the airways.
Our results highlight flagellin as a candidate immunopotentiator for new therapeutic and prophylactic sublingual vaccines.

W6.09.03
A Dectin-1-assisted APC-targeting TLR9-agonist as an adjuvant

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Innate immune stimulators including TLR ligands have been shown to work as good vaccine adjuvant. One of these stimulators, CpG ODN is a potent innate immune activator through TLR9, and a promising vaccine adjuvant and anti-cancer drug candidate. β-glucans has been known to induce innate immune signaling through a C-type lectin receptor (CLR), Dectin-1. Recently, some studies showed CLR signaling inhibits TLR and IFNAR signaling. However, these studied did not fully elucidated correlation between TLR and CLR, especially type I IFN production and adjuvanticity.
In this study, we examined weather CLR signaling influences TLR-induced type I IFN production. Our data demonstrated that Zymosan Depleted, which acts as pure Dectin-1 ligand suppressed IFN-α production after CpG ODN stimulation. However, soluble Dectin-1 ligand (SPG) did not.
We have previously shown that SPG forms of triple helix structure with single stranded DNA using specific sequence. We examined bioactivity and adjuvanticity of CpG-SPG complex, in vitro and in vivo, respectively. Accordingly, our data demonstrated that stimulation of immune cells with CpG-SPG complex induced more IL-12 and IFN-α production than naked CpG ODN. In mice, CpG-SPG strongly induced both humoral and cellular immune responses in a TLR9 dependent manner, but partially dependent on Dectin-1. Furthermore, CpG-SPG complex showed a potent adjuvanticity against influenza vaccine. Finally, we demonstrated that humanized CpG-SPG complex enhanced IFN-α production from human PBMCs. Our results suggested that CLR signaling inhibits TLR signaling via Dectin-1 signaling and CpG-SPG complex is a superior vaccine adjuvant than naked CpG ODN to use in clinical.

W6.09.04
Adjuvants are the future of Vaccine Formulation. The case of Finlay Adjuvants

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Background. After Jenner/Pasteur most vaccinologists have been looking for specific antigens. Nevertheless, for unsolved-vaccine diseases adjuvants are determinant, and certainly, the future of these vaccine formulations. Current development of Immunology and related disciplines permits its rational design and characterization. **Aim.** To show resent advances using Proteoliposomes (PL) and its derivate Cochleate (Co) adjuvants for prophylactic and therapeutic vaccines. **Results.** AF (Adjuvant Finlay) namely AFPL and AFCo were design for parenteral and mucosal application, respectively. AFPL1 is derived from PL of *Neisseria meningitidis* serogroup B and adsorbed onto $Al(OH)_3$ in order to increase stability and safety. It has been widely used in humans in a prophylactic meningococcal vaccine where AFPL1 was an essential adjuvant. A comparable PL is also one of the components of 4CMenB Novartis vaccine. AFPL1 has been also evaluated as an adjuvant for a therapeutic/prophylactic allergen vaccine shifting the allergen-specific Th2 pattern to Th1/Tr1 in mice and a Phase I trial is ongoing. AFPL1 was used at preclinical stage with several other antigens. AFCo1, a Co derived from AFPL1, is a mucosal adjuvant. AFCo3 non-derived from AFPL1, has been tested as immunopotentiator in fish decreasing their mortality. AF are able to function in simultaneous primes by parenteral and mucosal routes, inducing also mucosal immune response. Lastly, AF overcomes the thymus-independence of plain bacterial polysaccharides inducing a Th1 polarization and memory immune response at least in mice. **Conclusion**. AF are very promising adjuvants for unsolved-vaccine diseases including mucosal approaches and therapeutic vaccines.

W6.09.05
Antigen trafficking within lymphoid organs and humoral immune response: the effect of MF59 adjuvant

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Antigen retention within lymphoid organs facilitates the encounter with cognate B cells. Subcapsular sinus (SCS) macrophages and follicular dendritic cells (FDCs) of lymphoid organs are described to have key role in the humoral immune response because of their ability to deliver antigen trapped in immune complexes (ICs) onto cognate B cells. Adjuvants are substances that enhance immune response by a variety of mechanisms that are still partially understood. Here we show that during an endogenous response to an injected antigen, MF59 enhances antigen retention by either SCS or medullary macrophages and afterwards by FDCs. In addition, we observed that MF59 reaches the SCS and medullary compartments of the draining lymph node and can be detected here for prolonged period of time, such as one or two weeks. Moreover, MF59 is engulfed by macrophages in the form of big vesicles. The antigen capture by macrophages is independent from the antigen-specific IgG titer and its enhancement correlates with the ability of MF59 to induce a strong early antigen-specific antibody production and generation of germinal

centers (GCs). We also demonstrated that the activation of FDCs is necessary but not sufficient to detect GC formation and that both antigen trapping by FDCs and generation of GCs requires IC formation and cell activation. Taken together all these findings strongly suggest that the antigen retention within lymphoid organs affects the quality of the humoral immune response. The elucidation of the way MF59 enhances antigen retention will help to improve the efficacy of emulsions as vaccine adjuvants.

W6.09.06
SBA-15 nanostructured silica adjuvant enhances immune responsiveness and modulates sensibility to bacterial toxins

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Silicon oxide particles formed of ordered nanopores (SBA-15) are promising adjuvant vectors. Here we explored how silica might act in the enhancement of phagocytosis, the recruitment of inflammatory cells and also in the modulation of toxicity after administration of exo or endotoxins encapsulated in SBA-15. Confocal microscopy and flow cytometry tests confirmed that the pre-incubation of FITC labelled ovalbumin with SBA-15 improved the phagocytosis of the protein by bone marrow derived DC from C57BL/6 mice. The recruitment of cells evaluated by flow cytometry after 24 and 72 hours of subcutaneous injection of SBA-15 showed the presence of inflammatory cells at the site of injection, especially mature DC. BALB/c mice intraperitoneally immunised (ip) with diphtheria toxin (1 µg/mL): SBA-15 (25 µg/mL) presented 100% of survival rate whereas all mice that received diphtheria toxin (1 µg/mL): Alum (25 µg/mL) died. However, BALB/c mice inoculated ip with LPS from Salmonella typhimurium (100 µg/mL) in SBA-15 (1000 µg/mL) showed a higher death percentage (55%) compared to the LPS (100 µg/mL):Alum (1000 µg/mL) group (36%). Though, both experimental groups were able to produce similar specific antibody titres indicating that SBA-15 worked well as immunological adjuvant, reducing the toxicity of the diphtheria toxin in immunisations but increasing the death of mice injected with LPS. These data confirm that SBA-15 acts as adjuvant for antibody responses and suggest that its effects may reflect enhanced availability of antigen without modifying its properties.

W6.10 Mucosal vaccines

IL6.10.01
Acute epithelial death plays a role in intrinsic defense against enteric bacteria

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The interactions of host and microbes, including those commensal and pathogenic, in the mucosal compartments are currently one of the hot topics in immunology field. Further, host cell death is critical to the fate
of bacterial infection, to host innate immune responses, and to disease outcome. “The demise of infected cells” plays a pivotal role in sacrificing damaged cells, eliminating pathogens, limiting microbial replication, and emitting alarm signals. Previous studies demonstrated that *Shigella* invasion induces both necrosis-like death in the epithelial cells and apoptosis-like death in the macrophages via a caspase-1-independent manner. In contrast, bacterial pathogens deploy multiple mechanisms to postpone host cell death and favor infection. Here we found that *Shigellae* can invade the terminal ileum of murine small intestine at 1 hour of the early phase but are rapidly cleared within 24 hours. At early phase, we found several histological changes without inflammation including epithelial shedding and cell death in the lamina propria and crypt. Dead products, including DNA and intracellular organelles, trapped *Shigellae* and then damaged tissue was mostly recovered within a day. These acute responses were general events against human-specific pathogens such as *E. coli* as well as murine-specific pathogens such as *Citrobacter rodentium*. Interestingly, the human intestine also shares this intrinsic defense. Therefore, we propose the concept of a general intrinsic mechanism that repels the initial introduction of enteropathogenic bacteria by sacrificing hazardous host cells. To overcome this defense mechanism and successfully disseminate, some pathogens may have evasion strategies

IL6.10.02
Novel HIV-1 IL-13Rα2 adjuvanted vaccines: High avidity mucosal and systemic CD8 T cells with greater protective immunity

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Our studies have demonstrated that compared to a purely systemic (intramuscular - i.m.) HIV-1 prime-boost immunisation, mucosal (intranasal - i.n.) immunisation can induce cytotoxic CD8 T cells (CTL) of higher avidity with lower IL-4/IL-13 activity and better protective immunity. Our recent studies, indicate that co-expression of IL-13Rα2 soluble or membrane bound receptors together with HIV-1 vaccine antigens in an i.n./i.m pox viral prime-boost vaccination modality can i) dramatically enhance HIV-specific systemic and mucosal CTL avidity and multi-functionality. ii) Also following a surrogate mucosal HIV-1 challenge induce excellent protective immunity similar to an IL-13 gene knockout animal. When how the transient inhibition of IL-13 could induce high avidity CTL were investigated, data revealed that i) CD8 T cell avidity is defined at the vaccination site the lung mucosae at very early stages of priming and ii) according to the antigen presenting cell subsets these vaccines induce or recruit. Moreover at the T cell level, the down-regulation of IL-4Rα densities on effector CD8 T cells and up-regulation of CD8α/β co-receptors play a critical role in modulating CD8 T cell avidity. Our findings not only offer exciting prospects for a future HIV-1 vaccine development but also many other chronic mucosal infections where high avidity CD8 T cells are required for protection.
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W6.10.01
Microbe-dependent proliferating IgA-producing plasma cells mediate early-phase robust intestinal IgA responses in mice

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Intestinal plasma cells (PCs) predominantly produce IgA, but their immunological complexity remains obscure. Here, we show that murine intestinal IgA PCs can be newly classified into two populations on the basis of CD11b expression, which cannot be discriminated by currently known criteria such as general PC markers, B-cell origin (e.g., B1 and B2 cells), and T-cell dependence/independence. $CD11b^+$ IgA^+ PCs require the lymphoid structure of Peyer’s patches, produce more IgA than $CD11b^-$ IgA^+ PCs, proliferate vigorously, and require microbial stimulation and IL-10 for their development and maintenance. The proliferating IgA^+ cells dominantly present in the $CD11b^+$ IgA^+ PCs mediate early-phase antigen-specific intestinal IgA responses induced by oral immunization with protein antigen. Indeed, preferential depletion of $CD11b^+$ IgA^+ PCs results in the significant impairment of intestinal IgA production against orally immunized antigens. These findings reveal the functional diversity of IgA^+ PCs in the murine intestine and possible target in the development of oral vaccine.

W6.10.02
Development a novel mucosal vaccine targeted to GP2 on Peyer's patch M cells

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Mucosal vaccines can induce systemic immunity and mucosal immunity, such as antigen-specific SIgA, on mucosal surface. By contrast, vaccines injected by syringe and needle induce only systemic immunity, not mucosal immunity. Accordingly, mucosal vaccines can protect from pathogens before their invasion across mucosal barrier. However, mucosal vaccines have some disadvantage; for example, oral vaccines need to resist gastric acid and digestive enzymes to reach mucosal immune tissues such as Peyer's patches (PPs). To overcome the problem, live attenuated pathogens are often used as oral vaccines since they can propagate to produce enough immunogenicity even if a small portion of the administered amount could reach the immune tissue. However, attenuated pathogens could give rise to virulent revertants causing adverse side effects. To circumvent the problem, we developed a novel mucosal vaccine targeted to GP2. GP2 is an uptake receptor on PP M cells for $FimH^+$ bacteria to induce subsequent mucosal and systemic immune responses. We constructed a fusion protein of the Fab fragment from anti-GP2 monoclonal antibody and streptavidin core (anti-GP2-SA). In this report, we show that anti-GP2-SA is able to bind to GP2 on M cells and is taken up into PPs. Concurrently, biotinylated antigen proteins are delivered to PPs with anti-GP2-SA. These results suggest that anti-GP2-SA may induce mucosal immunity and obviate the risk of infectious diseases as a mucosal vaccine.

W6.10.03
Molecular Uniformed Rice-based Oral Cholera Toxin B Subunit Vaccine without Plant-associated Sugar Modification Induces Toxin-specific Neutralizing Immunity in Mice and Macaques

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Plants have been used as expression systems for a number of vaccines. However, the expression of vaccines in plants sometimes results in unexpected modification of the vaccines by N-terminal blocking and sugar-chain attachment. Although MucoRice-CTB was thought to be the first cold-chain-free and unpurified oral vaccine, the molecular heterogeneity of MucoRice-CTB, together with plant-based sugar modifications of the CTB protein, has made it difficult to assess immunological activity of vaccine and yield from rice seed. By using a T-DNA vector driven by a prolamin promoter and a signal peptide added to an overexpression vaccine cassette, we established MucoRice-CTB/Q as a new generation oral cholera vaccine for humans use. We confirmed that MucoRice-CTB/Q produces a single CTB monomer with an Asn to Gln substitution at the 4th glycosylation position. The complete amino acid sequence of MucoRice-CTB/Q was determined by MS/MS analysis and the exact amount of expressed CTB was determined by SDS-PAGE densitometric analysis to be an average of 2.35 mg of CTB/g of seed. To compare the immunogenicity of MucoRice-CTB/Q, which has no plant-based glycosylation modifications, with that of the original MucoRice-CTB/N, which is modified with a plant N-glycan, we orally immunized mice and macaques with the two preparations. Similar levels of CTB-specific systemic IgG and mucosal IgA antibodies with toxin-neutralizing activity were induced in mice and macaques orally immunized with MucoRice-CTB/Q or MucoRice-CTB/N. These results show that the molecular uniformed MucoRice-CTB/Q vaccine without plant N-glycan has potential as a safe and efficacious oral vaccine candidate for human use.

W6.10.04
Advantage of a new intra-cheek immunization route for therapeutic vaccines of human papillomavirus-associated head and neck cancers

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Papillomavirus (HPV)-16 infection has been recently associated with oropharyngeal head and neck cancers (HNCs) that express the viral E6 and E7 oncoproteins. The licensed vaccines are efficient for the prevention of HPV infection, but not for established tumors. Therefore innovative therapeutic vaccines targeting HPV oncogenes are required. Recently, we have described the efficiency of therapeutic intra-dermic DNA vaccinations using plasmo-virus like particles carrying the E7 oncoprotein (pVLPs-E7) associated with electroporation to control the growth of TC1 tumors subcutaneously injected in the flank of C57Bl6 mice. The present study aims at comparing immune and anti-tumoral efficacy of different routes of vaccination with pVLPs-E7.
Here, we have used an orthotopic model of HPV-induced HNCs where TC1 cells were injected into the cheek of C57BL6 mice. Tumor growth and anti-E7 immune response were compared after intra-cheek (i.c.) or intra-dermal (i.d.) pVLP-E7 vaccinations. While i.d. route gave rise to better systemic anti-E7 cellular immune response than i.c. route, both routes elicited superposable local anti-E7 immune responses. Interestingly, only i.c. route elicited an anti-E7 humoral response. When vaccinations were performed in mice bearing well-established tumors, a better therapeutic effect was observed using i.c immunizations. Furthermore, anti-tumoral responses were optimized by combining vaccinations with TLR agonists (Imiquimod and CpG) as adjuvants.
Our data show that i.c. mucosal vaccinations with pVLP-E7 combined with adjuvants, evaluated in an orthotopic tumor model, appear to be a valuable therapeutic strategy for HPV-induced HNCs.

W6.10.05
Cross-strain protective immunity elicited by a live attenuated nasal spray influenza vaccine after prime-boost immunization

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Antigen drift and shift of influenza viruses requires frequent reformulation of influenza vaccines. This stresses the need for a universal influenza vaccine. In this study, mice were vaccinated with the trivalent live attenuated (LAIV, nasal spray FluMist) and inactivated (TIV, intramuscular injection FluZone) influenza vaccines using 1-3 dose prime-boost vaccination regimens. While vaccination with both LAIV and TIV elicited strong humoral responses, only LAIV induced significant protection against challenge with a non-vaccine strain: H1N1 PR8 influenza virus. Furthermore, protective immunity against PR8 was dose-dependent. Of note, IL-2 and IFN-γ cytokine secretion in lung alveolar fluids were significantly elevated in mice immunized with LAIV. Moreover, T cell depletion of LAIV-immunized mice compromised protection, indicating that T cell-mediated immunities were required. In contrast, passive transfer of sera from mice immunized with LAIV into naïve mice failed to protect against PR8 challenge. To verify these results, we performed virus neutralization assays in vitro and confirmed that LAIV did not induce cross-strain neutralizing antibodies against PR8 virus. Finally, we showed that 3 doses of LAIV also provided protection against challenge with two additional heterologous viruses: FM/47 (H1N1) and HK/68 (H3N2). These results support the potential use of the LAIV as a universal influenza vaccine under a prime-boost vaccination regimen.

W6.10.06
Safety evaluation of Neuraminidase as a targeting substance of allergen-loaded microparticles for oral food allergy treatment

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Background: We have reported that Neuraminidase (NA) from Vibrio cholera represents a promising novel functionalization substance of allergen-loaded Poly(D,L-lactide-co-glycolide) (PLGA) microparticles (MPs) due to specific binding to intestinal epithelial cells and enhanced uptake via M-cells. As NA is of bacterial origin, we aimed to assess the safety of orally applied NA-coated MPs in an ongoing Th2 biased immune response.
Methods and results: The enhanced binding of NA-coated, OVA-loaded MPs to CaCo2 cells was associated with a concentration dependent decrease of cell proliferation. In naïve animals no induction of mast cell mediator release was observed by oral gavages of NA-coated, allergen-loaded MPs suggesting biocompatibility. When splenocytes from naïve mice were stimulated with NA or NA-coated MPs, a release of IL-10 and IFN-γ was observed. To investigate the immunomodulatory capacity of NA-MPs, BALB/c mice were first intraperitoneally injected with ovalbumin (OVA) followed by two oral OVA challenges to induce a profound IgE-mediated, OVA-specific response. After 6 oral administration cycles with NA-coated MPs, the immune response of animals was re-evaluated. Whereas no significant changes in serum antibody levels were observed, we found significantly lower total and OVA-specific IgA levels in intestinal lavages in treated allergic mice than in control groups. When splenocytes from OVA allergic animals receiving NA-coated, OVA-loaded MPs were stimulated with OVA, significantly elevated levels of IL-10 and IFN-γ were measured.
Conclusion: Our data suggest that allergen entrapped in NA-functionalized MPs act immunomodulatory in this type I allergic BALB/c mouse model.

W6.11 Vaccination and immunotherapy for infectious diseases

IL6.11.01
Novel Immune-modifying Nanoparticles for the Treatment of Inflammatory monocyte-mediated Disease

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Infiltrating inflammatory monocyte-derived macrophages and dendritic cells play key roles in initiating and exacerbating widely disparate disorders, including viral encephalitis, multiple sclerosis, myocardial infarction, reperfusion injury and inflammatory bowel disease. However, no effective therapy specifically targeting these cells exists.
We investigated the novel use of nanoparticles as tools to modulate the behaviour of circulating inflammatory monocytes during myeloid lineage-mediated disease in vivo. Intravenously infused, negatively charged, polystyrene, nanodiamond, or biodegradable poly (lactic-co-glycolic) acid immune-modifying-nanoparticles (IMP) were cleared from the blood within 30 minutes and taken up in an opsonin-independent fashion by inflammatory monocytes, which were then sequestrated by the spleen, undergoing phosphatidylserine flipping, and caspase 3-mediated apoptosis.
In lethal flavivirus encephalitis, this intervention resulted in substantially reduced monocyte migration to the brain, abrogated by splenectomy, with up to 80% long-term survival and sterilizing immunity. Parallel studies in other macrophage-mediated disease models, e.g., PLP139-151-induced experimental autoimmune encephalomyelitis (EAE) and thioglycollate-induced peritonitis, confirmed this therapeutic effect, evidently mediated through the class-A scavenger receptor, MARCO, expressed by both circulating inflammatory monocytes and marginal zone macrophages in the spleen, since IMP co-localize with MZM and this effect is not seen in MARCO-deficient mice.
IMP infusion in rodent models of myocardial infarction and inflammatory bowel disease (dextran sodium sulphate-induced colitis) also markedly reduced acute monocyte accumulation at inflammatory foci, attenuated disease symptoms and was associated with tissue repair. Our data highlight the interplay between scavenger receptor, inflammatory monocyte and spleen functions, and support the translation of IMP for therapeutic use in inflammatory monocyte-mediated diseases.

W6.11.01
HIV CD4 based vaccine pre-immunization increases CD8 T cell responses and confers protection against challenge with a recombinant vaccinia virus encoding HIV Gag-Pol proteins

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A vaccine able to provide cognate help may efficiently prime CD8+ T cell responses establishing an appropriate immune control of HIV. Furthermore CD4 T cells responses also have been correlated with HIV infection control. Our group has identified a set of 27 conserved and promiscuous CD4 epitopes from 8 HIV-1 proteins. HIVBr27 encoding such epitopes was highly immunogenic in BALB/c mice inducing strong CD4+ T cell responses. Here we tested whether pre-immunization with HIVBr27 could provide cognate help and improve CD4 and CD8 T cell responses against other HIV proteins (Gag, Pol and Vif) correlated with HIV protection/disease control.In relation to the CD8 T cells, we observed an overall increase in 57% and 40% of the breadth and magnitude of the responses, respectively, when comparing the CD4 based vaccine pre-immunized group to the control (empty vector pre-immunization). Into the CD4 T cell compartment we noticed an increase of 3 and 2 times in the breadth and magnitude of the responses, respectively, comparing CD4 versus control primed group. We also observed a 10 times reduction on the PFUs after recombinant Gag-Pol vaccinia virus challenge as well as a 32 fold increase in the anti-p24 antibody levels in the HIVBr27 pre-immunized group when compared to the control one. These results highlight the importance of having a CD4 inducer based component as a primer for the global responses, impacting in both cellular and humoral immune response.

W6.11.02
Enhanced and accelerated memory CD8 T cell formation by immunization with MVA-CD40L

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Modified vaccinia Ankara (MVA) is a safe and promising viral vaccine vector that is currently investigated in several clinical and pre-clinical trials. In contrast to inactivated or sub-unit vaccines, MVA is able to induce strong humoral as well cellular immune responses. In order to further improve its CD8 T cell inducing capacity, we genetically adjuvanted MVA-BN® with the coding sequence of murine CD40L, a member of the tumor necrosis factor (TNF) superfamily. Immunization of mice with this new vector led to strongly enhanced primary and memory CD8 T cell responses. Concordant with the enhanced CD8 T cell response, we could detect stronger activation of dendritic cells and higher systemic levels of innate cytokines (including IL-12p70) early after immunization. Interestingly, acquisition of memory characteristics (i.e., IL-7R expression) was accelerated after immunization with MVA-CD40L in comparison to non-adjuvanted MVA. Furthermore, the generated CTLs also showed improved functionality as demonstrated by intracellular cytokine staining and *in*

vivo killing activity. Importantly, the superior CTL responses were able to protect B cell deficient mice against a fatal infection with ectromelia virus. Taken together, we show that genetic adjuvantation of MVA can change strength, quality and functionality of innate and adaptive immune responses.

W6.11.03
IL-4 and IL-13 regulates CD8 co-receptor expression to dampen the quality of anti-viral CD8+ T cell immunity following HIV-1 prime-boost vaccination

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$CD8^+$ T cells are important in controlling viral infections including HIV-1. We have previously shown that interleukin (IL)-4 and IL-13 can dampen the quality (i.e. avidity and polyfunctionality) of anti-viral $CD8^+$ T cells following pox virus infection and prime-boost vaccination. Therefore, we investigated how these cytokines act to regulate anti-viral $CD8^+$ T cell quality. We initially monitored the expression of T cell quality markers (i.e. CD2, CD11a, CD8, T cell receptor and lck) on anti-viral (HIV-specific) $CD8^+$ T cells using flow cytometry following pox viral prime-boost vaccination of BALB/c wild-type (WT), IL-4 $^{-/-}$, IL-13 $^{-/-}$ or signal transducer and activator of transcription 6 (STAT6) $^{-/-}$ mice against HIV-1. In these studies, only CD8 co-receptor expression was enhanced on HIV-specific $CD8^+$ T cells that developed in gene knockout mice compared to WT mice. Using *in vitro* CD8 blocking assays to limit CD8 co-receptor availability for $CD8^+$ T cells, we established that increasing CD8 co-receptor expression could indeed enhance the avidity and polyfunctionality of HIV-specific $CD8^+$ T cells following vaccination. Based on these findings, we have developed novel IL-13 inhibitor pox virus vaccines that can enhance the quality of HIV-specific $CD8^+$ T cells most likely via up-regulation of CD8 co-receptor following vaccination. Our findings suggest that IL-4 and IL-13 can reduce CD8 co-receptor expression to dampen anti-viral $CD8^+$ T cell quality. These findings have important implications for designing efficacious vaccines against not only HIV-1, but also other virus infections.

W6.11.04
Type 2 diabetes patients, both young and elderly, have optimal B cell responses to the seasonal influenza vaccine

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The humoral immune response to the seasonal influenza vaccine in young and elderly patients with type 2 diabetes (T2D) was measured. Immune measures included the in vivo serum response to the vaccine by hemagglutination inhibition (HAI) and ELISA in 22 patients (14 young, 8 elderly) and 65 healthy age-matched controls (37 young, 28 elderly). B cell-specific biomarkers of optimal vaccine response were measured ex vivo by switched memory B cells and plasmablasts and in vitro by activation-induced cytidine deaminase (AID) in stimulated cells. Markers of systemic and B cell-intrinsic inflammation were also measured. Results show that in vivo responses, as well as B cell-specific markers identified above, decrease with age in healthy individuals but not in T2D patients. This occurred despite high levels of B cell-intrinsic inflammation (TNF-α) in T2D patients, which was surprising as we had previously demonstrated this negatively impacts B cell function. We have preliminary data to indicate that the innate immune system of T2D patients is beneficially hyperactivated and we suggest that this may induce a direct stimulation of B cells. These results altogether suggest that valid protection against influenza can be achieved in T2D patients and proposed mechanisms are discussed. Work supported by NIH grants AG032576 (BBB) and AI096446-01A1 (BBB+DF).

W6.11.05
Fusion of HCV non-structural antigen to MHC Class II associated invariant chain enhances T cell responses induced by vectored vaccines in non-human primates

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Developing a vaccine against hepatitis C virus (HCV) infection is an important medical priority. Induction of robust T cell responses could provide effective immune control.
We have previously shown that adenovirus-vaccine encoding non-structural (NS) HCV proteins induces potent T-cell responses and efficacy studies in chimpanzees demonstrate that these responses can be protective against challenge. Our phase I trial based on prime/boost with a simian Adenoviral (ChAd3) and MVA expressing the HCV-NS antigen has demonstrated that this vaccine strategy is safe and highly immunogenic. However, even higher T cell responses might be required to achieve efficacy in at risk populations and to exert a therapeutic effect in chronically infected HCV patients.
In this study we assessed fusion of the HCV NS antigen to murine and human MHC Class II associated invariant chain (Ii) expressed by viral vaccine vectors for its ability to increase the induced T cell response in mice and non-human primates (NHP). A dramatic increase was observed in CD1 outbred mice where vaccination with ChAd3 expressing the fusion antigen resulted in a 10-fold increase in IFN- producing $CD8^+$ T cells. In NHP $CD8^+$ T cell responses were enhanced and accelerated with vectors encoding the antigen fused to the human or murine Ii. Further experiments are underway to elucidate the adjuvant mechanism of Ii.
These data showed for the first time that the enhancement induced by vector vaccines encoding antigen fused to Ii was not species-specific and translated from mice to non-human primates opening the way for testing in humans.

W6.11.06
Antigenization of bovine immunoglobulin variable regions for development of novel vaccines

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Immunoglobulin specificity and affinity is a function of the complementarity determining regions (CDRs) of the heavy and light chain variable regions. The CDRs interact with the antigen as they stick out of the beta-sheets formed by the framework regions. The CDRs as such are stable conformational structures of an Immunoglobulin. The molecular modeling of bovine heavy (V_H) and light (V_L) chain domains predicted that the V_L might only have a supportive role in antigen recognition in antibodies with exceptionally long CDR3H (>48 amino acids). It was hypothesized that bovine CDR3H could form the site for presentation of conformational B-cell epitopes in order to develop a vaccine. We have now expressed bovine V_H with a CDR3H of 61 amino-acids and V_L, as single chain variable fragment (scFv) with an 18 amino acid linker in *Pichia pastoris*. This scFv retained the (poly)specificity of the parent antibody in ELISA. This confirmed the conformational stability of the CDR3H region in this format. Previously a protein fragment containing a bovine herpesvirus-1 (BoHV-1) neutralizing epitope was identified in our laboratoy. The sequence encoding this fragment will be grafted into the CDR3H encoding sequence and expressed as scFv in *Pichia pastoris.* Confirmation of the epitope structure will be done in ELISA with recombinant scFv3-18L against BoHV-1, previously engineered in our laboratory and *in vivo* testing in a BoHV-1 susceptible host. Antigenization of recombinant bovine antibody fragments could provide a novel approach to develop safe targeted vaccines. [Supported by NSERC Canada]

W6.11.07
Identification of novel *Streptococcus pneumoniae* candidate vaccine antigen

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Mortality due to pneumococcal infections remains high worldwide, augmented by widespread antibiotic resistance in many pneumococcal strains. To identify protein antigens that may be involved in the development of natural immunity to *S. pneumoniae*, a pneumococcal cell wall protein-enriched extract was screened using 2-D gel electrophoresis and immunoblotting with sera obtained longitudinally from children attending day-care centers, frequently exposed to *S. pneumoniae*. We concentrated on proteins for which antigenicity increases with age, coinciding with decreased morbidity. The proteins from this group that do not share homology to human proteins and that are conserved among different *S. pneumoniae* strains were tested for their ability to elicit protection against *S. pneumoniae* challenge in animal models. *S. pneumoniae* proteins for which antigenicity increases with age (PPP, GtS, Nox, PsipB, FBA, TF and FtsZ) were amplified from TIGR4 strain, cloned, expressed in *E. coli*, and purified. Mice were immunized three times intranasally or subcutaneously with these proteins in the presence of adjuvant and challenged two weeks later. Nasopharyngeal and lung colonization levels were quantified 48hrs following bacterial challenge, and survival was monitored daily for seven days.
All seven proteins elicited protective immune responses in mice as determined by reduced nasopharyngeal and lung colonization, prolonged survival, and the ability of antibodies obtained from immunized mice to ex-vivo neutralize bacterial virulence in the intraperitoneal challenge model. Immunization with proteins that demonstrate age-dependent antigenicity recapitulates the development of natural immunity in children and elicits protective immune responses in mouse challenge systems.

W6.11.08
Diminished levels of pneumococcal polysaccharide (PPS) responding 'B1 like' cells and it's corelation with poor anti PPS response post PPV-23 in long term HAART cohorts

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Despite HAART's success in reducing the incidence of opportunistic infections, invasive pneumococcal disease burden continues to be 35 to 100 fold higher in HIV infected population as compared to HIV negative individuals. PPV-23, a capsular polysaccharide vaccine is recommended as a prophylactic measure in HIV positive individuals; however its serological benefit in this group is currently unclear. In humans, B cells that respond to pneumococcal challenge are poorly characterized unlike in mice where B1 and MZ B cells are known to play an effective role in pneumococcal clearance. We designed a novel method for identifying pneumococcal polysaccharide (PPS) responding B cells by conjugating vaccine serotypes to fluorescent molecules. Using this method, we investigated serotype 14 and 23F responding B cells in HIV cohorts on long term HAART. On day 7 post PPV-23 vaccine, we saw a surge of 14 and 23F specific CD20+IgM+CD27+CD43+CD5+/- 'B1 like' cells in the peripheral blood of immunized volunteers as compared to pre-vaccination day levels. The percentage of 14 and 23F responding B1 like cells in HAART cohorts were comparable to HAART naïve group; but significantly less compared to young healthy HIV negative PPV-23 immunized volunteers. This correlates with the diminished anti PPS antibody response observed in the HAART treated group as opposed to healthy adults who exhibit appreciable serological response to PPV-23.Based on preliminary results, HAART does not appear to fully reconstitute the functional B cell subset required for protection against S.pneumoniae.

W6.11.09
Chimeric flagellin as the selfadjuvanting antigen for antibody response against Helicobacter pylori

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Almost half of the world population is infected with Helicobacter pylori. In most hosts the infection is asymptomatic, however, a subset of patients develops gastritis, peptic ulcer, and even gastric cancer. These flagellated Gram-negative bacteria have evolved to escape immune clearance by avoiding detection of their flagellin by the Toll-like receptor 5 (TLR5). TLR5 recognizes amino- (N) and carboxy- (C) terminal conserved segment of flagellin of bacteria such as Escherichia coli. The central hypervariable region of flagellin is known to be antigenic, but it is not mandatory for TLR5 signaling.
With the aim to engineer an antigen which would retain the H. pylori-specific antigenicity, but would also gain the ability to activate TLR5 signaling pathway, we constructed a chimeric flagellin by replacing the N- and C-terminal segment of H. pylori flagellin with segments from TLR5 activating E. coli flagellin.
Chimeric flagellin folded correctly and activated TLR5 in cell cultures. The vaccination of mice with the chimeric flagellin resulted in increased serum IgG and IgA antibody response. We found that antibodies against chimeric flagellin recognized native flagellin from H. pylori lysate. Vaccination with chimeric flagellin provided mice with significant protection against H. pylori.
Our approach can be used for the development of functional vaccines against human pathogen H. pylori as well as against other flagellated bacteria that evade TLR5 recognition.
Flagellin could also be used as an adjuvant for development of other types of vaccines (e.g. against tumours) where synergistic effect of activation of innate immune response is desired.

W6.11.10
Improved assay for quantification of immunoreactive hemagglutinin for potency determination and stability analysis in trivalent influenza vaccines using isotope dilution mass spectrometry

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Vaccination is the primary strategy to reduce the morbidity and mortality associated with influenza virus. There is an important public health need to develop methods to improve vaccine quality and to decrease the time required to get a protective vaccine to the public. Currently, regulatory potency requirements for trivalent influenza vaccine (TIV) require hemagglutinin (HA) quantification based on the single radial immunodiffusion (SRID) assay, which is laborious, time-consuming and difficult to obtain reproducible results. We have developed a potency assay, immunocapture-isotope dilution mass spectrometry (IC-IDMS), to quantify immunoreactive HA.
IC-IDMS has been validated utilizing polyclonal antisera to strain-specific HA, provided by regulatory agencies as reagent standards for SRID. These antibodies coated on magnetic beads capture conformationally correct HA in purified bulk materials and final TIVs. The captured proteins are enzymatically digested, and evolutionarily conserved tryptic peptides are quantified for each HA subtype. IC-IDMS is also being adapted for use with monoclonal antibodies. Panels of mAbs have been generated, purified, and characterized for evaluation as capture antibodies. These reagents could alleviate the current requirement of annual production of strain-specific antisera.
IC-IDMS provides accurate and specific quantification of immunoreactive H1, H3, and B in TIV samples. IC-IDMS is valuable for evaluating binding specificity and efficiency of antibodies, can be used to determine sub-potent TIVs and to monitor the stability of final vaccine products. IC-IDMS has several advantages over SRID,

including higher throughput as well as improved precision, accuracy, selectivity, and sensitivity. IC-IDMS has the potential to significantly impact influenza vaccine testing.

W6.11.11
Necrotic cell vaccine against Hepatitis C

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Potent, broad and sustained cell-mediated immune response, resulting in effective memory has the potential to restrict HCV infection and eliminate virus-infected cells after challenge. Among the HCV non-structural (NS) proteins, NS3 is more conserved across the genotypes and early, multi-specific CD4+ helper and CD8+ cytotoxic T cell responses against NS3 have been associated with viral clearance and protection.
We developed a novel mouse dendritic cell (DC) line, stably expressing HCV g1b NS3. Overexpression of NS3 had no effect on the maturation of this DC cell line. NS3 DCs were then manipulated to induce cell necrosis and C57BL6 mice were vaccinated with live or necrotic NS3 DCs in a prime boost regimen. We report that a single vaccination with necrotic cells expressing NS3 resulted in a greater influx of necrosis-sensing, cross-presenting CD11+ CD8+ Clec9A+ DCs in lymph nodes draining the site of injection at early time points post-vaccination and greater levels of T cell activation. This in turn, resulted in broad and greater responses to NS3, induction of multifunctional NS3-specific T cells, increased cytokine production and proliferation by NS3-specific T cells after prime-boost vaccination. On the basis of these promising results, we suggest that necrotic cell vaccination provides an innovative new strategy for immune modulation and enhancement of vaccine effectiveness.

W6.11.12
Langerin negative dendritic cells promote potent CD8+ T-cell priming after skin delivery of a recombinant adenovirus-based vaccine using microneedle arrays

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Improving antigen immunogenicity and reducing dependence on the cold chain are key goals in vaccine development. To achieve this we have developed a simple dissolvable microneedle array (MA) delivery system, fabricated to confer properties that maintain the transduction competence of a live adenoviral vaccine vector within the microneedle matrix and provide tensile strength to enable skin penetration with subsequent vaccine delivery to DCs. Using recombinant Adenovirus (Ad5) vectors encoding either HIV-1 CN54 gag or ovalbumin in a biodegradable polymer matrix containing a disaccharide as preservative, we addressed both the utility of this approach in eliciting $CD8^+$ T cell immunity and the DC populations critical to programming this response.
Dried AdHu5 M.A. immunization in B6 mice induced $CD8^+$ T cell expansion and multifunctional cytokine responses equipotent with conventional injectable routes of immunization. Intravital imaging of langerin-eGFP and CD11c-eGFP reporter mice, using flurochrome-linked dextran included with Ad5 in the needle matrix, demonstrated fluorescent M.A. cargo distributed both in the epidermis and dermis, with acquisition by dermal CD11c+ DC. Analysis of skin draining LNs after Ad5 vectored MA immunization, found no evidence for significant accumulation of $CD11c^+MHCII^{hi}Lang^+CD8^{neg}CD103^{neg}$ Langerhans cells or presentation of Kb/SIINFEKL complexes by $Lang^+$ DCs. Strikingly, we found a critical role for $CD11c^+$ MHC II^{hi} $CD8a^{neg}$ $EpCAM^{neg}$ $CD11b^+$ $Lang^{neg}$ DCs in priming the CD8+ T cell response, while $Lang^+$ DCs were dispensable.
These data shed new light on the early events following dermal vaccine delivery and have important implications for the development of viral vectored vaccines.

W6.11.13
Targeting of antigen via XCR1, the lineage marker for cross-presenting dendritic cells, elicits potent CD8 cytotoxicity in vivo

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Current vaccines essentially elicit protective neutralizing antibodies. However, some pathogens like mycobacteria, hepatitis C virus, Plasmodium, or HIV can only be cleared by cytotoxic T cells. We have recently shown that the chemokine receptor XCR1 is selectively expressed on cross-presenting dendritic cells (DCs), the key players in the induction of CD8+ T cell cytotoxicity. Based on this finding we have now established model systems for antigen targeting into cross-presenting DCs via XCR1. To this end, a mAb to murine XCR1 (MARX10) or XCL1, the natural chemokine ligand for XCR1, were fused to ovalbumin using recombinant techniques. Neither targeting reagent activated DCs when injected without adjuvant. A single i.v. injection of small amounts (1-2.5 µg) of either targeting reagent, applied together with LPS, efficiently induced proliferation and expansion of antigen-specific CD4+ and CD8+ T cells. Both targeting systems generated potent CD8+ T cell killing capacity in naïve C57BL/6 mice, when tested using an in vivo kill assay and an aggressive tumor model system. Both targeting strategies were highly specific as neither proliferation nor cytotoxicity was detected in XCR1-deficient mice. In clear contrast, injection of soluble ovalbumin was ineffective in all assay systems used. We thus could demonstrate that targeting of antigen into murine cross-presenting DCs via XCR1 induces a potent antigen-specific cytotoxic CD8+ T cell response in vivo. Since XCR1 is also selectively expressed on human cross-presenting CD141+ DCs, these targeting strategies will be transferable to the human, enabling the development of novel preventive and therapeutic vaccines.

W6.11.14
miR-21 confers resistance against CVB3-induced myocarditis by inhibiting PDCD4-mediated myocyte apoptosis

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The participation of microRNAs (miRNA) in physiopathologic processes of cardiovascular diseases endows them with the feasibility as potential targets for clinical intervention. Herein, key myocardial miRNA involved in the murine myocarditis following Coxsackievirus B3 (CVB3) infection was screened and its contribution to the disease development was investigated. It was found that myocardial miR-21 level was significantly reduced during the process of viral myocarditis and was negatively related to the extent of CVB3-induced myocardial inflammation and injury. When retrieving miR-21 expression by injecting pMDH-miR-21 via hydrodynamic injection on day 1 post infection, viral myocarditis was significantly alleviated as evidenced by less body weight loss, less myocardial injury and elevated survival rate. Further study revealed that miR-21 conducted its protective role by reducing myocyte apoptosis via inhibiting the expression of its target gene-programmed cell death 4 (PDCD4). In conclusion, in vivo up-regulating miR-21 expression could protect CVB3-induced myocarditis by repressing PDCD4-mediated myocyte apoptosis .It indicates that miR-21 intensively regulates the pathogenesis of viral myocarditis, and might be used as a novel therapeutic target for the future treatment of CVB3-induced myocarditis.

W6.12 Organ transplantation

W6.12.01
Monitoring indirect allorecognition in renal transplant recipients

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After allogeneic transplantation, T-cells can ,recognize the donor HLA molecules in two different manners: intact donor HLA-on donor antigen presenting cells (APC, direct pathway) and donor-derived

HLA peptides restricted by self-HLA on recipient APC (indirect pathway). Most strategies to monitor alloreactive T-cells are restricted to cells with direct specificity.
We have set up an experimental model to measure indirect reactivity, using HLA-typed dendritic cells or PBMC as antigen presenting cells. For antigen loading we used cell lines expressing a single HLA antigen or HLA monomers. As a readout CD8 T cell clones recognizing HLA-A2 (direct) or CD4 T cell clones recognizing HLA-A2-derived peptides restricted by HLA-DR1 (indirect) were used.
HLA-A2 molecules from cells/fragments did not results in indirect presentation but were able to activate the CD8 T cells (semi-direct presentation). However, using HLA monomers, a specific and dose dependent induction of IFN-γ production and proliferation was observed with the indirect CD4 T cell clone. This method was applied to renal transplant recipients 1-2 years post transplantation receiving kidneys with multiple HLA-I mismatches. PBMC from recipients were incubated with mismatched monomers for up to 72h. A specific IFN-γ response was observed with one of the monomers, confirming presence of T cells with indirect alloreactivity.
In conclusion we have developed a system to monitor indirect T-cell alloreactivity which should be further exploited in a larger cohort of transplant recipients and allows a more detailed analysis of qualitative aspects of this T cell response.

W6.12.02
In vitro and in vivo characterization of IL-9 producing alloreactive T cells

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Alternative mechanisms of acute allograft rejection are related to T helper type 2 (Th2) alloimmune responses and are often associated with eosinophil infiltration and interleukin (IL)-9 production. In this regard, the existence of a recently described type of helper T cells, which is characterized by the secretion of IL-9, might be relevant. Therefore, the differentiation of alloreactive IL-9 producing cells was investigated.
Stimulation of CD4+ T cells from BALB/c mice with allogeneic bone marrow derived dendritic cells (DCs) from C57BL/6 mice in the presence of IL-4 and TGF-b induced IL-9 production in up to 8% of the T cells with 50% of the cells co-expressing IL-4, although it hast been reported that 'Th9' cells do not express cytokines attributed to other T helper cells. The differentiation strongly depended on the presence of TGF-b, as generation of IL-9 producing T cells showed a concentration dependency and was inhibited by neutralizing TGF-b. LPS stimulated DCs did induce more IL-9 producing alloreactive T cells compared to TNF-a stimulated DCs. Experiments concerning the expression of characteristic surface molecules revealed that IL-9 secreting cells neither express T1/ST2, a previously described Th2-associated marker, nor the IL-4 receptor. In vivo, IL-9 producing T cells are rather of CD44-low phenotype and can be found in the spleen following allogeneic skin transplantation. The contribution of IL-9 producing alloreactive T cells to graft rejection as well as the functional differences between IL-9 single and IL-4/IL-9 single producing cells need to be further investigated.

W6.12.03
Experimental chronic lung allograft damage

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The success of human lung transplantation is limited by chronic lung allograft damage resulting in bronchiolitis obliterans syndrome (BOS). Acute rejection episodes and infections are important risk factors and seem to play major pathogenic roles. The aim of this study is to characterize a relevant experimental model for human BOS.
The Fischer 344 to Lewis rat strain combination was used for orthotopic left lung transplantation. Isografts were performed in Lewis rats. Recipients were treated with ciclosporin for 10 days. Lipopolysaccharide or vehicle was instilled into the airways 28 days after transplantation to mimic bacterial infection. Grafts were monitored using computed tomography and recipients were sacrificed on days 28-90. Graft histopathology was compared to lungs from patients, which were re-transplanted due to end-stage allograft dysfunction.
Three months after transplantation, the overall histopathology of rat lung allografts treated with lipopolysaccharide closely resembled human grafts with end-stage chronic pulmonary allograft damage, characterized by vasculopathies, fibrosis, leukocytic infiltrates, and a spectrum of mild to severe bronchial remodeling. By real-time RT-PCR, we demonstrated an induction of the TLR signaling system in allografts compared to isografts. Furthermore, chemokines CXCL9 and CXCL10 were induced in rat lung allografts before and after application of lipopolysaccharide. These chemokines are of prognostic value for human BOS.
In conclusion, we established an experimental model, which includes the most important risk factors, pivotal aspects of the pathogenesis and histopathological hallmarks of human BOS. This model will be helpful to elucidate the pathogenesis of BOS and to develop new therapeutic approaches.

W6.12.04
Soluble FGL2 induces tubular epithelial cells apoptosis in renal allograft rejection

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Background. Acute rejection (AR) hinders renal allograft survival. Tubular epithelial cells (TECs) apoptosis contributes to premature graft loss in AR, while the mechanism remains unclear. Soluble fibrinogen-like protein 2 (sFGL2), a novel effector of regulatory T cells (Tregs), induces apoptosis to mediate tissue injury. In this study, the role of sFGL2 in AR was investigated both *in vivo* and *in vitro*.
Materials and Methods. Serum level of sFGL2 and the peripheral percentage of $CD4^+CD25^+Foxp3^+$ Tregs were measured in renal allograft recipients with AR or stable renal function (n = 30). Human TECs were stimulated with sFGL2, TNF-α or PBS *in vitro*, and investigated for apoptosis by flow cytometry with Annexin V/PI staining. Apoptosis-associated genes expression in TECs were further assessed. Approval for this study was obtained from the Ethics Committee of Fudan University.
Results. Serum level of sFGL2 was significantly increased in the AR patients in correlation with peripheral Tregs. *In vitro*, sFGL2 remarkably induced TECs apoptosis, with a significant up-regulation of pro-apoptotic genes, including *CASP-3*, *CASP-8*, *CASP-9*, *CASP-10*, *TRADD*, *TNFSF10*, *FADD*, *FAS*, *FASLG*, *BAK1*, *BAD*, *BAX* and *NF-KB1*, without significant changes in anti-apoptotic genes, including *CARD-18*, *NAIP*, *BCL2*, *IKBKB* and *TBK1*.
Conclusion. sFGL2, an effector of Tregs, induces TECs apoptosis in renal allograft rejection through a comprehensive facilitation of both extrinsic and intrinsic apoptotic pathways and a modulation of regulatory signalings towards the pro-apoptosis profile. Our study suggests sFGL2 a crucial mediator in the pathogenesis of allograft rejection and provides novel insights into the role of Tregs in AR.

W6.12.05
Association of splenic $CD11c^+$ B-cell population with long-term graft tolerance in mouse cardiac transplantation model

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Organ transplantation is currently the effective method to treat end-stage organ failure. Strong immuno-suppressive drugs significantly improve graft survival. However, their toxic complications prevent long-term patient survival. Therefore, induction, maintenance, and

forecast of transplantation tolerance have been the hot topics of the field. In clinic liver and kidney transplantations, γδ-T cells and B-cell-associated proteins are found to be associated with long-term graft acceptance. In animal models, there have been no objective cellular/molecular patterns to indicate graft tolerance or rejection yet. Anti-CD154/DST treatment is used in our BALB/c-to-C57BL/6 mouse cardiac transplantation model to induce graft tolerance, which can last for at least 180 days. We found that the presence of splenic $CD11c^+$ B cells in long-term tolerant recipients is associated with graft tolerance. The $CD11c^+$ B-cell population emerges from day90 post tolerance induction and peaks around day150, suggesting different tolerance maintenance mechanisms at different tolerant stages. Graft rejection leads to decrease of the $CD11c^+$ B-cell population. The $CD11c^+$ B cells express more CD19 molecules. The $CD11c^+$ B-cell population shows several signatures of B-cell tolerance, such as immature $IgM^{high}IgD^{low}$ B-cell profile and higher expression of PDL-1 and PDL-2, compared to the $CD11c^-$ B-cell population. However, we do not observe high expression of CD5 and IL-10 expression in the $CD11c^+$ B-cell population. Our observations suggest the existence of cellular/molecular patterns of long-term graft tolerance in animal models. These studies may result in a sensitive, easy, and objective criterion for tolerance prediction in animal models and clinical transplantations.

W6.12.06
Tolerance induction to immunogenic grafts via antigen-specific Tregs without the need for chronic immunosuppression

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Regulatory T cells (Tregs) play an important role in the induction of allospecific tolerance. However tolerance in solid organ transplantation by mere transfer of Tregs has been difficult. Besides this the stability of the differentiation phenotype of Tregs has recently been questioned.
We therefore aimed in generating large numbers of stable allospecific Tregs from naive T cells by retroviral transduction with Foxp3. These were tested in an immunogenic skin transplantation model.
We established a system of transduction of mouse T cells with ecotropic retroviruses expressing Foxp3 and Thy1.1 as a surface marker to follow up transduced T cells. Alloantigen-specific T cells were isolated and transduced with Foxp3. Alloantigen-specific Foxp3 T cells showed high expression for Treg markers. They could suppress a MLR and could be expanded in vitro while maintaining their Treg phenotype. These Tregs prevented skin graft rejection without the need for chronic immunosuppression and recipients showed allospecific tolerance. Alloantigen-specific Tregs were far more potent than polyspecific Tregs. Mechanisms of tolerance were graft specific homing, expansion and long-term persistence of Tregs within the graft. In fact, tolerance could be transferred with re-transplantation of the tolerant graft onto secondary recipients.
The results prove that large numbers of stable alloantigen-specific Tregs can be generated from a polyclonal repertoire of naive T cells. This is the first time that allotolerance was achieved in a non-lymphopenic transplant model using skin grafts in an immunogenic strain combination. Therefore, antigen-specific Tregs might have a huge therapeutic potential after solid organ transplantation.

W6.13 Bone marrow transplantation and graft versus host disease

W6.13.01
PI3K-independent signal contributes to ICOS-mediated T-cell costimulation in acute graft-versus-host disease in mice

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We and others have previously shown that ICOS plays an important role in inducing acute GVHD in murine models of allogeneic bone marrow transplantation (BMT). ICOS potentiates TCR-mediated phosphoinositide 3-kinase (PI3K) activation and intracellular calcium mobilization. However, ICOS signal transduction pathways involved in GVHD remain unknown. Here we examined the contribution of ICOS-PI3K signaling in the pathogenic potential of T cells using a knock-in mouse strain, ICOS-YF, which selectively lost the ability to activate PI3K. We found that when total T cells were used as alloreactive T cells, ICOS-YF T cells caused less severe GVHD compared to ICOS-WT T cells but they induced much more aggressive disease than ICOS-KO T cells. This intermediate level of pathogenic capacity of ICOS-YF T cells was correlated with similar levels of IFN-γ producing CD8 T cells that developed in the recipients of ICOS-WT or ICOS-YF T cells. We further evaluated the role of ICOS-PI3K signaling in CD4 vs. CD8 T cell compartment using GVHD models that are exclusively driven by CD4 or CD8 T cells. Remarkably, ICOS-YF CD8 T cells caused disease similar to ICOS-WT CD8 T cells whereas ICOS-YF CD4 T cells behaved very similarly to their ICOS-KO counterparts. Consistent with their *in vivo* pathogenic potential, CD8 T cells responded to ICOS ligation *in vitro* by PI3K-independent calcium flux, T-cell activation, and proliferation. Thus, in acute GVHD in mice, CD4 T cells heavily rely on ICOS-PI3K signaling pathways; in contrast, CD8 T cells can utilize PI3K-independent ICOS signaling pathways, possibly through calcium.

W6.13.02
$CD11b^+$ NK cells are the main effector NK cell subpopulation that impairs GVHD

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Clinical studies exploiting the impact of Natural killer (NK) cells during bone marrow transplantation (BMT) have provided promising results. Murine NK cells can be separated along their expression of CD27 and CD11b and CD117 (c-kit). However, the functional relevance of the distinct NK subsets in graft-versus-host-disease (GVHD) has not been investigated so far. We have established different protocols for expansion of murine NK subpopulations. The four different NK cell subsets provide differences in their genomic, phenotypic and functional properties. Our data clearly demonstrate that CD11b+ NK cells express multiple genes of cytotoxic pathways and develop the highest tumorlytic capacity. We observed up to 60% tumor lysis by CD27- CD11b+ NK cells compared to 40-45% by CD27+ CD11b+, about 25% by CD27+ CD11b- and 10% by c-kit+ CD11b- NK cells. Interestingly, CD27+ NK cells provided the highest IFN-γ production upon incubation with tumor cells and/or IL-2. We further analyzed the migratory capacity and tissue homing of these NK subsets. CD11b+ NK cells migrate to the GVHD target organs, whereas CD27+ NK cells preferentially home to the bone marrow. Finally, we investigated the role of distinct NK subpopulations in the development of GVHD in a MHC mismatched BMT mouse model.
In summary, our study outlines that only the CD11b+ NK cells provide GVHD protection. These new insights are highly relevant for the selection of the optimal NK cell preparation in the field of cellular therapy.

W6.13.03
In vitro-generated myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GVHD) while preserving the graft-versus-tumor (GVT) effect

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Allogeneic bone marrow transplantation (BMT) is a curative treatment modality for hematologic malignancies, since alloantigen-specific donor T cells eliminate residual tumor cells (graft-versus-tumor (GVT) effect). However, these T cells are also responsible for the induction of graft-versus-host disease (GVHD) by attacking alloantigen expressing recipient tissue. Therapeutic goal in BMT is the inhibition

of GVHD while maintaining the GVT-effect. Myeloid-derived suppressor cells (MDSCs), a heterogenic population of myeloid precursors, suppress T cell activation and might therefore be a putative therapy in GVHD prophylaxis.
MDSCs were generated by culturing BM cells in the presence of GM-CSF and G-CSF. These in vitro-generated CD11b+Gr-1+ MDSCs suppressed allogeneic T cell proliferation in vitro. To test, whether MDSCs inhibit GVHD, B6-derived MDSCs were co-transplanted with B6-derived allogeneic BM and spleen cells (SC) into lethally irradiated B6.bm1 or B6D2F1 mice. In a dose dependent manner, MDSCs significantly suppressed clinical and histological GVHD and improved survival up to 70 % - 100 %, depending on the BMT-model used. Transplanted MDSCs homed into lymphoid and GVHD target organs. However, they did not interfere with phenotype and cytotoxicity of allogeneic T cells. Importantly, co-transplantation of MDSCs did not interfere with the GVT-effect, since syngeneic tumor cells were still efficiently eradicated after MDSC co-transplantation.
In summary, transplantation of in vitro-generated MDSCs efficiently prevented clinical and histological GVHD while preserving T cell-mediated tumor cytotoxicity, suggesting that MDSC co-transplantation represent a useful cellular GVHD-therapy.

W6.13.04
Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease

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The placenta protects the developing fetus from the mother's immune system. Fetal membranes have previously been used to treat severe burn injuries, indicating that these tissues can promote wound healing. We have previously found that fetal membrane cells (FMCs) isolated from term placenta prevent alloreactivity *in vitro*. FMCs share many features with bone marrow-derived mesenchymal stromal cells (MSCs), which we previously introduced to treat severe acute graft-versus-host disease (GVHD). Here, we tested FMCs for treatment of steroid-refractory acute GVHD. After two passages in culture, approximately 10^9 FMCs were obtained from one single placenta. The FMCs were positive for CD29, CD44, CD73, CD90, CD105, CD49d, PD-L1 and PD-L2, but were negative for hematopoietic and epithelial markers. Microsatellite polymorphism analysis showed that FMCs were of maternal origin. All FMCs used showed normal karyotype. Nine patients who had undergone hematopoietic stem cell transplantation and who had developed steroid-refractory grade III-IV acute GVHD were given 0.9-2.8 x 10^6 FMCs/kg at 15 infusions. Median age was 57 years. Two of eight evaluable patients had a complete response and four had a partial response, giving an overall response rate of 75%. Two patients showed no response at all. Three patients are alive from 11 to 26 months after HSCT. One patient is well and two have chronic GVHD. To conclude, we have developed a protocol for generation of large quantities of stromal cells with immunosuppressive capacities that are accessible without any invasive procedures. FMCs may be successfully used for immune modulation and tissue repair.

W6.13.05
CMV reactivation and kinetics of Natural Killer (NK) cell reconstitution in 439 patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT)

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NK-cells are the first subset to recover after allo-HSCT, and play a major role in early immunity. Relatively small cohort studies suggest that the kinetics of NK-cell reconstitution and cytomegalovirus (CMV) reactivations are closely intertwined. NK-cells that express the activating receptor NKG2C seem crucial in the resolution of CMV episodes, even in the absence of T-cells. Nevertheless, the alleged ability of NK-cells to control CMV infection still remains to be corroborated on larger cohorts.
We aimed at prospectively investigating the kinetics and profiles of NK cell reconstitution after allo-HSCT together with CMV reactivation patterns.
Freshly collected samples from 439 consecutive recipients were analyzed at months M3, M6, M12 and M24 post-transplantation in order to characterize NK-cell subsets.
Data were analyzed with respect to conditioning regimen, source of stem-cell, underlying disease, occurrence of GvHD, and profiles of CMV reactivation.
We showed, from multivariate analysis, that the cell-source was the major factor influencing the reconstitution of immature CD56bright NK subset (P=.03 at M3). Higher numbers of infused CD34+ cells were associated with higher CD56dim NK-cell counts from M3 to M12. The cumulative incidence of chronic GvHD was strongly associated to CD56dimNK-cell counts above 70/mm3 at M3. CD56bright NK-cell counts below 12/mm3 at M3 were associated with higher incidence of CMV reactivation between M0 and M3. Moreover, the numbers of CMV reactivations and CD56bright NK-cell counts were inversely correlated between M3 and M6.
These date highlight the interest to evaluate immune reconstitution at the subset rather than at the global level of NK-cells.

W6.13.06
CD8+ T-cells expressing the homing receptors CCR7 and CD62L mediate the pathogenesis of graft-versus-host disease

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Graft-versus-host disease (GVHD) represents one of the major complications associated with allogeneic stem cell transplantation. The pathogenesis involves migration of the transplanted donor naïve T-cells into the secondary lymphoid organs in the recipient, which is mainly steered by CD62L and CCR7. Therefore, we aimed to study whether the expression of CD62L and CCR7 in cytotoxic CD8+ T-cells is associated with GVHD.
This single center study included 27 donor-recipient pairs. All patients had reached at least 100 days after SCT. Samples from the apheresis product (donor) were phenotyped by flow cytometry (CD45, CD3, CD4, CD8, CD62L, CCR7). CD62L and CCR7 expression in CD4+ and CD8+ T-cells were compared between patients who developed GVHD (n=12) and those who did not (n=15).
The patients who developed GVHD were transplanted with a slightly higher number of T cells (mean 241 vs. 201*106/kg; p=0.17) and CD8+ T cells (92 vs. 71*106/kg; p=0.10) compared to patients without GVHD. Interestingly, the GVHD group received a significantly higher number of CCR7+CD8+ T-cells (52*106/kg, p=0.010) and CD62L+CD8+ T-cells (39*106/kg, p=0.041) compared to the no GVHD group (29 and 25*106/kg, respectively). The GVHD group received also a higher number of CD62L+CCR7+CD8+ T-cells compared to no GVHD (30 vs. 17*106/kg, p=0.033). In contrast, no differences were seen in the absolute number of transplanted CD4+ (p=0.51), CD4+CCR7+ (p=0.31), CD4+CD62L (p=0.99), or CD4+CCR7+CD62L+ (p=0.81) T-cells.
Our results indicate that CCR7+ and CD62L+ expressing CD8+ T-cells mediate pathogenesis of GVHD and are potential therapeutical targets for the disease.

W6.14 Immunosuppressive and tolerogenic treatments

W6.14.01
Immunological outcomes during 1 year of treatment with the anti-interleukin-12/23 p40 monoclonal antibody ustekinumab for psoriasis

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Psoriasis is a chronic inflammatory disease mediated by cytokine-producing T helper (Th) 1 and Th17 cells. Ustekinumab, a biotherapy targeting the IL-12/23 p40 subunit is highly efficient in patients with moderate-to-severe plaque psoriasis.
The TRANSIT study (NCT01059773), a 52-week, randomised, open-label trial in patients with moderate-to-severe plaque psoriasis and inadequate response to methotrexate, assessed the safety and efficacy of two methotrexate-to-ustekinumab transition strategies.
At week 52, 77% of patients achieved clinical benefit (75% reduction in psoriasis area severity index compared with baseline [PASI 75]) and 83% had only minimal residual lesions (PASI≤5). The systemic immunological effects of ustekinumab during 1 year of treatment were analysed in a subgroup of patients (n=117) by monitoring the immune phenotype and function of circulating leukocytes at baseline, week 12 and week 52. The results show that ustekinumab induced only mild changes in circulating leukocytes at week 52 compared with baseline: i) slight increases in the numbers of T and B cells (both $p<0.001$); ii) increases in the numbers of regulatory T cells ($p=0.004$); iii) decreases in the numbers of natural killer (NK) cells ($p<0.001$). More importantly, the immune functions tested were not substantially altered, including: i) NK cell cytotoxicity; ii) mitogen- and recall antigen-induced T cell proliferation; iii) IL-17, interferon-γ, tumour necrosis factor (TNF)-α, and IL-22 secretion; iv) frequency of Th1 and Th17 cell precursors.
Collectively these data show that, for the patients studied, ustekinumab treatment did not impair systemic immune functions.

W6.14.02
Induction of immune tolerance by antigen loaded erythrocytes: Innovative therapy for therapeutic protein allergy

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Introduction:
Pompe disease is a rare inherited metabolic disorder caused by deficiency of the enzyme acid alpha-glucosidase. Currently, the unique approved therapy is the intravenous administration of a recombinant enzyme called alglucosidase-alpha (AGA). However, due to its immunogenicity, the life-long treatment and the frequency of injections, the use of AGA leads to severe adverse reactions and/or reduced therapy efficacy. In this context, our aim is to reduce / abolish the specific humoral response to antigen (Ag) using the properties of erythrocytes to deliver specifically the Ag to antigen-presenting cells and to induce antigen-specific immune tolerance.
Methods:
AGA was entrapped by a hypotonic dialysis process in erythrocytes treated with [bis(sulphosuccinimidyl)] suberate (BS3) to stimulate erythrocyte phagocytosis and favor organ targeting. To demonstrate that our technology can prevent the immunization to AGA, mice were intravenously injected three times with AGA entrapped in RBCs before being sensitized to AGA using Cholera Toxin (CT) as adjuvant. Control animal received injections of unentrapped AGA.
Results:
We demonstrated that AGA delivered by BS3-treated erythrocytes is able to induce immune tolerance. A strong decrease in specific humoral response was observed despite three humoral stimulations with AGA and CT (more than 80% of IgG decreased). Furthermore, this tolerance induction is maintained at least for 2 months and the mice remain fully responsive to CT stimulation.
Conclusion:
We have developed a promising strategy to inhibit protein-specific humoral responses. This therapeutic approach of tolerance induction could offer multiple applications in patients developing hypersensitivity to therapeutic proteins.

W6.14.03
Therapeutic role of the novel negative checkpoint regulator VISTA in murine autoimmune disease models

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Therapeutics targeting negative checkpoint regulators such as CTLA-4 and PD-L1 has been effective in treating human cancer and to a lesser extent autoimmune diseases. We have recently discovered the novel checkpoint regulator designated V-region Immunglobulin-containing Suppressor of T cell Activation (VISTA) bearing homology to the extracellular domain of PD-L1.
This is the first study focused on the therapeutic efficacy of VISTA-Ig, a fusion protein in murine autoimmune models of Multiple Sclerosis (Experimental Autoimmune Encephalomyelitis (EAE)) and Systemic Lupus Erythematosus (SLE).
In EAE mice, prophylactic and therapeutic treatment with VISTA-Ig significantly reduced disease severity compared with control-Ig treated mice in vivo. In the lupus prone mouse strains NWBW-F1 and NZM2410, therapeutic treatment with VISTA-Ig prevented the onset of proteinuria, dramatic weight loss and increased regulatory T cells in contrast with control-Ig treated mice. Clinical analysis of kidneys from the VISTA-Ig treated group showed minimal to mild glomerulonephritis compared with mice treated with control-Ig. Collectively these studies demonstrate that VISTA-Ig is a novel effective therapeutic in murine autoimmune diseases.

W6.14.04
Interleukin-7 receptor blockade by monoclonal antibody to IL-7Rα induces islet allograft tolerance and long-term skin allograft survival through inhibition of T cell reconstitution

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Using a pancreatic islet allograft model, we show that anti-IL-7Rα mAb given every other day from 3 weeks before graft to 4 weeks post-graft induced graft tolerance (graft survival >180d compared to 21d in untreated mice, $p<0.001$) and maintained an abrogation of both cellular and humoral alloimmune responses long time after the cessation of treatment. In a stringent skin allograft model, IL-7R blockade given after T cell depletion by anti-CD4 and anti-CD8 mAbs doubled graft survival to 58d versus 30d with depletion alone ($p<0.0001$), and when low-dose tacrolimus was added, two-third of mice accepted skin graft for at least 90d. IL-7R blockade following T cell depletion inhibited T cell reconstitution and resulted in: (1) 4 to 10-fold reduction in the absolute number of total T cells, CD4+, CD8+ T cells, and CD44hiCD62Llo memory T cells, (2) moderate increase in CD4+FoxP3+ Treg frequency, (3) abrogation of both cellular and humoral alloimmune responses as shown by IFNγ Elispot, MLR-3H thymidine and DSA measurement, (4) inhibition of skin graft leukocyte infiltrate, (5) diminution of intragraft expression of TH1, TH2, and TH17 cytokines, chemokines, and chemokine receptors (all $p<0.05$). Interestingly, a 5-week treatment with anti-IL-7Rα mAb did not decrease cellular and humoral anti-viral immune response in mice previously infected with an adenovirus vector. Taken together, we demonstrate for the first time that IL-7R blockade has strong tolerizing effect and synergizes with other immunosuppressants to induce long-tem graft survival even in a stringent allograft model and therefore might be a clinically relevant therapy in transplantation.

W6.14.05
Control of autoimmunity by Treg-inducing liver sinusoidal endothelial cells

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Background & Aims: The lack of treatments that specifically suppress the immune response to disease-relevant self-antigens is a major obstacle for the efficient therapy of autoimmune diseases such as multiple sclerosis. Previously, we have shown that the liver favors the generation of autoantigen-specific CD4+ Foxp3+ regulatory T cells (Tregs) that could suppress autoimmune disease (Lüth S et al., J Clin Invest. 2008 Oct;118(10):3403-10). Here we aimed to identify the Treg-inducing cells in the liver, and explore whether Treg-induction by liver cells could be harnessed for the treatment of autoimmune disease.
Methods: Antigen-specific Treg-induction by various liver cell types was analyzed in vitro and Treg functionality was assessed in vivo in mouse models of multiple sclerosis. For the treatment of autoimmune disease, we developed a carrier system for delivery of autoantigen peptides to Treg-inducing liver cells in vivo.
Results: We show that liver sinusoidal endothelial cells (LSECs) efficiently induce the TGF-β -dependent conversion of CD4+ Foxp3- non-Tregs into CD4+ Foxp3+ Tregs. To harness LSECs for Treg-based treatment of autoimmune disease, we engineered a nanoparticle carrier that facilitates selective and efficient delivery of autoantigen peptides to LSECs in vivo. In two independent mouse models of multiple sclerosis, only a single administration of nanoparticles loaded with autoantigen peptides provided complete disease protection and effective therapy of established disease.
Conclusions: LSECs are potent inducers of antigen-specific Tregs. The selective delivery of autoantigen peptides to LSECs by nanoparticles enables effective treatment of autoimmune disease.

W6.14.06
Induction of immunosuppression by mesenchymal stem cells

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Mesenchymal stem cells (MSCs), also called multipotent mesenchymal stromal cells, are believed to exist in almost all tissues as a key cell source for tissue repair and regeneration. Our recent studies showed that MSCs are strongly immunosuppressive *in vivo* and *in vitro* in both animals and humans. This immune modulatory property of MSCs is not innate, rather induced by by IFNγ and the concomitant presence of any of three other proinflammatory cytokines, TNFα, IL-1α or IL-1β. These cytokine combinations provoke the expression of high levels of several chemokines and inducible nitric oxide synthase (iNOS) by MSCs. These chemokines drive T cell migration into proximity with MSCs, where T cell responsiveness is suppressed by local high concentrations of nitric oxide (NO). This cytokine-induced immunosuppression was absent in MSCs derived from $iNOS^{-/-}$ or $IFN\gamma R1^{-/-}$ mice. Blockade of chemokine receptors also abolished the immunosuppressive effect of wild type MSCs. In vivo administration of wild-type MSCs, but not $IFN\gamma R1^{-/-}$ or $iNOS^{-/-}$ MSCs, prevented GVHD, liver cirrhosis and Parkinson's disease, Human MSCs demonstrated dramatic therapeutic effect in patients suffering from end-stage liver disease. Since mesenchymal stem cells (MSCs) have extensive proliferative capacity in vitro, and could be expanded to sufficient quantity for in vitro and in vivo investigations, we believe that further studies on their immune modulatory properties will provide critical information for proper clinical applications of these valuable cells.

W6.15 Immunopharmacology

IL6.15.01
Modelling human inflammation *in vitro* with human blood monocytes

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The use of human primary cells *in vitro* is becoming the preferred choice for obtaining valid and meaningful immunopharmacological data, in particular when addressing inflammation, as animal models have proved largely unreliable and transformed cell lines do not reliably reproduce the response of primary cells.
The human innate/inflammatory response in a tissue can be modelled *in vitro* by culturing human normal monocytes isolated from blood in conditions that resemble the recruitment from blood into the inflamed site, then the encounter with the inflammatory agents, and eventually the conditions promoting tissue repair and homeostatic regulation upon resolution. The interindividual variability of the monocyte response proved very low, underlining the robustness of the model. Changing the culture conditions could also allow us to reproduce the conditions of persistent inflammation characteristic of chronic inflammatory diseases, for instance in rheumatoid arthritis, or those of elderly individuals. Since reacting/adapting to the changing microenvironment is typical of immune responses, the full development of the reaction should be examined in the *in vitro* models, in order to indentify possible anomalous reactivity.
These kinds of models can be used for different scopes, including
1. identification of subtle kinetic or dynamic changes or markers that distinguish a normal inflammatory response from a persistent pathological reaction;
2. evaluation of the immunomodulatory effects of microorganisms and other agents, alone or in combination;
3. screening of anti-inflammatory compounds;
4. assaying the immunosafety of drugs, contaminants, or engineered nanoparticles.
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IL6.15.02
Molecular mechanisms associated to a new combined therapy for multiple sclerosis

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Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system. While there have been significant advances in therapies targeting immune system dysfunction, there is an urgent need for developing drugs aimed at protecting demyelination and axonal loss as part of the pathogenic mechanism of MS. In our study, we used the experimental autoimmune encephalomyelitis (EAE) model induced in C57BL6 mice, by immunization with MOG35-55 peptide. We studied the molecular mechanisms involved in the effect of C-Phycocyanin (C-Pc), a natural antioxidant derived from *Spirulina platensis* (a blue - green algae) and beta IFN, on EAE. The study involved clinical evaluations, measurements of regulatory T cells in the spleen, effector and regulatory cytokines by qPCR in the brain and serum by Bioplex, and analyses using Transmission Electron Microscopy. Results showed a reduction of clinical signs, the induction of CD4 + Foxp3 + in the spleen and the reduction of effector cytokines, such as IL-17 in the

brain and serum. Also, ultra-structural analyses demonstrated that C-Pc was able to remyelinate axons, and the gene expression profile of Microarray studies identified common and specific processes modulated by C-Pc and beta IFN. This suggests the need of a combined Multiple Sclerosis therapy with two components targeting the main steps of the MS pathogenic mechanism, one related to immune dysfunction and the other directed towards neurodegenerative changes. Our study, consistent with current approaches on the use of combined therapies, constitutes an attractive and potentially effective strategy for future clinical trials in MS.

W6.15.01
L-GILZ forms a complex vith p53 and mdm2 and suppresses tumor growth through p53 activation

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Long Glucocorticoid-Induced Leucine Zipper (L-GILZ) is a newly identified isoform of the better characterized GILZ, a glucocorticoid (GC)-induced gene, which plays an important role in GC-induced immunomodulation. GILZ interacts with many signaling proteins, such as NF-kB, Ras and Raf, inhibiting downstream signaling pathways and affecting cell cycle, apoptosis, and differentiation. L-GILZ interacts with Ras and contributes to cell differentiation control. The p53 transcription factor regulates the expression of genes crucial for biological processes such as apoptosis, cell proliferation, metabolism, senescence and cell repair. Activation of p53 can suppress neoplastic transformation, inhibiting the growth of mutated or damaged cells. Notably, p53 binding proteins, such as MDM2, limit p53 activity thus regulating p53 stress response. We here demonstrate that L-GILZ activates p53 and induces inhibition of cell proliferation and tumor cell growth. In particular, over-expression of L-GILZ in (*p53*+/+) HCT116 human colorectal carcinoma cells, results in inhibition of cell proliferation, induction of apoptosis and suppression of growth of xenograft in mice. Moreover, expression of p21 and PUMA is increased in L-GILZ-transfected (*p53*+/+) but not in (*p53*-/-) HCT116 cell line. A direct interaction of L-GILZ with both p53 and MDM2 appears to regulate p53 activation. In fact, we demonstrate that the binding affinity of L-GILZ for MDM2 is higher than that for p53 and that L-GILZ disturbs p53/MDM2 complex formation thus making p53 available for downstream gene activation. These findings reveal L-GILZ as a novel p53 regulator and as a candidate for new therapeutic anticancer strategies targeting p53.

W6.15.02
Bringing together the potential of immune cells and the power of new drugs to target cancers by nanoparticles

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RITA (Reactivation of p53 and Induction of Tumour cell Apoptosis) is a compound activating p53, a key tumor suppressor gene defective in 90% of tumors. RITA treatment induces ULBP2 expression on tumors leading to a higher NK cells susceptibility (Li et al. Cell Cycle 2011).
To enhance the pharmacological effect of RITA on tumour cells a novel drug delivery strategy will be developed, by functionalizing the shell of RITA-loaded nanoparticles with immunogenic peptides thus enabling combinatorial effects consisting of RITA as well as NK cell mediated antitumor responses in vivo.

PEG-PLA copolymer micelles with a hydrophilic, non-immunogenic corona made of poly (ethylene glycol) (PEG) and a hydrophobic biodegradable poly(lactide) (PLA) core have enormous potential as long-circulating drug carrier systems. “Tree shaped” copolymers made of a PEG trunk and two or four arms of PLA have been synthesized. The interactions of the copolymers, previously functionalized with fluorescent agent, at single cells and population levels by using confocal analysis have been evaluated. PEG-PLA linear (AB), A(B)2 and A(B)4 enter inside the cells between 2 and 24 hours.
Our data indicate that the copolymer PEG-PLA A(B)2 is get efficiently internalized by HEK 293T since its nanoparticles can be found inside the cytoplasm. In the time course experiments, increased number of cells displays signs of copolymer internalization with substantial accumulation of foci present at 24 hrs; no staining was observed on the cell membranes and nuclei. Effects of PEG-PLA linear on lymphocyte cell subsets and their cytotoxicity towards tumour targets will be discussed.

W6.15.03
Sub-visible particles in protein therapeutics - are they responsible for the induction of unwanted immune responses?

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Sub-visible particles in protein therapeutics have received increasing attention in recent years because they are believed to play a major role in the induction of unwanted immune responses. In particular amyloid-like protein particles could be associated with an activation of the innate immune system as was demonstrated for Amyloid beta peptides. Currently available technologies such as Micro-Flow Imaging and Nanoparticle Tracking Analysis are suitable for quantification of sub-visible particles but have limited capability for more in-depth characterization of the nature of these particles.
We developed a novel flow-cytometry-based technology that combines quantification and in-depth characterization of sub-visible particles over a size range of 0.75µm- 75µm. This technology allows assessment of the amount, size and nature of sub-visible particles. The inclusion of the fluorescent dye 4-(dicyanovinyl)-julolidine (DCVJ) which binds to cross-beta-sheet structures in amyloid-like protein particles, provides important information on the nature of the particles.
We used the new technology to assess amount, size and nature of sub-visible particles in a variety of *in vitro* generated sub-visible particle preparations originating from different therapeutic proteins. We also included non-protein particles in the analysis. Concomitant to particle analysis we used cellular *in vitro* assays to assess the potential of different protein particles to trigger the innate immune system. We believe that the combination of sub-visible particle characterization with the assessment of potential immune stimulatory activities can provide new insight into important mechanisms regulating the induction of unwanted immune responses by protein therapeutics.

W6.15.04
Assessing immune toxicity of engineered nanoparticles: modulation of inflammation in a novel in vitro model based on human primary monocytes

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The potential effects of engineered nanomaterials on innate/inflammatory responses need to be considered when assessing nanosafety issues, since the alteration of the normal progress of inflammatory defence response may cause pathological derangement even in the absence of overt toxicity.
To investigate the interference of nanomaterials with the normal development of the inflammatory response, a human monocyte-based *in vitro* model has been developed and proved to realistically

reproduce the human inflammatory reaction. $CD14^+$ human blood monocytes were sequentially stimulated with different combinations of agents and conditions (CCL2, LPS, TNFα, IFN-γ, 37°C *vs*. 39°C), so as to mimic the entire course of a physiological inflammatory reaction (initiation, development, eventual resolution). A model of persistent inflammation has been also designed to investigate the possible modulation of with pathological inflammation by nanoparticles.
Monocytes were exposed to Au nanoparticles (diameter 10 nm, 1.28 µg/ml, 0.16 $cm^2/10^6$ cells, endotoxin-free) during the course of the *in vitro* inflammatory reaction. Gene expression and protein production of inflammatory and anti-inflammatory cytokines were analysed at different times during the course of the inflammatory response.
Preliminary results show that Au nanoparticles do not significantly affect the course of either the physiological or the persistent inflammatory response in the *in vitro* monocyte-based models, indicating that these particles do not pose a significant risk for human health.
This study thus proposes the use of a valid and representative model for human inflammation, for realistically investigating the effects of nanoparticles on inflammatory/innate immune responses, predictive on immuno-nanorisk for human health.
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W6.15.05
Protein kinase Cδ regulates inducible nitric oxide synthase expression through IRF1

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In inflammation, pro-inflammatory cytokines and bacterial products induce the production of high amounts of NO by inducible nitric oxide synthase (iNOS) in inflammatory and tissue cells. NO is an effector molecule in innate immunity, and it also has regulatory and pro-inflammatory/destructive effects in the inflammatory process. Protein kinase Cδ (PKCδ) is an important signaling protein regulating B lymphocyte functions, but less is known about its role in innate immunity and in the regulation of expression of inflammatory genes in activated macrophages and tissue cells. In the present study we investigated the role of PKCδ in the regulation of iNOS expression in inflammatory conditions.
Down-regulation of PKCδ by siRNA and inhibition of PKCδ by rottlerin suppressed NO production and iNOS expression in activated macrophages and fibroblasts. PKCδ targeted siRNA and inhibition of PKCδ by rottlerin suppressed also the expression of transcription factor IRF1. Accordingly, down-regulation of IRF1 by siRNA reduced iNOS expression in activated macrophages. In addition, inhibition of PKCδ showed anti-inflammatory effects in carrageenan induced paw inflammation in mice as did iNOS inhibitor L-NIL.
These results show, that PKCδ up-regulates transcription factor IRF1, possibly through activation of transcription factor STAT1, and by that mechanism enhances the expression of iNOS in cells exposed to inflammatory stimuli. In addition, inhibition of PKCδ was found to have anti-inflammatory properties also in vivo. Taken together, these results suggest that PKCδ inhibitors hold anti-inflammatory properties in vitro and in vivo, making PKCδ a potential target for anti-inflammatory drug development.

W6.15.06
Molecular mechanisms associated to a new combined therapy for multiple sclerosis

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Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system. While there have been significant advances in therapies targeting immune system dysfunction, there is an urgent need for developing drugs aimed at protecting demyelination and axonal loss as part of the pathogenic mechanism of MS. In our study, we used the experimental autoimmune encephalomyelitis (EAE) model induced in C57BL6 mice, by immunization with MOG35-55 peptide. We studied the molecular mechanisms involved in the effect of C-Phycocyanin (C-Pc), a natural antioxidant derived from Spirulina platensis (a blue - green algae) and beta IFN, on EAE. The study involved clinical evaluations, measurements of regulatory T cells in the spleen, effector and regulatory cytokines by qPCR in the brain and serum by Bioplex, and analyses using Transmission Electron Microscopy. Results showed a reduction of clinical signs, the induction of CD4 + Foxp3 + in the spleen and the reduction of effector cytokines, such as IL-17 in the brain and serum. Also, ultra-structural analyses demonstrated that C-Pc was able to remyelinate axons, and the gene expression profile of Microarray studies identified common and specific processes modulated by C-Pc and beta IFN. This suggests the need of a combined Multiple Sclerosis therapy with two components targeting the main steps of the MS pathogenic mechanism, one related to immune dysfunction and the other directed towards neurodegenerative changes. Our study, consistent with current approaches on the use of combined therapies, constitutes an attractive and potentially effective strategy for future clinical trials in MS.

W6.16 Biomarkers and clinical profiling of human immune responses

W6.16.01
An autoantibody profile for SLE diagnosis using the ImmunArray iCHIP™

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Systemic lupus erythematosus (SLE) is a chronic, recurrent, potentially fatal multisystem inflammatory disorder mainly affecting women. SLE patients produce antibodies to many different self-antigens. Nevertheless, currently used serological markers for SLE are lacking in specificity and/or sensitivity. SLE is diagnosed through a combination of clinical and laboratory criteria with four out of 11 criteria developed by the American College of Rheumatology. Accurate and timely diagnosis of SLE is important because treatment can reduce morbidity and mortality. The aim of this study was to develop an improved diagnostic test by measuring and multiplexing specific autoantibody reactivities in SLE patients. Autoantibody reactivity profiles in serum samples collected from 97 SLE patients within three years of the diagnosis were compared with those of 56 healthy controls. Autoantibody profiles were determined using the ImmunArray iCHIP™ - a proprietary protein microarray technology that allows the display of antigens representing a wide range of SLE-associated biochemical pathways on a single chip. Using this novel platform, SLE patients could be differentiated from healthy subjects by a relatively small subset of auto-antigens and Epstein Barr Virus (EBV) antigens. The autoantibody reactivity profile that allowed SLE diagnosis with high sensitivity and specificity displayed differential response to known SLE-specific antigens, such as single-stranded DNA and EBV, and to several novel ones. Validation of this profile in additional patient samples showed good performance. An antibody profile for SLE diagnosis using a single multiplexed chip was successfully developed. This profile may differentiate between SLE patients and healthy individuals.

W6.16.02
Identification of potential biomarkers and therapeutic targets for Multiple Sclerosis by M2 proteomics.

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Despite extensive research, multiple sclerosis (MS) remains a disease that lacks a definitive diagnostic test. Even after a diagnosis of MS has been established, currently it cannot be reliably predicted whether and when disease relapses will occur, thus patients may undergo years of unnecessary treatments. Additionally, because the current treatments for MS can produce dramatically different outcomes in individual patients, there is a critical need to develop biomarkers for treatment efficacy and resistance. We have recently developed a novel quantitative Microwave & Magnetic (M^2) proteomics method to quantitatively measure changes in proteome expression during experimental autoimmune encephalomyelitis (EAE), the standard murine animal model of MS. Our data revealed significant consistent changes in the proteome over the disease course. Changes in the expression of protein isoforms correlated either with time (days after EAE induction) or disease severity (clinical score). Interestingly, we have identified unique changes in the proteome prior to the clinical symptoms onset. Those pre-onset changes may allow us to predict the subsequent severity of the disease. Last, we have identified that glucocorticoid-drugs (GCs) have an effect on these changes with a strong statistical correlation to EAE severity and more importantly, these changes closely mirror disease progression independent of any cytokine response to the GCs. Thus, homologous human biomarkers may be useful to accurately and reliably predict disease initiation, clinical severity and GCs drug efficacy. Importantly, these biomarkers illuminate some of the key mechanisms that contribute to the disease and may be useful to develop new therapeutic targets for MS.

W6.16.03
The alarmins S100A8 and S100A9 as promising targets for non-invasive molecular imaging of phagocyte activation during inflammation

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The proinflammatory alarmins S100A8 and S100A9 are released by activated phagocytes at sites of inflammation. Due to their favorable kinetics S100A8/S100A9 (calprotectin) could be characterized as a very early and sensitive biomarker in a broad spectrum of acute and chronic inflammatory disorders like rheumatoid arthritis, allergies, inflammatory bowel or lung diseases. The purpose of our study was to avail these findings for *in vivo* imaging of inflammation with the perspective of monitoring disease activity in clinically relevant disorders.
S100A9-antibody was coupled to the fluorescence dye Cy5.5 for optical imaging studies by Fluorescence Reflectance Imaging (FRI). We investigated various mouse models of inflammatory and infectious diseases using anti-S100A9-Cy5.5 antibodies. S100A9 knock-out-mice served as additional controls to prove specificity. In the contact dermatitis model injection of anti-S100A9-Cy5.5 resulted in contrast to noise ratios (CNR) which were more than ten-fold higher compared to those of knock-out-mice and also significantly higher after injection of IgG-Cy5.5 without relevant specificity. With this specificity-proven marker we monitored inflammatory models of different immunological etiology, e.g. collagen-induced arthritis (CIA) or experimental leishmaniasis. In both models molecular imaging of S100A9 provides a sensitive and specific method of non-invasive monitoring even in subclinical disease stages. In addition, S100A9 is the first biomarker with a prognostic value for the Th1/Th2-driven inflammatory process in experimental leishmaniasis even weeks prior to the clinical outcome. In conclusion S100A9 has the potential to monitor disease activities *in vivo* for many other inflammatory diseases associated with a high phagocyte activity.

W6.16.04
Fractalkine ligand-receptor axis dictates prognostic outcome in human colorectal cancer

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In the tumor microenvironment chemokines affect cancer progression, influencing the composition of the tumor milieu and regulating metastatic processes. In this study we investigated the expression and role of the CX3CL1-CX3CR1 axis in 170 specimens of human colorectal cancer (CRC). We found that CX3CL1 and CX3CR1 were expressed by neoplastic cells at primary tumor sites, while - in marked contrast- expression at metastatic sites (lymph nodes and liver) was significantly reduced. High expression of both ligand and receptor by primary tumor cells (positive-axis tumors) was associated with better patient prognosis, compared to negative-axis tumors and with a reduced risk of developing metachronous metastasis. At inflammatory/tumor sites CX3CL1 has been associated with increased recruitment of immune cells; in our study, tumor expression of CX3CL1 did not correlate either with the density of CD3+ nor with CD68+ cells at the tumor invasive front. Since CX3CL1 is a trans-membrane molecule and affects cell-cell interaction, we hypothesized that double expression of the two molecules increased the adherence among cancer cells, preventing tumor dissemination. By using mixed populations of CX3CL1- or CX3CR1-transduced tumor cells, we observed that ligand-induced engagement of receptor-positive tumor cells resulted in enhanced adhesive activity and lower migratory ability in vitro. Furthermore, in a mouse spleen-liver model of metastasis, double expression of CX3CL1 and CX3CR1 significantly reduced the number of liver metastasis. In conclusion, tumor CX3CL1-CX3CR1 expression is associated with lower degree of disease recurrence and distant metastasis; thus, this chemokine axis is a potential biomarker with prognostic utility in human CRC.

W6.16.05
Tumor-specific adaptive immune responses to primary and metastatic ovarian tumors

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The presence, abundance, population, and diversity of Tumor Infiltrating Lymphocytes (TILs) have been identified as prognostic indicators in several cancers. Emerging cancer therapeutics including immunomodulators and adoptive T-cell therapy highlight the need to better understand and track this population of T cells, especially the need to understand if the adaptive immune response is tumor specific or general. We have developed a method to amplify, using a multiplex PCR approach, and deeply sequence rearranged T-cell receptor Beta (TCRB) chains. We use this assay to characterize TIL populations of primary and metastatic tumors and matched blood. We sequenced 10 primary and metastatic ovarian tumors collected from 5 patients. Each tumor was divided into a grid pattern with 8-22 sections (tumor size dependent). For each tumor section we collected data on the number, diversity, and the unique CDR3 sequences carried by the TILs, to characterize the intra-tumor heterogeneity of TIL count, diversity, and T cell clone overlap. In addition, we sequenced the immune repertoire from a paired peripheral blood sample to identify if the immune response was tumor specific. We found that the immune response within peripheral blood was significant and quantifiably different than the tumor repertoire. TIL repertoire within sections of a tumor showed high similarity to each other. These data indicate that the TCR repertoire of the ovarian tumor environment is tumor specific and homogeneous.

W6.16.06
Unique TCR signature of a novel cross-reactive CMV-specific T cell receptor: potential clinical implications

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Human cytomegalovirus (CMV) reactivation contributes to significant post-transplant clinical complications. Cross-reactive virus-specific T cells that co-recognise specific HLA alloantigens expressed by the donor allograft may evoke clinically relevant immune reactivity. We identified a novel HLA-A*02:01-restricted cytotoxic T-cell lymphocyte (CTL) with dual specificity towards the CMV-specific epitope (NLVPMVATV) and HLA-B27 subtypes. We examined the T cell receptor (TCR) repertoire encompassing conventional and cross-reactive virus-specific CTLs in a healthy individual and following an episode of post-transplant CMV reactivation in a lung transplant recipient (LTR; HLA-B27 allograft). Autologous peptide-pulsed A*02:01/NLV-specific CTLs were *in vitro* expanded, specificity confirmed by tetramer. Cross-reactive NLV-specific CTLs were identified as IFN-g-secreting following stimulation with HLA-B27 allele-expressing cell lines. Paired TCR variable alpha and beta chains from CDR3 were characterised using novel single-cell multiplex nested RT-PCR. In healthy individual, conventional NLV-specific TCR repertoire was dominated by a single TCR clone (81%; TRAV35TRAJ42_TRBV12-4TRBJ1-2). However, upon stimulation with HLA-B*27:09, cross-reactive NLV-specific TCR repertoire significantly skewed to a different clonotype (70%; TRAV3TRAJ31_TRBV12-4TRBJ1-1). This cross-reactive TCR clonotype was also identified in a LTR, increasing dramatically from pre-transplant (62%) to becoming the sole TCR (100%) prior to a clinically relevant episode of CMV reactivation. Functional activation by HLA-B27 molecules and NLV-specificity was confirmed by expressing LTRs cross-reactive TCR genes in a SKW3 T cell line. The public nature of the unique cross-reactive TCR signature can be used to monitor T cell dynamics during active viral infection in allogeneic transplant recipients and determine their ability to cause destructive immunopathology.

W7.01 Imaging of the immune system

IL7.01.01
In vivo imaging of intrahepatic effector CD8 T cell dynamics

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Effector CD8 T cells play a critical role in viral clearance and liver disease during HBV infection. Accordingly, understanding the signals that modulate how these cells home to the liver, recognize antigen and exert effector functions within this organ is of paramount importance.
Through the use of intravital imaging and unique mouse models of HBV pathogenesis, we found that, in contrast to most microvascular beds in which leukocyte adhesion is restricted to postcapillary venules, >90% of intrahepatic effector CD8 T cells arrest occurs within liver sinusoids. This early intrahepatic accumulation is independent of selectins, integrins, chemokines and MHC/TCR interaction but involves platelets and CD44-hyaluronic acid interaction. Indeed, blocking CD44 on platelets (but not on CD8 T cells) prevented early intrahepatic CD8 T cell accumulation. Our results suggests a model whereby platelets - after adhering to liver sinusoidal endothelial cells and becoming activated - provide a preferential surface onto which CD8 T cells arrest their run within the hepatic microcirculation.
After their initial arrest, effector CD8 T cells exhibit an intrasinusoidal crawling behavior that is inhibited by antigen recognition. Indeed, by setting up an immunofluorescence staining that allows us to identify IFN-γ-producing cells relative to their position within liver sinusoids, we found that effector CD8 T cells become IFN-γ positive while still intravascular, suggesting that recognition of hepatocellular antigens occurs in a diapedesis-independent manner. Specifically, effector CD8 T cells were found to recognize hepatocellular antigens by extending protrusion through sinusoidal fenestrae and this process is required to allow T cell extravasation.

IL7.01.02
Intracellular control of central versus effector memory T cell migration and activation

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New imaging techniques including twophoton microscopy (2PM) and mesoscopic imaging have uncovered the dynamic interactions between lymphocytes and antigen-presenting cells and the large-scale rearrangements of lymphoid tissue during inflammation. Here, we will discuss factors orchestrating central versus effector T cell migration in vivo, with a focus on G-protein-coupled receptors and their intracellular effectors. To this end, we use 2PM to follow the dynamic behavior of antigen-specific T cells in lymph nodes (“central memory T cells”) and salivary glands (“effector memory T cells”) during the memory phase of an antiviral immune response. In both tissues, central and effector memory T cells show robust migration, albeit at slower speeds and more complex cellular shape in salivary glands. Furthermore, both effector and central memory T cells maintain their ability to rapidly engage with CD11c+ antigen-presenting cells. The absence of the Cdc42 guanine exchange factor DOCK8 and pharmacological inhibition of the Rho effector ROCK resulted in impaired effector memory T cell migration in salivary glands, presumably owing in part to its greater stromal tissue complexity as compared to lymph nodes. Taken together, these findings suggest tissue-specific roles for intracellular signaling modules, which control tissue-specific T cell function.

W7.01.01
Lateral distribution of the T cell receptor and membrane lipid detected by high-resolution secondary ion mass spectrometry

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The engagement of the T cell antigen receptor (TCR) by a specific peptide-MHC ligand initiates a trans-membrane signaling that activates the T cell. An important event in T cell signaling is the recruitment of signaling proteins to the cell membrane and the assembly of a multi-protein (and lipid) signaling complex. Although it has been suggested that lipid/cholesterol composition plays an important role in the regulation of surface signaling complexes, direct visual evidence on lipid/cholesterol participation in the formation of these complexes is still lacking. Here, we characterize the clustering of TCR within T cell and model membranes by secondary ion mass spectrometry (NanoSIMS) with a spatial resolution of 50 nanometers or better. Quantitative information about the chemical composition of membrane complexes can be obtained through the use of orthogonal isotopic labeling of each signaling molecule. Analysis of nanoSIMS images shows the accumulation of TCRs into 60-150 nm clusters/islands within the membrane of an activating T cell and we further attempt to reveal the composition and lateral distribution of TCR and other membrane components, including cholesterol, within model membranes. Because these membrane structures may be shared with many other cell types, we believe the insights we gain in this system will be generalizable to other examples of cell surface receptor signaling.

W7.01.02

Role of colonic lymphoid patch dendritic cells in patrolling the colonic lumen, transporting antigen, and imprinting homing of T cells to the colon

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Viral transmission occurs largely through mucosal surfaces, but neither mechanisms of mucosal immune surveillance nor mechanisms of imprinting of T cell homing to the colon are well understood. Because CX3CR1$^+$ dendritic cells (DCs) that extend processes into the gut lumen are not mobile and cannot transport antigen, an explanation for antigen surveillance was sought. Here, we show that migratory CD103$^+$CD11b$^+$ DCs actively patrol the colonic lumen, phagocytose microorganisms, and transport them to colonic lamina propria. Recruitment of DCs to the colonic lumen depends on CCL20. These results resolve a paradox that DCs that could acquire antigen could not transport it, and explain pathogen sampling from the gut lumen. We also examined the ability of colonic DCs to imprint T cell homing to the colon. In contrast to small intestinal DCs that induce T cell homing to the small intestine, we find large intestinal DCs induce homing preferentially to the colon. They express lower levels of RALDH, responsible for RA production, than small intestinal DCs and thus induce only alpha4beta7 but not CCR9. Further, whereas beta7-deficient CD8$^+$ T cells were unable to home to the colon or small intestine, as expected, CCR9-deficient T cells still homed well to the colon but not to the small intestine. Thus, the signals for homing to the two compartments are different and homing to the large intestine is imprinted by the specialized DCs residing there. Understanding these functions may allow more rational design of mucosal vaccines targeted to the relevant compartment.

W7.01.03

Comprehensive confocal imaging and 3D computer analysis of blood and lymphatic vascular channels across entire lymph nodes

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Lymph nodes (LNs) are highly organized organs of the adaptive immune system. An intricate network of blood and lymphatic vascular channels serves as entry and exit routes for lymphocytes but their overall structure and dynamism during LN activation remain poorly understood. By using a new automated imaging system which enables extended tissue imaging of whole lymph nodes at confocal resolution, we aim to gain new spatial information on the LN topology and make structural changes measurable through computer analysis. Fluorescently labeled vascular systems of murine LNs were imaged at 2 µm pixel resolution in their entirety of up to 8 mm^3 and analyzed using in-house designed software tools. High-resolution volume images of the blood and lymphatic networks alone provided new insights into their labyrinthine arrangement in LN subcompartments, while additional computer reconstruction allowed sophisticated measurements in 3D. As a first step, we calculated the blood vessel network volume, individual segment diameters, and the distribution of distance to the nearest blood vessel. Current experimentation is employing measurements of the vessel diameters to revisualize the blood vessel network, allowing functional elements such as the feed arteriole and putative high endothelial venules to be extracted and analyzed in detail. Subsequently, we plan to investigate LNs at different stages of the immune response to gather more information on the remodeling processes of vascular networks in response to increased lymphocyte traffic. Ultimately, we wish to elucidate the topological relationship and dynamism of structural elements in LNs and draw a comprehensive map of LN anatomy.

W7.01.04

Skin patrol by tissue-resident CD8+ T cells

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Recent work has demonstrated that following the clearance of infection, a stable population of memory T cells remains present in peripheral organs and contributes to the control of secondary infections. However, little is known about how tissue-resident memory T cells behave in situ and how they encounter newly infected target cells.
Here we demonstrate that antigen-specific CD8$^+$ T cells that remain in skin following Herpes Simplex Virus infection show a steady state crawling behavior in between keratinocytes. Spatially explicit simulations of the migration of these tissue-resident memory T cells indicate that the migratory dendritic behaviour of these cells allows the detection of antigen expressing target cells in physiologically relevant time frames of minutes to hours. Furthermore, we provide direct evidence for the identification of rare antigen-expressing epithelial cells by skin-patrolling memory T cells in vivo. These data demonstrate the existence of skin patrol by memory T cells and reveal the value of this patrol in the rapid detection of renewed infections at a previously infected site.

W7.01.05

Single cell analysis of molecular networks involved in T-cell activation and signalling

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Our immune system must be able to rapidly fight against pathogens, but at the same time be tightly regulated to prevent harmful responses. This intricate balance is controlled in part by T lymphocytes. Targeting T cells has the potential to revolutionize therapeutics, however a better quantitative understanding of molecular processes controlling the functions of these cells is still required.
T cell signaling is tightly regulated by a set of molecular networks that involve families of transcription factors called Nuclear Factor kappaB (NF-kappaB) and Nuclear Factor of Activated T-cells (NFAT). Using live-cell imaging we previously showed that in response to cytokine stimulation the NF-kappaB system undergoes nuclear-to-cytoplasmic oscillations, which might control downstream gene expression (Science, 324:242, 2009, PNAS 107:11644, 2010). However, the precise role of this dynamic behaviour in T cell signalling remains unresolved.
Here we use systems biology approaches involving cutting-edge live-cell-imaging to investigate activation and signaling of Jurkat and primary CD4+ T cells in response to cytokine and T-cell receptor stimulation. Single-cell NF-kappaB and NFAT dynamics are correlated with patterns of downstream gene expression, including Interleukin-2, which is indicative of complex dynamical control of T lymphocyte function. This new single-cell picture of T cell biology will move us towards better therapeutic strategies for inflammatory disease.

W7.01.06
Dynamic expression of co-stimulatory and co-inhibitory receptors regulates termination of T cell-DC interaction during CD8 T cell activation

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Multiphoton intravital microscopy (MP-IVM) of the lymph-node (LN) has revealed that activation of naïve CD8 T cells by interaction with antigen (Ag)-laden dendritic cells (DCs) occurs in three distinct phases. The first phase of short-transient T cell-DC interactions is followed by a second phase of intense communication between T cells and DCs, characterize by long-stable interactions and reduced motility. After phase 2 termination, T cells re-acquire motility, shorten their interaction with DCs, start proliferating and exit the LNs. Whilst transition from phase 1 to phase 2 has been well characterized, the mechanisms responsible for termination of phase 2 are unknown. Systematic analysis of T cell expression profiles suggested that phase 2-3 transition is actively controlled by molecular mechanisms which involved a timed regulation of co-stimulatory and co-inhibitory molecules, such as CD28 and PD-1. Using MP-IVM of the LN, we studied the interstitial motility of PD-1-/- T cells. When compared with wild type T cells, PD-1-/- T cells have extended phase 2 defined as longer T cell-DC interaction and reduced motility. Whether and how PD-1/PD-L1 axis synergizes with other co-stimulatory and co-inhibitory receptors is under evaluation. Altogether these data provide evidences of a new molecular mechanism based on dynamic regulation of co-stimulatory and co-inhibitory receptors, which control T cell activation and may fundamentally shape the ensuing T cells response.

W7.02 Systems and theoretical immunology

W7.02.01
Methodological applications of host-pathogen data profiles conserved in Evolution

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The hypothesis that properties well conserved in Evolution may provide informative methods was evaluated in avian and mammalian species infected by viral, parasite, and bacterial agents. Three data levels were investigated: (i) non-structured data (percentages of lymphocytes, neutrophils, and macrophages), (ii) 'basic' data structures (interactions between two or more cell types, expressed as ratios), and (iii) 'high-level' structures (three-dimensional [3D] relationships).
In all studies, non-structured data did not differentiate disease-positive (D+, or observations in which a microbe was isolated) from disease-negative (D-, or microbial-negative) groups: D+ and D- data distributions overlapped. In contrast, when 'high-level' data structures were investigated in 3D space, all studies displayed a single and circular line of observations, which distinguished D- from D+ observations and also provided temporal information. However, when 'basic' data structures (the components of 'high-level' structures) were tested individually, they failed to discriminate D- from D+ observations.
Findings were consistent with Systems Biology/Evolutionary Biology theory: 'emergence' (the quintessential property of biological systems) does not depend on any one elemental factor, but may be revealed when such factors are assembled into and integrated with structures of higher level. Because 'emergence' revealed its robustness, infectious disease research may be facilitated by methods that, by focusing on data structure, may reveal properties well conserved in Evolution. Such methods may possess higher predictability than methods that, regardless of the number of individuals tested or factors investigated, do not and cannot capture phenomena conserved over millions of years.

W7.02.02
Higher expression of genes related to B cell activation in female spleen cells

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Vertebrate immune responses in males and females are dimorphic, where female immunity to infectious agents is usually more efficient but carry an increased incidence of autoimmune diseases. Although it has been suggested that such bias could be due to the positive effect of oestrogens on immune responses, the molecular basis for these changes are, as yet, unclear. We compared gene expression of healthy young adult male (baseline) and female (study subject) C57BL/6 mouse splenocytes, which had comparable cell ratios of T cells, B cells (B-1, B-2 and Bmz), and macrophages. Total spleen RNA (purity >1.9 260/280 OD) were used to produce cDNA and cRNA that was hybridised to Affymetrix 430 2 Complete Genome Mouse chips. Only data with twofold differences (increase or decrease) after normalisation (Qspline) were considered and were fit by means of the R package. False positives (False Discovery Rate) was 5% (adjusted $p<0.05$). Overexpressed genes grouped by gene ontology belonged to several categories, several of them immunologically relevant, including B cell activation (GO:0042113), among others. Overexpressed B cell activation genes included signalling proteins and receptors (**Bank1**, **Swap70**, **Cr2**, Prkcb, Pik3ap1, **CARMA1**, PTPN6, CD79a, CD22, CD40, Fcrla, Fcer2a, Ly6d), transcription factors (**Bcl11a**, Ebf1, FoxP1), etc. (Ighv14-2). Underlined gene names were confirmed and validated in B57BL/6 and Balb/c mice by quantitative RT-PCR and are being functionally examined. Polymorphisms of Bank1 and Cr2 have been linked to autoimmune diseases in humans.

W7.02.03
Functional proteomic interrogation of immune cell crosstalk and the effects of cytokine-targeted inhibitors using Single Cell Network Profiling (SCNP)

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Single cell network profiling (SCNP) is a multiparametric flow cytometry-based assay that measures simultaneous changes in multiple intracellular signaling proteins in response to modulators, providing a functional measure of pathway signaling in single cells without cell subset isolation. SCNP was applied to interrogate immune cell crosstalk and effects of targeted cytokine inhibitors upon crosstalk.
Healthy donor peripheral blood mononuclear cells (PBMCs) were modulated with Super Antigen (SAg) to drive immune cell cross talk via cytokine release and direct cell contact. Signaling kinetics in JAK-STAT, NFkB, MAPK and PI3K pathways were measured in monocytes, B cells and CD3+ CD4+/- T cells.
Within 45 minutes of SAg modulation multiple signaling pathways were selectively activated in monocytes. By 6 and 24 hours monocyte signaling resolved while lymphocyte signaling emerged. IL-2, TNFa and IL-6 inhibitors were used to deconstruct pathway specific contributions to cellular activation, identifying functional consequences of targeted cytokine blockade across cell subsets. IL-2 neutralization reduced PI3K pathway activation and p-STAT5 induction in T cells, while IL-6 blockade abrogated p-STAT3 induction in CD4+T cells, and TNFa neutralization reduced NFkB signaling in CD4- T cells.
Collectively these data demonstrate the ability of SCNP to better define disease pathophysiology and its abrogation, via interrogation

of immune cell crosstalk, dissection of pathway specific activity of targeted cytokine inhibitors, and holistic evaluation of the effects of targeted therapeutics on immune cell signaling. Expansion of this analysis to additional autoimmune and oncology-relevant modulators, signaling pathways, cell subsets and targeted therapeutics is in progress.

W7.02.04
Comparison of functional immune signaling profiles in peripheral blood mononuclear cells (PBMC) from rheumatoid arthritis (RA) patients versus healthy donors (HD) using Single Cell Network Profiling (SCNP)

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Prognostic and predictive biomarkers are lacking in RA. SCNP is a multiparametric flow cytometry-based assay that simultaneously measures changes in multiple intracellular signaling proteins in response to modulators providing a functional measure of pathway activity in single cells.
SCNP of 42 nodes (modulation/intracellular readout) within 21 immune cell subsets was performed on PBMCs from 181 RA patients collected before initiating new treatment, either MTX or biologic agent, within the national Treatment Efficacy and Toxicity in Rheumatoid Arthritis Database and Repository (TETRAD), and 10 age- and gender-matched healthy donors. TETRAD participants were 86% female and 76.5% Caucasian. All met ACR classification criteria for RA and mean Disease Activity Score on 28 joints (DAS28) was 4.77±1.40 [SD]. Using half the donors as a training set, multiple variations in signaling responses in discrete cell subsets associated with donor characteristics (e.g. healthy vs. RA, disease activity) were identified, and will be subsequently confirmed in the remaining donors (test set).
Specifically, TNFα signaling was lower in monocytes in most RA samples while analysis of T cell subsets identified significant differences with opposing directionality in IL-6 signaling as compared to healthy: RA helper T cell subsets had decreased IL-6→p-STAT1/3; cytotoxic T cell subsets showed increasing responsiveness to IL-6; central memory cytotoxic T cells had a significant increase in IL-6→p-STAT1. The clinical significance of these observations will be investigated with relation to RA pathophysiology and treatment response. These data reveal the functional biology associated with RA pathophysiology and enable the identification of potential prognostic and predictive biomarkers.

W7.02.05
The role of microRNAs in regulatory T cell function

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The transcription factor FOXP3 is essential for the formation and function of regulatory T cells (Tregs) as demonstrated by the autoimmune disease in scurfy mouse and IPEX patients. However, little is known about the molecular basis of human FOXP3 function or the relationship between direct and indirect target genes in human Treg. We have performed a genome wide analysis of human FOXP3 target genes in natural Treg using chromatin immunoprecipitation array (ChIP-on-chip) combined with gene expression profiling and micro RNA profiling. These experiments identified 63 micro RNAs that are potential human FOXP3 target genes. We first analysed these miRs for differential expression, and a subset are up regulated in Treg. When we used lentivirus shRNAi to knock down FOXP3 expression in human Treg we observed that the miRs expression levels were reduced, suggesting regulation by FOXP3. We next identified putative miR target genes in our human Treg gene signature that are themselves down regulated, and analysed their expression in FOXP3 ablated Treg vs FOXP3 sufficient Treg. These experiments identified a number of genes that are direct targets of the miRs that are dependent on FOXP3, and suggest that these target genes may be tightly regulated as part of the human Treg gene network. The chromatin remodelling gene SATB1 is tightly repressed to maintiant Treg function. We are now investigating other miR and FOXP3 target genes in human natural vs induced reguatory T cells.These results provide new insights into the mechanism of action of FOXP3 in human Treg.

W7.02.06
A bioinformatics pipeline to uncover regulatory modules and their condition-specific regulators in human monocyte-to-macrophages differentiation and polarization

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In healthy organisms, inflammation is the first line of defense against infections and other damage. Monocytes and macrophages are key players of this process. Since a deregulation of the activity of these cells is at the basis of several pathological conditions, elucidating the molecular mechanisms of monocyte/macrophage activation represents a major step to study inflammatory disorders and, eventually, develop new therapeutic strategies. However, these mechanisms and their interplay during monocyte/macrophage activation and M1/M2 polarization remain poorly characterized. Here we report the application of computational methods to reconstruct gene regulatory networks and decipher transcriptional modules in an in vitro model of physiological inflammation. Specifically, gene expression profiles of human blood monocytes, from normal healthy donors and exposed to combinations of factors reproducing normal inflammatory conditions, have been monitored during a time course of 48 hours and transcriptional data analyzed using an ad-hoc bioinformatics pipeline aimed at reconstructing gene regulatory modules. The computational process starts with the identification of clusters of co-regulated genes whose expression changes during the time course. Through enrichment analysis and scanning analysis of transcription factor (TF) binding site motifs, we identified subsets of inflammation related genes and TFs that can be considered as process controllers and, thus, further used as candidates to reconstruct regulatory modules. The bioinformatics analysis identified several member of the interleukin-1 family as master regulators of specific regulatory modules linked to the inflammation process.

W7.03 Structural biology in immunology

IL7.03.01
Structural basis of viral escape in Influenza

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A high mutation rate in RNA viruses facilitates viral replication and escape from immune system surveillance. We used a reverse genetics approach to probe the mechanism underlying immune escape for influenza virus-specific CD8+ T cells responding to the immunodominant NP366 epitope restricted to the H2Db MHC molecule. All engineered mutants with a substitution at a critical residue (methionine at position 6, P6M) evaded recognition by wild-type DbNP366-specific CD8+ T cells. The mutants NP-M6I and NP-M6T were associated with a substantial “hole” in the naïve TCR

repertoire against these endogenous epitopes, and thus minimal primary responses. We solved the structures of the NP-M6I, NP-M6T and compare them with the NP-wt as well as the NP-M6W peptides. The structures revealed that the "hole" in the repertoire against those mutants could be due to altered conformation of a key residue (His155) in the MHC-I binding cleft, offering us a molecular mechanism for viral escape.

W7.03.01
Crystal structure of adenovirus E3-19K protein bound to HLA-A2 reveals mechanism for immunomodulation

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The cell-surface presentation of viral antigens by MHC class I molecules is critical for eliminating host infected cells. In turn, viruses have evolved various strategies to interfere with antigen processing and presentation. The adenovirus (Ad) E3-19K protein binds to and retains MHC class I molecules in the endoplasmic reticulum (ER), thereby suppressing anti-Ad activities of CD8+ T-cells. E3-19K is a type I transmembrane glycoprotein that comprises a N-terminus ER-lumenal domain, a transmembrane domain, and a C-terminus cytosolic tail. The ER-lumenal domain of E3-19K associates with the ER-lumenal domain of MHC I, while the dilysine motif in the cytosolic tail of E3-19K provides the signal for localization of the E3-19K/MHC I complex in the ER. We will describe a novel rescue refolding strategy that allowed formation of the Ad serotype 2 (Ad2) E3-19K/HLA-A2 complex, from which we recently grew crystals diffracting to 1.95 Å resolution. Ad2 E3-19K binds to the N-terminus of the HLA-A2 groove, contacting the α1-, α2-, and α3-domains and β2m. Ad2 E3-19K has a unique structure comprised of a large N-terminal domain, formed by two partially overlapping β-sheets arranged in a V-shape, a C-terminal α-helix and tail. The structure reveals determinants in E3-19K and HLA-A2 that are important for complex formation; conservation of some of these determinants in E3-19K of different Ad species and MHC I of different HLA loci suggests a universal binding mode for all E3-19K proteins. Our structure offers explanations for the mechanism by which E3-19K modulates antiviral cellular immunity.

W7.03.02
Structural insight into HLA DQ8 restricted TCR selection in celiac disease

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Celiac disease (CD) is almost exclusively associated with the HLA alleles DQ2 and DQ8, either alone or in combination. CD is characterized by an inflammatory T-cell response to dietary gluten that leads to a progressive degradation of the gastrointestinal lining. Gluten specific CD4+ T-cells present in the lamina propria of the small intestine respond to a number of DQ2 and/or DQ8 resticted, glutamine and proline rich epitopes and preferentially recognize deamidated peptides. Sequence analysis of T-cell receptors (TCRs) isolated from such gluten reactive T cells from different HLA-DQ8+ CD patients revealed an antigen dependent bias in Vα- and Vβ-chain usage (TRAV26-2 and TRVB9-1 for DQ8-glia-a1), as well as a high prevalence of non-germline encoded arginine residues within either the CDR3α or the CDR3β region of the TCRs. We solved five high-resolution crystal structures of TCRs recognizing the deamidated DQ8-glia-a1 epitope. The structural comparison of the ternary complexes revealed a striking degree of similarity in the overall docking modes of the TCRs on DQ8-glia-a1, as well as in the positioning of the characteristic CDR3 arginine residues. Using alanine substitution mutants of a TRAV26-2/TRVB9-1 TCR we demonstrated the importance of arginine in CDR3α and of two TRVB9-1 germline encoded residues for DQ8-glia-a1 recognition. In summary, our data points towards the presence of common structural drivers for TCR selection in CD.

W7.03.03
Divergent T cell recognition of a lengthy epitope bound to Major Histocompatibility Complex class I molecule

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The surface expression of T cell receptors on CD8+ T cells allows them to recognise short peptide antigens between 8 to 10 amino acids in length, presented by class I major histocompatibility complex molecules (MHC-I). Interestingly, longer epitopes (>10 amino acids) have also been shown to play essential role in the cellular immunity, yet how these antigens are recognised by T cell receptors remains under-explored. Our present study investigated two distinct T cell clones (CA5 and SB47) recognising a 13mer viral peptide (named LPEP) presented by the MHC class I molecule; HLA B*3508. Using biophysical (surface plasmon resonance), cellular and crystallographic approaches, we investigated the interaction between the CA5 and SB47 TCRs against the HLA-B*3508-LPEP complex. The crystal structure of the TCR-pMHC complex revealed that, whilst the CA5 TCR focused centrally to the super-bulged peptide antigen and struggled to contact the MHC surface, the SB47 TCR was able to avoid the bulged peptide landscape and engaged the extreme N-terminal part of the MHC-I surface. These structural differences between the CA5 and SB27 TCR-pMHC complexes have provided novel insights into the lengthy antigen detection by CD8+ T cells as well as shaping our understanding towards the fine-specificity of MHC restriction.

W7.03.04
The constant region affects antigen binding of antibodies to DNA by altering secondary structure

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We previously demonstrated an important role of the constant region in the pathogenicity of anti-DNA antibodies through modulation of immunoglobulin affinity and specificity. To determine the mechanisms by which the constant region affects autoantibody binding, a panel of isotype-switch variants (gG1, IgG2b, IgG2a) was generated from the PL9-11 IgG3 anti-nuclear antibody, which differed only in their constant region. The affinity of the PL9-11 antibody panel for histone antigens was measured by surface plasmon resonance (SPR). Tryptophan fluorescence was used to determine the wavelength shift of the antibody panel upon the addition of DNA and histone (2A and 2B) antigens. Finally, circular dichroism spectroscopy was used to measure changes in secondary structure corresponding to antibody/antigen interactions. SPR analysis revealed significant differences of histone 2A/2B binding affinity between members of the PL9-11 panel. The wavelength shifts of tryptophan fluorescence emission were found to be dependent on the antibody isotype, while increasing salt concentrations lowered K_D values. Circular dichroism analysis determined that changes in antibody secondary structure content differed between the PL9-11 IgG isotypes upon antigen binding. Thus, the antigen binding affinity of anti-nuclear antibodies is dependent on the particular constant region expressed. Moreover, the effects of antibody binding to antigen on tryptophan fluorescence, as well as secondary structure shifts, were also constant region dependent. Alteration of secondary structures influenced by constant regions may explain differences in fine specificity of anti-DNA antibodies between antibodies with similar variable regions, as well as cross-reactivity of anti-DNA antibodies with non-DNA antigens.

W7.03.05
Structural insight on the recognition of surface-bound opsonins by the integrin αI-domain of complement receptor 3

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Complement is the first line of defense against invading pathogens and bridges the innate and the adaptive immunity. Complement activation leads to proteolysis of the central complement component, C3, yielding an anaphylatoxin C3a and an opsonin C3b. The latter

becomes covalently bound to the target surface via its reactive thioester. C3b is further processed into iC3b and C3dg by factors I and H and finally to C3d by other plasma proteases. These fragments are ligands for complement receptors expressed on immune cells. Amongst these, integrin receptors CR3 (CD11b/CD18, αMβ2, Mac-1) and CR4 (CD11c/CD18, αXβ2) are essential for the recognition and phagocytosis of immune complexes. Until now, a systematic comparison of the binding selectivity amongst C3 fragments for these receptors has been lacking. Furthermore the CR3 binding-site on the C3 molecule has not been identified so far.
By using cell-adhesion and SPR assays, we have identified the minimal binding domain of C3 for CR3. Surprisingly, these results reveal a difference in selectivity towards C3 fragments for CR3 and CR4, since CR3 preferentially binds to C3d. Furthermore, we report the crystal structure of the CR3 I-domain in complex with C3d at 2.8 Å resolution. This structure provides the first atomic model for CR3 binding to iC3b, C3dg or C3d and explains the lack of binding to C3b. In addition, our structure suggests a simultaneous binding of CR3 and another receptor, CR2, to C3 fragments, which might be important for trafficking of opsonized antigen in lymph nodes between B-cells and follicular dendritic cells.

W7.03.06
Structural insights into S100 proteins recognition and signal transduction by the Receptor for Advanced Glycation End-products (RAGE)

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RAGE is a pattern recognition receptor from the immunoglobulin superfamily that recognizes endogenous, danger-associated patterns. All these extracellular ligands accumulate in diseased tissues. Their binding to RAGE ectodomain triggers downstream signaling events that activate NFκB and other transcription factors, promoting pro-inflammatory molecules release. This maintains a high inflammatory state and imposes a massive stress on the cells, ultimately leading to tissue damage rather than healing and recovery. RAGE blockade has therefore been considered as a promising therapeutical strategy in various pathological settings. However, after a decade of efforts put in that direction, the results are still unclear, due to the fact that RAGE-mediated signaling has also a protective effect in tissue homeostasis and resolution of inflammation.
To determine the structural basis for RAGE signaling and to be able to design specific, efficient modulators of the receptor, we have pursued the structure determination of several RAGE:ligand complexes. We have obtained the crystal structure of the complex between human RAGE ectodomain and murine S100A6 at 2.3 Å resolution. This structure shows how ligand binding induces a novel dimeric conformation of RAGE, that appears to be suited for signal transduction and effector binding to the receptor intracellular domain. Surprisingly, the structure reveals a new, unexpected conformation for the dimeric S100A6. Modeling and bioinformatics suggest that this might be a common feature for other S100 proteins upon RAGE binding. This S100 conformation might therefore constitute a promising target for drug design of specific inhibitors with therapeutical potential for treating e.g. cancer and neurodegenerative diseases.

W7.05 Evolution of the immune system and comparative immunology

IL7.05.01
Antigen Mediated Positive and Negative Selection of Developing Chicken B Cells

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Cell surface immunoglobulin (sIg) expression is required for the productive colonization of follicles in the avian bursa of Fabricius. We have used retroviral gene transfer to introduce chimeric sIg receptors where the specificity determining region of lamprey variable lymphocyte receptors (VLRs) is fused to the chicken sIgM constant region (VLR:Tm). While expression of phycoerythrin (PE) specific VLR^{PE}Tm supported B cell development in the absence of endogenous sIgM, expression of VLR^{HEL}Tm with specificity for the self-antigen Hen Egg Lysozyme (HEL) leads to B cell deletion. Similarly i.v. injection of PE into VLR^{PE}Tm transduced embryos resulted in deletion of VLR^{PE}Tm expressing B cells.
Expression of mCD8alpha:chIgalpha receptors also supports bursal colonization by B cell precursors. Co-expression of TL, a natural ligand of mCD8alpha, however resulted in deletion of CD8alpha:Igalpha expressing B cells. Deletion required signaling through the mCD8alpha:Igalpha receptor as mutations in the Igalpha ITAM abrogated TL induced deletion. Thus negative selection requires signaling downstream of sIg.
In chicks transduced with VLR^{PE}Tm, VLR^{PE}Tm expressing bursal B cells disappeared within the first 10 days after hatch. In contrast, intrabursal introduction of PE into bursal follicles through the gut maintained the presence of VLR^{PE}Tm expressing B cells. Intrabursal PE further enhanced the emigration of bursal VLR^{PE}Tm expressing B cells to the periphery, including both short lived and longer lived VLR^{PE}Tm expressing B cells. This demonstrates that gut derived antigen can mediate positive selection of antigen specific chicken B cells and supports a role for antigen in gut associated B cell development.

IL7.05.02
Phenoloxidases and cytotoxicity in ascidians: an overview

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Phenoloxidases (POs) belong to a family of copper-containing proteins (including also hemocyanins) widely distributed among invertebrates. They are able to convert polyphenols to quinones and induce cytotoxicity through the production of reactive oxygen species, a fundamental event in many immune responses. In ascidians, PO activity has been described and studied in both solitary and colonial species and the enzyme is involved in inflammatory and cytotoxic reactions against foreign cells or molecules, and in the formation of the cytotoxic foci which characterize the nonfusion reaction of botryllid ascidians. Expressed genes for putative POs have been recently identified in the solitary ascidian *C. intestinalis* (CiPO1 and CiPO2) and the compound ascidian *Botryllus schlosseri*.
Multiple sequence alignments evidenced the similarity between the sequences of ascidian POs and crustacean proPOs whereas the analysis of the three-dimensional structure reveals high similarity with arthropod haemocyanins, which share common precursors with arthropod proPOs. *Botryllus* and *Ciona* POs grouped in the same cluster, and all of them share the full conservation of the six histidines at the two copper-binding sites as well as of other motifs, also found in arthropod haemocyanin subunits, involved in the regulation of enzyme activity. *In situ* hybridisation indicated that the *Botryllus* PO is transcribed inside morula cells, a characteristic cytotoxic haemocyte type.
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W7.05.01
Generation of B lymphocytes and immunoglobulin diversity in Bos taurus

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We have characterized B lymphopoiesis, germline IGLV, IGKV and IGHV repertoires and the mechanisms of the generation of the primary immunoglobulin repertoire in cattle.
B lymphopoiesis is active in fetal bovine bone marrow and lymph nodes, proceeding through CD79α+IgM- pre-B cells expressing RAGs and surrogate light chain. TdT is selectively expressed in fetal

bone marrow B cells, indicating diversification of the recombining Ig genes by junctional mechanisms.
During the last trimester of fetal development the immature B cells populate the ileal Peyer's patch, generating oligoclonal lymphoid follicles with extremely rapid B cell turnover. AID is strongly expressed in the follicles, associated with hypermutation of the IGHV genes before exposure to external antigens. AID is also expressed in the spleen, but the ileal Peyer's patch is likely quantitatively more significant to the generation of the diversified B cell pool due to the higher proliferation rate.
B lymphopoiesis is practically absent in the adult, possibly due to changes inherent to the hematopoietic lineage. Thus, cattle rely on the peripheral B cell pool generated in fetal and early postnatal period for the rest of the life.
The germline repertoire of Ig gene segments is very limited in cattle. There are a maximum of 33 functional paralogous light chain V genes dominated by a single subgroup, and a maximum of 20 functional paralogous heavy chain V genes belonging to a single clan II subgroup. Thus, junctional diversity and hypermutation are essential in the generation of sufficient primary immunoglobulin repertoire.

W7.05.02
Atlantic cod displays an unusual TLR repertoire - a Gadidae-specific feature?

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Atlantic cod has an unusual immune system compared to other vertebrates. A loss of major histocompatibility complex class II (MHC-II) is accompanied by a large expansion of MHC-I and an unusual repertoire of Toll-like receptors (TLR). Further, Atlantic cod lacks homologs of the mammalian surface TLRs 1, 2, 6 (and 10) in addition to having large gene expansions in TLR7, 8, 9 and 22. These features indicate an evolutionary adaption.
We have performed a complete characterization of TLR genes in Atlantic cod. Here we present a novel TLR, TLR25, previously reported only in Channel catfish and demonstrate that Atlantic cod has teleost derived surface TLRs: TLR14, 22 and 25. Within the individual gene expansions we find sites of diversifying selection that could account for the differential expression patterns for individual gene copies.
By examining several members of the gadidae family we found that the lack of mammalian surface TLR homologs is consistent with the findings in Atlantic cod. We further found that the gene expansion patterns are conserved except for TLR25 that seems to be specific for Atlantic cod and are currently investigating species belonging to the phylogenetically more distant gadiformes order.

W7.05.03
Evolution of antigen recognition by Vγ9Vδ2 T cells

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Vγ9Vδ2 T cells carry TCR with eponymous V gene products and Vγ9Jγ1.2 rearrangements, which recognize pyrophosphate containing metabolites of host and microbial isoprenoid synthesis (phosphoantigens) such as IPP and HMBPP. It is generally perceived that these T cells are restricted to higher primates including humans. The aim of this study is to test for existence of such cells in other mammalian taxa and to use this knowledge to understand the molecular basis of their TCR-mediated activation. Analysis of public databases revealed fragments of Vγ9 and Vδ2 like genes in at least one clade of the Eutheria subgroups (Euarchontoglires, Laurasiatheria, Xenarthra, Afrotheria) suggesting the common emergence of Vγ9 and Vδ2 genes and placental mammals. In six species full length Vγ9 and Vδ2 like sequences including RSS were found: 1. Horse 2. Cow 3. Alpaca 4. Sloth 5. Bottlenose dolphin 6. Grey mouse lemur. The first three were further analyzed by (RT) - PCR and sequencing. Horse and cow showed Ig domain disrupting mutations or stop codons, respectively, but alpaca (Vicugna pacos) blood mononuclear cells revealed the presence of Vγ9 & Vδ2 containing TCR chains. Further cloning indicated absence of somatic hyper mutation (SHM) in these V genes and indicated a dominance of functional Vγ9Jγ1.2 rearrangements. Full length Vγ9 & Vδ2 containing TCR chains have been amplified. Attempts to clone, express and analyze the function of the presumed alpaca Vγ9Vδ2 TCRs are in progress along with analysis of those Vγ & Vδ genes which were recently reported to posses SHM in Camelus dromedaries.

W7.05.04
***Drosophila* Helical factor (Hf) and helical cytokine evolution: from *in silico* approaches to functional evidence**

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Cytokines have been first discovered in human and their existence in invertebrates was only confirmed in the recent past, when molecular biology and functional studies lead to the discovery of cytokines in insects, crustaceans and tunicates. Nonetheless, fundamental conundrums pertaining the evolution of cytokines, *e.g.*, which is their origin and how their differentiation have proceeded, remain unanswered principally because of their primary sequence variability.
Drosophila helical factor (Hf) is the first soluble factor predicted to be an helical cytokine in invertebrates by an on-purpose developed algorithm. I*n vitro* and *in vivo* experiments have demonstrated that Hf expression increases after an immune challenge and the activation of the kinase dTAK1, homologue of the vertebrate MAP kinase MAP3K7. Confocal microscopy demonstrated that Hf has a cytoplasmic localization in *Drosophila* cultured hemocytes, and in accordance with early *in silico* predictions Hf is released after a bacterial challenge. The recombinant peptide rHf stimulates the expression of the antimicrobial gene *defensin* in absence of any further immune challenge, suggesting the involvement of Hf in the induction of *Drosophila* antimicrobial peptides.
By starting with *in silico* analyses and then experimentally verifying the computational predictions we have tackled the difficult task of the evolution of helical cytokines, demonstrating the importance of molecular fold recognition algorithms to find structurally conserved molecules when sequence variability hampers the application of sequence similarity-based investigations.

W7.05.05
Phenoloxidases and cytotoxicity in ascidians: an overview

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Phenoloxidases (POs) belong to a family of copper-containing proteins (including also hemocyanins) widely distributed among invertebrates. They are able to convert polyphenols to quinones and induce cytotoxicity through the production of reactive oxygen species, a fundamental event in many immune responses. In ascidians, PO activity has been described and studied in both solitary and colonial species and the enzyme is involved in inflammatory and cytotoxic reactions against foreign cells or molecules, and in the formation of the cytotoxic foci which characterize the nonfusion reaction of botryllid ascidians. Expressed genes for putative POs have been recently identified in the solitary ascidian *C. intestinalis* (CiPO1 and CiPO2) and the compound ascidian *Botryllus schlosseri*.
Multiple sequence alignments evidenced the similarity between the sequences of ascidian POs and crustacean proPOs whereas the analysis of the three-dimensional structure reveals high similarity with arthropod haemocyanins, which share common precursors with arthropod proPOs. *Botryllus* and *Ciona* POs grouped in the same cluster, and all of them share the full conservation of the six histidines at the two copper-binding sites as well as of other motifs, also found in arthropod haemocyanin subunits, involved in the regulation of enzyme activity. *In situ* hybridisation indicated that the *Botryllus* PO is

transcribed inside morula cells, a characteristic cytotoxic haemocyte type.
This research was supported by the Italian MIUR (PRIN 2010-2011).

W7.05.06
The secondary structure of the pre-mRNA encoding the Antarctic fish Igμ chain drives its atypical splicing pattern

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Teleosts inhabiting the cold seawater of the Antarctic continental shelf (-1.87 °C constant throughout the year) underwent major modifications of their morphology, ecology and biology to adapt to the cooling of the environment. Our study was aimed at investigating Antarctic teleost Immunoglobulin μ heavy chain since, due to the plasticity of its gene locus, it is appropriate to identify the modifications occurred during the adaptive evolution. For this purpose, we sequenced both the membrane and the secreted form of IgM from 14 Antarctic and non-Antarctic species belonging to the suborder Notothenioidei. The primary mRNA splicing for the membrane form was found to be atypical in the majority of Antarctic species, because it led to exclusion of the entire CH2 and CH3 constant exons, and to inclusion of 39-nucleotide exons encoding an unusually long extracellular membrane-proximal domain. Genomic DNA analysis revealed that each 39-nucleotide exon fall within a long sequence that is antiparallel to an upstream region. By combining computational, molecular biology and physicochemical approaches, we demonstrated that the antiparallel regions of Igμ chain are responsible for the secondary structure of pre-mRNA, and, in turn, for the splicing mechanism. In fact, the presence of a long double-stranded structure was predicted by computational tools and confirmed by the physical analysis of a synthetic RNA fragment reproducing part of the primary transcript. By Atomic Force Microscopy the length, the thickness and the persistence length of RNA were determined. The double-stranded structure was demonstrated to be temperature dependent by Circular Dicroism spectroscopy analysis.

W7.07 Veterinary immunology

IL7.07.01
***In vitro* evaluation of the anti-inflammatory control actions of interferon-alpha in swine**

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Interferon-alpha (IFN-α) shows potent immunomodulatory properties, which underlies its use for successful low-dose oral treatments of diverse viral infections and immunopathological conditions. The studies on oral administration have been hampered by the lack of recognized *in vitro* models, reproducing the *in vivo* control action of IFN-alpha over inflammatory cytokine responses. Owing to the above, the aim of our study was to validate IPEC-J2 (a continuous cell line of porcine intestinal epithelial cells) as reporter system of the above properties of IFN-alpha. Three different experimental conditions (oxidative stress, inflammatory response and amplification of lymphoid cell signals) were selected to evaluate the effects of porcine recombinant IFN-alpha$_1$ (rIFN-alpha), two natural porcine IFN-alpha preparations (nIFN-alpha) and human lymphoblastoid IFNs. The IFNs under study showed significantly different control actions in IPEC-J2 cells at either low or moderate concentrations (1 and 100 U/ml, respectively). In particular, rIFN-alpha was shown to down-regulate interleukin (IL)-8, IL-1beta, TNF-alpha and beta-defensin 1 genes either directly, or indirectly through second messengers released by IFN alpha-treated lymphoid cells. Concerning IL-6, only second messengers from IFN alpha-treated lymphoid cells could regulate the expression of this cytokine in IPEC-J2 cells. Also, the supernatant of IFN alpha-treated tonsil cells (100 U/ml) caused a significant decrease of IL-8 gene expression and protein secretion ($P < 0.05$). The release of second messengers from IFN alpha-treated tonsil cells went along with a positive regulation of IgA and antibacterial peptides. Our findings confirm an important role of type I IFNs in the regulation of the inflammatory response.

W7.07.01
A novel multi-stage subunit vaccine against paratuberculosis induces significant immunity and reduces bacterial burden in tissues

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Effective control of paratuberculosis is hindered by lack of a vaccine preventing infection, transmission and without diagnostic interference with tuberculosis. We have developed a novel multi-stage recombinant subunit vaccine in which a fusion of four early expressed MAP antigens is combined with a MAP protein expressed in latent infection (FET11 vaccine). FET11 vaccine proteins were formulated with CAF01 adjuvant and injected to MAP challenged calves at two different ages. 28 calves divided into two FET11 vaccine groups, a commercial vaccine and a control group were used in the study and followed for a year. The FET11 vaccine induced a significant T cell response against constituent vaccine proteins characterized by a high percentage of CD4+ T cells and participation of polyfunctional CD4+ T cells. Of the two different age groups, late FET11 vaccination conferred protective immunity characterized by a significant containment of bacterial burden in gut tissues compared to non-vaccinated animals. There was no cross-reaction with bovine tuberculosis in vaccinated animals. This novel multi-stage vaccine has the potential to become a marker vaccine for paratuberculosis.

W7.07.02
Bovine viral diarrhea virus actively replicate in bovine monocyte-derived dendritic cells and use them as viral reservoirs

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Bovine viral diarrhea virus (BVDB) causes immune-suppression and persists in the host for long periods of time. The mechanisms underlying these behaviors have not been completely elucidated. The aim of this work was to study the interaction between BVDV (Type 1, cp) and bovine DC. Bovine DC-CD11b+ were differentiated in culture from purified CD14+ peripheral-blood monocytes and treated with infectious or inactivated virus for one hour, in the presence or absence of specific antibodies. Pre-incubation with anti E2 antibodies complete block DC infection. In the absence of anti E2 antibodies, the virus infected and rapidly replicated in DC. Negative strand RNA and viral non-structural protein NS3 were detected as soon as 3 hours post-infection. Extracellular virus increased 2 logs/IDTC50 titers in 6 hours. Infection down-regulated the expression of MHC-I, II and co-stimulatory molecules, that were not recovered by treating with mitogens. Synthesis of pro-inflammatory cytokines was not impaired. Infected DC were also unable to present FMDV antigens to T-cells. Infected DC remained inactivated and did not undergo apoptosis. The ability of BVDV to actively replicate in DC, keeping them alive and arrested in an immature state could be an evidence of a mechanism of the virus to evade the immune system while mantaining persistent infections. It may also explain the complete lack of adaptive responses observed in cattle up to three weeks post infection.

W7.07.03
Novel peptide-MHC tetramers to characterize the specificity of the CD8+ T cell response against Theileria parva

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The *Theileria parva* parasite is able to transform bovine T lymphocytes resulting in a lethal lymphoproliferative disorder in cattle. A better understanding of the cytotoxic T lymphocytes (CTL) response elicited during the "infection and treatment method" vaccination will shed light on the mechanism of protective immunity against *T. parva* and help develop a better recombinant vaccine. Peptide-MHC tetramers have been extensively used in human and mouse studies to decipher T cell responses but remains a novel approach in bovine immunology. In the present study, we describe the creation of "one-pot, mix and read" peptide-MHC tetramers for the enumeration and phenotypic characterization of bovine T cells specific towards *T. parva* CTL epitopes. A set of eight bovine MHC class I heavy chains and corresponding known *T. parva* CTL epitopes have been used to generate peptide-MHC tetramers. These reagents were first validated using a bank of bovine CTL lines which are specific towards *T. parva* CTL-epitopes. Each of the tetramers was successful in binding to their respective CTL as observed by flow cytometry. Recent experiments demonstrated the capacity of these tetramers to stain *ex vivo* cells isolated from *T. parva*-infected cattle. The data show that these reagents can be used to generate, "on-demand", any peptide-MHC class I tetramers to evaluate cellular immune responses in cattle to any infectious diseases where CTL play a role. In the future, these tetramers will be used in a reverse immunology strategy to identify new *T. parva* CTL epitopes that can be used as novel vaccine candidates.

W7.07.04
Dendritic cell tracking in pigs

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Cellular therapies using immune cells are being increasingly applied in clinical trials. The success of cellular therapies depends in part on accurate delivery of cells to target organs. In dendritic cell (DC) therapy, in particular, delivery and subsequent migration of cells to regional lymph nodes is essential for effective stimulation of the immune system. Thus, the design of an optimal DC therapy would be facilitated by technologies for monitoring DC trafficking by different methods. Domestic pigs are closely related to humans and represent an excellent animal model for immunological studies. The aim of this study was to investigate the possibility to use the pig as a model for DC tracking *in vivo*.
Porcine monocyte derived DC (moDC) culture with superparamagnetic iron oxide (SPIO) particles was standardized considering SPIO concentration and culture mortality. Phenotyping (upregulation of SLA-II, CD80/86), cytokine production (TNF-α, IL-6 ELISA) and mixed lymphocyte reaction assay (moDCs:PBLs) confirmed that SPIO-moDC culture were similar as mock moDCs and fully functional for the *in vivo* tracking. After subcutaneous inoculation, SPIO-moDC migration was confirmed by magnetic resonance imaging (MR 0,2 T) at 24, 48 hours and by Perls staining of draining lymph nodes. Moreover, after one dose of different RHDV-VLPs-pulsed moDCs specific local responses in the draining lymph node cells were confirmed using ELISPOT IFN-γ. Additionally, systemic PBMCs were able to elicit specific responses to RHDV-VLP antigen.
This study has shown the possibility to use pigs as a suitable animal model to test DC trafficking with the aim of improving cellular therapies.

W7.07.05
Development of an ELISA method to diagnose infected poultries

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Influenza A viruses are enveloped viruses within the family Orthomyxoviridae and are further classified into subtypes depending upon their surface glycoproteins. Vaccination programs for the control of avian influenza (AI) in poultries have limitations due to the problem of differentiating between vaccinated and virus-infected birds. We have used NS1, the conserved nonstructural protein of influenza A virus, as a differential diagnostic marker for influenza virus infection. Experimentally infected poultry were evaluated for the ability to induce antibodies reactive to NS1 recombinant protein. The NS1 protein of influenza A is a nonstructural protein expressed in large amounts in virus-infected cells, but it has not been detected in virions. In this project two conserved amino acid sequences of NS1 protein were selected on the basis of their high degrees of antigenicity, as antigen to develop an ELISA method. ELISA could be used to screen the infected and vaccinated sera due to their titer of antibody.

W7.07.06
A role of myeloid precursor cells (MDSCs), p-STAT 3 and lymphocyte Treg in development of immunosuppression in dogs with skin and mammary cancer

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Recent studies have indicated an important role of myeloid precursor cells (progenitor cells that express the Gr1 antigen also called MDSCs) in tumor progression and metastasis.
Thus, the aim of our study was to assess the number of MDSCs (CD11b+/Gr1+), T_{reg} (CD4+/CD25+/FoxP3+) as well as white blood cells panel (CD3/CD4/CD8) in dogs at various clinical stage of skin and mammary cancer (n=50) in comparison to healthy patients (n=20). Moreover, we have assessed the number of MDSCs, T_{reg}, as well as p-STAT3 and VEGF-C expression in canine skin and mammary tumour tissues (benign, malignant and metastatic; n=150).
We have shown that in dogs at the late stadium of disease (advanced tumour with present metastases) the number of circulating MDSCs was significantly higher whereas the number of circulating T_{reg} was significantly lower. The number of other lymphocytes (CD3+, CD4+ and CD4/CD8) was also significantly lower.
On the other hand, the numbers of MDSCs and T_{reg} in metastasizing tumour tissues as well as p-STAT3 and VEGF-C expression were significantly higher.
Our results indicated a significant immunosuppression in patients at advanced clinical stage of cancer disease. Moreover, our results indicated that MDSCs may play an important role in immunosuppression by influence on T_{reg} (their number in blood decreases due to their accumulation in cancer tissue where they cause local immunosuppression) and in promotion of angiogenesis due to phosphorylation of STAT3 (via VEGF-C).
This study was supported by the grant no. IP2011027171 from the Ministry of Science and Higher Education.

POSTER PRESENTATIONS

P1.01 Granulocytes

P1.01.01
A defect in granulopoiesis in perforin-deficient mice

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Perforin is a major component in granules of cytotoxic T and Natural Killer lymphocytes, which are active against tumor cells and intracellular pathogens. We previously reported dexamethasone-induced upregulation of eosinophilopoiesis in bone-marrow cultures from different mouse strains, and observed that perforin-deficient bone-marrow lacked this response to dexamethasone. To explore the possible role of perforin in granulopoiesis modulation, we evaluated baseline and glucocorticoid-stimulated counts of total nucleated cells, neutrophils and/or eosinophils in: a) freshly harvested bone-marrow; b) bone-marrow cultured with IL-5 or GM-CSF, with or without dexamethasone. Steady-state bone-marrow cell counts were significantly reduced in perforin-deficient animals compared to wild-type controls. Dexamethasone upregulated IL-5-stimulated eosinophilopoiesis in a dose-dependent-manner in wild-type, but not perforin-deficient mice. In semi-solid cultures, dexamethasone upregulated GM-CSF-stimulated colony formation in wild-type (but not in perforin-deficient) bone-marrow. Intraperitoneal (i.p.) administration of dexamethasone upregulated eosinophilopoiesis and neutrophilopoiesis in wild-type (but not in perforin-deficient) bone-marrow. Dexamethasone induced thymic involution in both mouse strains, confirming that dexamethasone absorption led to similarly active systemic levels. To reconstitute the dexamethasone response in perforin-deficient mice, wild-type T lymphocytes (and their subsets) were transferred from naïve donors into perforin-deficient recipients intravenously, and the latter were subsequently injected with dexamethasone i.p., 24h before bone-marrow harvest and total/differential counts. Total T lymphocytes from wild-type (but not from perforin-deficient) donors reconstituted the response to dexamethasone for both eosinophils and neutrophils in perforin-deficient recipients. Furthermore, neutrophilopoiesis was upregulated when CD4-depleted T lymphocytes were transferred. Together, these data suggest a novel function of perforin in baseline and glucocorticoid-stimulated granulopoiesis. **support:** CNPq-FAPERJ-CAPES.

P1.01.02
Different anti-HSP70 antibodies detect different dynamics of intracellular HSP70 content in heat shocked human neutrophils

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The dynamics of stress-induced changes of intracellular HSP70 content and the mechanisms of stress-induced HSP70 release are still poorly described. We analyzed the dynamics of HSP70 content in human neutrophils in response to heat shock. The cells were stained intracellularly by a panel of six anti-HSP70 mAbs elaborated in our laboratory. Flow cytometry analysis demonstrated a substantial dependence of registered pattern of stress-induced HSP70 dynamics on mAbs used. In particular, in the cell samples stained by antibodies recognizing C-domain of HSP70 molecule, the hyperthermia-induced dynamics was characterized by an increase of HSP70 intracellular level immediately after the treatment and by considerable decrease of the cell HSP70 content in 15-30 min after heat shock followed by growth of intracellular level of HSP70 for next several hours. In contrast, we did not observe the initial phase with intracellular HSP70 level growth when we used for the protein staining the antibodies recognizing N-domain of HSP70. We also did not detect this stage with HSP70 increase by using Western blot analysis. We suggest that the registered distinction between patterns of dynamics obtained with different mAbs can reflect the involvement of HSP70 in the processes of hyperthermia-induced intracellular protein aggregation. Registered in our experiments the decrease of intracellular HSP70 content can be connected with HSP70 extracellular release. The short-term decrease of intracellular level of HSP70 at the initial phase of cell response to stress was confirmed by Western blotting normalized to β-actin.
This work was supported by RFBR, grants # 12-04-31578 and # 12-04-32065

P1.01.03
Role of CD63, CD11b and CD14 as potential markers of granule release induced by propionate in bovine neutrophils

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Short-chain fatty acids (SCFA) are produced in the rumen of cattle by bacteria, being the major energy source in ruminants. Propionate is one of the main SCFA and additional to the nutritional function can induce several neutrophil functions via calcium (Ca2+) release, such as reactive oxygen species production and intracellular pH changes. Recently, we demonstrated that propionate induces granule release. In the present work we assess the role of putative granule markers such as CD63, CD11b and CD14 on azurophil, gelatinase granules release and secretory vesicles.
Neutrophils isolated from blood samples of healthy heifers were used. Degranulation induced by propionate was recorded through the release of the neutrophil enzymes myeloperoxidase (MPO) by absorbance, lactoferrin by ELISA, matrix metalloprotease-9 (MMP-9) by zymography and alkaline phosphatase by absorbance. The changes of CD63, CD11b and CD14 expression induced by propionate were analyzed by flow cytometry. The stimulation of neutrophils with 0.3 mM propionate induced the release of lactoferrin and MMP-9 as revealed by ELISA and gelatin zymography, respectively. Propionate at 30 mM induced the release of MPO and alkaline phosphatase as demonstrated using an enzymatic assay. 30 mM of propionate induced an increase in forward scatter parameter and increased CD63, CD11b and CD14 expression. We proposed that CD63, CD11b and CD14 are associated with the release of azurophil and secretory vesicles in bovine neutrophils.
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P1.01.04
Photoconversion and in vivo imaging reveal a role for neutrophils in regulating adaptive immune responses

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Rapid cellular communication between the site of injury and the secondary lymphoid organs, where adaptive immune responses are initiated, is essential for successful defense against pathogens. We used photoconversion to identify the cells that first leave the site of bacterial infection in the skin and migrate to secondary lymphoid organs to initiate immune responses. Unexpectedly we found that at the early stages of the response neutrophils were the main immune cells to egress from the site of inflammation.
Analysis of the phenotype and function of skin-egressing neutrophils in the draining lymph nodes showed that these neutrophils are activated and primed for interacting with other immune cells. Surprisingly however, neutrophils suppressed rather than activated adaptive immune cells.
Lastly we used two-photon microscopy in live mice to analyze the mechanisms of neutrophil migration from the skin to the lymph nodes and found that this migration most likely occurs via the lymphatic vessels.
In conclusion our data highlight a role for neutrophils in orchestrating early immune responses. These findings have potential implications in management of many inflammatory conditions.

P1.01.05
Different role of nitric oxide on adhesiveness and apoptosis of inflammatory granulocytes after burn injury

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Nitric oxide (NO) is an important mediator of inflammatory reaction. Our previous studies showed increased production of NO at the wound site after burn injury. At the same time we found decreased adhesiveness and apoptosis rate of inflammatory granulocytes (Gr) ex vivo and in cultures. The aim of this study was to investigate the influence of locally originated NO on adhesion and apoptosis of inflammatory Gr at the wound site after burn injury. Inbred Albino Oxford rats were subjected to non-lethal, full-thickness burn injury. Immediately after injuring, sterile polyvinyl sponges were implanted subcutaneously on the borderline of the burned skin in order to obtain inflammatory Gr. Control group were animals implanted with sterile sponges only. In vivo application of aminoguanidin (AG), a specific inhibitor of inducible nitric oxide synthase (iNOS), resulted in increased apoptosis rate of inflammatory Gr from control animals, while this treatment was ineffective in burn injured animals. In regard to Gr adhesiveness, in vivo AG treatment had an opposite effect manifested by stimulation of inflammatory Gr adhesion from burn injured rats, while adhesiveness of Gr from control animals was unchanged. Cumulatively, these results indicate that locally produced NO contribute to the inhibition of Gr adhesiveness without significant influence on apoptosis rate of these cells at the wound site after burn injury.

P1.01.06
Glucocorticoid-induced leucine zipper (GILZ) promotes apoptosis in vitro in neutrophil-like cells

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Glucocorticoid-induced leucine zipper (GILZ) is an ubiquitous potent anti-inflammatory protein whose expression is induced by glucocorticoids, TGF-β or IL-10 in haematopoietic cells. We and others have evidenced that GILZ regulates inflammation -involved signal transduction pathways, such as AP-1, NF-kappaB or FOXO. Moreover, we recently detected GILZ expression in blood neutrophils of patients suffering from acute respiratory distress syndrome (ARDS).
The objective of this study was to evaluate the role of GILZ in human neutrophils apoptosis. For this purpose, we used neutrophil-like PLB-985 differentiated cells. We generated GILZ overexpressing cells stably transfected with the human gilz gene. These cells were then differentiated in neutrophil-like cells using all trans-retinoic acid (ATRA) and dimethylformamid (DMF). We measured CD11b expression, oxidative burst, phagocytosis capacity and verified the presence of multi-lobed nuclei. Differentiated PLB-985 cells were then characterized for their spontaneous and activation-induced apoptosis using PMA through SubG1 analysis and AnnexinV/7AAD staining. We found that GILZ overexpression led to an exacerbated apoptosis, characterized by caspase 3, 9 and 8 activation and loss of mitochondrial potential. These results suggested that GILZ-induced apoptosis involved the mitochondrial pathway. Interestingly, apoptosis of PLB-985 GILZ clones was associated with a downregulation of Mcl-1, whereas BID or BIM were not involved.
This model suggests that in neutrophils, GILZ could participate in the control of apoptosis, essential to the resolution of inflammation in vivo. Further investigations of GILZ functions in neutrophils are ongoing both in vitro and ex vivo during ARDS.

P1.01.07
In vivo induction of Neutrophil Extracellular Traps by Mycobacterium tuberculosis

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Background.- A novel mechanism of cellular death, NETosis, was described in neutrophils, it is characterized by the expulsion of chromatin nets admixed with microbicidal granular proteins in which microorganisms, including bacteria, protozoa and fungi, get trapped and killed in vitro. A variety of microorganisms, are able to induce NETs in vitro. Neutrophils infected in vitro with M. tuberculosis H37Rv (Mtb) also produce NETs.
Aim of the study.- To investigate if Mtb is also able to induce NETs in vivo. Materials and methods.- Guinea pigs were intradermally inoculated with Mtb and the production of NETs was investigated. NETs were looked for in cryostat sections of the skin stained for DNA, histones, elastase, myeloperoxidase and acid-fast bacilli. Heat-killed Mtb (HK-Mtb), as well as M. lepraemurium and BCG were also investigated as possible NET-inducers.
Results. - Mtb resulted in an excellent NET-inducer; NETs were identified from 30 min after inoculation and reached maximal development 4 to 6 h thereafter. NETs were positive for DNA, histone, elastase, and myeloperoxidase, just as it has been described for NETs in vitro and they were linked to ROIs as deduced from the in situ reduction of NBT. Viable and HK-Mtb, M.lepraemurium and BCG, were all equally efficient as NET-inducers. Conclusion. - NETs production does not seem to be a particular response to infection with certain microorganisms; it is rather a general response of neutrophils to infection and occurs at the latest stages of phagocytosis; nevertheless NETs may have some beneficial effect in the control of tuberculosis.

P1.01.08
Activation of phagocytosis by a low molecular fraction (below 5 kDa) from cord blood in leukocytes after hypothermic storage or cryopreservation

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It is known that after hypothermic storage (+4^{o}C) and cryopreservation (-196^{o}C) the phagocytic activity of human donor blood leukocytes and the NBT test indices significantly decrease, which is primarily attributed to disturbances in cell energy metabolism. This study indicated that the cord blood low molecular fraction (CBF) in the concentration of 0.15 mg/ml stimulated the engulfing and digesting activities of phagocytes, while the comparator agent Actovegin had a similar effect in the concentration of 1.50 mg/ml. The investigation of neutrophil capacity for producing reactive oxygen species by NBT test showed that CBF led to a significant rise in the number of NBT-positive cells both after hypothermic storage and after cryopreservation. Actovegin significantly increased this index after cryopreservation of leukocytes, but did not affect the number of NBT-positive cells after hypothermia. CBF and Actovegin were proved to promote restitution of the functional activity of neutrophils in the presence of a glycolysis inhibitor - sodium iodoacetate (1mM). It was discovered that neither CBF nor Actovegin influenced the phagocytic activity of neutrophils during co-incubation with a microtubule polymerization inhibitor -colchicine (100 μM). There is evidence on CBF-induced alkaline phosphatase activation in native and frozen-thawed leukocytes. CBF was found to stimulate glucose transport from incubation medium into leukocytes, which appears to be one of the major mechanisms of its action.

P1.01.09
Chemerin aggravates DSS-induced colitis associated with defective protective innate responses

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Chemerin is characterized by a chemoattractant protein present in various inflammatory sites. Elevated circulating chemerin was recently found in patients with inflammatory bowel disease (IBD), however, the role of chemerin in IBD remains unknown. In this study, we examined a role for chemerin in the development of a mouse model of IBD induced by DSS. We found that administration of exogenous chemerin aggravated the severity of DSS-induced colitis, which was characterized by higher clinical disease scores, extensive mucosal damage and significantly increased expression of pro-inflammatory cytokines in the colons. The exacerbated colitis was associated with impaired early colonic infiltration of neutrophils and decreased colonic expression of IL-4 and M2 macrophage-associated genes. In vitro study further demonstrated that chemerin directly inhibited IL-4-induced M2 macrophage polarization. Significant up-regulation of chemerin levels were found in the colons from DSS-exposed mice and UC patients. Moreover, the chemerin levels appeared to correlate significantly with UC disease severity. Our results suggest that chemerin promotes DSS-induced colitis by impairing the protective innate responses and may contribute to the pathogenesis of IBD.

P1.01.10
Neutrophils as potential biomarkers in the differentiation of multiple sclerosis and neuromyelitis optica

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Neutrophils are the most abundant leukocytes in human peripheral blood, and provide a first line of defence against bacterial and fungal pathogens. They are known to play a critical role in acute inflammation, but their involvement in chronic inflammatory and autoimmune diseases is just about to emerge. The presence of neutrophils is a characteristic feature of lesions in neuromyelitis optica (NMO), but not multiple sclerosis (MS), although both conditions are chronic inflammatory demyelinating diseases of the central nervous system (CNS). In this study we aimed to examine the phenotype and functionality of neutrophils in peripheral blood of NMO and MS patients in order to examine the potential for these immune cells to be used in the differentiation and diagnosis of NMO and MS. Preliminary results indicate that neutrophils in both NMO and MS patients show a more primed phenotype compared to healthy controls (HC), based on a higher expression of the surface markers TLR2, CXCR1 and the fMLP receptor. Additionally, MS and NMO patients appear to differ with respect to neutrophil adhesion and migratory capacity, as well as in effector mechanisms such as degranulation and oxidative burst: relative to HC these features are increased in MS patients, but down-regulated in NMO patients. These data highlight the importance of neutrophils as potential cellular markers to discriminate between NMO and MS. Moreover, our results suggest a neutrophilic involvement in the pathogenesis of these two inflammatory conditions.

P1.01.11
Kinase activity profiling of polymorphonuclear cells in critically ill patients

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Background:
Sepsis is a complex clinical condition that arises from multiple interacting tissues and cell-specific host response mechanisms. Kinases play an integral role in the transduction of intracellular signalling cascades and regulate a diverse array of biological processes essential to immune cells. Polymorphonuclear cells (PMNs) play an important role in host defense against infection, but also contribute to collateral tissue damage during sepsis.
Aim and Methods:
The current study explored signal transduction events in PMNs purified from peripheral blood (>90% pure) of patients with sepsis admitted to the Intensive Care Unit (ICU). Sepsis patients were compared with critically ill patients admitted to the ICU for non-infectious disease and age and gender matched healthy controls. PMN kinase activities were determined in cell lysates using kinase activity arrays enabling the analysis of 1024 consensus sequences of protein kinase substrates.
Results:
Based on the profiling data MAPK pathways (including ERK1/2) shared common activities between SIRS and sepsis patient, however these pathways did differentiate from the controls. SIRS samples exhibited more differences (e.g. in DNA damage and AKT signalling) compared to control samples than samples from sepsis patients.
Conclusion:
As expected, according to our profiling data there is a difference in kinome activity between healthy and diseased states. SIRS exhibited more differences in kinome profile compared to control than sepsis. Both profiles exhibited inflammatory and stress MAPK signalling.

P1.01.12
The role of neutrophils upon infection with the protozoan parasite Leishmania mexicana in C57BL/6 mice

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Neutrophils are massively and rapidly recruited to sites of Leishmania infection, and growing evidence suggests that these innate immune cells play a role in the development of the adaptive immune response. In response to infection with the protozoan parasite L. mexicana, C57BL/6 mice develop small chronic unhealing cutaneous lesions with persistent parasites. Only a weak L. mexicana-specific immune response develops, as revealed by low dendritic cell activation and poor differentiation of CD4+ Th1 cells. Following parasite inoculation, a first wave of neutrophil activity peaks at one hour, and they return 10 days later at the site of infection, as followed by live in vivo imaging. Here we investigated the early role of neutrophils upon infection with L. mexicana. To this end, we either transiently depleted neutrophils for the first days of infection with one single injection of the 1A8 mAb prior parasite inoculation, or we infected the recently generated neutropenic Genista mice. The development of an increased inflammatory lesion was observed in the neutrophil depleted mice compared to control mice, and the early

absence of neutrophils was associated with an increased CD4+ Th1 cell immune response in the draining lymph nodes, characterized by increased levels of IFNγ and TNFα. Altogether, these results demonstrate that during the first days of infection, neutrophils contribute to the immune hyporesponsiveness observed following L. mexicana infection, and indirectly contribute to the control of excessive inflammation

P1.01.13
Intercellular ROS signaling in granulocyte-mediated tumor cell destruction

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The involvement of granulocytes in the immune response against cancer is not well understood. Depending on the oxidative burst of granulocytes and cytokine milieu in which they act, granulocytes may play either an inhibitory or stimulatory role in tumor growth. Reactive oxygen species (ROS) released during oxidative burst of granulocytes can induce lipid peroxidation (LPO) resulting in the destruction of biomembranes. However, the intercellular ROS signaling in granulocyte mediated tumor cell destruction is unclear. Therefore, in the present work we have studied the relationship between plasma inflammatory mediators and oxidative burst of granulocytes in tumor growth. The involvement of LPO-derived aldehydes (i.e. 4-hydroxy-2-nonenal (HNE) and acrolein) in granulocyte-tumor response was further evaluated. Finally inhibitory studies were used to study the importance of certain ROS in redox signaling pathways on tumor cell destruction. The results obtained, revealed a significant differences in inflammatory mediators in animals with tumor regression/progression (e.g. IL-17 and TGFβ). Also we have found that coordinated action of HNE/acrolein and granulocytes stimulates intracellular tumor cell ROS formation and have effect on TLR4 expression. Moreover, inhibitory studies revealed possible main intercellular signaling pathway in granulocyte mediated tumor cell destruction. Still, further research is required to clarify the relationships between inflammation and cancer and to define new potential targets for granulocyte mediated malignant destruction.

P1.01.14
Cyclophilin A stimulates recovery of the hematopoietic and the immune system of the organism after radiotherapy

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Radiotherapy is accompanied with severe side effects: damage and death of hematopoietic cells and decrease of leukocytes in blood. Preparations, commonly used for restoration of the hemogram after such treatment, are colony stimulating factors, but their application is limited. Search of new factors, promoting neutralization of side effects after radiotherapy, is of great practical interest.
Previously in our laboratory it was shown, that Cyclophilin A (Cyp A) (a 18 kD protein) is a chemoattractant for stem cells and progenitors of different lineages, localized in bone marrow (Khromykh L., 2007). It was also shown that Cyp A induces differentiation of stem cells in granulocytes and thrombocytes (unpublished data). These findings suggest that the studied protein can promote recovery of the hematopoietic and the immune systems of the organism after radiotherapy.
We have shown that Cyp A stimulates restoration of bone marrow in mice post sublethal irradiation, promoting reconstitution of stem cells and granulocytopoiesis. Post irradiation Cyp A stimulates migration of bone marrow stem cells, committed toward granulocytes, in blood. On the third week post sublethaly irradiation Cyp A promotes full restoration of T lymphocytes in mice spleens that exceed 2-fold the percent of these cells in control irradiated animals. The level of humoral response in mice on the third week post irradiation was restored to the level of non-irradiated mice under Cyp A treatment. Thus, our data suggest that Cyp A is a factor taking part in recovery of the hematopoietic and the immune systems of the organism after radiotherapy.

P1.01.15
Effect of colchicine on expression of inflammation-related genes in neutrophils from healthy subjects and patients with familial Mediterranean fever

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Despite the long-standing use of antimitotic drug colchicine in the treatment of familial Mediterranean fever (FMF) the precise mechanisms of its action in polymorphonuclear neutrophils (PMNs) remain largely unknown. Colchicine is a microtubule depolymerizing agent which arrests microtubule assembly and inhibits many cellular functions. FMF is a hereditary autoinflammatory disorder characterized by abnormal activation of PMNs and their unprovoked influx into the affected sites. This study aimed to analyze transcriptional events elicited by colchicine in PMNs isolated from the peripheral blood of 16 FMF patients in the remission period and 11 healthy control subjects by determining mRNAs expression of 8 selected genes using quantitative real-time RT-PCR. Colchicine exposure (1μg/ml) elicited up-regulated expression of IL-8 and IL-1β genes in FMF (13-fold and 2.7-fold, $p<0.05$, respectively) and healthy (3-fold and 6.5-fold, $p<0.05$, respectively) PMNs, and at the same time in down-regulated caspase-1 in FMF PMNs (3-fold, $p<0.05$). In FMF PMNs treated with colchicine, mRNAs of IL-8 (51-fold, $p<0.01$) and c-FOS (7-fold, $p<0.05$) were elevated compared to cells from control subjects. By contrast, caspase-1 mRNA was decreased in FMF PMNs compared control cells (1.6-fold, $p<0.05$). Thus, we provide in vitro evidence of colchicine modulatory role on expression of inflammation-related genes at the transcriptional level. The up-regulation of IL-1β and IL-8 suggests that colchicine can, besides its well-known anti-inflammatory properties, exhibit pro-inflammatory features. Finally, suppressed caspase-1 expression by colchicine could represent a therapeutic target and may be implicated in effective therapy of FMF.
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P1.01.16
Cross-talk between neutrophils and Pseudomonas aeruginosa biofilm. Role of MPO-halide system products.

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Formation of biofilm is observed in a majority of chronic bacterial infections such as chronic sinusitis, periodontal diseases and chronic wounds. These infections are persistent as they show increased resistance to antibiotics and host defence system. Interestingly, it has been shown *in vitro* that interaction between biofilm and neutrophils may result either in destruction of bacterial biofilms or in facilitation of biofilm formation. Contribution of MPO-halide system products to these opposite neutrophil-biofilm interactions is not clear. Taurine chloramine (TauCl) and taurine bromamine (TauBr) are the physiological products of activated neutrophils, resulting from the reaction between taurine with hypochlorous acid (HOCl) and hypobromous acid (HOBr), respectively. It has been shown *in vitro* that taurine haloamines exert anti-microbial properties against planktonic form of various bacteria. Moreover, clinical studies have shown that both haloamines are effective in the local treatment of skin and mucosa chronic infections. Nevertheless, it has not been tested yet whether they can kill bacteria hidden in biofilm or disrupt biofilm structure. In this study we have investigated the capacity of TauCl

and TauBr to inhibit *in vitro* the formation of *P.aeruginosa* biofilm. Our results suggest that TauBr is able to inhibit *in vitro* the formation of *P.aeruginosa* biofilm but it cannot destroy the preformed biofilm and effectively kill hidden bacteria. However, TauBr anti-biofilm properties are enhanced in the presence of DNase. In contrast to beneficial effect of MPO-halide system products, the addition of dead neutrophils to preformed biofilm enhanced *P. aeruginosa* development.

P1.01.17
Role of 5-lypoxygenase in the regulation of eosinophilopoiesis

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Leukotriene D_4, a product of 5-lipoxygenase, is a potent stimulator of eosinophilopoiesis in bone-marrow cell culture with IL-5 in BALB/c naïve mice. In sensitized and challenged (S/C) BALB/c mice, a similar upregulation of eosinophilopoiesis is observed in bone-marrow cells cultured with IL-5 alone, raising the issue of 5-LO contribution to this upregulation. Diethylcarbamazine is known as an effective inhibitor of leukotriene synthesis, although its mechanism of action is unclear. We showed that diethylcarbamazine suppresses eosinophilopoiesis in S/C animals, both *in vivo* and *in vitro*. We here evaluated the effect of leukotriene synthesis blockade on hematopoiesis in allergic mice, and compared it with diethylcarbamazine effects by analyzing: a) 5-lipoxygenase-deficient mice (ALOX) and wild-type controls (PAS) S/C; b) BALB/c mice S/C pretreated with MK886 (5-lipoxygenase activating protein inhibitor) and controls; c) ALOX and PAS mice S/C pretreated with diethylcarbamazine. We analyzed eosinophil numbers in bone-marrow (freshly collected or cultured with IL-5) and in peritoneal fluid of these mice. Blockade of 5-LO in OVA-challenged BALB/c mice by MK886: a) abolishes the challenge-induced increase in bone-marrow eosinophil numbers; b) inhibits challenge-induced eosinophil migration to the peritoneal cavity. In OVA-challenge ALOX mice: a) challenge-induced eosinophil migration into the peritoneal cavity is reduced; b) the challenge-induced increase in bone-marrow eosinophil numbers is abolished. Furthermore, diethylcarbamazine treatment suppressed eosinophilopoiesis in OVA-challenged PAS mice, but not in ALOX mice. These results suggest an important role for 5-LO in challenge-induces hematological changes and in the mechanism of action of diethylcarbamazine on the bone-marrow.
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P1.01.18
The p38-MSK1 signaling cascade influences cytokine production through bZIP transcription factors in human neutrophils

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Neutrophils influence innate and adaptative immunity by generating numerous cytokines and chemokines whose regulation depends on transcription factors NF-κB and C/EBP. Here, we describe the critical involvement of CREB factors (CREB1, ATF-1) in this functional response, as well as the upstream signaling components. Neutrophil stimulation with physiological agonists (LPS, TNF) led to a rapid increase in the phosphorylation, DNA binding activity, and chemokine promoter association of CREB1 and ATF-1. These responses occurred downstream of the TAK1-p38-MSK1 signaling axis, as did the phosphorylation and promoter association of another bZIP factor, C/EBPβ. Accordingly, inhibition of TAK1, p38 MAPK, or MSK1 interfered with cytokine generation in neutrophils. Likewise, overexpression of a dominant negative CREB1 mutant (K-CREB) or of a point mutant (S133A) resulted in a decreased ability of human neutrophil-like PLB-985 cells to generate inflammatory cytokines (CXCL8, CCL3, CCL4, TNFα). These studies unveil several potential molecular targets that could be exploited in the context of several chronic inflammatory diseases that prominently feature neutrophils and their products.

P1.01.19
Down-modulation of activated human neutrophil by LMW-fucoidan: role of microparticles

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Background
During migration, neutrophils (PMN) interact with several mediators, which can lead to their activation, interfering with cell survival and inflammation resolution. Fucoidans are sulfated polysaccharides which are able to inhibit selectin-mediated events, inhibiting PMN rolling, a crucial step to inflammation resolution. A low-molecular-weight-Fucoidan (LMW-Fuc) fraction extracted from the brown algae *Ascophyllum nodosum* exhibits potent antithrombotic and proangiogenic properties, although its effects on inflammatory cells are still unknown. So we aimed to evaluate LMW-Fuc effect on activated PMN.
Results
LMW-Fuc inhibited PMN migration induced by LPS, fMLP or migration of PMN primed with LPS and further challenged to fMLP. Corroborating this data, in LPS/fMLP-activated PMN, LMW-Fuc attenuated the induced alterations on actin cytoskeleton dynamics and inhibited AKT phosphorylation. We also observed that LMW-Fuc was able to accelerate apoptosis of activated PMN and, corroborating this result, LMW-Fuc prevented Bad degradation (an AKT target) induced by LPS/fMLP treatment. Then we showed that LMW-Fuc was able to inhibit extracellular, but not the intracellular ROS production induced by LPS/fMLP treatment. Furthermore, in stimulated PMN LMW-Fuc inhibited microparticle (MP) release, which are the key actors of extracellular ROS production in these cells, mainly, as we observed, by its property to carry p47. Finally, we demonstrated that PMN MP was also able to induce extracellular ROS in macrophage, also in a p47-dependent mechanism, demonstrating a paracrine role for MP.
Conclusion
Thus LMW-Fuc presents a potent ability to attenuate PMN activation that might be potentiated by its ability inhibiting MP release.
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P1.01.20
IL-4 derived from basophil play a pivotal role in cysteine protease-induced natural helper cell-mediated lung inflammation

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Cysteine proteases including plant derived papain and mite derived protease, Derp1 are known to be a potential trigger for allergic inflammation by barrier disruption leading to elevation of IL-33 mediated TH2 cytokine circuit in the absence of acquired immune cells. IL-33 released from the disrupted lung epithelial cells induced IL-5 and IL-13 production from lung natural helper (LNH) cells, resulting in eosinophilic airway inflammation. However, in present study, we found that papain stimulated basophils has a crucial role in the LNH cell mediated eosinophilic inflammation as a source of TH2 cytokine that controls activation and expansion of LNH cells. Conditional deletion of basophils in Bas-TRECK system, caused a resolution of the papain-induced eosinophilia and mucus formation in lungs. Similar recovery from eosinophilic airway inflammation was found in the mice lacking the IL-4 production specifically in basophils, 3'UTR KO mice, indicating basophil derived IL-4 had a critical role to induce LNH function. IL-4 not only enhanced the expression of eotaxin, IL-9 and IL-13 in LNH cells which is required for eosinophil attraction, but also extended a cell growth. These results demonstrated that papain-induced IL-4 production from basophils play a pivotal role to control the expression of eotaxin, IL-9 and IL-13 in LNH cells, subsequently leading to eosinophil mediated airway inflammation in lungs.

P1.01.22
The Dectin-1 ligand Beta-glucan triggers neutrophil extracellular trap (NET) formation via a signaling pathway requiring Src-family kinases and Syk

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Neutrophil extracellular traps (NETs) are formed by fibers of chromatin and granule constituents that are released upon neutrophil stimulation. Strong evidence supports a role for NETs in host defenses against different pathogens. However, a role for NETs in inflammation-based pathologies has been also reported. We investigated formation of NETs in response to beta-glucan, a yeast surface component recognized by the C-type lectin receptor Dectin-1. We found that beta-glucan was as potent as phorbol myristate acetate (PMA) in stimulating NET formation in human neutrophils. PP2, a Src-family kinase (SFK) selective inhibitor, inhibited NET formation in response to beta-glucan, but not PMA. Consistently with the view that reactive oxygen species (ROS) play an essential role in NET formation, PP2 suppressed ROS formation in response to beta-glucan, but not PMA. Additionally, a new generation Syk inhibitor PRT-060318 markedly inhibited NET formation and ROS generation in response to beta-glucan in human neutrophils. Neutrophils from mice with the genetic deficiency of the SFK members Hck and Fgr or Hck, Fgr and Lyn formed NETs and generated ROS in response to beta-glucan. However both the SFK inhibitor PP2 and the Syk inhibitor, PRT-060318, inhibited NET and ROS formation in SFK-deficient neutrophils. This is the first evidence that the beta-glucan receptor Dectin-1 triggers NET formation. The evidence that targeting SFK and Syk inhibits NET formation highlights a possible new strategy to control NET formation in inflammation-based and autoimmune diseases.

P1.01.23
Effects of proton pump inhibitors and H2-antagonists on neutrophil oxidative metabolism in patients with gastric and duodenal ulcer bleeding

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Background. Despite the progress in pharmacotherapy and endoscopic management of bleeding 20-30% of patients demonstrate the recurrent bleeding. One of the reasons of it may be inflammation propagation related with dysfunction of neutrophils (Nph).
The goal of this research was to estimate the effects of the most common antisecretory drugs as proton pumps inhibitors (PPI) and H2 antagonists (H2A) on Nph oxidative metabolism.
Material and methods: Characteristics of bleeding were defined in 124 patients by clinical, laboratory and endoscopic criteria according to Forrest class. In total, 63 patients were treated with PPI, and 61 with H2A. To assess Nph oxidative metabolism, superoxide production was measured using the nitroblue tetrazolium reduction test (NBT) in isolated neutrophils at 1st, 3d and 7th day of therapy.
Results. NBT reduction rate was significantly higher in patients with active bleeding (F1a) than that in patients with sustained haemostasis ($p<0.05$). It has been shown that intensity of superoxide radicals' production by Nph was higher in patients who were admitted at 12-24 hours than at 3-6 hours after first clinical manifestation of hemorrhage ($p<0.05$). Administration of PPI was associated with decrease the NBT reduction rate ($p<0.01$), whereas H2A administration was associated with stimulation of NADPH-oxidase activity ($p<0.05$) in neutrophils.
Conclusion. Administration of PPI and H2A not only affect gastric acid production but also change innate immunity mechanisms since NADPH-oxidase activity of Nph is related with pagocytosis and lifespan. So antisecretory drugs can modulate the acute inflammatory reaction by cells kinetics in ulcer region.

P1.01.24
IgE-armed basophils and their skin-infiltration are essential for acquired tick resistance

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Ticks transmit various pathogens to humans and animals, causing serious infectious diseases such as Lyme disease. Many animal species manifest resistance against tick feeding in re-infestation, and this resistance contributes to the prevention of pathogen transmission to the host. We previously demonstrated that basophils infiltrate the tick-feeding sites during the 2nd infestation, and that basophil depletion abolishes the acquired tick resistance. Adoptive transfer experiments revealed the importance of Ig-Fc receptors on basophils for the acquired protective immunity against tick-feeding. However, it remains elusive which class of antibodies is involved in the protection. Both FcγRIII and FcεRIα are highly expressed on basophils. We found that mice deficient for FcεRIα but not FcγRIII failed to manifest the acquired tick resistance, indicating the pivotal role of IgE but not IgG. Intriguingly, adoptive transfer of serum isolated previously-infestation mice, failed to confer the resistance naïve wild type mice, suggesting that anti-tick IgE alone is not sufficient for the manifestation of acquired tick resistance. We found little or no infiltration of basophil to tick feeding sites of recipient mice, and the pretreatment of naïve mice with both immunized serum and IL-3 lead to the acquisition of tick resistance, concomitantly with the basophil infiltrate the tick-feeding sites. These findings suggest that IgE-armed basophils and their skin-infiltration are essential for acquired tick resistance.

P1.01.25
IL-17A differential effects in murine bone marrow granulopoiesis in presence of GM-CSF or IL-5 in vitro

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Interleukin (IL)-17A plays diverse roles in autoimmunity and allergy. Its best known functions are stimulatory and selective for the neutrophil lineage. However, IL-17A effects on granulopoiesis remain in completely understood. We examined IL-17A effects on eosinophil and neutrophil production from cultured murine bone-marrow (BM). In IL-5-stimulated liquid cultures, IL-17A suppressed eosinophilopoiesis. by contrast, IL-17A enhanced GM-CSF-stimulated eosinophilopoiesis. Downregulation of IL-5-stimulated eosinophilopoiesis by IL-17A was prevented by addition of zVAD-fmk (a terminal caspase inhibitor) and by aminoguanidine (a selective iNOS inhibitor). In cultured BM from iNOS- or IL-17AR-deficient mice, suppression of IL-5-stimulation eosinophilopoiesis was undetectable, contrasting with wild type controls. In wild-type BM (C57BL/6 or BALB/c), synergism was detectable when IL-17A and sodium nitroprusside (a NO donor) were present together, whereas either agent, added separately to the culture, had no effect on BM from both iNOS-deficient and wild-type donors. By contrast, the upregulation of eosinophilopoiesis in wild-type GM-CSF-stimulated BM cultures by IL-17A was iNOS-independent. In progenitor (clonal) semi-solid cultures seeded with GM-CSF, colony formation was decreased by 2 ng/ml (but not by 0.2 ng/ml) IL-17 A. Together, these results suggest divergent mechanisms of action for IL-17A as a regulator of BM granulopoiesis, depending on the cytokine environment, which may reflect the involvement of different subsets of target cells in hemopoietic tissue, which are broader for GM-CSF (multilineage stimulus) than for IL-5 (eosinophil-selective stimulus), at different steps. Funding-CNPq-FAPERJ-FIOCRUZ

P1.01.26
Effect of testosterone on the activity of inflammatory cells

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Immunomodulation by sex hormones have both physiological and pathological implications in several processes. However, the role of androgens in controlling the inflammatory response is just begun to be dissected. Our initial goal was to analyze the influence of testosterone on prostatic inflammation. Wistar rats were castrated and treated with vehicle(C) or testosterone at 0.75mg/rat/day(C+T) or 2.5mg/rat/day(C+TT) and then infected with 106CFU E. coli. At 2d postinfection, C+TT exhibited greater neutrophil invasion and lower levels of TLR4 and β–defensin1 in the prostate compared to C (ANOVAp<0.01) by western blot. An intense E.coli immunolabeling occurred, with numerous bacteria undergoing partial digestion by neutrophils, demonstrated by electron microscopy. All these changes were remarkable at 5th day in C+TT and C+T correlating with a greater bacterial growth (p<0.01vs.C). Conversely, C group exhibited a lower amount of neutrophils.
The effects of castration(C) on the recruitment and ex vivo activation of peritoneal neutrophils were subsequently analyzed. The amount of neutrophils, 4h post-injection of thioglycollate, and the production of ROS were lower in C(p<0,05vs.C+T), determined by cytometry and fluorescence of H2DCFDA after 20nM PMA activation. Coincidentally, the coincubation of peritoneal neutrophils with E.coli (1:1) resulted in a lower production of ROS by C (p<0.01). However, this group showed a better bactericidal activity after 30min(p<0.01vs.C+T). In agreement, the antiandrogen Flutamide reduced the synthesis of IL-10 by peritoneal granulocytes in response to LPS.
These results indicate a complex regulation of the early stages of the infectious/inflammatory process by androgens suggesting a better outcome in the absence of testosterone.

P1.01.27
Reveiling the death pathway leading to eosinophil cytolysis

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Eosinophil cytolysis could be considered as a mode of degranulation, leading to the release of intact granules, so-called clusters of free eosinophil granules (cfegs). It has been referred to as “state of utmost activation”, however, leads to eosinophil death. The appearance of cfegs has been demonstrated in eosinophilic tissues in different diseases.
The mechanisms underlying this activation-induced form of cell death in eosinophils remain unclear. Here, we propose a cascade of events, involving adhesion, granule fusion processes, reactive oxygen species (ROS) production, and earlier degranulation steps, leading to a distinct morphology characterized by cytosolic vacuolization. This cascade of events involves amplification steps and leads to cell death, which is characterized by loss of granule, vacuole, plasma, and nuclear membrane integrity. On a molecular level, we demonstrate a signalling cascade, involving the β2-integrin Mac1, phosphatidylinositol 3-kinases (PI3K), p38 mitogen-activated kinase (MAPK), receptor-interacting serine/threonine-protein kinase 1 (RIPK1). Major effectors in the progression of cytosolic vacuolization to cytolysis are furthermore ROS, which are mitochondrial as well as NADPH oxidase derived. Taken together, we report an adhesion-triggered programmed cell death pathway in eosinophils associated with the release of cfegs that represents at least one signalling cascade leadingto the well known morphological phenomenon of eosinophil cytolysis.

P1.01.28
Basophils control T cell responses and limit disease activity in Experimental Murine Colitis

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Basophilic granulocytes have been recognized as important inducers of T helper cell 2 (Th2) responses. Using the colitis model of adoptive transfer of CD4+ CD62L+ T cells into lymphopenic hosts we analyzed how basophils regulate T helper cell responses and modulate disease activity. Transferred T helper cells rapidly proliferate, produce large amounts of interleukin 3 (IL-3) and expand the number of basophils in an IL-3 dependent manner. Depletion of basophils with two different antibodies substantially upregulated intracellular Th1 cytokine expression in transferred T cells at day 8. This increased Th1 cytokine expression persisted until the end of the experiment. Basophil depleted mice showed exacerbation of colitis with more severe loss of weight, stronger histological damage and colonic leukocyte infiltration combined with an increased production of proinflammatory cytokines. We show that basophil derived IL-4 and IL-6 downregulates prdouction of interferon gamma, IL-2 and tumor necrosis factor alpha in T helper cells. These data show a beneficial role of basophils in a T cell driven model of autoimmunity.

P1.01.29
Sympathoadrenergic modulation of human neutrophils

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Background: Catecholamines (CA) are mediators of the central nervous system and of the sympathetic branch of the autonomic nervous system involved in the genesis of the stress response. The chronic stress seems to have a negative effect on the cardiovascular system. The cells of the immune system (IS) express adrenergic receptors (AR) and are sensitive to stimulation with adrenergic agents. Among the cells of IS involved in cardiovascular diseases, the PMNs has a key role. We will investigate the presence in PMNs of a catecholaminergic system and the role played by CA to influence the functions involved in cardiovascular diseases.
Materials and Methods: PMNs were obtained from healthy subjects and ARs expression were assayed by real-time PCR. ROS levels were detected by spectrofluorimeter and the β_2 integrin expression by flow cytometry. The migration was evaluated by Boyden chamber and CA by HPLC.
Results: PMNs expressed detectable levels of mRNA for tyrosine hydroxylase as well as for several subtypes of ARs. Stimulation with fMLP increased α_{1A}-, α_{2A}-, β_1-, β_2- and β_3-ARs. Adrenaline alone was unable to affect ROS, migration and β_2 expression, while coincubation with fMLP revert the fMLP- induced increase of these parameters.
Conclusions: Adrenaline seems to modify some functions of activated PMNs, suggesting that the sympathetic branch of ANS is able to influence PMN functions.

P1.01.30
Role of neutrophils in neovessel formation in atherosclerotic lesions: preliminary evidence in patients with carotid plaque

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Background: Atherosclerosis is a chronic inflammatory process of the arterial wall. Angiogenesis plays a pivotal role in the development of atherosclerotic plaque instability. Polymorphonuclear cells (PMN) are present in atherosclerotic lesions and recent evidence suggests their involvement in plaque formation and destabilization.

Materials and Methods: PMN from venous blood of healthy subjects (HS) and patients with carotid plaque (Pt) were isolated by density-gradient centrifugation, while PMN from plaque were obtained by immunomagnetic sorting. Phenotypic assay of PMN is performed by means of real time PCR and ELISA.
In order to perform the neovessel formation assays we use human umbilical vein endothelial cells (HUVEC).
Results: 30 Pt and 10 HS were enrolled. PMN in carotid plaques were preferentially located in close proximity to neovessels. No differences was observed in IL-8 and elastase mRNA expression between HS and Pt, while VEGF mRNA levels was higher in Pt with respect to HS. No differences was measured in elastase production from PMN both in HS and in Pt; while for the VEGF production, we observed a major production in HS with respect to Pt . Preliminary data about neovessel formation assay show an effect of PMN-activated supernatant to stimulate capability of HUVEC cells to form neovessel.
Conclusions: Clarifying the role of PMN in the development and progression of the atherosclerotic plaque will likely provide novel insights into the cellular and molecular mechanisms contributing to the pathogenesis of ATH.

P1.01.31
Blockage of histamine receptor 2 ameliorates the progression of sepsis in NOD diabetic mice

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Infections are a common and serious complication of diabetes and a well-recognized cause of mortality of diabetic individuals. Neutrophils act as first-line-of-defense cells and a significantly lower chemotaxis has been found in PMNs of diabetic patients. The failure of neutrophil migration to the infection sites is associated with poor outcome in sepsis. Some studies have shown that mast cells (MCs) play a deleterious role in severe sepsis by inducing systemic release of pro-inflammatory mediator, contributing to the impairment of neutrophil migration and ultimately mortality. In this study, we examined whether blockage of histamine receptor 2 (H2R) interfere with the progression of sepsis induced by cecal ligation and puncture (CLP) in NOD diabetic mice. Diabetic mice undergoing mild sepsis (MS) exhibited 100% mortality within 24 hours, whereas around 40% of the treated diabetic mice survived upon 7 days. Correlated with decrease the mortality rate, H2R blockade also prevented the failure of neutrophil migration and systemic bacterial dissemination after MS. Furthermore, organ damage markers are elevated in diabetic mice serum and treatment normalizes this levels. These results suggest that histamine released by MCs plays a detrimental role in sepsis control during diabetes and that H2R blockade could be a novel potential therapeutic option to reduce lethality from sepsis in diabetic host.

P1.01.32
Entry of Staphylococcal Superantigen-Like Protein 11 into neutrophils attenuating their recruitment

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Immune evasion is an important part of *Staphylococcus aureus* pathology. Normally the host eradicates the bacteria through innate defences primarily mediated by neutrophil phagocytosis. However, *S. aureus* evades these important immune defences by inhibiting components involved in chemotaxis, opsonisation, phagocytosis and bacterial damage. One family of 14 virulence factors, known as the Staphylococcal Superantigen-Like (SSL) proteins, are involved in innate immune evasion. The conserved exotoxin SSL11 targets cells by binding to cell surface glycoproteins expressing trisaccharide sialyllactosamine and is rapidly internalised. As sialylation is an important glycosylation for many crucial immune molecules, it is likely that SSL11 is essential in aiding *S. aureus* immune evasion mechanisms. For this reason, the interaction of SSL11 with myeloid cells is being investigated. Using microscopic techniques, it has been identified that SSL11 disrupts the motility of neutrophils to opsonised bacteria but does not inhibit the ability of the neutrophil to phagocytose them. Internalisation of SSL11 into the cell is active and dependent on clathrin receptor-mediated endocytosis. SSL11 also causes potent neutrophil aggregation as a consequence of SSL11 dimerisation. This is likely to be central in aiding the survival of the bacterium by preventing neutrophil recruitment.

P1.01.33
Effect of exercise on cytokine production by neutrophils

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Purpose/Objective. It is known that neutrophils are important source of cytokines. Neutrophils take direct part in reaction of whole body on physical activity. We aimed to study a cytokine production by neutrophils in sportsmen.
Material and Methods. The capacity of neutrophils to produce cytokines IL-1b, IL-1RA,IL-6, IL-8, IL-10, TNF-α, MIF-1β and VEGF A was studied in 20 sportsmen with low level of training loads, and 22 highly qualified sportsmen of various specialization (12 bicyclists and 10 runners) with high level of training loads on different stages of a year training cycle. The untrained volunteers served as the control. ELISAs were used to measure cytokines.
Results. Neutrophils of sportsmen with low level of training loads produced the increased amount IL-1β, IL-1RA, IL-6, IL-8. Cytokine-secreted activity of neutrophils of highly qualified sportsmen did not differ from control, except for IL-8, but depended from specialisation and intensity training loads at various stages of a year training cycle. Spontaneous secretion VEGF A by neutrophils of bicyclists in the competitive season has been increased ($p<0,05$). It is found positive correlation of spontaneous production IL-1RA and IL-8 by neutrophils with cortisol in control ($r=0,59$ and $r=0,82$, respectively). Regular exercises reduce dependence of cytokine secretion by neutrophils from stressor factors.
Conclusion. Collectively, our data show different effects of exercise on capacity of neutrophils to produce cytokines. These differences, probably, reflect various degree of manifestation of adaptive processes in sportsmen.
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P1.01.34
Role of nanoparticles in apoptosis and cytoskeleton rearrangement in human leukemia eosinophil like AML 14.3D10 cells

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Although inflammation is a toxic effect frequently reported following nanoparticle (NP) exposure, the direct interaction of NPs with human granulocytes, key players cells in inflammation, is not well documented. Recently, we demonstrated that several NPs, including TiO_2, ZnO, CeO_2, AgNPs of 20 nm ($AgNP_{20}$) and of 70 nm ($AgNP_{70}$) can alter the biology of human neutrophils. In the present study, we decided to determine whether or not these NPs can possess potential cytotoxic effects and alter the biology of eosinophils using the human leukemia eosinophil like AML 14.3D10 cell line. Low cytotoxicity was associated with the tested NPs as assessed by trypan blue exclusion and LDH liberation assays at 100 µg/ml. We also show that NP induce apoptosis at different potencies as determined by cytology, by flow cytometry using FITC-annexin-V and by DNA degradation. In addition, we show that NPs induce the processing of caspases-3, -4 and -9, as well as the degradation of some cytoskeletal proteins. Finally, using an ELIS-Array assay, we report here that NPs induce cytokine and chemokine secretion in AML 14.3D10 cells. Taken together, our results indicate that human eosinophils are important target to NPs that can alter their cell physiology, including, at least, apoptosis, caspase processing, degradation of cytoskeletal proteins, and cytokine/chemokine production.

P1.01.35
Orai1-mediated Ca2+ influx independent of SOCE is essential for LPS and C5a-induced neutrophil migration in acute inflammation

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The pattern of Ca^{2+} mobilization serving neutrophil migration is distinct in case of different chemoattractants. Migration, elicited by chemoattractants such as C5a and fMLP, depends on extracellular Ca^{2+}. However, this is not the case with MIP2-elicited migration. In vitro, Orai1 is required for C5a and fMLP-elicited migration but not for the Ca^{2+} influx independent MIP2-elicited migration. Elevation of the cytosolic levels of calcium post stimulation with C5a, which is due to Ca^{2+} influx, was substantially reduced in Orai1 deficient neutrophils. These data suggest the involvement of Orai1 in Ca^{2+} influx, serving neutrophil chemotaxis. These findings were recapitulated in vivo, in *Orai1*$^{-/-}$ bone marrow chimeric mice, in the context of agonist-induced peritoneal recruitment of neutrophils. Moreover, in case of LPS-induced peritonitis, where neutrophil recruitment largely depends on C5a, reduced numbers of neutrophils were thrived from the peritoneal cavity of *Orai1*$^{-/-}$ bone marrow chimeric mice as compared to wild type transplanted mice. Dispelling the strongly entrenched perception of Orai1 as a store-operated Ca^{2+} entry (SOCE) mediator, we provide several lines of evidence demonstrating that Orai1 requirement comes in a non-SOCE manner. We show that Orai1 is not involved in neutrophil SOCE. Although STIM1 is the master mediator of neutrophil SOCE, STIM1 deficient neutrophils exhibited efficient migration in vitro and in vivo. Finally, application of YM58483, with a regiment abrogating SOCE, was of no consequence in neutrophil migration.

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P1.01.36
Soluble molecules from Trypanosoma cruzi are able to induce NETosis from human neutrophils in the absence of classical apoptosis and necrosis signals

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NETs (Neutrophil Extracellular Traps) have been described as a new mechanism of the microbicide neutrophils to quickly capture and kill many pathogens, including bacteria, fungi and parasites. NETs are formed during active cell death, named NETosis. New data indicate that this novel mechanism of cell death differs from previously known types of cell death, including apoptosis and necrosis. Here, our aim was to investigate if NETosis process induced by soluble molecules from Trypanosoma cryzi (T.cruzi) parasite occurs in the absence of apoptosis and necrosis signals. In order to answer our question, human neutrophils were isolated from blood by using sedimentation gelatin method and co-cultivated with different doses of soluble extract from T.cruzi parasite. The end of incubation we evaluated the cellular permeability, phosphatidylserine externalization and DNA degradation by flow cytometry. Images acquired and analyzed have been demonstrated that soluble molecules from T.cruzi induced NETosis on human neutrophils in dose-dependent manner. This release was similar to positive control (fMLP 10-6M) and opposite to negative control (RPMI only). However, any tested dose (0.1-50 µg/mL) was able to induce significantly cellular permeability, phosphatidylserine externalization or DNA degradation in those neutrophils after 1-4 hours of incubation. Together, our preliminary results suggest soluble molecules from T.cruzi are able to induce NETosis process from human neutrophils in the absence of classical apoptosis and necrosis signals. Extend this study will assist in to further understand this novel antimicrobial mechanisms of neutrophils, mast cells and eosinophils more recently.

P1.01.37
Role of PI3K, PKC and ERK on neutrophil extracellular trap release induced by Leishmania amazonensis

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Neutrophils discharge to the extracellular medium web-like structures comprised of chromatin and proteins (NETs), which bind and kill microbes. Reactive oxygen species (ROS) and ERK phosphorylation are required for NETs induction by some stimuli, but the full process is still unclear for many others. We reported that Leishmania amazonensis (La) induces NETs release and that these fibers ensnare and kill this parasite. Here our aim is to further identify signaling pathways behind La-induced NETosis. Hence, neutrophils isolated from healthy donors were treated with selective inhibitors prior to the interaction with La, and NET-DNA measured in the culture supernatant. Our results show that PI3K, PKC and ERK selective inhibitors induced significant inhibition over NETs release. These results were confirmed by decondensed chromatin observed by DNA staining pattern under fluorescence microscopy. ERK phosphorylation after La-neutrophil interaction was abolished by the selective inhibitors. ROS generation, measured with specific probes, was abolished by PKC and reduced over 60% by ERK inhibitors. Both PI3Kδ and PI3Kγ inhibitors, reduced over 50% La-induced NETs release and we detected a 97% reduction of ROS generation only with the PI3Kγ inhibitor. Also, PI3Kγ inhibition diminished ERK phosphorylation. Depletion of intracellular calcium decreased 40% La-induced NETosis, but not ROS generation. Therefore, we suggest that PI3Kγ together with PKC controls ROS generation and that PI3Kγ is upstream of ERK phosphorylation on NETs signaling pathway induced by La. PI3Kδ and Ca2+i did not affected ROS generation but reduced NET generation, which might indicate activation by a different pathway. Supported by:FAPERJ, CNPq, CAPES.

P1.01.38
Modulation of human neutrophil functions with antioxidants in vitro

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Background and aims. Signalling in neutrophils from soluble stimules resulting in degranulation and a respiratory burst has not been understood yet. A number of signal transmitters such as tyrosine and serine/threonine kinases (Abe et al, 2009) are sensitive to the redox status of granulocytes. The role of an antioxidant, cationic plastoquinone derivative containing charged phosphonium, was investigated in the model of degranulation of human neutrophils. Well-known antioxidants such as N-acetylcystein (NAC) and Trolox, ROS-scavengers, Tiron and TEMPOL, were used as controls.
Methods. Human neutrophils were purified from the blood of healthy donors using a standard protocol. The respiratory burst induced by fMLP was measured using luminol-dependent chemiluminescence. Hydrogen peroxide was quantified by flow cytometry using DCFH-DA. The exocytosis of specific and azurophil granules was determined from the expression of CD66b and CD63, respectively, in a flow cytometric assay.
Results. It was shown earlier that cationic plastoquinone derivatives possess strong antioxidant activity at a nanomolar concentrations and show pronounced prooxidant activity at higher concentrations. In this study it was demonstrated that at concentrations of 1-100 nM, it reduced degranulation of fMLP-activated neutrophils in a dose-dependent manner and inhibited intracellular hydrogen peroxide and luminol-enhanced chemiluminescence. NAC and Trolox decreased ROS generation and the exocytosis of neutrophils in a concentration-dependent manner.
Conclusions. Our results indicate that antioxidants of different origins can modulate neutrophil activity. Their use in the treatment of inflammatory diseases is discussed.

P1.01.39
Critical roles of DOCK2 and DOCK5 in neutrophil chemotaxis, ROS production, and NETs formation

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Neutrophils are highly motile leukocytes and play important roles in the innate immune response to invading pathogens. Upon infection, neutrophils rapidly migrate to the site of infection, phagocytose bacteria, and kill them by producing reactive oxygen species (ROS). All these neutrophil functions are critically dependent on Rac, a member of the small GTPases that cycle between GDP-bound inactive and GTP-bound active states. However, the upstream regulators controlling Rac activation in response to chemotactic cues and other stimuli are not fully understood.
DOCK2 and DOCK5 are mammalian homologues of Caenorhabditis elegans CED-5 and Drosophila melanogaster Myoblast City. Although DOCK2 and DOCK5 do not contain the Dbl-homology domain and the pleckstrin homology domain typically found in guanine nucleotide exchange factors (GEFs), these molecules bind to nucleotide-free Rac and catalyze GTP-GDP exchange reaction through their DHR-2 domain. In this study, we show that DOCK2 and DOCK5 coordinately regulate various neutrophil functions. We found that fMLP- and PMA-induced Rac activation are almost completely lost in neutrophils lacking both DOCK2 and DOCK5, resulting in severe defects in neutrophil chemotaxis, phagocytosis, and ROS production. In addition, double KO mice exhibited a defect in formation of neutrophil extracellular traps (NETs), the extracellular structures composed of chromatin and granule proteins that bind and kill microorganisms. Although NETs play an important role in protective immunity, NETs are also involved in inflammatory response and autoimmunity. Therefore, DOCK2/DOCK5-mediated Rac activation would be a therapeutic target controlling NETs-mediated immune-related disorders.

P1.01.40
Untouched isolation of functionally unaffected neutrophils from whole blood within 20 minutes

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Human neutrophils are commonly obtained by density gradient centrifugation. Commonly used methods such as Ficoll-Paque gradients in combination with dextran sedimentation, discontinuous Percoll or Polymorphprep gradients are exceedingly time-consuming and frequently result in impure neutrophils which are contaminated by other leukocytes, mainly eosinophils.
With the new MACSxpress technology, untouched human leukocyte subsets can be isolated from up to 30 ml of anticoagulated whole blood. Erythrocyctes are aggregated and sedimented, while non-target cells are removed by immunomagnetic depletion with MACSxpress Beads, yielding untouched target cells of high purity.
MACSxpress-enriched neutrophils had average purities of 93% with yields of ~97% (n=14). ROS (reactive oxygen species) generation and expression levels of the surface markers CD11b, CD62L and CD181 (CXCR1) of MACSxpress-isolated neutrophils were comparable to cells isolated with density gradient centrifugation. In addition, separated neutrophils maintained efficient phagocytic capacity, which was assessed by uptake of opsonized bacteria and showed typical migration behavior toward fMLP. Cell viability was unaffected, as demonstrated by flow cytometric analysis of AnnexinV staining.

P1.02 Mast cells

P1.02.01
Role of mast cells and histamine in local reaction induced by *Potamotrygon motoro* stingray venom in mice

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Stingray accidents cause an intense pain followed by edema, erythema and necrosis formation. The therapy is based on administration of analgesic, antipyretic and anti-inflammatory drugs. The aim of this work was to verify the participation of mast cells and histamine on the inflammatory reaction induced by injection of *Potamotrygon motoro* venom (PmV). Mice were pre-treated with either cromoglycate, a mast cell inhibitor, or the histamine antagonists promethazine, cimetidine and thioperamide at selected time intervals before venom injection. PBS pre-treatment was used as control. PmV (8 µg/ 30 µL) or PBS were injected into mouse footpad to evaluate edema formation (0.25, 0.5, 1, 4, 24 and 48 h) and cell recruitment (4 h). Presence of mast cells was confirmed by histological analysis. Pre-treatment with cromoglycate diminished edematogenic activity in all time periods analyzed, reaching 62 % of reduction at 30 min. A decrease of edema formation was also noticed in animals treated with promethazine or thioperamide after PmV injection. Moreover, treatment with cromoglycate, promethazine, or thioperamide diminished local PmV-induced leukocyte infiltration, mainly of neutrophils (15, 20 and 30 % respectively). Cimetidine neither affects edema nor cell influx induced by PmV. Besides, thioperamide also reduced lymphocyte numbers. This is the first report demonstrating participation of mast cells and histamine in the local inflammatory response induced by freshwater stingray venom. The use of antihistaminic may be a new strategy for ameliorate the treatment for stingray envenomation. Supported by FAPESP, CAPES and INCTTox.

P1.02.02
Cross reactivity of IL-3 between mouse and rat in the induction of mouse bone marrow derived mast cells

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Both mouse and rat are classified as rodent and amino acid sequence homology (AASH) between mouse and rat shows more than 90% in most protein constituents of equivalents, with some except. The AASH is 96% in stem cell factor (SCF), however, that is 54% in IL-3 between both species. IL-3 is one of the important stimulators of hematopoietic stem cell. The addition of mouse IL-3 (mIL-3) alone or with mouse SCF (mSCF) to bone marrow cell culture (BMCC) induces mouse bone marrow derived mast cells (mBMMC). Mast cells are the important immune cells found even in shark that is the species regarded as an evolutionarily early stage animal. Rat IL-3 (rIL-3) induced proliferation of mBMMC at 7 week BMCC prepared with both mIL-3 and mSCF, not at 3 week. The BMMC proliferation induced by rIL-3 was enhanced by mSCF, but decreased by rat SCF. These results suggested both that rIL-3 deliver certain signal through mIL-3 receptor to allow mBMMC activation and that the change of rIL-3 from mIL-3 was not established simultaneously with SCF modification in evolution from mouse to rat.

P1.02.03
OX40L-STIMulated inhibition of mast cell calcium influx

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In the complex network of immune interactions, the amount of information available on the functional interplay between mast cells (MCs) and T regulatory cell (Tregs) is going to keep increasing.
Among the mysteries in this field is the nature of the inhibitory mechanism exerted by Treg on MC FcεRI-dependent extracellular Ca2+ flux. This process occurs on a single cell level through the OX40-OX40L axis without modifying Ca2+ mobilization from intracellular stores. To gain more insight into this mechanism we explored the relocation at lipid raft domains of STIM1, a transmembrane protein that mediates Ca2+ influx after depletion of intracellular Ca2+ stores by gating of store-operated Ca2+ influx channels (SOCs).
As visualized by confocal microscopy, the physical interaction with a single Treg leads to the inhibition of STIM1 relocation into lipid rafts. Additional findings suggest OX40L triggering as an underlying mechanism for the STIM1 defect observed, by a marked modification of lipid rafts organization.
In addition, both confocal and raft analysis showed aberrant STIM1 distribution upon antigenic stimulation in OX40L-deficient MCs.
Although their role in MC biology is not well understood, TRPC channels have been reported to contribute to Ca2+ influx. Here, preliminary results show their coupling to STIM1 dynamics and to OX40L engagement.
Taken together, these findings identify STIM1 as a critical mediator of OX40L "tuning" action on MC Ca2+ influx, but further investigations are required in order to have a more complete understanding of this inhibitory mechanism.

P1.02.04
Augmenting the effects of vaccine adjuvants by targeting mast cells

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Replacement vaccine adjuvants with better potency are desired especially in the face of a global resurgence of infectious diseases. Mast cells can be exploited for developing novel adjuvants by virtue of their potential in secreting cytokines that favour adaptive immunity.
Our research has focused on a detoxified cholera toxin (CT)-derived adjuvant, CTA1-DD which is a fusion protein consisting of the Al subunit of CT linked to a synthetic dimer of fragment-D of *Staphylococcus aureus* protein A (DD). CTA1-DD itself has been demonstrated to be a potent mucosal adjuvant in various immunization models. As the DD domain can unspecifically bind immunoglobulins, we proposed that IgG and CTA1-DD can form complexes that may activate mast cells through FcγRIIIA, an activating Fc receptor expressed on connective tissue mast cells (CTMC). In deed, CTA1-DD in combination with normal mouse IgG induced TNF-α production from mouse CTMC, resulting in greater antigen-specific immune responses than when CTA1-DD was used alone as an adjuvant in nasal immunization. Furthermore, FcγRIIIA-expressing CTMC were observed to reside in mouse nasal submucosa. Mice deficient in mast cells or FcγRIIIA, or treated with a CTMC inhibitor consistently expressed compromised antigen-specific immune responses to intranasal immunization adjuvanted with CTA1-DD/IgG complexes. Replacement of amino acid 7, arginine, by lysine (CTA1-R7K-DD) abolishes the adjuvant effect completely. Yet, CTA1-R7K-DD complexed with IgG could activate CTMC thus partially restoring the adjuvant function.
In conclusion, the effects of mucosal adjuvants can be further enhanced by engaging mast cells through adequately manipulating the adjuvant formulation.

P1.02.05
Generation of mast cells from murine fetus liver and transcriptome analysis using next generation sequencer; a comparison of the profiles between bone marrow derived mast cells with fetus liver derived mast cells

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Embryonic lethal due to a gene manipulation provoke severe difficulty in the analysis of protein function, although conventional or conditional knockout techniques are widely used for exploring the roles of proteins in various cells. We generated mast cell-like cells from mouse early fetus liver (FLMCs), and compared the mRNA expression pattern of FLMCs with that of bone marrow-derived mouse mast cells (BMMCs).
The FLMC was generated from liver of C57BL/6 mouse embryos at embryonic age 12-13, and the RNA was purified by commercial kit. Messenger RNA expression pattern was analyzed using Illumina Genome Analyzer IIx. Transcripts were classified according to whether the mRNA in both BMMC and FLMC increased or not.
The coefficient of correlation between both transcripts revels in BMMCs and FLMCs was about 0.96. Approximately 95% of all mRNA we analyzed was expressed at similar levels on both types of non-stimulated cells. On both IgE cross-linked BMMC and FLMC, the changed expression was observed in about 0.7% mRNA of above-mentioned 95% mRNA. The changed mRNA belonged to the category of angiogenesis, cell cycle control, hormones, immune responses, and signal transduction. Meanwhile, approximately 5% of all mRNA we analyzed was expressed at significantly different levels between non-stimulated BMMC and FLMC; they involved mRNAs for chemokines, interleukins and their receptors.
FLMCs can be used as a powerful tool for the elucidation of proteins function in mast cells, even if the fetal lethal caused by a gene deletion has occurred.

P1.02.06
Mast cells induce invariant Natural Killer T cells to cytokine secretion and proliferation via CD1d: Another path to asthma?

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Increasing evidences show that beyond their role as effectors during allergic reactions, mast cells (MCs) can function as antigen presenting cells that activate diverse T cell populations. Recently, the role of invariant Natural Killer T cells (iNKT) in allergic asthma has become more evident, where they contribute to the development of Th2 responses. During allergic asthma, MCs-released mediators induce the recruitment and regulation of diverse innate lymphocytes, such as iNKT. Therefore it appears very likely that MCs interact with iNKT cells in the lung during the course of allergic asthmatic reactions.
In our study we analyzed the MCs and iNKT interaction. We found that mouse peritoneal MCs (PMC) express surface CD1d, which is upregulated *in vivo* after a single intraperitoneal administration of the prototypical glycolipid alpha-Galactosylceramide (aGalCer). In contrast to primary cells, in cultured peritoneal (CPMC) and bone marrow-derived MCs (BMMC) CD1d was located mostly intracellularly. However, after the induction of IgE-mediated degranulation, CD1d relocated to the cell surface at similar levels to those found *in vivo*. Moreover, when cultured in the presence of aGalCer and upon degranulation, the CD1d-aGalCer complex was detectable on the surface of BMMC, thus enabling the activation of iNKT by MCs. When co-cultured *in vitro*, mast cells loaded with aGalCer indeed induced the release of Il-4, IL-13 and IFN-gamma and proliferation of iNKT cells.
Our data provide the first evidence of the involvement of MCs in responses to glycolipids. And notably, reveal a yet unidentified scenario where MCs likely modulate iNKT cell activation and homeostasis.

P1.02.07
Mast cell CYP27B1 hydroxylase activity is required for 25OH vitamin D_3-induced suppression of IgE-mediated activation

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Mast cells (MCs) have gained notoriety based on their detrimental pro-inflammatory contributions to IgE-mediated allergic disorders, such as asthma and life-threatening anaphylaxis. We have determined that the pro-inflammatory properties of MCs can be reduced by application of 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), the biologically active form of vitamin D_3 (VitD_3). A number of immune cell types have the ability to produce endogenous 1α,25$(OH)_2D_3$ from the predominant inactive VitD_3 metabolite in circulation, 25-hydroxyvitamin D_3 (25OHD_3), due to expression and activity of the hydroxylase enzyme CYP27B1. In this study, we explored if 25OHD_3 can modulate IgE-dependent MC activation. Our data show, for the first time, that MCs express intracellular CYP27B1 and that 25OHD_3 can be converted to significant levels of 1α,25$(OH)_2D_3$ by MCs. In addition, MC-CYP27B1 activity is required for 25OHD_3 -induced inhibition of IgE-mediated MC-TNF and IL-6 production *in vitro*. By employing a mouse IgE-mediated MC-dependent passive cutaneous anaphylaxis (PCA) model, as well as four mouse groups with different cutaneous MC profiles in the ears, including WT mice, MC-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice and C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice engrafted with either WT or *CYP27B1*$^{-/-}$ MCs, we found that topical 25OHD_3 application significantly reduced the magnitude of PCA-associated ear swelling in the WT MC engrafted group but not in mice that received *CYP27B1*$^{-/-}$ MCs. Taken together, these findings provide evidence that MCs are capable of utilising 25OHD_3 to generate biologically active 1α,25$(OH)_2D_3$, and thereby extends the modus operandi of VitD_3 (via both 25OHD_3 and 1α,25$(OH)_2D_3$ metabolites) to efficiently negatively regulate IgE-mediated MC activation.

P1.02.08
IgE-mediated allergic responses in human mast cells in humanized NOG mice expressing human IL-3 and GM-CSF

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Humanized mice reconstituted with human immune systems are essential to study human immune reactions in vivo and are expected to be useful for studying human allergies. However, application of this technology in the study of human allergies has been limited, largely due to the poor development of human myeloid cells, especially granulocytes and mast cells, which are responsible for mediating allergic diseases, in conventional humanized mice. In this study, we developed a novel transgenic strain, NOD/Shi-scid-IL2rγnull (NOG), bearing human interleukin (IL)-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) genes (NOG IL-3/GM-Tg). In this strain, a large number of human myeloid cells of various lineages developed after transplantation of human CD34+ hematopoietic stem cells. Notably, mature basophils and mast cells were markedly increased in peripheral blood (PB), bone marrow (BM), spleen and bronchoalveolar lavage (BAL) of Tg mice, and these cells expressed a significant amount of high affinity IgE receptor. These humanized NOG IL-3/GM-Tg mice developed human mast cell-mediated passive cutaneous anaphylaxis (PCA) reactions when administered anti-4-hydroxy-3-nitrophenylacetyl (NP) IgE antibodies (Abs) and NP. More importantly, combination of the serum from Japanese cedar pollinosis patients and the cedar pollen extract also elicited strong PCA responses in the mice. Thus, our IL-3/GM-Tg mice are the first humanized mouse model to enable the study of human allergic responses in vivo and are excellent tools for preclinical studies of allergic diseases.

P1.02.09
Roles of basophils and mast cells infiltrating the lung in murine asthmatic responses induced by multiple antigen challenges

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Mast cell hyperplasia has been observed in the lungs of mice with experimental asthma, but few reports have studied basophils. Here, we discriminated and quantified mast cells and basophils in the lungs in a murine asthma model, determining if both cells were increased by multiple antigen challenges, and assessing the roles of these cells in asthmatic responses. Sensitized Balb/c mice were challenged intratracheally with ovalbumin 4 times. Mast cells and basophils in enzymatically digested lung tissue were detected by flow cytometry. An anti-FcεRI monoclonal antibody, MAR-1, was administered i.p. during the multiple challenges. The numbers of both mast cells (IgE+ C-kit+) and basophils (IgE+ C-kit− CD49b+) increased in the lungs after three challenges. Treatment with MAR-1 completely abolished the increases; however, a late-phase increase in specific airway resistance (sRaw), and airway eosinophilia and neutrophilia were not affected by the treatment, although the early-phase increase in sRaw was suppressed. MAR-1 reduced antigen-induced airway interleukin (IL)-4 production. Basophils infiltrating the lungs clearly produced IL-4 after antigen stimulation in vitro; however, histamine and murine mast cell protease 1 were not increased in the serum after the challenge, indicating that mast cell activation was not evoked. In conclusion, both mast cells and basophils infiltrated the lungs by multiple intratracheal antigen challenges in sensitized mice. Neither mast cells nor basophils were involved in late-phase airway obstruction in this model, although early-phase airway obstruction was mediated by basophils. Targeting basophils in asthma therapy may be useful for an early asthmatic response.

P1.02.10
Immune suppression following exposure to UV-radiation is associated with increases in mast cell densities in primary and secondary lymphoid tissues

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Ultraviolet (UV) radiation causes skin cancer, in part, by suppressing adaptive immune responses. One way UV achieves this is by altering the traffic of mast cells around the body. Exposing C57BL/6 mice to UV 4 times per week for 20 weeks resulted in significant increases in mast cell densities in the skin, lymph nodes and spleen. The density of mature mast cells was also higher in the bone marrow (but not thymus) of UV exposed mice as assessed by flow cytometry. In contrast, the frequency and number of T cells, B cells and DC were unaltered by UV in these primary lymphoid tissues. Mast cell accumulation in peripheral sites such as the skin and lymph nodes is associated with a UV-induced increase in CXCL12. Therefore, to determine whether the increase in bone marrow mast cells was due to changes in CXCL12, we isolated mRNA from bone marrow cells after 6, 12 and 16 weeks of irradiation. However, chronic UV did not significantly increase CXCL12 levels in the bone marrow suggesting another mechanism is involved in UV-induced mast cell increases in the bone marrow. To that end, we have discovered that exposure to chronic doses of UV results in an increase in mast cell progenitors in both the bone marrow as well as peripheral blood. These findings explain not only the increase in mast cells within bone marrow, but also those observed at peripheral sites.

P1.02.11
Intravenous injection of particulate antigens larger than the intracellular gap of endothelial cells can induce mast cell-mediated systemic anaphylaxis

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IgE-dependent systemic anaphylaxis is the most severe manifestation among mast cell-mediated hypersensitivity reactions. Mast cells are found throughout the body, particularly in a perivascular area and beneath epithelia. In blood vessels, endothelial cells are continuously connected by tight junctions, and the size of intercellular gaps is approximately 3.0 nm, limiting the passage of large molecules, for example, albumin (7 nm) and ovalbumin (5 nm). In spite of this fact, we unexpectedly found that allergen-conjugated microspheres with a 3,000 nm diameter could induce systemic anaphylaxis with drastic reduction in the body temperature when intravenously injected into mice that had been sensitized intravenously with allergen-specific IgE. The intravenous injection of allergen-conjugated microspheres also elicited a passive cutaneous anaphylactic reaction in mice sensitized subcutaneously with allergen-specific IgE. Of note, mast cell-deficient mice failed to induce both systemic and subcutaneous anaphylaxes, indicating the critical role for mast cells in the reactions. These results are consistent with the idea that perivascular mast cells can capture blood-circulating particulate antigens through the vessel wall and are activated to induce anaphylaxis.

P1.02.12
The development of oral tolerance is inhibited by TLR2 agonists but not by IgE-mediated mast cell activation

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Food allergy is linked to defective oral tolerance. Many factors have been implicated in this process. TLR2 activators are found in many foods, including processed meats, cheese and chocolate. In our study, we have investigated the role of mast cells and TLR2 in the development of oral tolerance. Mice were provided ovalbumin (OVA) in drinking water for one week and the extent of oral tolerance assessed following systemic immunisation (day 8) and boost (day 22) with alum precipitated OVA. Antigen specific IgE, IgA and IgG subclass responses were assessed by ELISA. In some experiments, TLR2 (Pam 3CSK4, FSL-1) or TLR4 (LPS) agonists were provided, three times by gavage, during the initial week of tolerance induction.
Neither two strains of mast cell deficient mice, nor TLR2 deficient mice, showed defective oral tolerance responses, compared with matched, wild type controls. Oral tolerance induction was associated increased antigen specific regulatory T cells in the mesenteric lymph node and spleen ($P<0.01$). IgE mediated mast cell activation also had little impact on oral tolerance development . In contrast, oral TLR2 agonist treatment significantly impaired suppression of humoral responses to foods upon systemic challenge ($P<0.05$ for anti-OVA IgE and IgA). Similar oral treatment with LPS did not modify oral tolerance responses.
In conclusion, neither TLR2 nor mast cells are necessary for oral tolerance induction. However, oral TLR2 activation, but not TLR4 activation, reduces the development of tolerance, these findings could have important implications for the mechanisms of development of food allergy.

P1.02.13
Notch1 signaling confers antigen-presenting functions on mast cells

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Notch proteins (Notch1-4) are a family of transmembrane receptors that play important roles in the determination of cell fate. We have found that Notch1 and Notch2 are constitutively expressed on mouse mast cell surface. However, little is known about the roles of Notch-mediated signaling in mast cells. Here, we show that mast cells acquire antigen-presenting functions by Notch1-mediated signaling. Bone marrow-derived mast cells (BMMCs), which were generated *in vitro* by culturing bone marrow cells isolated from BALB/c mice with IL-3 and stem cell factor, were cocultured with Notch ligand-expressing CHO cell lines for 5 days. Flow cytometric analysis showed that the cell surface expression of MHC class II and a costimulatory molecule OX40 ligand was induced in BMMCs by stimulation with Notch ligand. The inducible expression of MHC class II depends critically on the expression of the class II transactivator (CIITA)-type III mediated by Notch1 signaling. Furthermore, Notch ligand-stimulated BMMCs directly activate antigen-specific $CD4^+$ T cells by antigen presentation *in vitro*. The ability to activate T cells was markedly elevated by sensitizing BMMCs with antigen-specific IgE. These findings suggest that mast cells acquire antigen-presenting cell functions by Notch1-mediated signaling and may contribute to the maintenance and/or augmentation of antigen-specific immune responses.

P1.02.14
Candida albicans activates mast cells in vitro

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Mast cells are long-lived cells that mature and reside in peripheral tissues such as skin and mucosae. They are equipped with several receptors that can recognize many microorganisms and can produce a great diversity of mediators. Recently, mast cells have been considered as sentinels of the immune system and they contribute to the elimination of pathogens. Candida albicans is a dimorphic fungus that usually behaves like a commensal but in cases like immunodeficiencies it can turn into a pathogen. The immune response against C. albicans is already described and many cell types that are important for the elimination of C. albicans have been described. However, the role of mast cells in this infection is still unclear.
We demonstrated that both hyphae and yeast induce mast cell degranulation. Moreover, mast cell activation was also reflected with cytokines production like TNFα IL-1β, RANTES, MIP-1α and MIP-1β. Finally, we observed that mast cells also produced ROS after stimulation with both phases of C albicans.
Our data suggest the importance of mast cell in the infection with C. albicans. It seems that the function of these cells is to initiate the inflammation, to recruit cells like neutrophils and monocytes with its mediators, and to induce maturation of T lymphocyte trough the production of RANTES. In conclusion these results support the hypothesis that mast cells are key cells to initiate the immune response against C. albicans.

P1.02.15
Involvement of transcription factors PU.1, GATA1 and GATA2 in the expression and function of human FcepsilonRI on mast cells

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The high affinity IgE receptor, FcepsilonRI, which is composed of three subunits, alpha-, beta- and gamma-chains, plays an important role in IgE-mediated allergic responses. We have previously identified several transcription factors including GATA-1, GATA-2 and PU.1 as transcriptional regulator of mast cell-specific gene expression. In the present study, we analyzed the involvement of PU.1, GATA1, and GATA2 in FcepsilonRI expression in the human mast cell line LAD2 and primary human mast cells using siRNA and chromatin immunoprecipitation (ChIP) assay to verify our previous findings. Transfection of siRNAs against PU.1, GATA1, and GATA2 into LAD2 cells caused significant downregulation of cell surface expression of FcepsilonRI. Quantification of the mRNA levels of alpha, beta and gamma revealed that PU.1, GATA1 and GATA2 siRNAs suppressed

the alpha transcript, whereas the amount of beta mRNA was reduced in only GATA2 siRNA transfectants. In contrast, gamma mRNA levels were not affected by any of the knockdowns. ChIP assay showed that significant amounts of PU.1, GATA1 and GATA2 bind to the promoter region of *FCER1A* (encoding FcepsilonRIalpha), and that GATA2 binds to the promoter of *MS4A2* (encoding FcepsilonRIbeta). These knockdowns of transcription factors also suppressed the IgE-mediated degranulation activity of LAD2. Similarly, all of three knockdowns suppressed FcepsilonRI expression in primary mast cells, especially PU.1 siRNA and GATA2 siRNA that target FcepsilonRIalpha and FcepsilonRIbeta, respectively.
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P1.02.16
Identification of a molecule critical for degranulation of mast cells and anaphylactic reaction

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Anaphylaxis is a life-threatening allergic reaction. IgE-associated anaphylaxis is mainly mediated by mast cells that express FcεRI, the high-affinity receptor for IgE, on their surface. Crosslinking of FcεRI-bound IgE with multivalent antigens induces degranulation of mast cells, with concomitant secretion of chemical mediators.This process involves dynamic rearrangement of microtubules, yet the signaling cascade linking FcεRI to microtubule dynamics is not fully understood. In this study, we show that a member of DOCK family proteins plays a key role in degranulation of mast cells and anaphylactic reaction. By generating knockout (KO) mice, we found that FcεRI-mediated degranulation of mast cells was severely impaired in the absence of this molecule. While the KO mice exhibited a resistance to systemic and cutaneous anaphylaxis, transfer of wild-type mast cells restored the susceptibility, indicating that mast cells are primarily involved in these anaphylactic reactions. Imaging analysis using GFP-tagged C-terminal fragment of adenomatous polyposis coli (APC) also revealed that microtubule dynamic are impaired in the KO mouse-derived mast cells. Since DOCK family proteins act as guanine nucleotide exchange factors (GEFs) through the DHR-2 domain, we expected that the GEF activity would be important for this regulation. Interestingly, however, the expression of the mutant lacking DHR-2 domain completely restored degranulation response in the KO mouse-derived mast cells. Thus, we have identified a novel signaling molecule that links FcεRI to microtubule dynamics during mast cell activation.

P1.02.17
C. albicans phagocytosis by mast cells via Toll like receptor-2: protection or immune escape?

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Candida albicans (C. albicans) is a fungus commonly found in the mucosa of the gastrointestinal tract, including the mouth, and may cause candidiasis, especially in immunosuppression. Mast cells reside in the subepithelial region and actively participate in innate immunity, like one of the first lines of defense against *C. albicans.* The recognition of this fungus can occur via different receptors, *e.g.* Toll-like receptor 2 (TLR2), present on the surface of mast cells. Thus, this study evaluated in vitro the relationship of the phagocytosis, intracellular oxide nitric production by mast cells challenged with *C. albicans* and the involvement of TLR2 in these mechanisms. For this, Murine bone marrow cells (BMMC) wild type (TLR2+/+) or knockout (TLR2-/-) were cultured in presence of stem cell factor and interleukin-3. After, BMMCs were challenged with FITC-labeled *C. albicans* by 60 minutes and the phagocytosis analyzed by confocal laser scanning microscopy. Furthermore, intracellular oxide nitric production was measured used the DAF-FM diacetate and spectrophotometer. Our results showed that BMMC TLR2+ /+ phagocytose *C. albicans* but did not showed overproduction of the oxide nitric after stimulation with fungus, in relation with the basal production. However, in the absence of the TLR2, the oxide nitric production is increased, while the phagocytosis is reduced, in comparison with wild mouse. Therefore, we conclude that the *C. albicans* recognition by TLR2 results in the increased phagocytosis, but without oxide nitric stimulus. This can represent an escape way of the fungus of the defense mechanisms developed by mast cells via TLR2.

P1.02.18
Deciphering the molecular immunobiology of anaphylactic shock - and the de novo paradigm shift

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Anaphylactic shock (AS) is a cardiovascular collapse caused by an acute, potentially lethal immune reaction primarily induced by the elevated immunoglobulin-E (IgE) antibodies that signal through the high affinity FcεRI receptor to release inflammatory mediators from immune cells such as mast cells. The AS is potently mediated through various intracellular signalling molecules such as Phospholipase Cγ (PLCγ), Phosphoinositide-3-Kinase (PI3K), Mitogen Activated Protein Kinases (MAPKs), Sphingosine Kinases (SPHKs), etc., These molecules induce calcium mobilization, nuclear factor kappa B (NFkB) activation, cytokine and eicosanoid secretion, and degranulation. These processes are essential for the initiation of peripheral vasodilatation, vascular permeability, leakage of plasma, and intravascular volume depletion eventually leading to cardiovascular collapse. various research groups around the globe has made a huge effort to better identify and understand the mechanisms responsible for the induction of anaphylaxis and AS to develop potential novel therapeutic modalities to effectively tackle sudden onset of AS. Much of this effort has been channelized toward the development and investigation of animal models since the development and availability of genetically modified mice have been very much economical and reliable to deduce the function of a gene in a particular molecular pathway necessary, either to enhance or attenuate the AS. In this poster presentation, we discuss various molecular immunological processes with an additional emphasis on the role of nitric oxide and a de novo paradigm shift in the immunobiology and molecular therapeutics of AS.

P1.02.19
Mast cell derived-histamine promotes the recruitment and immunoregulatory activity of myeloid derived suppressor cells

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Unresolved inflammation increases myelopoiesis, resulting in bone marrow release of a heterogeneous group of mononuclear (CD11b+Ly6Chigh) and polymorphonuclear (CD11b+Ly6Ghigh) cells, known as myeloid derived suppressor cells (MDSCs). We demonstrate that while each population of MDSCs deferentially regulates the immune response, both require mast cells (MCs). In the absence of MCs, MDSCs failed to promote B16 melanoma metastasis. This tumor-promoting property was limited to Ly6C+ MDSCs. In our parasitic model, mice infected with Nippostrogylus brasiliensis (Nb) and adoptively transferred (AT) with Ly6G+ MDSCs had enhanced parasite clearance. Upon AT, MDSCs preferentially migrated to the liver, which is the site of interaction with MCs, proliferation, and activation of MDSCs. This differential activity of Ly6C+ and Ly6G+ MDSCs as well as trafficking to the liver was abrogated in MC deficient mice. We next elucidated the contribution of MC-released histamine to this interaction. MDSCs expressed

histamine receptor 1 (HR1) and histamine enhanced MDSC survival and expansion. Additionally, histamine upregulated Arg1 and iNOS enzyme expression, both of which are utilized by MDSCs to inhibit T cell activity. To confirm this in our parasitic model, we utilized cetirizine, an HR1 antagonist. HR1 blockade decreased MDSC activity, migration to the liver, and parasite clearance. Lastly, we analyzed the mechanism by which histamine promotes MDSC activity. Our data thus far suggests that histamine induces Stat3 signaling in Ly6C+ MDSCs and Stat5 Ly6G+ MDSCs. Taken together, our studies indicate that in the absence of MCs as well as inhibition of histamine signaling, MDSCs are unable to exert their effects.

P1.02.20
The potential role of mast cells in the C-protein induced myositis model

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Background: Mast cells (MCs) function as immune sentinel cells in the tissue. The well-known cellular infiltrate in inflammatory myositis mainly consists of macrophages and lymphocytes that produce numerous inflammatory cytokines and chemokines augmenting inflammatory cell infiltration and damage of myofibers. The role of tissue-resident MCs has not been well studied in inflammatory myositis.
Objective: To study the phenotype and role of MCs in a murine myositis model.
Methods: C-protein induced myositis (CIM) was induced with human skeletal C-protein fragment in 8 week-old female MC-deficient SASH mice and C57BL/6 controls. Quadriceps muscles of both legs were harvested at day 21. Toluidine blue stain was used to identify degranulating MCs in the muscle tissue. Immunohistochemistry was performed to detect mouse mast cell protease (mMCP)-1, -4, -5, -6 positive MCs.
Results: MCs were mainly located in the connective tissue between fascicles showing a connective tissue MC phenotype. The density of MCs and percentage of degranulating MCs per 5 high power field (HPF) were increased in CIM tissues. MC deficient mice had lower histology score of muscle inflammation compared with controls. The difference mainly attributed to abrogated invasiveness of inflammatory infiltrates into fascicles in MC deficient mice.
Conclusion: Connective tissue-type MCs in skeletal muscles are activated upon CIM induction. MCs facilitate tissue invasion of inflammatory infiltrates into muscles in the CIM model.
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P1.02.21
IgE by itself affects, directly and indirectly, mast cell effector functions

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Recent findings have implied that IgE by itself may modulate mast cell (MC) response, however detailed data are limited. Therefore, the aim of this study was to evaluate the influence of IgE alone on various aspects of mast cell biology. The study was conducted *in vitro* on mature rat peritoneal MCs. We examined effect of IgE alone on FcεRI expression (flow cytometry) and MC migratory response (Boyden microchamber assay). IgE-induced MC-derived mediator release, such as histamine (spectrofluorometric method), cysteinyl leukotrienes (cysLTs) and TNF (ELISA) was also assessed. We stated that IgE alone transiently up-regulated FcεRI expression. Spontaneous as well as TNF- and CCL5-induced migratory response of IgE-primed MCs was highly increased, as compared with native MC migration. Moreover, IgE alone triggered MCs to preformed mediator release, cysLTs generation and *de novo* TNF synthesis; in synergy with TLR2, but not TLR4 ligands, IgE was found to amplified cysLTs generation. Importantly, IgE was effective only at concentrations ≥ 3 μg/mL. Molecular basis investigation using an array of specific inhibitors showed that Src kinases, PLC/A2, MAP kinases (ERK and p38) and PI3 kinase were entirely or partially involved in IgE-induced MC response. In conclusion, we documented that IgE alone not only enhances surface FcεRI level and mediates/influences preformed and *de novo*-synthesized MC mediator release but also strongly affects MC migratory response. These observations may provide new insight into MCs activity in conditions where elevated IgE level is observed, including allergic diseases. This study was supported by the Medical University of Łódź (Grant 502-03/6-164-01/502-64-037).

P1.02.22
Mast cells play a crucial role in non-IgE mediated experimental allergic enteropathy

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Allergic enteropathy (AE) is a clinical phenotype of food allergies. Detailed pathomechanisms for AE are still not known. To investigate cellular and molecular mechanisms for AE, we established a murine model. BALB/c mice were sensitised with ovalbumin (a major egg white allergen) plus ALUM and fed egg-white diet (EW-diet). The mice developed acute inflammation in the jejunum accompanied by a strong induction of OVA-specific IgE antibodies by short-term feeding of the allergenic diet for 7 days, and presented chronic inflammation by further long-term feeding of the diet totally for 28 days. To assess the involvement of IgE antibodies in the jejunamal inflammation, we used mice deficient of FcεRI, the high affinity IgE receptor. In the FcεRI deficiency, acute inflammation was still induced by the short-term EW diet, while resolution of inflammation was observed after the long-term EW diet. Interestingly, high levels of mouse mast cell protease 1 (mMCP-1) were detected in the jejunum of both WT and FcεRI-deficient mice on the short-term EW diet. A treatment using an anti-c-kit monoclonal antibody to abolish mast cell function attenuated the induction of intestinal inflammation in WT and FcεRI-deficient mice. The results suggested that (i) mast cells play a crucial role in both IgE- and non-IgE mediated AE, and that (ii) IgE antibodies are involved in the induction of chronic AE. Our findings could contribute to establish a therapeutic strategy for AE.

P1.02.23
Mast cells support in vitro differentiation of Th17 cells

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Th17 differentiate from CD4+ naive cells following activation within specific cytokine milieu. Some of the cytokines are expressed by mast cells. It also known that T cells are capable to activate mast cells by cell to cell contact. Taking into consideration that in certain pathological conditions such as rheumatoid inflammatory immune response is characterized by activation of both Th17 and mast cells we decided to investigate interaction of mast cells and Th17 cells. Naïve CD4+ cells isolated from peripheral blood were cocultured with mast cell lines HMC-1 and LAD2. Cells were activated with anti-CD2;CD3;CD28 Ab in medium alone or in the presence of cytokines and production of cytokines was analyzed by ELISA. We observed that in the presence of mast cells activation of CD4+ lymphocytes with anti-CD2,CD3,CD28 Ab alone was sufficient to obtain in 5 days long culture significantly higher IL-17 production than that observed in cultures of CD4+ stimulated with Ab in the presence of cytokines. Presence of mast cells has also resulted in enhancement of IL-17 production in cultures stimulated with Ab and cytokine cocktail. Separation of mast cells from T cells by microporous membrane significantly decreased but not abolished the mast cell mediated

effect on IL-17. In conclusion mast cells support differentiation of CD4+ cells toward IL-17 producing Th17 cells in a process that in part requires direct cell to cell contact. Activation of Th17 cells in the presence of mast cells may generate different pattern of cytokine production than activation of each these cell types separately.

P1.02.24
Maturation of mast cells by Wnt family proteins

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Mast cells play an important role in the pathogenesis of allergic diseases. Immature mast cells migrate into peripheral tissues from the bone marrow and undergo complete maturation in the peripheral tissues. The tissue microenvironment affects the process of the maturation of mast cells. However, maturation mechanism of mast cells is poorly understood. Previous studies showed that co-culture of bone marrow-derived mast cells (BMMCs) with feeder cells, such as Swiss 3T3 fibroblasts and OP9 cells, in the presence of stem cell factor facilitated morphological and functional maturation toward a connective tissue-type mast cells (CTMCs)-like phenotype. To investigate whether maturation of mast cells requires physical contact between mast cells and feeder cells, BMMCs were co-cultured with feeder cells in the Transwell apparatus. Our results indicated that both soluble factors and cell-adhesion molecules, which are derived from feeder cells, might play an important role in mast cell maturation. Quantitative RT-PCR analysis revealed that several Wnt genes expressed in both Swiss 3T3 fibroblasts and OP9 cells. To determine whether Wnt signal play a role in the maturation of mast cells, we compared the degree of mast cell maturation of the BMMCs that were treated with or without Wnt protein. The expression levels of CD81 protein and histidine decarboxylase (HDC) mRNA, and activity of mast cell-specific protease, were elevated in BMMCs treated with Wnt protein. These results suggest that Wnt signals can promote the maturation of BMMCs into CTMCs. This study provides important insights into molecular mechanisms of the maturation of mast cells.

P1.03 NK cells and NK receptors

P1.03.01
Sp1 transcription factor is involved in the regulation of NKG2D ligands in tumor cells and during tumor Progression

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MICA/B and ULBPs are tumor-induced ligands for NKG2D, an activating receptor expressed by NK and T cells, which is crucially involved in the recognition and elimination of cancer cells. MICA and ULBP1 are overexpressed in tumor cells; however the mechanisms involved in their expressions are poorly defined. Herein we study the regulation of NKG2D ligands expression in tumor cells and during tumor progression. We characterized that Sp1 and Sp3 transcription factors are the main regulators of NKG2D ligands expression in tumor cells. The induction of tumor progression by TGF-β stimulation, by GSK-3β inhibition and by Snail1 over-expression in several tumor cell lines was associated with strong up-regulation of MICA/B and ULBP1-3 expressions. Transcriptional studies showed that the overexpression of Snail and the inhibition of GSK-3B increased Sp1 transcription factor activity. Moreover, pharmacological and RNA interference experiments demonstrated that the induction of NKG2D ligands expression during tumor progression was dependent on the induction of Sp1 transcription factor activity. Accordingly with an increase of NKG2D ligands expression in tumors cells, these cancer cells were more susceptible to NKG2D-mediated killing by NK cells. In conclusion, our experiments demonstrate that Sp1 is the main regulator of NKG2D ligands expression in cancer cells. We also show that tumor progression activates NKG2D-dependent immune response against carcinoma cells and this induction of NKG2D-mediated response was mainly mediated by the increase of Sp1 transcription factor activity in the tumor cells.

P1.03.02
A novel immunological approach to prevent HCV recurrence in liver transplantation

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HCV chronic infection accounts for over 180 million of patients worldwide and represents the leading indication for liver transplantation. HCV liver reinfection universally occurs upon transplantation driven by the remnant virus still circulating in the peripheral blood and sustained by the immunosuppressive regimen associated to the anti-rejection therapy. Following an initial dramatic decrease, HCV-RNA viremia rapidly increases early after transplantation, peaking already at day 3-4 post-operation.
In the acute phase of infection NK cells strongly respond to HCV by killing infected hepatocytes in a cell-cell contact manner or via release of soluble factors such as IFN-gamma. However, HCV escapes innate immunity and establishes chronic infection by inhibiting/outnumbering NK cells. Here we describe an innovative immunological approach for the prevention of HCV recurrence based on the infusion of activated NK cells in recipients promptly after liver transplant. High doses of NK cells will be injected in recipients within the first 16-48 hours post transplant to exploit the viremia reduction that follows the anhepatic phase. Patients will receive multiple infusions of NK cells to outnumber HCV virions before its rapid expansion. NK cells will be manipulated in vitro to preferentially direct towards the expression of IFN-gamma. Homing to the liver IFN-gamma+ NK cells should clear HCV infection without being aggressive on target cells. IFN-gamma+ NK cells might represent an appealing tool to cleanse HCV infection without destroying liver tissue suggesting a role for this cells as efficient therapeutic to adjuvant liver transplantation to prevent HCV relapse.

P1.03.03
CD56+ cells in demyelinating polyneuropathies

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Acquired autoimmune demyelinating polyneuropathies include Guillain-Barré Syndrome (GBS) and Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), which differ in the etiology (GBS usually develops as a complication of infectious processes, immunopathogenesis, course duration, methods of treatment and therefore call for differential diagnosis. Were observed 68 patients with demyelinating polyneuropathies by flow cytometry who were divided into two groups: 42 GBS patients and 26 - with CIDP. Expression of cell-surface markers such as CD3+/CD56+/CD4+, CD3+/CD56+/CD8+, CD3+/CD56+/DN, CD3-/CD16-/CD56+, CD16+/CD56+/CD158a,h+, CD16+/CD56+/CD94+, CD16+/CD56+/NKG2D+, CD16+/CD56+/NKp46+ were assessed by multiparametric FACS. It was found that in GBS unlike in the CIDP the fall of NKT number was not present but reliably reduced the NK cell number. In all patients CD4+ NKT increased 10 and more times, subpopulation CD8+ NKT significantly decreased. The changes in NK cell in GBS patients were accompanied by a decline in their expression of KIR (CD158a,h), NKG2D, NKp46. The fall of natural cytotoxicity of receptor content (NKp46) in 1,8 fold on NK membrane is observed only in GBS while negative shift in receptor expression of antibody dependent cytotoxicity is observed in all manifestations of demyelinating polyneuropathies. It is expected that the observed immunopathogenetic features with NKT and NK cells participation can help in differentiation of GBS from CIDP.

P1.03.04
Assessment of natural killer receptors and activity in severe and moderate Chronic Fatigue Syndrome

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Abnormalities in the immune system are a common finding within sufferers of Chronic Fatigue Syndrome (CFS). Research has consistently found compromised Natural Killer (NK) cell function in CFS subjects. The mechanism of the reduced NK function is unknown and however this may potentially be related to the presence of NK receptors. It is also unknown whether reduced NK function is further compromised within CFS subjects who are very severely affected by the disease. The purpose of this study was to examine NK activity and Killer Immunoglobulin-like Receptors (KIRs) in moderate (MFS) and severely affected CFS (SFS) subjects in comparison to controls. CFS subjects were characterised as either moderate or severely affected based on health and quality of life questionnaires. Severe subjects were housebound. Blood samples were collected s from controls (n=9; 50.9 $\pm$ 2.5 years), MFS (n=9; 49.9$\pm$ 3.7years) and SFS subjects (n=9; 42.3 $\pm$ 3.9 years). Using flow cytometry NK cells were measured for their activity based on their ability to lyse K562 cells and cell surface expression of KIRs. ANOVA was the statistical method used to assess all data collected. Preliminary data from CFS subjects showed significant reductions NK cell activity and an increase in KIR3DL1 in CFS subjects when compared to the controls. Results suggest that CFS patients have further compromised immune function, particularly reduced NK cell activity, when compared to controls. Additionally, increased levels of KIR, particularly KIR3DL1 in CFS patients, may potentially explain why these patients have a decrease in NK lysis.

P1.03.05
Host response to murine CMV in class I MHC disparate mice reveals primacy of licensed NK cells in viral control

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Murine cytomegalovirus (MCMV) resistance in MA/My mice requires the class I MHC Dk molecule and inhibitory receptor Ly49G2+ NK cells. In MCMV-sensitive C57L mice without Dk, Ly49G2+ NK cells fail to deliver viral control. Expression of a Dk genomic transgene in C57L fully reverses MCMV-sensitivity. As a cognate ligand of Ly49G2, Dk licenses the NK subset for effector activity. Together the data suggest that Ly49G2+ NK cells restrain MCMV infection. However, the role of licensed NK cells in viral control is poorly defined. Hence, we combined classical genetics with flow cytometry to interrogate the NK cell response to MCMV infection in a diverse cohort of 200+ MA/My x C57L hybrid mice disparate for Dk. To identify NK cell features essential to viral control, we performed trait comparisons in R using Pearson's correlation test followed with a Bonferroni correction for multiple tests, hierarchical clustering and principal component analysis. The data show that Ly49G2 receptor display on naïve blood NK cells significantly predicted low viral burden. More remarkable, the frequency of licensed Ly49G2+ NK cells rapidly and selectively expanded in infected Dk mice and therefore represented the best possible index (of 109 traits examined) of viral control. From the data, we infer that licensed Ly49G2+ NK cells efficiently detected missing-self MHC cues on virus-infected targets, which elicited NK subset expansion and target cell killing. MHC polymorphism therefore regulates NK licensing and the early detection of viral targets by defined NK subsets, which ultimately determines their capacity to deliver innate viral control.

P1.03.06
ADAM10 contributes to MICA/B shedding from multiple myeloma cells in response to genotoxic stress

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The MHC class I-related chain MICA/B ligands for the activating receptor NKG2D can be shed from tumor cells and their presence in sera is associated with impaired NK and CTL effector functions and disease progression. We showed that multiple myeloma (MM) cells up-regulate surface expression of NKG2D ligands after treatment with low doses of chemotherapeutic drugs and become more susceptible to NK cell lysis. Here, we investigated whether drug treatment of MM cells could result in MICA/B release. Soluble MICB was up-regulated after drug treatment and the ADAM10 specific inhibitor GI254023X dramatically reduced MICB shedding in a dose-dependent manner and was accompanied by a concomitant increase of cell-surface MICB. Similar results were obtained by shRNA-mediated gene silencing. Remarkably, chemotherapeutic agents increased ADAM10 expression either in MM cell lines or in *ex-vivo* malignant $CD138^+/CD38^+$ plasma cells. Regarding MICA, since its polymorphism can affect the shedding process, we investigated the effect of chemotherapeutic agents on the release of two allelic variants of MICA: MICA*019 and MICA*008 which represent the prototype of the long and short form of the MICA alleles, respectively. Similarly to MICB, drug treatment stimulated MICA*019 shedding with an ADAM10 dependent mechanism while didn't affect MICA*008 release. Our findings indicate that chemotherapeutic drugs not only enhance MICA/B surface expression on MM cells, but also promote their shedding. The MICA genotype and the relative contribution of membrane associated vs soluble MICA/B in NKG2D dependent mechanisms of NK cell recognition of MM cells, should be considered to design NK cell-based immunochemotherapeutic approaches.

P1.03.07
Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors

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Natural killer cell (NK cell)-based immunotherapy of cancer is hampered by the transient effector function of NK cells. Recently, mouse IL-12/15/18-preactivated NK cells were shown to persist with sustained effector function in vivo. Our study investigated the antitumor activity of such NK cells. A single injection of syngeneic IL-12/15/18-preactivated NK cells, but neither naive nor IL-15- or IL-2-pretreated NK cells, combined with irradiation substantially reduced growth of established mouse tumors. Radiation therapy (RT) was essential for the antitumor activity of transferred NK cells. IL-12/15/18-preactivated NK cells expressed high levels of IL-2Ralpha (CD25), and their rapid in vivo proliferation depended on IL-2 produced by CD4+ T cells. IL-12/15/18-preactivated NK cells accumulated in the tumor tissue and persisted at high cell numbers with potent effector function that required the presence of CD4+ T cells. Human IL-12/15/18-preactivated NK cells also displayed sustained effector function in vitro. Further results on human cytokine activated NK cells will be presented and responsible mechanisms will be discussed. Our study provides a better understanding for the rational design of immunotherapies of cancer that incorporate NK cells. Moreover, our results reveal an essential role of CD4+ T cell help for sustained antitumor activity by NK cells linking adaptive and innate immunity.

P1.03.08

N6-isopentenyladenosine, an endogenous isoprenoid end product, directly affects cytotoxic and regulatory functions of human NK cells through FDPS modulation

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N6-isopentenyladenosine (iPA) is a naturally occurring nucleoside with an isopentenyl moiety derived from mevalonate pathway, and well established anti-tumor activity. In analogy to the unique specificity for phosphoantigens like isopentenyl pyrophosphate (IPP), shown by human Vγ9Vδ2 T cells, here we report for the first time the ability of iPA to selectively expand and directly target human natural killer (NK) cells. Interestingly, submicromolar doses of iPA stimulate resting human NK cells and synergize with IL-2 to induce a their robust activation ex vivo with significant secretion of RANTES and MIP-1α and a large increase in TNF-α and IFN-γ production when compared with IL-2 single cytokine treatment. Moreover iPA promotes NK cell proliferation, upregulates the expression of NK cell activating receptor NKp30, as well as CD69 and CD107a expression. Accordingly this phenotype correlates with significant greater cytotoxicity against tumor targets. At the molecular level, iPA leads to a selective potent activation of MAPK signaling intermediaries downstream IL-2 receptor. The effect results at least in part from the fine modulation of the farnesyl diphosphate synthase (FDPS) activity, the same enzyme implicated in the stimulation of the human γδ T cells. The iPA-driven modulation of FDPS, can cause an enhancement of post-translational prenylation essential for the biological activity of key proteins in NK signaling and effector functions, such as Ras. These unanticipated properties of iPA provide additional piece of evidence of the immunoregulatory role of the intermediates of the mevalonate pathway and open novel therapeutic perspectives for this molecule as an immune modulatory drug.

P1.03.09

NK cells in dasatinib-treated chronic myeloid leukemia patients are terminally differentiated effector cells lacking homing and KIR receptors

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Dasatinib is a BCR-ABL1 inhibitor with important immunomodulatory effects, including mobilization of NK cells in the peripheral blood. Our aim was to analyze whether the dasatinib-induced mobilization correlates with a characteristic trafficking and differentiation phenotype in NK cells.
Peripheral blood samples were obtained from 9 healthy donors and 14 dasatinib-treated CML patients before their daily drug dose and phenotyped with a comprehensive flow cytometry panel. The patients had been using dasatinib for 1-60 months (mean 17).
Dasatinib-treated CML patients have equal proportions of NK cells (CD3negCD56+) of total lymphocytes as healthy (mean 19.5 vs. 11.1%; p=0.51). NK cells from patients expressed lower proportions of the trafficking receptors CCR7 (6 vs. 30%; p=0.007), CD62L (25.6 vs. 50.4%; p=0.037), and CD11b (23.9 vs. 79.5%; p=0.0002). Further, a lower frequency of the activation and proliferation markers CD27 (5.1 vs. 19.5; p=0.009), CD28 (0.4 vs. 3.3; p=0.001), and CD45RO (1.7% vs. 6.2%; p=0.030) were observed in the patients. Also, the patients had a lower frequency of NK cell receptors; NKp30 (33.4 vs. 75.7%; p=0.003), CD158i (9.1% vs. 32.9%; p=0.023), CD158d (1.1% vs. 10.9%; 0.007), NKG2D (67.8% vs. 87.4%; p=0.013), NKG2A (19.4% vs. 42.7%; p=0.023), NKB1 (6.3 vs. 18.4%; p=0.005) and CD161 (29.2 vs. 68.9%; p=0.002). Conversely, the frequency of CD57+ (61.6 vs. 35.3; p=0.018) NK cells was higher in patients than in healthy donors.
In summary, NK cells in dasatinib-treated patients are similar to chronically activated NK cells, characterized by terminally differentiated effector cells lacking homing and KIR receptors.

P1.03.10

Induction of functional NK receptors on peripheral blood CD8+ T cells

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It has been shown for some time that a small subset of CD8+ T cells can express NK receptors (NKR) under specific conditions. However, the signals that might lead to that acquisition, the expression pattern and the role of NK activating receptors in T cells is a barely explored field in tumor immunology. We showed that IL-15 could induce or up-regulate certain activating receptors on peripheral blood CD8+ T cells, after long-term culture. Importantly, besides the acquisition of an NK-like phenotype, we found those receptors to be functional, namely leading to significantly increased levels of degranulation and IFN-gamma production after triggering. Those results suggest IL-15 as a signal capable of driving CD8+ T cells into a differentiation program towards the acquisition of functional NK receptors. Therefore, by potentially displaying dual innate and adaptive immune functions, CD8+ NKR+ T cells appear as promising for immunotherapy strategies against cancer.

P1.03.11

Receptors of NK cells and lymphocytes subsets in patients stage II and III breast cancer

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BACKGROUND. Breast Cancer is a global health problem. It is the most frequently diagnosed cancer in women, accounting for approximately 30% of all cancer diagnosed and about 16% of all cancer deaths. Breast cancer is a heterogeneous disease with a wide range of clinical presentations. NK cells are important during immunity response in cancer. There are different molecular and genetic factors; however, it remains to be elucidated whether immunological factors could be intervening in the clinical stage.
OBJECTIVE. Evaluate NK cell activating receptors and peripheral T and B cell percentage in patients stage II and III breast cancer.
METHODS. Breast cancer biopsies and peripheral whole blood were taken from all participants with prior written informed consent. Participants with breast cancer stage II and III histologically confirmed were enrolled in the study. NKp30, NKp46 and NKG2D receptors were measured by flow cytometry gating peripheral CD56+ cell population. Lymphocyte subsets were identified by stain of CD3+CD4+, CD3+CD8+, and CD19+ markers and were represented as positive cell percentage.
RESULTS. Fluorescence median intensity of NK cell activating receptors did not show any stastical diference in both groups. However, we observed that NKp46 tended to decrease.
CONCLUSIONS. Our preliminar results revealed a tendency to decrease in NKp46. To evaluate with more confidence the role of these receptors and cells it will be necessary to increase the patient in the study to demonstrate whether NK cell activating receptors could actually represent an useful immunological factor in breast cancer.

P1.03.12
An approach to the immunophenotypic signature of selected CD4+NKG2D+ T cell subsets

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Background. Why do some CD4+ T cells exhibit cytotoxic behavior? This has been an important question resolved, in part, by the fact that these cells express the activating immunoreceptor NKG2D, which has traditionally been restricted to NK and CD8+ T cells. Contradictory evidence has tagged, however, this particular population with an immuno-suppressive role in cancer patients. These apparently confounding data have led to the proposal that two different CD4+NKG2D+ T cell subsets might be operating. Currently, there is still a poor immunophenotype description of these cells. Objective. To better characterize the phenotype expressed by the CD4+NKG2D+ T cell subset in healthy volunteers. Methods. Multicolor flow cytometry was used for exploration of TCRalpha/beta, CD28, CD158b, CD45RO, HLA-DR, CD161, and CD107a on gated CD3+CD4+NKG2D+ T cells. ELISA was used to quantify TNF-alpha, IL-15, IL-10 and IFN-gamma. Results. The gated CD3+CD4+NKG2D+ T cell subset effectively defines a “classical” population of TCRalpha/beta-expressing CD4+ T cells in healthy individuals (n=30), which is not higher than 3% of the total CD3+CD4+ T population. The expression of CD45RO suggests a memory and/or activated state. The activating markers HLA-DR, CD161, and CD107a were heterogeneously expressed. The costimulatory receptor CD28 was also expressed on this subset. Interestingly, five individuals showed an increased number of CD3+CD4+NKG2D+ T cells, which were mostly negative for both CD28 and activating markers. Conclusions. The present results reveal the existence of two separate CD4+NKG2D+ T cell subsets defined by the coexpression or absence of CD28, which might indicate a functional dichotomy within this particular subset.

P1.03.13
HMPV infection, immune evasion and its elimination

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Human Metapneumovirus (HMPV) has become one of the main viral pathogens responsible for acute respiratory tract infections in children but also in the elderly and immuno-compromised patients. The pathogen most closely related to HMPV is human respiratory syncytial virus (HRSV), the most common cause of bronchiolitis and pneumonia in young children. HMPV has been classified into two main viral groups A and B and has a seasonal distribution in temperate countries with most cases occurring in winter and spring. Previous works showed that HMPV induces strong innate immune response in BALB/c mice as compared with RSV. In our study, we interested to test the HMPV reactivity to Natural Killer cells. As a start of this work we detected that HMPV infects A549 cells. Following this infection, cell-surface expression of CEACAM1 was detected 48 hours post infection. Our results also show an increase in MHC-Class I levels in the infected cells. Unlike previous results that show an increase of the inhibitory ligands, we also observed an increase of the activating NK receptor-(NCR1) ligands. Our finding raise a question what is the mechanism by which two opposite ligands are activated?.

P1.03.14
Neuroblastoma-derived transforming growth factor (TGF) β1 modulates the chemokine receptor repertoire of human resting Natural Killer Cells.

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In this study we showed that chemokine receptor repertoire of human resting Natural Killer (NK) cells is altered by neuroblastoma (NB) cell conditioning. In particular, NB-conditioning resulted in the upregulation of CXCR4 and CXCR3 in all NK cells and in down-regulation of CX_3CR1 in the $CD56^{dim}$ subset. The expression of other chemokine receptors such as CXCR1 and CCR7 remained unaltered. NB-conditioned medium was analyzed for the presence of up to 50 soluble mediators using a Multiplex Assay. This analysis proposed a number of NB-derived soluble factors being possibly involved in the modulation of CXCR4, CXCR3 and CX_3CR1 in NK cells. Ab-mediated blocking of these factors in NB/NK cell cocultures revealed that the modulatory effect was dependent on TGFβ1. Moreover rTGFβ1 (and rTGFβ2) induced a chemokine receptor repertoire identical to that of neuroblastoma-conditioned NK cells. The TGFβ1 appears to work in a dose-dependent way since low-intermediate amounts of the cytokine were sufficient to modulate CXCR4, CX_3CR1 and CXCR3 expression while high amounts were necessary to down-regulate the expression of the NKp30 activating receptor. Finally, the analysis of NK cells from patients with stage 4 NB suggests that neuroblastoma conditioning could exert, *in vivo*, an immune modulatory similar to that emerged from *in vitro* experiments. Altogether our data propose a novel tumor escape-mechanism based on the modulation of chemokine receptors that play pivotal roles in NK cells bone marrow homing, egress or recruitment into peripheral tissues.

P1.03.15
Related Human Cytomegalovirus Genes US18 and US20 Target MICA for Lysosomal Degradation

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NK cells play a crucial role in controlling human cytomegalovirus (HCMV) infection. The activating receptor NKG2D is expressed by all NK cells and binds 8 different ligands: MICA and MICB, and ULBP 1-6. In its turn, HCMV is known to encode at least 3 genes that target NKG2D ligands (i) UL16 sequesters MICB, ULBP1, 2, 4 and 6 in the ER (ii) UL142 retains MICA in the Golgi and (iii) the microRNA miR-UL112 suppresses MICB expression . Our studies using viral deletion mutants revealed that HCMV encoded functions, in addition to UL142, that targeted MICA. MICA expression during virus infection could be rescued by by lysosomal inhibitors. The function was first mapped to the US18-22 gene region by screening HCMV mutants with deletions in genomic ‘blocks’, and then to both US18 and US20 using a panel of Ad recombinants. The two genes were capable of acting independently, yet in combination clearly co-operated in both the suppression of MICA expression and NK cell activation. US18 and US20 are both members of the US12 gene family. All 12 members of the US12 family are arranged consecutively on the genome and constitute an ancient viral genomic expansion or ‘accordion’ potentially selected to enhance HCMV immune evasion. This gene family is defined by 7 transmembrane-spanning domains and have homology with the cellular TMBIMs (transmembrane bax-inhibitor 1 motif-containing proteins), excepting US19. This study provides the first insight into the function of the US12 gene family and potentially for its expansion in primate cytomegaloviruses.

P1.03.16
Abundant stage-dependent Ly49E expression by liver NK cells is not essential for their differentiation and function

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The NK receptor Ly49E has several unique characteristics. Unlike most NK receptors, Ly49E is highly expressed on fetal NK cells, whereas expression is decreased on bone marrow-derived NK cells in adult mice. To investigate a possible role for Ly49E in NK cell differentiation and function, we have generated an Ly49E KO mouse. Our results show that bone marrow and splenic NK cells are present in normal numbers in Ly49E KO mice, expressing an unaltered panel of NK receptors and differentiation markers. Furthermore, cytokine production and cytotoxicity by these cells is unaffected. Surprisingly, WT DX5-negative liver NK cells express high Ly49E levels, and this both in fetal and adult mice. $Ly49E^{+}DX5^{-}$ liver NK cells transferred into *Rag-2*$^{-/-}$*/gc*$^{-/-}$ mice maintain high Ly49E expression in the liver and differentiate into $DX5^{+}$ NK cells in spleen and bone marrow. Ly49E expression is not crucial for liver NK cell differentiation during ontogeny, as the $DX5^{-}/DX5^{+}$ ratio, the NK receptor repertoire, and the granzyme B and TRAIL levels are comparable in Ly49E KO versus WT mice, except for lower TRAIL expression on $DX5^{-}$ liver NK cells in 20-day-old mice. The TRAIL-, perforin- and FasL-mediated cytolysis by liver NK cells is unaffected in Ly49E KO mice. Collectively, we show that in addition to high Ly49E expression on fetal NK cells versus low Ly49E expression on conventional NK cells in adult life, Ly49E remains highly expressed on DX5-negative liver NK cells. However, Ly49E expression does not have a crucial role in differentiation and/or function of these NK cells.

P1.03.17
Inhibition of Glycogen Synthase Kinase-3 increases NKG2D Ligand MICA expression and sensitivity to NK cell-mediated cytotoxicity in Multiple Myeloma cells: role of STAT3.

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Engagement of NKG2D and DNAM-1 receptors with their ligands on lymphocytes plays an important role for anticancer response and represents an interesting therapeutic target for pharmacological modulation. In this study, we investigated the effect of inhibitors targeting the Glycogen Synthase Kinase-3 (GSK3) on the expression of NKG2D and DNAM-1 ligands in human MM (Multiple Myeloma) cells. GSK3 is a serine-threonine kinase point of convergence of numerous cell-signaling pathways, able to regulate the proliferation and survival of cancer cells, including MM. We found that inhibition of GSK3 is able to up-regulate MICA protein surface, mRNA expression and promoter activity in MM cells without significant effects on the expression of MICB and DNAM-1 ligand PVR/CD155. Moreover, exposure to GSK-3 inhibitors renders myeloma cells more efficient to activate NK cell degranulation and trigger NK cell-mediated cytotoxicity. We could exclude that two transcription factors specifically inhibited by active GSK3, β-catenin or Heat Shock Factor-1 (HSF-1), are involved in the up-regulation of MICA expression by using RNA-interference or viral transduction of constitutive active forms. On the contrary, inhibition of GSK3 correlated with a significant downregulation of STAT3 activation, a negative regulator of MICA transcription. Both Tyr^{705} phosphorylation and in vivo binding of STAT3 on MICA promoter are reduced by drugs targeting GSK3; in addition, overexpression of a constitutively active form of STAT3 significantly inhibits MICA upregulation. Thus, we provide evidence that regulation of the NKG2D-ligand MICA expression may represent an additional mechanism supporting the anti-myeloma activity of GSK3 inhibitors, and suggest their possible immunotherapeutic value.

P1.03.18
Do NK cells play a role in the possible association between natalizumab treatment and the development of melanoma?

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Natalizumab (NTZ) is an anti-alfa4integrin (CD49d) antibody approved for treatment of multiple sclerosis (MS). A possible association between NTZ treatment and higher risk of melanoma is under debate. As NK cells (NK), which contribute to controlling melanoma development, express CD49d, we assessed if NK-mediated killing of melanoma cells and melanoma-driven NK migration are impaired upon exposure to NTZ. CD49d and its receptor, CD106, were similarly expressed on NK freshly isolated or in-vitro expanded (bulk NK) from healthy donors (HD). Their expression did not differ between HD and untreated MS patients; both were also expressed on melanoma cells. Upon exposure to NTZ, CD49d expression on bulk HD NK decreased, but NK cytotoxic activity towards melanoma, measured by degranulation (staining for CD107a), was not affected, either when NTZ was added to bulk NK/melanoma co-cultures or when bulk NK were pre-exposed to NTZ for 12 hours. Bulk NK generated from NTZ-treated MS patients retained the decrease on CD49d expression observed before in-vitro expansion. Preliminary data on bulk NK generated from MS patients before and after treatment indicate that NTZ treatment did not affect the cytotoxic activity of NK from these patients towards melanoma, suggesting that the possible increase in melanoma incidence in NTZ-treated MS patients might not be related to impairment of NK cytotoxic activity towards melanoma. Consequently, we are investigating the possibility that NTZ treatment might impair the ability of NK to migrate towards melanoma and are developing an endothelial barrier model to evaluate the effect of NTZ on melanoma-driven NK migration.

P1.03.19
Elucidating the role of the natural killer cell receptor, Ncr1, in controlling melanoma metastases using in vivo reflectance confocal microscopy

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Background: Natural killer (NK) cells play a crucial role in early host responses against viruses, bacteria and malignancies mainly by killing of infected/transformed cells. NK cell activity is controlled by various inhibitory and activating receptors. Among the latter are proteins such as the natural cytotoxicity receptors [NCR (NKp44, NKp30 and NKp46)]. NKp46 is expressed almost exclusively on NK cells, and it is the only NK cell receptor with a mouse orthologue, the Ncr1.
Several publications have demonstrated the critical role of NKp46/Ncr1 in the eradication of various tumors including melanoma. However, the mechanism by which Ncr1 operates in-vivo is yet unclear. In-vivo reflectance confocal microscopy (RCM) is a novel technology enabling non-invasive visualization and mapping of time kinetics of changes in the structure of malignant skin lesions.
Objectives To investigate the mechanism by which Ncr1 is involved in melanoma development In-vivo using RCM.
Materials and methods: Using B16F10.9 tumors and an Ncr1 knockout mice we investigated the development of primary and metastatic melanoma by various in vitro and in vivo assays that includes visual inspection and RCM.
Results: Ncr1 was found to control tumor size and metastases in a dose dependent manner. Interestingly, high frequency of metastases was observed in the absence of Ncr1 even when the primary tumors demonstrated similar size features. Absence of Ncr1 also correlates to more aggressive structural RCM indices of the developing tumors in the absence of Ncr1.
Conclusions: Ncr1 is involved in the structural modulation of B16 melanoma and consequently in its metastases development.

P1.03.20
The receptor KIR3DS1 expressed by NK cell is associated with HCV viral clearance

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Background: HCV treatment, leads to a massive activation of the innate immunity response due to the fact that IFN-α is a potent activator of NK cells. NK cell inhibitory receptors (KIRs) are associated either with activating or inhibiting NK cell action. The objective was to evaluate the influence of the presence of KIR-3DS1 on HCV treatment response in HIV/HCV genotype 1 co-infected patients.
Methods: KIR, HLA-B and the IL28B gene were genotyped. Reductions of plasma HCV RNA levels between baseline and week 1, week 2 and week 4 were analyzed for IL28B genotype and KIR3DS1. Rapid and sustained virological response (RVR and SVR) rates were also analyzed.
Results: Sixty HIV/HCV genotype 1 co-infected patients were included. Patients with KIR3DS1 and Bw4 had higher rates of HCV viral decline than those who were not carriers of KIR3DS1 (week1: p=0.01; week2: p=0.038; week 4: p=0.03). Patients carrying KIR3DS1/Bw4 had higher rates of RVR and SVR than those not carrying KIR3DS1 (RVR: 46.15% *versus* 17.02%, p= 0.012; SVR: 63.6% *versus* 13 26.5%, p = 0.031). With respect to patients carrying the IL28B-CC genotype, those with KIR3DS1/Bw4 had greater rates of HCV viral clearance (week1: p < 0.001; week2: p= 0.01; week 4: p = 0.02), RVR (%) and SVR (%) than those not carrying KIR3DS1.
Conclusion: Our results show that the KIR3DS1/Bw4 genotype has a positive effect on HCV viral clearance during the first weeks of Peg-IFN/RBV treatment in HCV/HCV co-infected patients bearing genotype 1, and higher RVR and SVR rates.

P1.03.21
Measurement of natural killer cell apoptotic inducing lytic proteins in peripheral blood mononuclear cells and isolated natural killer cells

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Natural Killer (NK) cells are immune cells responsible for protecting the body from infection and malignancy. Cytotoxic NK cells contain secretory granules which store and release apoptotic inducing proteins such as perforin, granzyme A (GrzA) and granzyme B (GrzB). The aim of this study was to measure lytic proteins in samples of peripheral blood mononuclear cells (PBMCs) and isolated NK cells to determine if there is a difference in lytic protein expression. PBMCs from 19 healthy participants (mean age: 31±7.2SD) were isolated using density gradient centrifugation and negative isolation was used to separate the NK cells. Intracellular staining determined the content of perforin, GrzA and GrzB via flow cytometry in control and K562 stimulated samples. Statistical analysis included a repeated measures analysis of variance and significance was set at p<0.05. No significant changes were identified in lytic protein content in the control and K562 stimulated samples between the PBMCs and isolated NK cells. A significant increase in lytic proteins was observed in the isolated NK cells. The lymphocyte composition of the PBMC samples may account for this increase. NK cells only comprise of 15±8% of total circulating lymphocytes in the PBMC sample. Compared to the number of NK cells present in the isolated sample, there were fewer NK cells in the PBMC sample, translating to reduced levels of lytic proteins. Measurement of lytic proteins provides a direct measurement of NK cytotoxic activity and it may be beneficial for identifying cytotoxic activity deficiencies in the clinical setting.

P1.03.22
Functional NK cells developed from human hematopoietic stem cell in human interleukin-2 transgenic NOG mice

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We generated human interleukin-2 (hIL-2) transgenic NOD/Shi-*scid IL2rg*^{null} (NOG) mouse. We investigated human lymphocyte development *in vivo* in this hIL-2 transgenic NOG (hIL-2-NOG) strain after xeno-transplantation of human hematopoietic stem cells (HSC).
hIL-2-NOG and NOG mice were irradiated and intravenously inoculated with 2-5 x 10^4 cord-blood derived human CD34$^+$ HSC. After HSC transplantation, human cells in the peripheral blood and the various immune tissues of these HSC transferred mice were analyzed. Various developmental stages of human NK cells were detected, and especially, mature CD56$^+$ NK cell population was rapidly increased in hu-HSC-hIL-2-NOG mice. Human NK cells of hu-HSC-hIL-2-NOG mice consisted of both CD56^{high}CD16$^+$ and CD56^{high}CD16$^-$ cell populations, whereas most NK cells in human peripheral blood were CD56^{dull}CD16$^+$. The human NK cells of hu-HSC-hIL-2-NOG mice expressed various NK cell-specific surface markers involving activating and inhibitory receptors, such as NKp46, CD94, NKG2A, NKG2D and KIRs (Killer cell Immunoglobulin-like Receptors). Regarding cytotoxic activity of NK cells against K562, killing activity of purified human NK cells from hu-HSC-hIL-2-NOG were lower than that of normal human NK cells, but enhanced following stimulation with human cytokine for 2 days. Production of cytotoxic granule (perforin and granzyme A) and IFNg was detected in human NK cells of hu-HSC-hIL-2-NOG mice. Tumor growth in hu-HSC-hIL-2-NOG mice subcutaneously transplanted K562 was suppressed, but in hu-HSC-NOG mice was not.
These data suggest that hIL-2-NOG mice will become a useful tool for analyzing human NK cell development and function in vivo.

P1.03.23
Multi-step defect in NK cells and acute myeloid leukemia interaction

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Natural Killer cells (NKs) are candidates for acute myeloid leukemia (AML) immunotherapy since the demonstration of their ability to reduce relapse. Most cytotoxic activity of NKs is assured by direct killing via the release of lytic granules content into the contact area with target cells. However, down-regulation of activating receptors on NKs associated with a reduced cytotoxicity was shown in AML patients.
In this work in vitro study using AML cell lines (HL60 and THP1), AML patients' blasts and NKs from healthy unrelated individuals showed that receptors down-regulation requires NK/target contact and is associated with conjugate formation.
Contact-to-death kinetics in NK/target conjugates were calculated in time-lapse experiments where dying tumor cells were detected by emergence of a nucleic acid staining. Comparison between the highly NK-susceptible target K562 and HL60 showed no difference.
NKs synapses formed with AML or K562 cells were evaluated in conjugates showing opposite flat cell membranes and F-actin polymerization. NK cells showed proper recruitment of adhesion molecule LFA-1. However, we observed defective polarization of lytic granules with AML cell lines and patients' blasts, independently of HLA class-I expression. In addition, reduced recruitment of the adaptor protein CD3ζ suggested a defective activating signaling.
Attempts to correct NK/AML synapse defects using IL-2 or IL-15 activated NKs augmented conjugate formation but did not affect granules polarization. Unlike pre-treatment of AML cell lines with the immunomodulating molecule lenalidomide which significantly increased the lytic granules polarization. This last result justifies further studies to use immunomodulating drugs to improve NK-cell immunotherapy efficacy.

P1.03.24
Differential requirements of activating and inhibitory Ly49 receptors in recognition of MCMV-infected cells

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NK cells have a prominent role in control of cytomegalovirus (CMV)infection during the time preceding the induction of the adaptive immune response. In order to prevent recognition by CD8 T cells, CMVs encode proteins that down-regulate expression of MHC-I molecules from the cell surface, which render infected cells prone to 'missing-self'-mediated killing by NK cells. We have shown that murine CMV (MCMV) developed a mechanism to prevent NK-cell activation by escaping 'missing-self' recognition. MCMV m04 protein binds to newly synthesized MHC-I molecules and escorts them to the cell surface to engage NK-cell inhibitory Ly49 receptors. Moreover, we provided evidence that several activating Ly49 receptors also depend on presence of m04 for their own recognition of infected cells. However, presence of m04 is necessary, but not sufficient for the recognition of infected cells by these activating Ly49 receptors. Beside m04, another unidentified viral factor encoded by gene in the region of m167-m170 is required for their triggering. Transcriptome analysis of m167-m170 region revealed the presence of a single, antisense, highly abundant 1.7 kb long transcript, which encodes protein of 17 kDa that corresponded to ORF m169. So far, we have no evidence that m169 protein represents the third component of MCMV required for m04/MHC-I-dependent recognition of infected cells by activating Ly49 receptors. Our results indicate that activating and inhibitory Ly49 receptors have differential requirements for m04/MHC-I-dependent recognition of MCMV-infected cells, suggesting more stringent conditions for allowing the activation of NK cells.

P1.03.25
Spinal Cord Injury-induced Immune Depression Syndrome - the role of NK and T cells

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The nervous and the immune systems interact in a bidirectional way. The nervous system "hardwires" the lymphatic organs and the immune system relays information to the CNS through cognate receptors.
This interplay between these "megasystems" becomes evident in case of lesions in the CNS as well as spinal cord injury (SCI). Following such an insult a downregulation of the immune system has been observed. This phenomenon is called CNS Injury-Induced Immune Depression Syndrome (CIDS) (Meisel 2005). In the frame of SCI, a fluctuation of several leukocyte subclasses has been observed during the first week after acute lesion both in humans and experimental model. This downregulation of the immune system is thought to underlie the increased susceptibility of these patients to infectious diseases. Nevertheless, this susceptibility remains elevated in SCI patients beyond the acute period, being the main cause of morbidity and mortality among SCI patients during the post-acute period post-lesion.
Based on the hypothesis that a functional deficit in certain classes of leukocyte subpopulations might be underlying the increased susceptibility to infections, NK cells have been identified as potential mediators of this immune depression.
Using a SCI transection model in rat, we perform a longitudinal analysis of the fluctuations of peripheral blood and immune relevant organs T and NK cells as well as immunophenotypic changes and capacity to produce IFN-γ and TNF-α along time. These measurements are performed by flow cytometry in 5 different time points: on day 1, 3, 7, 14 and 28 after spinal cord contusion.

P1.03.26
Cytomegalovirus regulation of mouse PVR

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Cytomegaloviruses (CMVs) are well known for their capacity to regulate the expression of cellular ligands of different immune receptors. Human CMV blocks the surface expression of CD155 (PVR) molecule, which serves as a ligand for activating receptors CD226 (DNAM-1) and CD96 (TACTILE), conserved between mice and humans and both expressed on NK cells and T cells. PVR is also a ligand for the inhibitory receptor, TIGIT. Here we demonstrate that mouse CMV (MCMV) negatively regulates PVR.
We showed significant upregulation of PVR mRNA after MCMV infection. In contrast, surface PVR was downregulated on infected cells, indicating the active role of MCMV in the control of PVR surface expression. We have identified two viral gene regions responsible: deletion of either m154 or m20 resulted in the rescuing of PVR on the surface. When both regions were deleted, upregulation of surface PVR was observed. Our results also indicated that MCMV inhibitors affect the maturation of PVR, rather than its active removal from the surface. We showed that m20 gene region is responsible for the ER retention of PVR, whereas the mechanism by which m154 affects the expression of PVR remained elusive. Both mature and retained PVR protein forms are subject to the proteasomal degradation route. We found that the transcriptional profile of m20 region is very complex, with multiple overlapping transcripts. We showed at least 3 transcripts which probably share 3' terminus and are differentially temporally expressed. The lack of any of the PVR regulators results in viral attenuation in vivo.

P1.03.27
Regulatory roles of novel NK-like CD56^{bright}CD11c^{+} cells on expansion of γδ T cells and memory CD8+ T cells

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Human γδ T cells and memory CD8+ T cells have recently received much attention in cancer immunotherapy. However, it is well known that the responses of γδ T cells to antigenic stimulation are diminished in vitro as well as in vivo in some cancer patient. In this study, we examined the cellular mechanism underlying the regulation of human γδ T cells expansion and memory CD8+ T cells, focusing on the effect of IL-18 on CD56^{bright}CD11c^{+} cells. IL-18 and IL-2 induced the generation and proliferation of CD56^{bright}CD11c^{+}cells in the culture of CD14^{+} monocytes and CD56^{int}CD11c^{+}cells even in the absence of antigenic stimulation. The NK-like CD56^{bright}CD11c^{+}cells had the capacity to promote expansion of zoledronic acid (ZOL)-stimulated γδ T cells,and also to support the proliferation of CD8+ T cells. On the other hand, pDCs and IFN-α appeared to suppress the development of CD56^{bright}CD11c^{+} cells, finally leading to impaired expansion of γδ T cells and suppressed the proliferation of CD8+ T cells. The addition of mature CD56^{bright}CD11c^{+} cells to the culture overcame the regulatory effect of pDCs. These results demonstrate that CD56^{bright}CD11c^{+} cells and pDCs are involved in the regulation of γδ T cells and memory CD8+ T cells responses to antigenic stimulation. The manner of positive regulation by NK-like CD56^{bright}CD11c^{+}cells were dependent on IL-18.

P1.03.28

Structure of the human activating receptor NKp65 bound to its keratinocyte ligand KACL (CLEC2A) reveals basis for genetically linked recognition in the natural killer gene complex

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The natural killer gene complex (NKC) encodes numerous C-type lectin-like receptors that govern the activity of natural killer (NK) cells. While some of these receptors (Ly49s, NKG2D, CD94/NKG2A) recognize MHC or MHC-like molecules, others (Nkrp1, NKRP1A, NKp80, NKp65) instead bind C-type lectin-like ligands to which they genetically linked in the NKC. To understand the basis for this recognition, we determined the structure of human NKp65, an activating receptor implicated in the immunosurveillance of skin, in complex with its NKC-encoded keratinocyte ligand KACL. Whereas KACL forms a homodimer resembling other C-type lectin family dimers (Ly49, CD69), NKp65 is monomeric. The KACL homodimer engages NKp65 bivalently, such that each KACL subunit constitutes an independent binding site for NKp65 and each NKp65 molecule makes identical contacts with KACL. This bivalent binding mode is completely distinct from those utilized by NKG2D, CD94/NKG2A or Ly49A to recognize MHC or MHC-like ligands. The structure explains the exceptionally high affinity of the NKp65-KACL interaction (KD = 6.7 × 10-10 M) compared with other interactions involving cell-cell recognition molecules, which may compensate for the monomeric nature of NKp65 to achieve NK cell activation. The NKp65-KACL complex provides the first binding toplogy template for genetically linked recognition within the NKC that can now be applied to other NKC-encoded receptor-ligand pairs (Nkrp1f-Clr-g, Nkrp1d-Clr-b, NKRP1A-LLT1, NKp80-AICL).

P1.03.29

Crosstalk between Dendritic Cells and NK cells in the Immune Response against the dimorphic fungus Paracoccidioides brasiliensis

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In a previous study we have demonstrated that NK cells can participate in the immune response in human Paracoccidioidomycosis (PCM). Recently, it have been shown that dendritic cells (DCs) and NK cells can interact, increasing their activities. The aim of this study was to evaluated whether DCs stimulated by Paracoccidioides brasiliensis (Pb) yeast cells are able to interact with NK cells, and how this interaction influence the activation, cytokine production and cytotoxic activity of NK cells against Pb. Our results showed that monocytes-derived DCs stimulated by Pb yeast cells produce cytokines (IL-12, IL-18, IL-23 and membrane associated IL-15), which are important for the activation of NK cells. NK stimulated by these cytokines or cocultured with DCs (previously stimulated by Pb yeast cells) presented increased expression of activation markers (CD25 and CD69), direct fungicidal capacity and augmented production of cytokines such as IFN-gamma and TNF-alpha. Furthermore, the interaction of DCs and NK cells leaded to the proliferation of NK cells (particularly the subpopulation expressing high levels of CD56 - CD56bright). These effects were dependent on the production of IL-15 and IL-12 by DCs, once the blockage of these cytokines abolished these responses. In clonclusion, our results demonstrate that the crosstalk between DCs and NK cells can influence the activity of NK cells, augmenting their cytotoxic activity and cytokine production, and hence can modulate the subsequent acquired immune response observed in PCM.

P1.03.30

Interleukin-21 promotes the differentiation of a population of natural killer lymphocytes expressing CD86 and HLA-DR with the ability to activate naive CD4 T cells

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Natural killer (NK) cell cytotoxic functions are involved in viral and cancer immunity. Moreover, NK-derived IFN-γ modulates CD4 T cell responses by promoting IL-12 production by dendritic cells and TH1 polarization. Here, we investigated the potential role of IL-21 in NK and CD4 T lymphocyte cross-talk.
In a first set of experiments, NK cell from healthy donors were cultured with IL-2 or IL-2 and IL-21 during 7 days. The transcriptome was analyzed and an increase of co-stimulatory molecules, CIITA and MHC class II molecules was observed. In addition, an over-expression of CD62L was observed suggesting an immunomodulating role of these cells. The expression of CD86 and HLA-DR on NK cells induced by IL-21 is dependent on the STAT3 pathway.
Surprisingly, these NK-CD86+ cells express NKp46 and NKp30 but are devoid of cytotoxic functions although they derived from the highly cytotoxic CD56bright NK cell population. Then, the ability of NK-CD86+ to activate CD4 T lymphocytes was investigated. NK-CD86+ cells were loaded with peptides and cultured with specific CD4 T cell clones. Interestingly, NK-CD86+ cells promote a particularly strong proliferation of specific CD4 T cells. Moreover, we have also shown that NK-CD86+ cells provide an effective co-stimulatory signal for naive CD4 T cell activation. In addition, these CD4 T cells activated in the presence of NK-CD86+ cells are maintained in central memory status. Unexpectedly, transwell experiments showed that this proliferation is not dependent on cell-cell contacts. Altogether, these results identified a new immunomodulatory NK cell population.

P1.03.31

NK cell reconstitution following manipulated or un-manipulated haploidentical hematopoietic stem cell transplantation in leukemia patients

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We analyzed the NK cell repertoire in leukemia patients transplanted from haploidentical donors comparing different graft preparations:
a) CD34+ or TCRαβ/CD19-depleted grafts in pediatric patients.
b) un-manipulated bone marrow in adults with post-transplant high dose cyclophosphamide (PT-CY BMT) to prevent graft vs host disease (GvHD).
Genetic, phenotypic and functional analyses have been performed to study the NK cell repertoire in the donor and in the recipient post-transplant.
At the 1st month post-transplant, NK cells in CD34+ grafts predominantly displayed an immature phenotype. Differently, in TCRαβ/CD19-depleted grafts we observed a high proportion of NK cells with a mature phenotype (CD56dim, KIR+/NKG2A- and CD57+) and a detectable alloreactive NK cell subset, when appropriate, suggesting the persistence of mature donor NK cells present in the graft; T lymphocytes were TCRγδ+.
We observed a fast immune reconstitution after PT-CY BMT: 1 month post-transplant, NK and T cells were 38% and 62% of lymphocytes, respectively. NK cells appeared phenotypically immature: CD56bright, CD94/NKG2A+, while CD16+, KIR+ and CD57+ cells were less represented. However, these NK cells displayed a normal cytolytic activity revealed by degranulation assay upon K562 stimulation. A more mature NK cell phenotype, could be frequently observed only several (6-12) months after transplant.
These preliminary data suggest that, in patients transplanted with TCRαβ/CD19-depleted cells, mature donor-derived NK cells, including alloreactive NK cells, persist at 1st month. Longer follow-up and larger group of patients are necessary to correlate these grafts

with clinical benefits in the control of leukemia relapse and infections and limited incidence of GvHD.

P1.03.32
Simultaneously expression of NKG2DL MICA/MICB and NKG2D receptor on human cervical cancer cells

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Cancer cells are known to secrete the stress molecules MICA and MICB that activate cytotoxicity by lymphocytes and NK cells through their NKG2D receptor as a mechanism of immunological defense. However, there is not know if cancer cells can also express this receptor as a possible mechanisms of depletion of NKG2DL MICA/MICB molecules and thus interfere with their immune recognition. The aim of this study was to analyse the expression of MICA and MICB and NKG2D receptor on human cervical cancer cells. CALO, INBL and Hela cell lines were evaluated by ELISA, Western Blot, FACS and immunocytochemestry to their capacity to express and secrete MICA and MICB and to be induced to proliferate by these molecules as well as to express their receptor NGK2D. Those cell lines produce and secrete the stress MICA and MICB as shown by Western Blot of lysed cells and by ELISA of their conditioned media. By Western Blot and flow cytometry we found that these cells also express the receptor NKG2D. When CALO, INBL and Hela were cultured with recombinant MICA and MICB they exhibited a dose dependent induction for their proliferation. By immunocytochemistry we also found that these cells express NKG2D. Our results show that tumor epithelial cells (non-leukocyte cells) can also express NKG2D receptor that was thought to be exclusive of NK and cytotoxic lymphocytes and could be considerate as a possible mechanism of immunological escape and of tumor growth induction.
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P1.03.33
Characterisation of chicken NK cell receptors

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Natural Killer (NK) cells are crucial for resistance to many diseases. Like mammals, chickens have two types of putative NK cell receptors (NKR), lectin-like or immunoglobulin (Ig)-like receptors.
There are only a few putative lectin-like NKR genes in the chicken genome, of which perhaps only one gene, BNK, present in the classical major histocompatibility complex (MHC), is an NKR. The putative ligand for BNK is Blec, another lectin-like gene in the MHC region next to BNK. BNK and Blec are most similar to NKR-P1 and its ligands LLT1 and clr, respectively. Using a bead binding assay, we identified a cell line which naturally expresses the BNK ligand that also stimulates a BNK reporter line. We now try to identify whether this ligand is Blec or not.
There are many chicken Ig-like receptor (CHIR) genes clustered on microchromosome 31 with features typical of human KIRs, LILRs and IgA Fc receptor. They have either one or two extracellular domains, and are activating, inhibitory, or bi-functional. We set up a typing method for CHIR-AB1 genes using reference strand-mediated conformational analysis (RSCA), originally used for high throughput genotyping of MHC genes. CHIR-AB1 genes are found throughout microchromosome 31, so they can act as genetic markers across the entire chromosome. We identified CHIR-AB1 haplotypes in experimental egg-layer lines, commercial broiler lines and a population of red jungle fowl, containing a variety of MHC haplotypes. We now investigate the effect of epistasis between MHC and CHIR on disease resistance and other traits.

P1.03.34
Dopaminergic D5 Receptor-mediated inhibition of NK cell proliferation and production of IFN-γ

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Dopamine is a catecholamine neurotransmitter which plays an important role in the regulation of the function of many effectory immune cells. However, there is no report yet regarding the possible regulatory role of DA in human NK cells. The present study investigated the expression of different dopamine receptors and the effect of their activation on human NK cells activity. D2, D3, D4 and D5 dopamine receptors were found to be expressed in resting NK cells. An increased level of these receptors was followed by IL-2 activation, with the predominant enhancement found for D5 receptor. The augmented expression of D5 receptor was correlated to the increased sensitivity of human activated NK cells to dopamine treatment and resulted in the immunosuppression of NK cells activity. In particular, we have observed a dose-dependent effect of dopamine that resulted in D5-mediated inhibition of IL-2 induced NK proliferation and IFN-γ production. IFN-γ synthesis under dopamine treatment was altered post-transcriptionally and dependent on the increased transcription of the known IFN-γ suppressor factor miR-29a in NK cells. We have also observed that the suppressive action of miR-29a is correlated to the D5-mediated inhibition of NF-κB transcription factors activity, in particular the blocking of nuclear translocation of p50 protein. Our findings demonstrated that both Il-2 induced proliferation and IFN-γ synthesis in human NK cells is regulated through D5 dopaminergic receptor, which may play an important role in physiological NK cells immunoregulation, as well as having a therapeutic value in diseases with uncontrolled NK cells activity.

P1.03.35
Helicobacter pylori increases NK cell response increasing ULBP2 surface expression in adenocarcinoma gastric cell lines

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ULBP2 is an inducible molecule expressed in stressed cells. This molecule is part of the NKG2D ligands (NKG2DL) and its recognition allows damage cells to be eliminated by Natural Killer (NK) cells. Modification in its expression by tumor cells is associated to immune evasion and tumor establishment. Helicobacter pylori (HP) infection on gastric cancer (GC) development has not been associated, but its influence in chronic inflammation has been established through TLR stimulation in innate immune response. Our aim was to describe the effect of HP in NKG2DL expression on gastric adenocarcinoma cell lines and evaluate its functional effect on NK cell response. For this purpose MKN-45 gastric adenocarcinoma cell line were incubated with Heat killed Helicobacter pylori (HKHP) and then were co-incubated with NK cells purified from healthy donors in order to performed a cytotoxic assay. ULBP-2 expression and NK cell activation were analyzed by flow cytometry. HP infection is highly prevalent and it has been associated to GC. In this work we demonstrate that HKHP induce ULBP2 surface expression in gastric adenocarcinoma cell lines, augmenting NK cell activation against them. By this way tumor cells could be triggering a chronic NK cell response, associated to NK cell anergy and immune evasion. Grant: Becas Apoyo Tesis doctoral 2411032, FONDECYT 1130330, and FONDEF CA12i10023, CONICYT.

P1.03.36
Adenosine produced by a CD38-mediated pathway in CD56^{bright} CD16^{-} NK cells inhibits autologous CD4^{+}T cell proliferation

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Recent studies have indicated that CD56^{bright}CD16^{-} natural killer (NK) cells play an important role in immunoregulation and autoimmunity by mechanisms that have yet to be elucidated. In this study, we

investigated the potential role of an extracellular nucleotide-metabolizing network that leads to production of adenosine (ADO), a multifunctional immunosuppressive nucleoside that binds to different specific receptors expressed by immune effector cells. The nucleotide-metabolizing ectoenzyme network includes the ADP-ribosyl cyclases CD38 and CD157, PC-1 [nucleotide pyrophosphatase/phosphodiesterase-1(ENPP-1)], CD39 (nucleoside triphosphate diphosphohydrolase-1) and CD73 (5'-nucleotidase). These ectoenzymes regulate the nucleotide/nucleoside balance and control the release of adenosine into the extracellular environment. We detected similar expression levels of CD38, CD39, CD73 and CD157 in peripheral blood $CD56^{dim}/CD16^{+}$ and $CD56^{bright}/CD16^{-}$ NK cells, whereas PC-1 was mostly expressed in $CD56^{dim}/CD16^{+}$ cells. $CD56^{bright}/CD16^{-}$ NK cells produced ADO when cultured in the presence of ATP, ADP (in progress), AMP or NAD^{+}. ADO inhibited the proliferation of autologous $CD4^{+}$ T cells. Pre-treatment with dipyridamole, which increases extracellular ADO by blocking ADO transporters, further inhibited $CD4^{+}$ T cell proliferation. On the contrary, ADO-mediated inhibition of autologous $CD4^{+}$ T cell proliferation was abrogated by blocking CD38 activity, but not by inhibiting CD39, CD73 or PC-1. These studies indicate that $CD56^{bright}/CD16^{-}$ NK cells can mediate immune regulation through ADO production, a process that requires the ADP-ribosyl cyclase CD38.

P1.03.37
Correlation between natural killer cells functionality and response to trastuzumab treatment in HER2+ breast cancer patients

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In the first decade of the 21st century, the model of breast cancer (BC) has been redefined from that of one disease to a heterogeneous group of related diseases. The approval of trastuzumab in 1998 has significantly improved patients' outcomes and opened the way for the beginning of advent of targeted approaches in breast cancer treatment. However, primary or acquired resistance to trastuzumab has been increasingly recognized as a major obstacle in the clinical management of this disease. The main objective of this project is to shed light on the mechanism responsible for the development of resistance to trastuzumab treatment in HER-2 positive breast cancer patients. We demonstrated that, after one year of trastuzumab administration, patients who became resistant to the treatment showed an impaired NK cells activity. Further we showed that NK cells activity is modulated through the control of immunoreceptors and kinase in a complex interaction between intracellular signaling pathways. In order to predict acquisition of trastuzumab resistance before treatment, we identified a different signature of immuno-receptors/kinase activation status related to the BC patients' outcome. These results opened the way to a more detailed therapy focused on patients' NK cells functionality.

P1.03.38
Modulation of activatory and inhibitory receptors of NK cells by CD137 costimulation

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Background: Activated T lymphocytes and NK cells express CD137 (4-1BB) a member of TNFR family however the extent of its contribution in NK cell biology is not fully revealed. Here we investigated the effect of CD137-CD137L interaction on the expression of stimulatory and inhibitory receptors of human activated NK cells.
Methods: The MCF-7 breast cancer cells were infected by recombinant non-replicative adenovirus vectors to express 4-1BBL transgene or GFP (as control). NK cells from Peripheral blood were negatively purified, activated with sub-optimal concentrations of IL-2 and IL-15 and cocultured with the infected MCF-7 cells. The expressions of activation markers (NKG2D, NCR2, CD69) and inhibitory receptor (NKG2A) were assessed afterward.
Result: Results showed significant up-regulation of the NK cell activatory receptors while the expression of the inhibitory receptor diminished consequent to 4-1BBL interaction. Compared to the NK cells activated with IL-2/IL-15, CD137 signal increased the expression of NKG2D from 35% to 42%, NCR2 from 23% to 39%, and CD69 from 50% to 59% (P =0.02). Although frequency of NKG2A expressing cells did not change significantly, normalized MFI of NKG2A expression on the NK cells reduced by CD137treatment (P=0.01).
Conclusion: CD137 costimulation may regulate NK cells' activity by differential governing the expression of activatory and inhibitory receptors.

P1.03.39
Natural killer cells in upper respiratory airway

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Natural killer (NK) cells are important lymphocytes for their anti-viral and anti-tumor functions. Accumulating knowledge suggests that NK cells in mucosal compartments are quite unique in their phenotype and function. However, little is known about NK cells in upper respiratory airway, especially in nasal cavity and salivary glands. Here, we present analysis of murine NK cells in nasal cavity and salivary glands.
In case of mice, NK cells in nasal cavity and salivary glands show unique expression pattern of surface markers including NK cell receptors and others related to maturation and activation. These NK cells show hyporesponsiveness to extracellular stimuli such as IL-12 and IL-18. However, nasal NK cells have shown to play indispensable role for nasal Influenza viral infection. As to salivary gland NK cells, their hyporesponsiveness to Cytomegalovirus infection has been reported. We have found other unique functions as well. In addition, we show some data obtained from human nasal and salivary gland samples.

P1.03.40
Expression of Ly6C defines two subsets of mature mouse NK cells

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NK cells are cytolytic and cytokine-producing effector cells that play crucial roles in the clearance of tumors and virus-infected cells. NK cells are classified into distinct populations according to the differentiation stages, but the roles of the each cell population and the factors that regulate the development and homeostasis of NK cells are unclear. In the present study, we report that mouse mature NK cells ($NK1.1^{+}/CD11b^{+}/CD3e^{-}$) in the spleen are subdivided into two populations based on the expression level of Ly6C, i.e. $Ly6C^{high}$ and $Ly6C^{low}$ subsets. We further show that these subsets are functionally distinct in their cytokine production and cytotoxic activity, i.e. IFNg production in response to IL-18 or IL-12 and cytotoxicity against the mouse T cell lymphoma Yac-1 were lower in $Ly6C^{high}$ NK cells than $Ly6C^{low}$ NK cells. In addition, $Ly6C^{high}$ NK cells were found in various tissues, including the liver, lung, peripheral blood and lymph node, but not in the bone marrow. Since NK cells develop in the bone marrow, $Ly6C^{high}$ NK cells were assumed to be differentiated from $Ly6C^{low}$ NK cells. Consistently, $Ly6C^{low}$ NK cells from the spleen were converted to $Ly6C^{high}$ NK cells upon transplantation. These results suggest that $Ly6C^{high}$ NK cells are mature cells in the peripheral tissues. While immune functions of the $Ly6C^{high}$ NK cells were lower than those of $Ly6C^{low}$ NK cells, $Ly6C^{high}$ NK cells became $Ly6C^{low}$ NK cells and produced more IFNg productions when stimulated with IL-15. These results collectively suggest plasticity of mature NK cells.

P1.03.41
Cervical cancer cells treated with EGCG inhibitor induces changes in IFN-gamma, TNF-alpha and perforin secretion in NK-92 cells

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Background: NK cells play an important role in virus-infected and tumor cells elimination. However the tumor cells are known to have several mechanisms as the expression of molecules that regulate the immune response like histidine decarboxilase (HDC). This enzyme may represent another resistance mechanism in cervical cancer cells.
Objectives: To analyze the HDC expression in HeLa, SiHa and C33A cervical cancer cells and to evaluate the IFN-gamma, TNF-alpha, granzyme-B and perforin NK-92 cells expression in tumor cells co-cultures.
Methods: The HPV+ HeLa and SiHa cervical cancer cell lines and HPV- C33A cancer cells were treated with EGCG (HDC inhibitor) and co-cultured with NK-92 cells at ratios 1:1, 1:10, 1:20 and 1:40. We evaluate the HDC expression in cancer cells and IFN-gamma, TNF-alpha, granzyme-B and perforin expression in NK-92 cells by flow cytometry.
Results: There are no significant changes in the expression of IFN-gamma, TNF-alpha, granzyme-B and perforin when the HeLa, SiHa and C33A cervical cancer cells were co-cultured with NK-92 cells.
Conclusions: The presence of the EGCG HDC inhibitor does not induce significant changes in IFN-gamma, TNF-alpha, granzyme-B and perforin expression in NK-92 cells, however there is a tendency to increase of IFN-gamma, TNF-alpha and granzyme-B expression when cervical cancer cells are treated with HDC inhibitor.

P1.03.42
Multidisciplinary approaches to enhance BRAF targeted therapy in Melanoma

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Mutations in the BRAF gene have been found in cancers, including melanoma in 40-50% of patients with melanoma disease. About 80% of the BRAF mutations carry the V600E mutation. These patients are treated with BRAF inhibitors alone or in combination with other cell signaling pathway inhibitors. However, a relapse of the disease is observed within 6-8 months after the commencement of the therapy. Some studies hint that NK cell based therapy can be effective in controlling melanoma. In this study multidisciplinary approaches were tested to understand the mechanism of Vemurafenib resistance by a BRAFV600E melanoma. Vemurafenib is a BRAFV600E inhibitor. Vemurafenib resistant melanoma cell lines were raised from the sensitive cell lines by drug treatment till 80-90% of cells showed resistance. Both the resistant and sensitive cell lines were tested in a NK cell cytotoxicity assay in vitro by standard 51Cr-release assay. The Vemurafenib resistant cell lines were found to have a reduced sensitivity to NK cell killing. Though there was no significant difference in expression of NK cell activating ligands on the surface between the resistant and sensitive cell lines, there was a tendency of higher expression of MHC class I on Vemurafenib resistant cell lines. BRAF exon15 sequencing shows no difference between Vemurafenib resistant and sensitive cell types. Experiments are in progress to understand the mechanism(s) of the reduced sensitivity of the Vemurafenib resistant cell lines to NK cell killing.

P1.03.43
KIR2DS1-dependent acquisition of CCR7 and migratory properties by human NK cells interacting with allogeneic HLA-C2+ DC or T cell blasts

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Natural killer (NK) cells may capture the CCR7 chemokine receptor from allogeneic $CCR7^+$ cells by a mechanism termed trogocytosis and acquire migrating properties in response to the CCL19/CCL21 lymph node chemokines. This event is negatively regulated by inhibitory KIRs and NKG2A. In this study, we analyzed the role of activating KIRs in the process of CCR7 uptake in NK cells interacting with different types of allogeneic $CCR7^+$ cells. Analysis of freshly isolated peripheral blood NK cells and NK cell clones revealed that in $KIR2DS1^+$ alloreactive NK cells, co-cultured with $HLA\text{-}C2^+$ $CCR7^+$ lymphoblastoid cell lines, the interaction of KIR2DS1 with HLA-C2 (its ligand) resulted in increased CCR7 uptake. We show that KIR2DS1 represents a remarkable advantage for the acquisition of CCR7 also from $HLA\text{-}C2^+$ allogeneic DC and T cell blasts because of its ability to override inhibition by NKG2A. These findings have important implications in haploidentical haematopoietic stem cell transplantation in which donor-derived (alloreactive) $KIR2DS1^+$ NK cells upon acquisition of CCR7 become able to migrate towards lymph nodes, where they may kill patient DCs and T cells, preventing graft-versus-host and host-versus-graft reactions.

P1.03.44
Regulation of NKG2D and DNAM-1 ligand expression in HCMV infected fibroblasts: role of the DNA damage response pathway and of viral replication

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Human cytomegalovirus (HCMV) evolved several strategies to evade NK and cytotoxic T cells, including the specific down-modulation of ligands of the activating receptors NKG2D and DNAM-1. Despite this, we believe that there is a window of opportunity to develop an adequate immune response, during which activating molecules can be expressed as a consequence of viral-induced stress response, and before immune-evasion events occurs. To this purpose we focused on MICA, ULBP3 (NKG2DL) and PVR (DNAM-1L) expression, and investigated the role of the DNA damage response (DDR) and its central mediator ATM, that is crucial in the regulation of NKG2DL and DNAM-1L expression. Human primary fibroblasts (HFF) infected with the AD169 strain significantly increased MICA, ULBP3 and PVR expression. However, if infected cells were treated with caffeine, an inhibitor of ATM/ATR kinases, ligand up-regulation was prevented, demonstrating that the DDR pathway was crucial in regulating these activating molecules also in the context of HCMV infection. Notably, DDR was necessary for HCMV productive replication as well, since little or no infectious virus was recovered from caffeine-treated cells. Ligand up-regulation was however observed to be HCMV replication-independent, as demonstrated by using Foscarnet, an inhibitor of viral DNA elongation, and related to HCMV-mediated exit from G0/G1 phases of the cell cycle. We are currently addressing the involvement of early viral proteins in ligand up-regulation capable of modulating early events responsible for an effective immune response.

P1.03.45
Deletion of IL-33 receptor reduces NK cell and CD8 T cell responses to herpesvirus infection

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Interleukin-33 (IL-33), a recently identified member of IL-1 cytokine family, serves as a ligand for the ST2 receptor (Schmitz et al, Immunity, 2005). Following pro-inflammatory stimulation, it is expressed in nonhematopoietic cells and released on cell necrosis as an alarmin. Although ST2 receptor is expressed by many cell types, its expression is highest on mast cells and activated T helper 2 (T_H2) cells where it participates in T_H2 immune responses by stimulating production of IL-5, IL-6, IL-13 and GM-CSF. NK and NKT cells also respond to IL-33 with increased IFN-γ production, suggesting its role in T_H1 immune response, as well (Smithgall et al, Int Immunol, 2008). Recently it was shown that IL-33 is necessary for CD8 T cell (CTL) responses to lymphocytic choriomeningitis virus (Bonilla et al, Science, 2012). We have studied the role of IL-33/ST2 signaling in murine cytomegalovirus (MCMV) infection and found that ST2-deficient mice had weaker NK cell and CTL responses to MCMV than control wild-type mice. These results suggest that IL-33 signals through ST2 receptor to amplify NK cell and effector T cell responses to virus infection. In spite of this, ST2-deficient mice showed normal MCMV control, indicating alternative mechanism induced in absence of ST2. Moreover, newborn ST2-deficient mice infected with MCMV showed much milder liver pathology compared to control mice, suggesting involvement of ST2 signaling in inflammatory response.

P1.03.46
Down-regulation of the NKG2D receptor is differentially controlled by MICA and ULBP2 ligands.

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The activating NKG2D receptor on human NK cells mediates “induced self recognition” in that its ligands are up-regulated by stressed or diseased cells. Evidence collected in the past years demonstrated that chronic exposure to NKG2DLs induces receptor down-modulation.
The aim of this study was to evaluate whether different NKG2D ligands (NKG2DLs), namely MICA and ULBP2, are equivalent in their capacities to down-modulate the surface receptor expression on human NK cells.
We analyzed the rate and kinetics of NKG2D down-modulation in primary cultured NK cells and in the NKL NK cell line upon stimulation with the Ba/F3 cell line stably overexpressing comparable levels of MICA or ULBP2 by FACS and fluorescence microscopic analysis. Although both ligands were able to reduce NKG2D expression, exposure to MICA over-expressing target cells resulted in a more rapid and efficient receptor down-modulation and in a more pronounced impairment of NKG2D-dependent cytotoxicity. MICA-experienced NK cells also showed a higher tyrosine phosphorylation of the ubiquitin ligase c-Cbl. Furthermore, ligand-induced receptor down-modulation affected the total NKG2D cellular levels, suggesting that the internalized receptor complexes were mainly subjected to degradation.
All together these results demonstrate that NKG2D down-regulation is influenced by the nature of its ligand and suggest a different contribution of the ubiquitin pathway in the control of NKG2D internalization and degradation in MICA- versus ULBP2-experienced cells.
Understanding the mechanisms of ligand-induced NKG2D down-modulation will be helpful to prevent evasion from NK cell-mediated immune response.

P1.03.47
Expression of ligands for NK cell receptors NKG2D and DNAM-1 on drug-induced senescent Multiple Myeloma cells depends on redox signaling

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Natural Killer (NK) cells represent a key component of anti-tumor immune response exhibiting cytotoxic functions and secreting several cytokines and chemokines. Particularly relevant for tumor cell recognition are the activating receptors NKG2D that binds MICA/B and ULBPs, and DNAM1 that recognizes nectin/nectin-like molecules (Nec2 and NecL5/PVR).
We have previously demonstrated that NK cells exhibit an enhanced cytotoxic activity against drug-induced senescent Multiple Myeloma (MM) cells due to an increased expression of NKG2DLs and DNAM1Ls. Little is known about the mechanisms underlying the up-regulation of these ligands induced by genotoxic agents.
The present study is focused on the identification of the molecular mechanisms underlying NKG2DL and DNAM-1L drug-induced expression on MM cells with particular attention to the role played by changes in the cellular redox state.
Our data indicate that low doses of doxorubicin and melphalan can trigger the activation of DNA Damage Response (DDR) and up-regulate NKG2DLs and DNAM1Ls, both at protein and mRNA levels, in an ATM/ATR and Chk1/2-dependent and p53-independent manner. Exposure of MM cells to free radical scavenger N-Acetyl-L-Cysteine (NAC) abrogates DDR activation and senescent associated-cell cycle G2M arrest, induced by doxorubicin and melphalan. Moreover, drug-dependent NKG2DLs and DNAM1Ls induction is inhibited by NAC on both MM cell lines and patient CD138+ malignant plasma cells. Finally, we have demonstrated the involvement of E2F1 transcription factor in the redox signaling-dependent induction of MICA and PVR genes following drug treatment.
The finding of new players regulating NK cell activating ligands on tumor cells might provide novel immunotherapeutic tools.

P1.03.48
Tumour-Priming of Natural Killer Cells

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Natural killer (NK) cells play an important role in host protection against pathogens and tumours by means of cell cytotoxicity and cytokine secretion. Similar to other lymphocytes, NK cells need to be primed in order to acquire their full effector functions, overcome any inhibitory signals and exhibit natural cytotoxicity against target cells. NK cells can be primed *in vitro* by incubation with an activating cytokine such as IL-2 or a target cell expressing the appropriate combination and intensity of ligands. We have data suggesting that cytokine priming of NK cells is physiologically different from tumour priming, and that CD2-CD15 interactions are part of the tumour-priming signal. Building on this work, we aim to complete NK cell activation profiles after cytokine and tumour stimulation, and identify the second component of the tumour priming signal by studying NK cell tumour interactions. Using this knowledge of the key components of NK cell activation, we aim to develop a protocol for the artificial priming of NK cells using antibody-coated beads. Translated in to the clinic, this novel approach would resolve some of the major issues in current standard therapies including complications in manufacturing and the dose limiting cytotoxicity.

P1.03.49
Association of activating kir genes with the late-onset crohn's disease

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Crohn's Disease (CD) is a chronic inflammatory disease affecting gastro-intestinal tract in humans. It is believed that the genetic make-up of an individual plays an important role in the development of this disease. Several genetic determinants implicated in innate immunity (e.g., NOD2/CARD15, IL-23R, etc) have been shown to be associated with CD. It is noteworthy that due to haplotypic differences, humans differ from each other .The receptors encoded by these genes tend to increase immune activation status of an individual. We report here that the activating KIR genes can be associated with the development of the late-onset (adult) CD in humans.We have performed a case-control study using two ethnically distinct cohorts of adult CD patients. The presence or absence of the activating KIR genes was determined by PCR using gene-specific primers. The data were analyzed using Chi-square, uni- and multi-variate logistic regression with STATA software (version 10).Our results show some changes of activating KIR genes in the adult CD patients compared with those of healthy subjects

P1.03.50
In vitro culture with IL-15 increases NK cell degranulation and enhances NK cell-mediated dendritic cell maturation

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Natural Killer (NK) cells may play an important role in the immune response against hematological malignancies as demonstrated in the setting of stem cell transplantation. However, abnormal NK cytolytic function is observed in several hematological malignancies, including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Several mechanisms are involved in this abnormal function including a decreased expression of NKp46, NKp30 and DNAM-1 activating receptors.
The aim of this study was to restore the expression of NK cell activating receptors that are downregulated in patients with hematological malignancies. Therefore, we have analyzed in vitro the effect of IL-15, IL-2 and TGF-β on NK cell phenotype and function in healthy donors and in AML and MDS patients. Our results showed that, similar to IL-2, IL-15 increased the surface expression of NKp30 whereas the expression of NKp46 and DNAM-1 was not affected. In contrast, TGF-β induced a decrease of NKp30 and did not affect the expression of NKp46 and NKG2D. IL-15 activated NK cells displayed a higher degranulation in response to the erythroleukemic cell line K562. We also observed an increased capacity of IL-15 activated NK cells to induce dendritic cell (DC) maturation probably due to the increased expression of NKp30, a receptor involved in NK-DC interactions.
In conclusion, our in vitro model supports the role of IL-15 regulating NK cell cytotoxic function and improving NK cell capacity to induce DC maturation. Further studies are required for integrating IL-15 activated NK cells into NK cell-based immunotherapeutic strategies.

P1.03.51
Immunosuppressed pre-metastatic niches are caused by hypoxic tumour-derived factors

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Hypoxia is a common feature and poor prognostic factor in many solid cancers including breast cancer. Within the primary tumour, hypoxia acts as a strong selective pressure that promotes angiogenesis, invasion and metastatic spread of tumour cells. The pro-tumourigenic properties of future metastatic sites are determined by the interactions between factors secreted by the primary tumour and bone marrow-derived immune cells, which drives formation of pre-metastatic niches before the arrival of tumour cells.
Here, we demonstrate in different immune competent, syngeneic orthotopic breast cancer models, that primary tumour hypoxia promotes pre-metastatic niche formation. Cell-free supernatant derived from hypoxic breast tumour cells results in increased bone marrow-derived cell infiltration into the lungs and leads to increased metastatic burden in breast and melanoma experimental metastasis models; suggesting reduced immune surveillance in pre-metastatic niches.
We define bone marrow-derived $CD11b^{+}/Ly6C^{med}/Ly6G^{+}$ myeloid and $CD3^{-}/NK1.1^{+}$ NK cell lineages as main constituents of the pre-metastatic niche, and show that NK cell cytotoxicity and maturity is decreased. The $CD11b^{+}/Ly6C^{med}/Ly6G^{+}$ population is defined as a granulocytic subset of myeloid cells with increased secretion of IL-1β and TNF; whose mobilisation and recruitment to the pre-metastatic niche is controlled by the systemic soluble factor MCP-1/CCL2 produced by hypoxic breast cancer cells. Furthermore, this subset shows potential to suppress NK cell function, creating an immune-suppressed environment allowing for tumour cell expansion and metastatic growth. This data demonstrates a pivotal role for tumour-derived factors to orchestrate the recruitment and activity of bone marrow-derived cells to create an immunosuppressed pre-metastatic niche.

P1.03.52
Characterization of activated and expanded natural killer cells for the use in cancer therapy

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Natural killer (NK) cells detect and eliminate tumor cells and can be used for immunotherapy of cancer. Ex vivo NK cell expansion is an option to improve their clinical utility (cell number, functionality) and here we investigated the effect of the expansion process on the NK cell phenotype.
Different mechanisms for NK cell activation and expansion were used, including co-cultivation with feeder cells or magnetic beads (MACSiBeads) loaded with antibodies against NK cell activating receptors. 500-3000-fold NK cell expansion after two weeks was reached by co-cultivation with feeder cells. Using MACSiBead-particles loaded with anti-CD2/CD335 antibodies resulted in an 8-47-fold NK cell proliferation. Primary and CD2/CD335 MACSiBead expanded NK cells of 24 donors were analyzed on the surfacome level by CSC technology in combination with total gene expression analysis. The combined proteotranscriptomic analysis revealed widespread qualitative and quantitative differences on the surfaceome level in-between primary and expanded NK cells. Genexpression analysis confirmed and amended the great differences between both populations. Out of the differentially expressed surface receptors in the gene expression and surfacome analysis, 30 markers were selected for investigation by flow cytometry. NK cells, expanded by loaded MACSiBeads or by co-cultivation with feeder cells, significantly change the expression of CD11b, CD39, CD45, CD54, CD56, CD71, CD83, CD109, CD314 and CD337.
Analysis of primary NK cells upon their activation and expansion ex vivo revealed significant differences indicating a change in their

function. Furthermore, the detected marker panels could be used for cellular barcoding of primary NK cells and their manufactured counterparts.

P1.03.53
Conserved cellular miRNA-viral mRNA interactions in influenza A/H1N1: a bioinformatical approach

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Influenza A virus (IAV) is a public health threat which causes epidemics and global pandemics. It encodes two major surface antigens: hemagglutinin (HA), which mediates viral entry into cells; and neuraminidase (NA), enables virion budding. Because of their targeting by the immune system, HA and NA are poorly conserved. MicroRNAs (miRNAs) are ~22 nt long non-coding RNAs which suppress gene expression post-transcriptionally by base pairing with a seed site on the targeted mRNA. Although viral miRNAs are known to target cellular genes, the existence of the opposite interaction between cellular miRNA and viral RNA remains a controversial issue. We decided to conduct a wide search to check whether seed sites of lung-expressed miRNAs are conserved in a high percentage of Influenza A/H1N1 HA and NA genes. For this purpose, we utilized the target prediction algorithm RNAHybrid, combined with our own script "miRNAflu", to process ~5500 HA and ~8600 NA sequences from various influenza A/H1N1 strains. This approach enables us to overcome the problem of genetic variation in the viral genes, and permits us to focus on interactions relevant to the vast majority of A/H1N1 influenza strains for further study and experimental validation. Our search has identified several candidate miRNAs, such as hsa-miR-34a, a tumor suppressor which was predicted to target the area encoding the proteolytic cleavage site of HA; and hsa-miR-15a, also a tumor suppressor, predicted to target viral NA.

P1.03.54
Improvement of NK cells functional activation after long-term IL-2 stimulation in vitro from ovarian neoplasia patients

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Introduction: NK cells are lymphocytes known by their ability to eliminate a variety of malignant cells without previous stimulation, in a process involving innate recognition by an array of stimulatory and inhibitory receptors. NK-like T lymphocytes has also been reported to eliminate tumor cells, but the process might involve innate or adaptive recognition. We evaluated the functional activation of NK and NK-like T cells, the expression of activating receptors DNAM-1, NKp30 and NKp44 (pre, short- and long-term IL-2 stimulated), from blood and ascites of ovarian neoplasia patients.
Results: NK functional activation of pre stimulated cells, seemed to be impaired as the disease develops, median/variation (Bng=13.86/ 2.25-21.78; Mlg=9.57/ 6.48-12.96; MlgMt=7.73/ 3.65-10.06; Asc=6.99/ 1.68-21.68). Short-term stimulation increased NK cells activation (Bng=24.19/ 9.52-57.03; Mlg=24.49/ 8.69-52.37; MlgMt=17.59/ 9.28-35.63; Asc=33.54/ 3.83-43.50). Long-term stimulation increased NK cells activation significantly ($p<0.001$) (Bng=58.04/ 40.39-74.48; Mlg=46.61/ 33.89-54.76). The percentage of the activating receptors on NK cells increased significantly ($p<0.05$) after long-term stimulation. NK-like T cells showed no activation on pre, short- and long-term stimulation.
Conclusion: The functional integrity of NK cells was impaired as ovarian malignancies develop. Long-term stimulation resulted in a higher number of functional NK cells compared to short-term, entitling this method for adoptive therapy. Long-term stimulation was efficient to up-regulate DNAM-1 activating receptor on NK cells, representing a way to overcome down-regulation demonstrated on patients with ovarian carcinoma. The lack of activation of NK-like T cells (pre, short- and long-term IL-2 stimulated) suggests that these cells are not activated through innate pathway but through adaptive pathway.

P1.03.55
Role of HLA-I molecules on NK cells' phenotype and functions

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TAP-deficiency is a rare disease characterized by chronic upper respiratory bacterial infections and granulomatous skin ulcers. It is caused by a mutation in the TAP-1 or TAP-2 gene, leading to a significantly low expression of MHC class I molecules on the cell surface, affecting the immune system functions notably Natural Killer (NK) cells. The interaction between NK cells' KIR (killer immunoglobulin-like receptors) and HLA class I molecules is known to play an important role in these cells' function and education. But the effect of HLA-I molecules on their receptors' acquisition is still not entirely elucidated.
Trying to give more insight to this question, we carried out a detailed analysis of different KIR, NKG2A, CD8 and CD57 in 7 TAP-deficient patients and a pannel of healthy donors (HD) using 15-color flow cytometry. Functional studies were performed (cytotoxicity, IFN-γ production and CD107a expression), followed by microarrays before and after their incubation with K562 cells.
Phenotyping showed a significant increase in KIR co-expression on CD8+NKG2A+ TAP-deficient NK cells compared to HD demonstrating an effect of HLA class I molecules' expression on their receptors' acquisition. Functional studies comfirmed NK cells' hyporesponsiveness in the studied patients except for one, whose functional NK cells present different recpetors' distribution compared HD.
As for the transcriptomic studies, microarrays revealed significant differences between HD and TAP-deficient NK cells before co-incubation, but a matched one after, an unexpected result for hyporesponsive NK cells.

P1.03.56
Impairment of natural killer cell function in HIV-1 infected individuals from Brazil

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In HIV infection, natural killer (NK) cells have shown potential in the control of viral replication and in the host protection against opportunistic infections. However, multiple studies have described several dysfunctions associated with NK cells in HIV-1 infected people. Thus, the objective of our work is to characterize the phenotype and function of NK cells from a group of Brazilian HIV-1 infected patients who have not started antiretroviral therapy. Control samples were obtained from healthy blood donors. Peripheral blood mononuclear cells were isolated from individuals by density gradient and NK cell frequency was determined by flow cytometry. NK cell was obtained by magnetic separation using CD56+ MicroBeads and its function was evaluated by cytotoxicity assay. Our preliminary results suggest a reduction in the frequency of NK cells (CD3-CD56+) from HIV-1 infected individuals compared to control ones. NK cell subpopulations determined according to CD56 and CD16 expression are also diminished in HIV-1 patients. NK cell from HIV-1 infected people showed lower percentage of cytotoxicity compared to control when analyzed in different effector:target cell ratio. Despite of having CD4+T cells above 300 cells/mm3, these HIV-1 patients showed impaired NK cell function that could contribute to their state of immunosupression. So, we suggest that by also analyzing NK cell function and phenotype, we could have a better perception of the individual immune defense system. Therefore, this study will contribute in the comprehension of immune mechanisms related to HIV-1 infection. Financial support: FAPESP.

P1.03.57
Influence of age and pro-inflammatory state on the frequency of NK cells subpopulations

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Several previous studies have show that NK cells are affected by the process of immunosenescence. In this study, we analyzed the effect of age and pro-inflammatory state on the frequency of NK cells subpopulations in elderly donors. We have also studied if CMV-infection affects the distribution of these subpopulations in young donors.
A total of 30 young donors (16 CMV-seropositive and 14 CMV-seronegative; age range 19-35) and 26 elderly donors (age range 72-91) were included. NK cell subpopulations defined by the expression of CD56 and CD16 were studied by multiparametric flow cytometry. CMV serostatus and the levels of C-reactive protein (CRP) were studied.
Our results did not show significant different in the frequency of NK cells from CMV+ and CMV- young donors, indicating that CMV infection seropositivity does not affect the frequency of NK cells in healthy young individuals.
An increased percentage of total NK cells was observed in elderly individuals compared with young. The percentage of CD56dimCD16+ subpopulation was maintained while the percentage CD56-CD16+ subpopulation was increased with age. The frequency of the CD56brightCD16- and CD56brightCD16low subpopulations was decreased. The frequency of CD56brightCD16- subpopulation in the elderly was inversely associated with the levels of CRP, indicating that the frequency of NK cell subpopulations changes with age and that these changes are more pronounced in elderly donors with pro-inflammatory state.

P1.03.58
NKG2D ligand expression in glomeruli correlates with renal NK cell infiltration in the MRL mouse model

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Natural killer (NK) cells are a major part of the immune system, and alterations in their activity have been correlated with various autoimmune diseases. The role of NK cells in the immunopathogenesis of systemic lupus erythematosus (SLE) is nonetheless poorly understood, with reports focusing mainly on NK cell reduction in the periphery of SLE patients. Using the MRL/MpJ and MRL/MpJlpr SLE-like murine models, we report here that NK cells are reduced only in the spleen and not in bone marrow of diseased mice. In addition, phenotypically immature NK cells accumulate in the spleen and bone marrow of diseased mice, which correlated with reduced degranulation of the CD107a protein. We also show a higher degree of NK cell infiltration in kidneys of diseased mice with a heightened glomerulonephritic state. The phenotype of kidney NK cells from diseased mice is more activated compared to healthy mice, suggesting a role for distinct microenvironments in different organs in regulating NK cells in this SLE-like model. Activatory receptor NKG2D ligands Rae-1 and Mult-1 were expressed in all MRL mouse glomeruli, and at higher levels in diseased mice. Increased NKG2D ligand expression in the glomeruli correlated with a higher number of NK and CD8+ cell glomerular infiltrate. These results suggest a role for NKG2D ligands and NK cells in the initiation and progression of glomerulonephritic lesions in SLE.

P1.03.59
Phenotypic and functional characterization of the CD56dimCD16lowNK cell subset in bone marrow and peripheral blood of healthy donors

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In this study, we have dissected the mature CD56 NK cell subsets based on the expression levels of CD16, one of the major NK cell activating receptor. We analyzed the phenotypic and functional profile of CD56brightCD16low, CD56brightCD16high, CD56dimCD16high and CD56dimCD16low NK cells in the peripheral blood (PB) and bone marrow (BM) from healthy donors who donated BM for transplantation. The results obtained indicated that CD56bright and CD56dimCD16low NK cells are more abundant in the BM with respect to the PB. Activating, inhibitory and cytokine receptors are expressed at lower levels on CD56dimCD16low NK cells with respect to CD56brightCD16low/high. CD56dimCD16low NK cells express higher levels of CXCR4, whereas have no detectable expression of CX3CR1 as compared to CD56dimCD16high NK cells. Unlike CD56dimCD16high, CD56dimCD16low NK cells, either from BM or PB, can release IFN-γ following cytokine stimulation and are the major cytotoxic NK cell population upon binding to K562 target cells as evaluated by degranulating or 51Cr release assay using sorted distinct NK cell subsets. CD56dimCD16low NK cells are able to degranulate also in response to NKp46 or NKp46 plus DNAM1 triggering although to a lesser extent with respect to CD56brightCD16high/low NK cells. Finally, sorted CD56dimCD16low NK cells acquire a CD56 and CD16 receptor profile similar to that of CD56brightCD16low/high following 7 day-culture in the presence of IL-12 plus IL-15.
All these data suggest that CD56dimCD16low NK cells are multifunctional cells that represent an intermediate differentiation stage between the CD56brightCD16low/high and CD56dimCD16high and can differentiate into CD56bright NK cells upon cytokine stimulation.

P1.03.60
CD28 and CTLA-4 are expressed by mouse NK cells and differentially control NK cell IFN-γ production in response to mature dendritic cells

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The receptors CD28 and CTLA-4 are important regulators of T cell activation, differentiation and survival. Triggering of CD28 co-stimulates T cell receptor-mediated signals. CTLA-4 is induced in T cells upon activation and inhibits T cell function. Accordingly, immunotherapies incorporating blocking anti-CTLA-4 mAbs improve the clinical outcome of certain cancer patients. The role of CD28 and CTLA-4 in NK cells is incompletely understood. Here, we show that activation of mouse NK cells with IL-2 leads to upregulation of CD28 and induction of CTLA-4. Other cytokines, such as IL-12 and TGF-β, further modulate CD28 and CTLA-4 expression in NK cells. Furthermore, using gene-deficient NK cells, we demonstrate that CD28 induces and CTLA-4 inhibits IFN-γ release in response to the recombinant ligand, B7-1, or to mature dendritic cells. Importantly, we show that mouse NK cells infiltrating solid tumors of different origin express CTLA-4 that could have implications for immunotherapies based on blocking anti-CTLA-4 mAbs.

P1.03.61
CRACC-CRACC interaction enhances NK cell-mediated liver injury *via* Kupffer cells

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CRACC is known as a critical activating receptor of NK cells. We previously reported a fulminant hepatitis model which is induced by Poly I:C/D-GalN and mediated by circulating NK cells. Since NKG2D is considered critical but not the only activating receptor in this model, we investigated the role of CRACC in this model. We found that CRACC was abundant on hepatic NK cells but with low degree of expression on Kupffer cells in normal condition. The CRACC expression on NK cells and also Kupffer cells was up-regulated remarkably by Poly I:C injection, and the ALT level of Poly I:C/D-GalN-treated mice was correlated to the increased CRACC mRNA expression of liver. After the expression of CRACC on Kupffer cells was specifically silenced by nano-particle encapsulated siRNA in vivo, the liver injury induced by Poly I:C/D-GalN was largely alleviated. In co-culture experiments, it was further verified that silencing CRACC expression or blockade of CRACC activation by mAb reduced the production of IFN-γ and TNF-α, a similar blocking effect on NKG2D-Rea1 recognition between NK cells and Kupffer cells by anti-NKG2D mAb. So, CRACC-CRACC interaction between circulating NK cells and resident Kupffer cells is also involved in Poly I:C/D-GalN-induced fulminate hepatitis.

P1.03.62
Immunoregulatory effects of Natural Killer cells in Multiple Sclerosis

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Objectives: Multiple sclerosis (MS) is an autoimmune neural disease with inflammation, demyelination, axonal/neuronal loss and gliosis. Although the ethiology of the disease is not defined clearly, there are findings indicate the contribution of the immune system. Natural Killer (NK) cells have immunoregulatory role in the pathogenesis of autoimmune diseases, but the actual role of NK cells in MS pathology is not clear. The frequency
and effector functions of NK cells in different MS subgroups were investigated.
Methods: Surface expression of CD3, CD4, CD8, CD19, CD16 and CD56 were detected in peripheral blood samples, and peripheral blood mononuclear cells were cultured for 24 and 72 h in the presence and absence of hrIL-2, hrIL-4 & hr IL-12. After 24 h, IFN-γ, IL-10 and IL-22 contents of $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$ NK cell subsets were measured by flow cytometry. NK cytotoxicity was measured using erythromyeloblastoid leukemia cell line K562, and the frequencies of NK cell subsets were detected after 72 h of culture.
Results: In comparison with healthy subjects, higher IL-10 and IL-22 levels in $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cell were detected in MS patients. Cytotoxic activity of NK cells was significantly decreased in untreated patients with relapsing-remitting MS and clinically isolated syndrome.
Discussion: Increased IL-10 secretion by NK cell subsets in MS patients revealed that IL-10 might have a suppressive effect such as inhibiting synthesis of pro-inflammatory cytokines. In addition, increased cytotoxic activity of NK cells in untreated patients may indicate that NK cells could be associated with autoimmunity.

P1.03.63
NK cell degranulation against human melanoma cell lines is correlated with the expression of NKG2D ligands

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The role of Natural Killer (NK) cells in immune surveillance against tumours has been recently underlined. Recent studies have demonstrated the expression of ligands for activating NK cell receptors in tumour cells from different histological origin. The fact that NK cells exert their cytotoxic function without previous sensitization to antigen places these cells as a key component of the immune response against cancer. Melanoma cells can be recognized and killed by NK cells, however previous studies did not clarify if there is a major activating receptor implicated in NK cell activation against melanoma. Here we analyze the role of different activating receptors in NK cell activation against a wide range of melanoma cell lines with different ligand expression levels. We found a positive correlation between NK cell activation and the expression on melanoma cells of MICA/B, ULBP2 and ULBP3, ligands for NKG2D, and CD155, a DNAM-1 ligand. Our results point to NKG2D as the most important NK cell activating receptor involved in NK cell response against this tumour, with a cooperation with DNAM-1 and Natural Cytotoxicity Receptors (NCRs). Thus, DNAM-1 and NCRs participated in the recognition of melanoma cell lines with low expression of NKG2D ligands. We have also observed that shedding of soluble forms of MICA/B constitutes an escape mechanism frequently used by melanoma cells to avoid NKG2D-mediated recognition. In conclusion, our results support the participation of multiple receptor-ligand interactions in NK cell response to melanoma with a pivotal role for NKG2D.

P1.03.64
Phenotypic Evaluation of NK and NKT-like cells in peptic ulcer and gastric adenocarcinoma caused by Helicobacter pylori infection

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Background: The role of mucosal immunity in developing Helicobacter pylori-mediated dyspeptic disorders such as peptic ulcer and gastric adenocarcinoma has been recently identified. The aim of this study was to compare the proportion of NK cells and NKT-like cells in dyspeptic disorders caused by H. pylori infection.
Materials and methods: In a case-control study, 27 patients with gastric carcinoma (GC), 25 patients with peptic ulcer (PUD), and 22 patients with non-ulcer dyspepsia (NUD) were enrolled. After endoscopic diagnosis, the proportions of NK cells subsets (CD3-CD56+ and CD3-CD16+), as well as NKT-like cells (CD3+CD56+) were determined using a four-color flow cytometry. The presence of CD8+ and CD8-NK cell subsets in peripheral blood of all groups was also determined. Data were analyzed by CELL QUEST software.
Results: The frequencies of CD3-CD16+NK, CD3-CD56+NK, and CD3+CD56+NKT-like cells in GC patients infected with H. pylori significantly were lowered than those in NUD subjects ($p<0.01$, $p=0.05$, and $p=0.02$, respectively). Further analysis showed that a high number of CD8-NK cells in the blood was CD56+ and CD16+ phenotypes.
Conclusion: Decreased numbers of NK Cell and NKT-like cell populations in patients with gastric adenocarcinoma compared to those with non-ulcer dyspepsia may confirm the role of these cells as effector cells in dyspeptic disorders caused by H. pylori infection.

P1.03.65
Fluorescent tagged NK cell receptors expressed in vivo using retrogenic mice to study the delivery of signals in NK cells

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NK cells display both activating and inhibitory receptors which recognize different ligands, and it is the integration of signals from these two classes of receptors that ultimately determines NK cell effector activity. Nevertheless, how exactly the signal integration occurs is still not well understood.
Our approach is to employ retrogenic mice to generate authentic primary NK cells expressing two fluorescent tagged receptors, NKG2D (short or long isoform) and Ly49A, prototypical activating and inhibitory NK cell receptors, respectively. The aim is to use NKG2D-GFP/Ly49A-RFP Forster resonance energy transfer (FRET) to study the co-localisation of these receptors in synapses at NK-target cell-cell contact interfaces. Also, we will test the consequences of altering ligand dimensions in the synapse formation and NK cell activation.
Our results show that GFP-NKG2D (short and long isoform) interacts with both adaptor molecules DAP10 and DAP12 in vitro, allowing efficient cell surface expression of fluorescent NKG2D. Also, we reconstituted irradiated C57BL/6 recipients with GFP-NKG2D transduced bone marrow cells from NKG2D-deficient C57BL/6 donor mice. Two weeks post reconstitution, we detected cell surface expression of GFP-NKG2D predominantly in NK cells. The functionality of the fluorescent receptors and their interaction with the adaptor proteins DAP10 and DAP12 are now being investigated in NK cells generated in vivo. Data on the functionality of the fluorescent receptors and the generation of NK cells in retrogenic mice will be presented.

P1.03.66
MiR-10b downregulates the stress-induced cell surface molecule MICB, a critical ligand for cancer cell recognition by natural killer cells

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Natural killer cells (NK) are a component of innate immunity well known for their potent ability to kill virus-infected or neoplastically transformed cells following stimulation of the NK cell receptor NKG2D. One of the various ligands of NKG2D is MICB, a stress-induced ligand that has been found to be upregulated on the surface of tumor cells. However, there is little knowledge about how this upregulation may occur or how it may be selected against in tumors as a mechanism of immune escape. Here, we report that the metastasis-associated microRNA (metastamir) miR-10b directly binds to the 3' untranslated region of MICB and downregulates its expression. Notably, antagonizing miR-10b action enhanced NKG2D-mediated killing of tumor cells in vitro and enhanced clearance of tumors in vivo. Conversely, overexpression of miR-10b downregulated MICB and impaired elimination of tumor cells. Together, our results define MICB as a novel immune target of miR-10b, implying a direct link between metastasis capability and immune escape from NK cells

P1.03.67
Phenotypic and functional assays based on NK cells to address the diagnosis of X-linked lymphoproliferative disease 1

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X-linked lymphoproliferative disease 1 (XLP1) is caused by mutations in SH2D1A (Xq25) encoding a 14kDa adaptor protein known as signaling lymphocyte activation molecule (SLAM)-associated protein (SAP). SAP is expressed in NK and T lymphocytes. When SAP is mutated, SLAM receptors (e.g. 2B4) recruit phosphatases leading to inhibitory instead of activating signaling. For diagnosis we combined SAP expression with cytotoxic assays to detect the paradoxic function of 2B4. We evaluated a group of twelve XLP1 patients in comparison to healthy controls. Intra-cytoplasmic staining of healthy PBL with anti-SAP 1C9 mAb showed a range of 4±36 mean ratio fluorescence intensity (MRFI). No staining was found in ten patients (MRFI=1). Interestingly, a weak staining (MRFI=3) was observed in one patient characterized by a missense mutation, described to shorten the half-life of the protein. Another patient showed a normal pattern of SAP expression (MRFI=8), as also confirmed by transiently transfecting HEK-293T cells with plasmid encoding this mutated SAP. A rapid functional assay was set-up using short term IL-2 activated PBMC effector cells against the FcγRc+ P815 target cells and anti-2B4 mAb (R-ADCC). Confirmatory assays was performed using purified NK effector cells expanded in culture. While in control cells anti-2B4 mAb always induced enhancement of killing, in XLP cells from all patients (including SAP+ sample) an inhibition was observed. Thus, 1C9 mAb can stain a mutated protein with abnormal function, indicating that both assays should be performed to correctly address the diagnosis. The status of female carrier was also studied.

P1.03.68
Establishment of a humanized mouse model to investigate the impact of mixed xenogeneic hematopoietic chimerism on the tolerance of human NK cells to pig cells

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Mixed hematopoietic chimerism permits durable tolerance of T, B and NK cells to xenoantigens in a rat to mouse bone marrow transplant model. However, it is unclear whether tolerance of human NK cells to pig xenoantigens can be induced by mixed hematopoietic chimerism. We assessed the tolerance of human NK cells towards pig cells in a humanized mice model with established pig and human mixed xenogeneic chimerism.
Pig and human mixed chimeras (MCs) were generated by injection of young pig bone marrow cells to irradiated pig cytokine (IL3, GMCSF and SCF) transgenic NOD-scid common gamma chain knockout (NSG) mice transplanted with cryopreserved human fetal thymic tissue under the kidney capsule. They received anti-CD2 antibody and human fetal liver CD34+ cells from the same donor. Hydrodynamic injection of plasmids encoding human IL-15 and Flt3L was performed to promote human NK cell reconstitution. About two weeks post-injection of plasmids, NK cells from MCs were analyzed for their cytotoxic responses to pig cells using a chromium release assay.
Expression of human IL-15 and Flt3L led to an increase in the total number of human NK cells in lymphoid tissues, including peripheral blood, liver, spleen and bone marrow on Day 12 post-injection. Splenic human NK cells from the plasmid-injected MCs were able to kill K562 cells and xenogeneic pig lymphoblasts with or without ex vivo stimulation by human IL-2. Preliminary data suggest that human NK cells from MCs are not tolerant to xenogenic pig cells in vitro following IL-2 activation.

P1.03.69
The human cytomegalovirus induces the CEACAM1 expression to escape immune elimination

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Human Cytomegalovirus (HCMV) employs numerous and diverse strategies to evade immune recognition and destruction. Here we show that HCMV induces CEACAM1 (Carcino Embryonic Antigen Cell Adhesion Molecule 1) expression on the cell surface of infected fibroblasts. The CEACAM1 induction was very rapid with induction of both short and long intracellular domain isoforms of CEACAM1, the latter comprising two ITIM (Immuno Tyrosine Inhibitory Motif) domains. Additionaly, the CEACAM1 upregulation was specific as it was not observed with fibroblasts infected with other dsDNA species such as Adeno and HSV viruses. Previous studies have established that CEACAM1 functions as an immunoinhibitory molecule via homotypic interactions and that those interactions suppress

CEACAM1 positive T cell and NK cell functions. Indeed we show that the upregulation of CEACAM1 on infected fibroblasts inhibited the cytotoxic activity of CEACAM1+ NK cells and further demonstrated that around 50% of all NK cells derived from various donors express CEACAM1 following activation. Finally we demonstrate that a component prepackaged into the HCMV viral particle directly triggers the CEACAM1 expression in infected cells and initial screening experiments ruled out several important viral components.

P1.03.70
The Rac activator DOCK2 regulates natural killer cell-mediated cytotoxicity through the lytic synapse formation

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Natural killer (NK) cells play an important role in protective immunity against viral infection and tumor progression, but also contribute to rejection of bone marrow grafts, via contact-dependent cytotoxicity. Ligation of activating NK receptors with their ligands expressed on target cells induces receptor clustering and actin reorganization at the interface, and triggers polarized movement of lytic granules to the contact site. Although activation of the small GTPase Rac has been implicated in NK cell-mediated cytotoxicity, its precise role and the upstream regulator remain elusive.
In this study, we show that DOCK2, an atypical guanine nucleotide exchange factor (GEF) for Rac, plays a key role in NK cell-mediated cytotoxicity. We found that, although DOCK2 deficiency in NK cells did not affect conjugate formation with target cells, DOCK2-deficienct NK cells failed to kill effectively leukemia cells in vitro and MHC class I-deficient bone marrow cells in vivo, regardless of the sorts of activating receptors. In DOCK2-deficient NK cells, NKG2D-mediated Rac activation was almost completely lost, resulting in a severe defect in the lytic synapse formation. Similar results were obtained when the Rac GEF activity of DOCK2 was selectively abrogated. These results indicate that DOCK2-Rac axis controls NK cell-mediated cytotoxicity through the lytic synapse formation.

P1.03.71
Dimerization of NKp46 receptor is essential for NKp46-mediated lysis: characterization of the dimerization site by epitope mapping

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NKp46 is a primary activating receptor of natural killer (NK) cells that is involved in lysis of target cells by NK cells. Previous studies showed that the membrane-proximal domain of NKp46 (NKp46D2) retained the binding of NKp46 to its ligands and is involved in lysis. We studied NKp46D2 by employing a peptide-based epitope mapping approach and identified an NKp46D2-derived linear epitope that inhibited NKp46-mediated lysis. The epitope, designated as pep4 (AA 136 to 155), interacted with NKp46 and lysis by NK cells was inhibited by the presence of pep4. Through modeling and mutagenesis we showed that it could be involved in NKp46 homodimerization. R145 and D147 contribute to the function of pep4 and R145Q mutation in recombinant NKp46 reduced its binding to target cells. At the cellular level, FRET analysis revealed that pep4 is indeed involved in dimerization of cell membrane-associated NKp46. We suggest that NKp46-derived pep4 site is part of the dimerization surface of NKp46 and that NKp46 dimerization contributes to NKp46-mediated lysis by NK cells.

P1.04 NKT cells

P1.04.01
CD2/CD3/CD28 stimulation augments Granzyme B-dependent tumor clearance of Ph+ B-Acute Lymphoblastic Leukemia by ex-vivo expanded human iNKT cells

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Innate invariant natural killer T (iNKT) cells are regulatory and cytotoxic and play critical roles in tumor surveillance. Here we report mechanisms to augment these effector functions in human CD3+Vα24+ iNKT cells. PBMC were stimulated with alpha-galactosylceramide (α-GalCer) and human IL-2 and IL-7. CD3+Vα24+ cells were sorted to >98% purity (day 7), co-cultured with irradiated allogeneic PBMC feeders with IL-2 and IL-7, and resorted at day 21 for functional assays including Luminex® 26-plex array, mRNA profiling, BADTA® cytotoxicity, and in vivo Nalm6 leukemia xenograft clearance under different iNKT stimulation conditions. iNKT cells robustly expanded (mean day 21 yield $6.7x10^6 \pm 0.8x10^6$ from $8.9x10^3 \pm 0.6x10^3$ day 0 iNKT) from n = 49 total blood donor apheresis units. Day 21 anti-CD2/CD3/CD28-stimulated iNKT cells expressed significant IL-4, IL-5, IL-13, GM-CSF and IFN-γ by Luminex® and high levels of GranzymeB and FasL by FACS and mRNA profiling. Day 21 iNKT cells displayed cytotoxicity against Ph+ Nalm6 B-ALL but not K562 myeloblasts in BADTA® assays which augmented by iNKT activation with anti-CD2/CD3/CD28 (mean±SEM 60.8%±2.8) versus α-GalCer (11.5%±5.0), when compared to unstimulated day 21 iNKT cells (11.0%±4.1). Inhibition of GranzymeB with Z-AAD-CMK abrogated iNKT cytotoxicity against Nalm6 in vitro (16.5% ± 2.8). Anti-CD2/CD3/CD28 stimulated day 21 iNKT cells without but not with Z-AAD-CMK pre-treatment induced clearance of Nalm6 xenografts by bioluminescent imaging compared to vehicle and unstimulated day 21 iNKT-treated controls. These findings identify a specific mechanism to optimize cytotoxic function of expanded human iNKT cells, identifying novel strategies to apply them in immunotherapy of high-risk B-ALL.

P1.04.02
Interaction between iNKT and B-1 cells after P.acnes adjuvant stimulus

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The invariant natural killer T lymphocytes and B1 cells participate in the early stages of the innate immunity migrating to inflammatory focus and modulating adaptative response. iNKT TCR recognizes glycosphingolipid presented via CD1d expressed in antigen presenting cells (APC), and is able to secrete a large number of cytokines. B1 are predominant in the peritoneal and pleural cavities, and simultaneously express macrophage and lymphocyte markers. B1 are able to derive into phagocitic cells, and also show APC function. The interaction between both cells is poorly understood, mainly under antigen or adjuvant stimuli. Killed-Propionibacterium acnes increases the absolute number of CD3+NK1.1+, B1 and macrophages in mice peritoneal cavity. Herein we studied the adjuvant effect of P. acnes on the interaction between B1 and iNKT. BALB/c, BALB/cXid and BALB/cJalpha18-/- were treated with one i.p. injection of killed-P. acnes or saline. After 24 hours, the peritoneal frequencies of B1 and iNKT were analysed by flow cytometry. We also evaluated CD1d expression on B1 and CD44 and CD69 in iNKT related with APC function and activation respectively.
P.acnes increased both iNKT and B1 cells in WT mice and also elevated iNKT CD69 expression when compared to controls. In absence of B1 bacteria didn't modify the absolute number of iNKT but increased its CD69 expression and decreased CD44 levels. In the absence of iNKT, P.acnes didn't increase the number of B1 or its CD1d expression. Our results suggest that the adjuvant effect of P.acnes in both iNKT and B1 depends on the interaction between them.

P1.04.03
Phenotypic and functional dynamics of invariant Natural Killer T ($CD3^+6B11^+$iNKT) cells in patients with tobacco-related oral carcinoma

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Invariant natural killer T (iNKT) cells are glycolipid-reactive T lymphocytes that share receptors and functions with natural killer (NK) cells, and reportedly play a pivotal role in various immune responses. We for the first time report abnormalities in iNKT cell, their proliferative response to lipid ligand, Th-like cytokine profile and antitumor response from patients with tobacco-related oral carcinoma (OSCC) using multi-color flow cytometry and MTT assays.
iNKT cells were significantly lower (p<0.004) in patients as compared to healthy donors and further showed significant decrease (p<0.01) in patients with late stage (III & IV) as compared to the early stage (I & II) tumors. The subsets analysis from peripheral blood revealed a marked decrease in $CD4^-CD8^-$ (double negative, DN) and concomitant increase in $CD8^+$ subset in patients as compared to healthy donors. OSCC patients showed impaired proliferative response to α-GalCer and reduced expression of IFN-γ and IL-4. However, *in vitro* activation with α-GalCer-pulsed DCs tended to restore both IFN-γ and IL-4-expression and increased cytotoxicity to human oral (SCC-4, KB) and breast cancer (MCF7) cell lines.
Results of the present study suggest that reduced frequency and skewed cytokine production by iNKT cells may be related to progression and dissemination of OSCC. Selective expansion and modulation of their functions by specific ligand/agonist may enhance their therapeutic potentials.

P1.04.04
Role of excessive thymic differentiation of RORgt+NKT17 cells in the pathogenesis of autoimmune diabetes

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Invariant NKT cells (iNKT) represent a unique subset of innate lymphocytes that play a crucial role in autoimmune diseases by exerting pro-inflammatory or regulatory functions. Conventional NKT cells act as regulatory cells in autoimmune diseases and their αGalCer-induced activation prevent pre-clinical models of Type 1 Diabetes (T1D) and Multiple Sclerosis (EAE). Recently, a new subset of NKT cells characterized by predominant secretion of IL-17 (NKT17 cells) and strong pro-inflammatory function was identified but its role in the pathogenesis of autoimmune diseases like T1D is still unclear. Here we show that the NOD mice have a significant increase in the percentages of NKT17 in the periphery (p<0.05) and in the thymus (p<0.001). The increase percentage of NKT17 in the NOD mice compared to control strains is particularly evident in the thymus where 30-40% of total αGC-CD1d-dim+ CD3+ NKT cells are IL-17+ (NKT17 cells) against 2-5% in the control strains. While conventional NKT cells down-regulate RORγt expression when maturing from stage 0 to stage 1 in the thymus, in the NOD mice a large percentage of thymic NKT cells maintain RORγt expression and are blocked in the maturation stage 1 (CD24-, CD44low). RORγt expression and NKT17 cell differentiation are negatively regulated by the transcription factor Th-POK. We are currently testing the hypothesis that epigenetic factors (miRNAs) that control Th-POK expression are altered in the NOD mice. Moreover, we are investigating whether the large number of NKT17 cells present in the periphery of NOD mice contributes to the pathogenesis of autoimmune diabetes.

P1.04.05
Interleukin-7 Receptor α-dependent thymopoiesis regulates thymic invariant natural killer T cell retention

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Invariant natural killer T cells (iNKT) represent an innate T cell lineage that differentiate in the thymus and are responsive towards lipid antigens. Seeding of the peripheral compartment is accompanied by gradual retention of mature iNKT cells in the thymus, however, the mechanisms governing thymic iNKT cell retention are poorly understood. We show that a chemotactic profile associated with active iNKT cell emigration is skewed towards retention in mice deficient for lymphotoxin β (LTβ). The requirement for LTβ was found to be non cell-autonomous and independent of LTαβ expression within the majority of mature hematopoietic and stromal cell lineages. Surprisingly, IL-7Rα-dependent fetal thymopoiesis was found to regulate iNKT cell retention in a manner coincident with secondary lymphoid organ formation. We propose that thymic iNKT cell retention in adult mice is a functional consequence of IL-7Rα-dependent thymopoiesis in the developing thymus organ.

P1.04.06
Difluoro substituted CD1d ligands as optimal adjuvants for the priming of multifunctional CD4 T cells

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The differentiation of naïve T cells to distinct effector populations is central to the development of effective immune responses. We developed an in vitro assay to study CD4 naïve T cell commitment to effector cell differentiation with the purpose of investigating the influence of different adjuvants. We analysed compounds known to have distinctive Th1:Th2 biasing influences and as well 2 novel CD1d glycolipid ligands, engineered for increased binding affinity and activation of human invariant NKT cells.
We studied the naïve T cell differentiation at day 10, in healthy controls following co-culture of whole blood with index adjuvants for 24 hours and transfer of WB supernatants to CD3/CD28 bead activated naïve CD4 T cells.
In the absence of adjuvant the default differentiation pathway was biased to Th1, with a Th1:Th2 ratio of 5:1 and a Th1:Th17 ratio of 25:1. In cultures with the toll like ligands R848 the Th1:Th2 ratio increased to 10:1 whilst the Th1:Th17 reduced to 20:1. The Th2 adjuvant alum generated a 1:1 Th1:Th2 ratio and 2:1 Th1:Th17 response. In the presence of the prototypic iNKT glycolipid, α-Galactosylceramide, a 3:1 Th1:Th2 and 2:1 Th1:Th17 response was seen. Finally, one of our novel difluoro glycolipid compounds (43) gave a unique profile with a 1:1 Th1:Th2 and 5:1 Th1:Th17 response whilst another (44) gave a enhanced pattern compared to α-GalCer with a 2:1 Th1:Th2 and 5:1 Th1/Th17 response. Biomarker correlates of novel adjuvants may provide a useful adjunct to vaccine design strategies for distinct pathogen targets requiring bespoke effector responses.

P1.04.07
Dynamics of iNKT cell-mediated provision of B cell help for antibody production

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Human invariant natural killer T (iNKT) cells can provide help for antibody production by B cells. We investigated the molecular interactions, ligand and cytokine requirements, and B cell subtypes affected during provision of human B cell help by expanded and

sorted $CD4^+$, $CD8\alpha^+$ and $CD4^-CD8\alpha^-$ iNKT cell subsets *in vitro*. All subsets of iNKT cells, in the absence of the iNKT cell agonist glycolipid, α-galactosylceramide (α-GC), induced IgG, IgM and IgA production by B cells within 10 days of co-culture. Antibody production was prevented by separation of the cells using transwell systems or by antibody blocking of CD1d, but not by blocking IL-4, IL-13 or CD40-CD154 interactions. Phenotypic analysis indicated that all iNKT cell subsets induced the expansion of marginal zone-like ($IgD^+IgM^+CD27^+$) unswitched memory B (MZB) cells but not switched memory B cells, suggesting that iNKT cells do not promote antibody isotype switching. All iNKT subsets induced the expression of CD40, CD69, and IL-21 receptor by B cells. A minority (~2%) of iNKT cells expressed follicular helper T cell (T_{FH}) markers including CXCR5, PD-1 and IL-21 but T_{FH} cells were not expanded by culture with B cells. Interestingly, addition of α-GC to the iNKT-B cell co-cultures did not lead to enhanced B cell activation in any of the above-mentioned assays. These data suggest that $CD4^+$, $CD8\alpha^+$ and $CD4^-CD8\alpha^-$ iNKT cells can activate and induce expansion of MZB cells and antibody production by isotype switched B cells by a mechanism that requires CD1d but not exogenous glycolipid antigen.

P1.04.08
iNKT cells can promote differentiation of B cells into cells with regulatory B cell phenotypes

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Human B cells express CD1d and therefore are likely to present glycolipid antigens to iNKT cells. We previously found that iNKT cells can provide help for antibody production by B cells *in vitro,* but that the iNKT cell agonist glycolipid, α-galactosylceramide (α-GC) was not required. Here we investigated if B cells could present α-GC to expanded iNKT cells *in vitro* and if α-GC-stimulated iNKT cells could reciprocally activate the B cells. Since iNKT cells comprise functionally-distinct subsets based on CD4 and CD8α expression, we analysed the effects of co-culturing sorted $CD4^+$, $CD8\alpha^+$ and $CD4^-CD8\alpha^-$ double negative (DN) iNKT cells with B cells. B cells were capable of presenting α-GC to $CD4^+$ and DN (and to a lesser degree $CD8\alpha^+$) iNKT cells resulting in IFN-γ, TNF-α, IL-4, IL-5 and IL-13 secretion by iNKT cells. However, B cells were 10-200-fold less efficient than DC at inducing cytokine production by iNKT cells. Culture of B cells with $CD4^+$ or DN iNKT cells resulted in the expansions of cells with regulatory B cell (B_{REG}; $CD1d^{hi}CD5^+$, $CD24^{hi}CD38^{hi}$) phenotypes and $CD4^+$ iNKT cells promoted IL-10 production by some B cells, without the need for α-GC. These results suggest that human iNKT cells can differentially control adaptive immune responses, depending on whether or not stimulatory glycolipid ligands are present. In the presence of α-GC, human B cells can weakly activate iNKT cells, resulting in Th1 and Th2 cytokine secretion. In the absence of α-GC, some iNKT cell subsets can promote differentiation of B cells into B_{REG} cells.

P1.04.09
Effective stimulation of invariant natural killer T cells by oligomannose-coated liposomes

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The invariant natural killer T (NKT) cells recognize glycolipid antigens presented by CD1d on antigen presenting cells, DCs, and α-galactosylceramide (αGC) has been used as an exogenous ligand for CD1d to stimulate NKT cells. Although systemic administration of αGC produces anti-tumor effects, soluble αGC induced anergy in NKT cells. Therefore, establishment of a method to deliver αGC adequately to DCs is required. Since DCs express number of mannose-recognized C-type lectins, decoration of αGC-formulated particles with oligomannose is promising approach for preferential delivery of αGC to DCs. However, such attempts have not yet been undertaken. The current study showed that oligomannose-decorated, αGC-formulated liposomes (αGC-OMLs) could activate NKT cells much more effectively both in vitro and in vivo than liposomes without coating oligomannose (αGC-BLs).

Splenocytes stimulated with αGC-OMLs in vitro produced higher levels of IFN-γ compared to those stimulated with αGC-BLs. The ratio of IFN-γ/IL-4 produced from an αGC-OML-treated cells was higher than those produced from αGC-BL-treated cells. αGC-OMLs were incorporated into splenic DCs and BMDCs more effectively than αGC-BLs. NKT cells stimulated with αGC-OML-treated BMDCs produced more significant levels of IFN-γ from NKT cells than αGC-BL-treated BMDC.
Systemic administration of αGC-OMLs led to modification of the kinetics of IFN-γ production in vivo. In addition, iNKT cells proliferated and expanded upon administration of αGC-OMLs much more extensively than with αGC-BLs or soluble αGC. Collectively, our results suggest that αGC-OMLs can be used as a preferential delivery system for lipid antigens to DCs to activate iNKT cells in vivo and ex vivo.

P1.04.10
Sterol carrier protein 2 is required for the maturation of iNKT cells and their stimulation by endogenous lipids

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The development and maturation of semi-invariant Natural Killer T (iNKT) cells rely on recognition of self-antigens presented by CD1d-restriction molecules within thymus. An important class of peroxisome-derived lipids are self-antigens required for generation of full iNKT cell repertoire.
The lipid transfer protein Sterol carrier protein 2 (SCP-2) shuttles lipids between peroxisomes, ER and mitochondria, and is involved in the synthesis of ether-bonded lipids. Mice lacking SCP-2 have reduced numbers of iNKT cells in thymus and in peripheral organs. Residual iNKT cells from Scp2-/- mice are functionally normal as well as the antigen-presentation capacity of thymocytes and dendritic cells. Endogenous lipids of Scp2-/- mice differ from those of wild-type mice and stimulate iNKT cells less efficiently than endogenous lipids from wild-type mice. The reduced iNKT cell number is the consequence of altered proliferation and increased apoptosis of maturing thymocytes causing partial block in selection processes. Thus, SCP-2 is important in shaping the lipid antigen repertoire required for the generation of normal numbers of iNKT cells.

P1.04.11
Octaarginine-modified liposomes enhance α-galactosylceramide based antitumor therapy

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Alpha-galactosylceramide (αGC), a lipid antigen presented on CD1d molecules, is predicted to have clinical applications as a new class adjuvant, because αGC strongly activates natural killer T (NKT) cells. Moreover, there is very little individual difference between T cell receptors of NKT cells, that is, the effect would be similar, irrespective of patient. Although αGC therapy has prominent advantages, an intravenous administration of soluble αGC did not result in measurable clinical benefits. Thus, an efficient delivery system is needed to achieve efficient αGC therapy by systemic injection. Here, we incorporated αGC into stearylated octaarginine-modified liposomes (R8-Lip) which is our original delivery system for vaccine, and investigated the effect of nanoparticulation. Unexpectedly, systemic administered R8-Lip incorporating αGC (αGC/R8-Lip) failed to improve the immune responses mediated by αGC compared with soluble αGC in vivo, although αGC/R8-Lip drastically enhanced αGC presentation on CD1d in antigen presenting cells in vitro. Thus, we optimized αGC/R8-Lip in vivo to overcome the inverse correlation. In optimization in vivo, we found that size control of liposome and R8-modification were critical for enhancement of the production of IFN-γ. The optimization led to accumulation of αGC/R8-Lip in the spleen and therapeutic effect against highly malignant B16 melanoma cells. The optimized αGC/R8-Lip also enhanced αGC presentation on CD1d in

antigen presenting cells and expanded the population of NKT cells. Herein, we shows that R8-Lip is a potent delivery system, and size control and R8-modification in liposomal construction are promising techniques for systemic αGC therapy.

P1.04.12
Roles of NKT cells in human gastric cancer

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Natural killer T (NKT) cells are a small subset of T lymphocytes that participate in both, innate and adaptive immune responses. Once activated, they release large amounts of cytokines that regulate cellular immune responses. Although NKT cells contribute to tumor immunosurveillance in several disease settings, they can also act as suppressors of anti-tumor immunity. In gastric cancer, the second most common cause of cancer death worldwide, the role of NKT cell functions still remains to be established. Using flow cytometry, we studied the frequency and cytokine production by circulating NKT cells in patients with gastric adenocarcinoma. The cytotoxic activity of NKT cells challenged with gastric adenocarcinoma cell lines was evaluated by CD107a degranulation assays. Patients with gastric cancer showed a significantly increased percentage of peripheral NKT cells. Upon PMA-Ionomycin stimulus, we detected a lower percentage of NKT cells that produce IFN-γ, while a higher frequency of NKT cells that secrete IL-10 was found in the blood of gastric cancer patients as compared to healthy donors. Peripheral NKT cells from patients presented compromised cytotoxic function towards gastric adenocarcinoma cells as compared to controls. Tumor infiltrating NKT cells could be detected, which also expressed IL-10. Our results suggest that, by promoting a tumor-associated suppressive microenvironment, the regulatory function of NKT cells in gastric cancer may dampen the anti-tumor immune response, thus contributing to tumor evasion. The mechanisms that trigger the regulatory effects of NKT cells in gastric adenocarcinoma are under our current investigation.
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P1.04.13
CD1d-restricted invariant natural killer T (iNKT) cells mediate airway inflammation through IL-25 and IL-18

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iNKT cells are characterized by their expression of an invariant T cell receptor (TCR) that recognizes glycolipid antigen presented by MHC class I-like molecule CD1d. Another distinguishing feature of iNKT cells is their promiscuous production of Th1, Th2, Th9 and Th17 cytokines. Here we identified iNKT cells consist from distinct functional subtypes based on the expression of IL-17 receptor B (IL-17RB), a receptor for IL-25/IL-17E: IL-17RB+ iNKT cells are “helper”-like cells and produce robust IL-13 and IL-9 with moderate IL-4, IL-10, IL-17A and IL-22 in response to IL-25, which is phenotypically and functionally distinct from classical IL-12-reactive IFN-γ-producing iNKT cells bearing CD122 without IL-17RB. In the in vivo experimental settings, IL-17RB+ iNKT cells play a crucial role in the induction of airway inflammation both of OVA (allergen) and of RSV (virus infection) model. Interestingly, however, the recruited and activated effector cells in a chronic phase were completely different between these two models: eosinophils and neutrophils in OVA, and macrophages in RSV. Through the elucidation of the mechanisms in a different type of activation of IL-17RB+ iNKT cells, IL-18 instead of IL-25 is responsible for the activation of these cells in RSV model, resulting in an acute and robust production of Th2 and Th17 cytokines. Our findings reveal that IL-17RB+ iNKT cells and leading effector cells as target cells of IL-25 and IL-18 in the pathogenesis of airway diseases, illustrating the clinical potential of severe exacerbation of developing asthma by IL-17RB+ iNKT cell-dependent cellular cascade targeted therapy in humans.

P1.04.14
Signal control of iNKT development and effector lineage differentiation

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The type I or invariant NKT iNKT) cells are among the first responders in various infectious and inflammatory settings and bridge innate and adaptive immunity. iNKT cells quickly producing numerous cytokines such as IFNγ, IL-17, IL-4, IL-13, and IL-10 to regulates innate and adaptive immune responses. iNKT cells contribute to the pathogenesis of allergy and asthma, autoimmune diseases, graft-versus-host disease, infections, cancer, and obesity as well as other diseases. While it is well established that the iVα14 T cell receptor and IL-15 receptor and numerous transcription factors are critically involved in iNKT cell development and effector lineage differentiation, the signaling events bridging the receptors and the transcription factors are poorly understood. We have recently demonstrated that diacylglycerol kinases α and ζ play crucial roles in iNKT cell development and that DGKα and ζ negatively control not only the well-known Ras-Erk1/2 and PKCθ-IKK-NFκB pathways but also mTOR signaling in T cells. We now reveal that mTOR signaling and its tight regulation play crucial roles in iNKT cells as deficiency of either mTOR or its regulator TSC1 profoundly impacts iNKT cell development and effector lineage differentiation.

P1.05 Innate lymphocytes

P1.05.01
Role of γδ T cells in neuronal injury

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Γδ T cells are an important innate source of IL-17. IL-17-producing γδ T cells were found at high frequency in brains of mice suffering central nervous system (CNS) injury. APC-derived IL-23 stabilizes and expands IL-17 production by γδ T cells. Toll-like receptors (TLRs) expressed in microglia, the brain’s resident macrophages, are involved in various CNS diseases. To investigate the crosstalk between microglia activated by TLRs, γδ T cells and neurons experiments in co-cultures of these cell types isolated from wildtype mice were performed. In a first step, IL-17+ γδ T cells generated in vitro were co-cultured with neurons. The presence of increasing numbers of IL-17+ γδ T cells but not supernatant alone or naive γδ T cells led to a time-dependent decrease of neuronal viability. Secondly, supernatants of microglia stimulated by ligands specific for TLR2, 4, 7 or 9 but not TLR3, induced activation of γδ T cells indicated by up-regulation of CD69 and CD25 as well as down-regulation of CD62L. Also, γδ T cells cultured with microglia-derived supernatant secreted high amounts of IL-17. Direct stimulation of γδ T cells with TLR ligands did not up-regulate CD69, CD25 or IL-17. In summary, TLR-activated microglia secrete factors promoting polarization towards IL-17+ γδ T cells. Direct cell-cell contact is necessary for neurotoxic effects mediated IL-17+ γδ T cells. The exact mechanism by which microglia recruit naïve and induce IL-17+ γδ T cells remains to be determined.

P1.05.02
IL-22 promotes bacteria-driven colon cancer

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There is now abundant evidence that chronic inflammation can promote tumorigenesis and patients with inflammatory bowel disease have an increased risk of developing colitis-associated cancer (CAC). Although inflammatory pathways that drive colitis are well-described

much less is known about the cellular and molecular mechanisms that induce and sustain cancerous lesions in the intestine.
Using a mouse model of CAC in which 129.RAG-/- mice were infected with Helicobacter hepaticus (Hh) and treated with the carcinogen 2-azoxymethane (AOM), we found that the treated mice develop invasive adenocarcinoma after 5 months, which was shown to be dependent on a colonic Innate Lymphoid Cell population (cILC). These cells accumulated in treated mice compared to uninfected controls and produced interleukin-17 (IL-17) and interleukin-22 (IL-22). Depletion of cILCs and blocking of IL-22 reversed established invasive adenocarcinoma and inflammation. Morever, we were able to show that IL-22 was driving STAT3 phosphorylation in colonic epithelial cells and promoted secretion of anti-microbial peptides as well as cell proliferation.
Transfer of regulatory T cells (Tregs) deficient for interleukin-10 markedly exacerbated the tumour-promoting inflammatory response suggesting dysregulated repair mechanisms may promote bacteria-driven cancer in a genetically susceptible background.

P1.05.03
B1 cells provide immunosurveillance of healthy and inflamed skin

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B1 cells are innate-like lymphocytes that provide important effector functions during infection and inflammation by rapidly secreting pro- and anti-inflammatory cytokines and efficiently mounting T cell-independent (TI) antibody responses. Despite their importance, however, the migratory paths, target organs, and trafficking molecules involved in B1 cell migration are poorly-defined. Our comparative studies in humans, mice, and sheep reveal that innate-like B1 cells are a so far uncharacterized part of the normal cutaneous immune system. In addition, we find that B1 cells, unlike conventional follicular B2 cells are efficiently deployed into the inflamed skin. Consistent with this, B1 cells and follicular B cells differentially express trafficking molecules. One such differentially expressed molecule is α4β1 integrin (VLA-4), which is required for B1 cell entry into the inflamed skin and other effector sites. As VCAM-1, the endothelial binding partner of α4β1 integrin, is upregulated in most inflammatory responses, the data suggest that B1 cells are universally recruited into sites of inflammation. In summary, the data support a model in which B1 cells, through the constitutive expression of inflammation-seeking homing molecules, are poised to migrate into barrier sites, such as the skin, and to sites of inflammation, where they rapidly provide innate effector functions, including production of TI antibodies, cytokines, and antimicrobial factors, and fulfill a so far unappreciated role in skin immunity.

P1.05.04
Activating receptor engagement elicits inflammatory signatures in RORγt+ innate lymphoid cells

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RORγt+ innate lymphoid cells (ILC) are crucial players of innate immune responses and represent a major source of IL-22, which has an important role in host defense against bacterial infection, mucosal wound healing, and intestinal epithelial cell homeostasis. Although expression of IL-22 by ILC was shown to be protective in inflammatory bowel disease (IBD), RORγt+ ILC can also produce pro-inflammatory cytokines and contribute to the pathogenesis of IBD. The signals required by RORγt+ ILC to express IL-22 and/or pro-inflammatory cytokines have been elucidated only partially. Here we show that RORγt+ ILC can directly sense the environment by the engagement of activating receptors (actR). Activating receptor triggering in RORγt+ ILC selectively activates a coordinated pro-inflammatory program, including TNF, while cytokine stimulation preferentially induces IL-22 expression. However, combined engagement of activating and cytokine receptors results in a strong synergistic effect. These data support the concept that actR+ RORγt+ ILC can be activated without cytokines and are able to switch between IL-22 or TNF production, depending on the triggering stimulus.

P1.05.05
The regulatory effect of IL-4 on the anti-tumor immune response mediated by human γδ T cells

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IL-4 has been shown to affect a number of immune processes such as Th cells differentiation and innate immune responses. However, the impact of IL-4 on γδ T cell responses remains unclear. In this study, we investigated the effects of IL-4 on the activation and proliferation of γδ T cell and the balance between Vδ1 T cell and Vδ2 T cell in human. We demonstrated that IL-4 inhibited the activation of γδ T cell in the presence of γδ TCR stimulation in a STAT6-dependent manner. However, IL-4 had growth-promoting effect on the activated γδ T cells. Furthermore, IL-4 polarized γδ T cells toward Vδ1 T cells, which in turn inhibited the growth of Vδ2 cells through the secretion of large amount of IL-10. In addtion, IL-4 can downregulate the expression of NKG2D of Vδ1 T cell. Compared to Vδ2 T cell, Vδ1 T cell secreted much less IFNγ and more IL-10 and had relatively low NKG2D expression in the presence of IL-4. Therefore, Vδ1 T cells bias weakened the overall anti-tumor immune response mediated by γδ T cells. Our results established for the first time a thoroughly negative regulatory role of IL-4 in γδ T cell-mediated anti-tumor immunity. Selective depletion of IL-4 or Vδ1 T cells in tumor tissue may be beneficial for tumor immune therapy.

P1.05.06
Intestinal epitherial NK cells produce α-defensin, but convetional NK cells do not

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NK cells are the innate lymphoid cells and retain functions such as anti-tumor and anti-viral activity. Recent studies show that $NKp46^+$ NK-like cells exist in lamina propria and play an important role to maintain gut homeostasis. There are several reports that NK-like cytolytic activity was confirmed in small intestinal IELs. However, the function of these NK-like IELs is still not known. This study aimed to reveal the role of NK-like IELs in the small intestine (si-NK). We prepared both si-NK and splenic NK cells (sp-NK) which defined as $CD3^-CD49b^+NK1.1^+$ cells from C57BL/6 mice. After fixation, we observed them under TEM. Thereafter we compared cytolytic activity of both NK cells. Finally, we compared the gene expressions of both NK cells by microarray. Morphologically, si-NK had more granules than sp-NK. The cytolytic activity of si-NK was lower than that of sp-NK. As a result of microarray, *Itgae* in si-NK (590-fold) and *vcam1* in sp-NK (1750-fold) were highly expressed respectively. No significant differences of gene expressions were found between si-NK and sp-NK in terms of cytolysis. A typical difference was the gene expressions of bactericidal peptides. The gene expressions of *Defa1* and *a3* in si-NK were much higher than those in sp-NK (1920 and 3350-fold) and furthermore, there were some cells stained with anti-αDef3 antibody in si-NK but not sp-NK subset. These results suggested that anti-microbial activity in addition to cytolytic activity is the new property of the function of si-NK.

P1.05.07
TCRγδ signal activation is required to overcome inhibition by ubiquitin ligase Cbl-b during γδT cell cytotoxicity

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T cell antigen receptor γδ (TCRγδ) and natural killer group 2, member D (NKG2D) are two crucial receptors for γδT cell cytotoxicity. Compelling evidences suggest that γδT cell cytotoxicity is TCRγδ dependent and can be costimulated by NKG2D. However, the molecular mechanism of underlying TCRγδ dependent activation of γδT cells remains unclear. In this study, we demonstrated that TCRγδ but not NKG2D engagement induced lytic granule polarization and promoted γδT cell cytotoxicity. TCRγδ activation alone was sufficient to trigger Vav1-dependent phospholipase C-γ1 (PLC-γ1) signaling, resulting in lytic granule polarization and effective killing, whereas NKG2D engagement alone failed to trigger cytotoxicity-related signaling due to the inhibitory effect of Cbl-b on Vav1. However, NKG2D ligation augmented the activation of γδT cell cytotoxicity through the Vav1-PLC-γ1 pathway. Vav1 overexpression or Cbl-b knockdown enabled NKG2D alone to induce γδT cell cytotoxicity. Taken together, these results suggest that the activation of γδT cell cytotoxicity requires a strong signal to overcome a signaling threshold set by the inhibitory effect of Cbl-b. Our finding provides new insights into the molecular mechanisms underlying the initiation of γδT cell cytotoxicity and likely implications for optimizing γδT cell-based cancer immunotherapy.

P1.05.08
Innate lymphoid cells mediate IL-33 dependent fibrosis

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Liver fibrosis, an accumulation of connective tissue due to an imbalance between production and degradation of extracellular matrix proteins, can be triggered by tissue damage by virtue of various causes like infection, drugs, metabolic disorders or immune attack. In case of persistent or recurrent damage signal fibrogenesis might progress towards cirrhosis, liver failure or hepatocellular carcinoma. Nowadays hepatic fibrosis is considered as a world-wide health problem, although no accepted anti-fibrotic agents are available in routine clinical use.
We found significant upregulation of IL-33, the alarmin-like cytokine in three independent models of mouse liver fibrosis: chemically induced (CCL4, TAA), infection related (S.mansoni) and cholestatic one (Bile duct ligation). Moreover analysis of human cirrhosis patients showed elevated secreted IL-33 in serum as well as protein level in liver. Using IL-33-/-, IL-33R-/- mice, a conditional IL-33 transgenic mouse strain and a novel DNA minicircle-based liver specific in vivo overexpression system, we demonstrate that IL-33 is sufficient to drive to excess extracellular matrix deposition in liver. This profibrotic function of IL-33 is mediated by IL-13-secreting liver resident type 2 innate lymphoid cells (ILC2). Moreover, we show that ILC2-derived IL-13 is acting through type II IL-4R-dependent STAT6 signaling, leading to hepatic stellate cell activation, and herewith represents the critical downstream cytokine of IL-33-dependent tissue remodeling and fibrosis. We suppose that IL-33 and its signal-associated pathways have considerable role in the development of hepatic fibrosis and therefore our findings may have important implications for therapeutic interventions across wide range of fibrotic diseases.

P1.05.09
Morphologic, flow cytometric, functional, and molecular analyses of S100B positive lymphocytes, unique cytotoxic lymphocytes containing S100B protein

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Little is known about the $S100B^+$ lymphocytes, which are unique human peripheral blood lymphocytes (PBL) containing the S100B protein. It has recently been shown that S100B is released from various types of $S100B^+$ cells and exhibits varied cytokine-like activities. In this study, we precisely characterized the $S100B^+$ lymphocytes of healthy adults with respect to the proportion in the whole PBL, immunophenotypes, function, and their S100B mRNA expression and also evaluated their S100B-releasing activity upon stimulation. $S100B^+$ lymphocytes were detected in all individuals examined, and the proportion of $S100B^+$ lymphocytes in the whole PBL ranged from 0.42 to 16.15% (mean 4.21%). In addition, two subtypes of $S100B^+$ lymphocytes, CTL subtype ($CD3^+$ $CD8^+$ $CD16^-$) and NK subtype ($CD3^-$ $CD8^-$ $CD16^+$) were detected. The majority of the CTL subtype of $S100B^+$ lymphocytes expressed the αβ-T cell receptor. Surprisingly, S100B mRNA was detected not only in $S100B^+$ lymphocytes, but also in every $S100B^-$ lymphocytes, although the expression levels of S100B mRNA in S100B- lymphocytes were much lower than those of $S100B^+$ lymphocytes. The CTL subtype of $S100B^+$ lymphocytes exhibited blastic morphological changes, proliferated and released S100B upon stimulation with PHA. The NK subtype of $S100B^+$ lymphocytes exhibited morphological NK activity when co-cultivated with NK-sensitive target, K-562 cells. Thus, the CTL subtype $S100B^+$ lymphocytes exhibit the biological characteristics of T cells, while the NK subtype $S100B^+$ lymphocytes exhibit the characteristics of NK cells. These results suggest that $S100B^+$ lymphocytes are a particular subtype of cytotoxic lymphocytes that play a unique role in anti-tumor immunity.

P1.05.10
Type I Interferon signaling in a mouse de novo glioma model

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We have previously demonstrated the protective role of type I interferon (IFN) signaling against glioma progression using a clinically relevant de novo mouse glioma model. However, the molecular factors responsible for inducing the IFN response (“sterile infection”) remain elusive. Here we report our initial findings on in vivo induction of IFN and its effects on glioma microenvironment. When we induced de novo glioma by Sleeping Beauty (SB) system in mice transgenic for IFNβ promoter-driven yellow fluorescence protein (YFP), robust YFP signals were detected in brain of the mice. Analysis of brain-infiltrating leukocytes showed CD11b+ myeloid cells expressed high levels of type I IFN. Furthermore, The CD11b+ myeloid cells upregulated expression levels of type I IFN by stimulating with glioma-derived genomic DNA via partially STING. In experiments using transgenic mice for loxP-tomato-loxP-EGFP/Mx1-Cre, whose cells turn from RFP+ to GFP+ after receiving IFN-signal, we observed approximately 50% of glioma-infiltrating lymphocytes turned into EGFP+ in the glioma microenvironment. Moreover, in CD4+ T-cells, EGFP+ cells expressed lower levels of Foxp3 and TGF-β1 than RFP+ cells. And in CD8+ T-cells, EGFP+ cells expressed higher levels of Tbx21 and IFN-γ and showed higher cytotoxicity than RFP+ cells. This suggests that type I IFN-signal would directly confer antitumor activity on T-cells in vivo. These demonstrate that use of the SB-induced de novo glioma model in these transgenic mice allow us to determine how type I IFN signals operate glioma development. Furthermore, these models will likely contribute to development of type I IFN-enhancing therapeutic strategies for gliomas.

P1.05.11
Insight into interaction innate immune response: Role for Asn40Asp allele of OPRM1 gene

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A functional immune system depends on the appropriate activation and proliferation of lymphocytes following antigen encounter, and this results in modulation of both innate (cellular) and acquired immune (humoral) responses. Present knowledge of interactions between opiates and the immune system is based on pharmacological studies and several mechanisms have been proposed for this interaction. Administration of opioids that bind to the classical mu opioid receptor has been shown to lead to unintended alterations in immune function, namely, immunosuppression. A growing body of evidence indicates that A118G polymorphism of the mu opiate receptor shows association with increased survival rates in breast cancer as well as use of opiate drugs for anticancer therapy. Recent evidence shows that activation of the opiate signaling plays a critical role in maintaining cell differentiation and loss of signaling results in cell proliferation. Taking a cue from the above report the present study was undertaken to understand the role of mu opiate signaling and the effect of A118G mutation (rs1799971) on cell proliferation. Our results show that an alteration of the allele from A to G leads to an arrest in cell cycle. As immune responses are a function of clonal expansion of immune cell types this mutation will result in an altered signaling causing arrest in cell cycle progression and hence lower immune response leading to immunosuppression. Indeed a decrease in circulating immunoglobulin levels and increased incidence of opportunistic infections in subjects following use of opioid drugs has been reported by several groups including ours.

P1.05.12
Human B-1 cell frequency and aging from cord blood to the elderly

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B-1 cells play a crucial role in innate immune responses. They are also involved in the pathogenesis of autoimmune diseases through producing natural auto-antibodies. The present study was aimed at determining the percentage of B-1 cells in blood samples of different Iranian age groups.
Blood samples were obtained from 7 separate groups comprising cord blood, under one year old, 2-12, 13-19, 20-39, 40-64 and over 65 years old normal individuals. The samples were assessed for the percentage of B-1 cells using two-color CD5/CD19 flowcytometry. Furthermore, the correlation between B-1 frequency and aging was evaluated using generalized additive model (GAM).
B-1 cell frequency was significantly higher in cord blood and blood samples obtained from under one year old infants compared with other groups. Under one year old infants possessed the highest levels of B-1 cells in their peripheral blood. Furthermore, B-1 cell percentage diminished with advancing age. This is the first study to present a detailed evaluation of B-1 cell frequency in seven human age groups and revealed that the percentage of B-1 cells in the human blood varies at different age intervals. Moreover, aging inversely affected B-1cell frequency so that the blood samples from the elderly contained the lowest number of B-1 cells which could result in a more susceptibility to certain diseases. A better understanding of age-dependent impacts on human B-1 cell frequency would shed new light on the exact role these cells play in human diseases.

P1.05.13
An innate-like CD4+ T cell expressing complement receptor 1

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CD35, also known as the C3b/C4b or immune adherence receptor, has the widest range of activities of any of the complement regulatory proteins and functions in immune complex clearance, inhibition of complement activation, phagocytosis and humoral immunity. Previous studies have shown that CD35 is on a minor (6 to 12%) population of human thymocytes and peripheral blood CD4+ T cells. It has not been determined whether this receptor marks a separate thymic-derived T cell lineage or whether its expression is inducible. Given the importance of both complement and T cells to immune homeostasis, we examined the basis for CD35 expression on CD4+ T cells. We found a small number (1 to 3%) of CD35+ cells in more than one subset of CD4+ T cells, with the largest number (4 to 8%) being present within the naïve cell population. Also, when labeled T cells were activated by TCR cross-linking, the division index was almost twice that of the CD35- T cells, with no intra- or extracellular expression of CD35 in the CD35- population. The faster response rate, coupled with the absence of inducible expression and less dependency on IL-2, suggests that CD35+ T cells are physiologically unique with innate-like capabilities. They may develop separately from conventional CD4+ T cells and play a prominent role during the induction phase of a T cell response to targets opsonized with C4b and C3b.

P1.05.14
The C-terminal region of the Activation Induced Cytidine Deaminase (AID) is required for the gene conversion and mediates the end joining during class switch recombination

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Activation-induced cytidine deaminase (AID) is essential factor for the class switch recombination (CSR) and somatic hypermutation (SHM) of Immunoglobulin genes. To complete CSR, it is speculated that the C-terminal region of AID is necessary for synapse formation between Sμ and an appropriate S region. Among several C-terminal mutants, P20 which has 34 amino acid insertion at 182th amino acid residue, preserves NES and shuttles between nucleus and cytoplasm. Moreover, P20-mediated SHM is observed, although CSR efficiency is remarkably low.
In order to gain insight into the mechanism of AID in DNA repair and find out which end joining (EJ) pathway is involved in P20 mediated CSR, we analyzed junction microhomology mediated by P20 between Sμ and Sγ1 regions of AID-/- spleen B cells infected by P20. Interestingly, we found that most of the sμ-sγ1 junctions in P20 cells favour longer microhomology compared to the wtAID over-expressed cells. This finding is strongly further supported by the reduced level of C-NHEJ factors and essential synapsis factors at the Sμ region in C-terminal mutant JP8Bdel cells.
Gene conversion (GC) is another event which requires AID. Therefore, we tested the involvement of the AID C-terminus region in GC. Our sequencing data using DT40 cells over-expressing P20 also shows that the C-terminal region of AID is required for the recombination step in GC.
Taken altogether, our results using the two different systems, spleen and DT40 cells, demonstrate that the C-terminal region of AID is required for efficient pairing during CSR and possibly homologous recombination.

P1.05.15
HIV infection inhibits the crosstalk between monocytes-derived dendritic cells and Vγ9δ2 T cells

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DCs act as sentinel cells against incoming pathogens and represent the most potent antigen presenting cells, having the unique capability to prime naïve T cells. Innate immune lymphocytes as Vγ9Vδ2 T cells are able to sense pathogens and it has been demonstrated that they induce DC maturation, in turn expanding functions of both innate and adaptive immunity. Activated Vγ9Vδ2 T cells are able to induce DCs maturation as evidenced by increased up-regulation of CD86, IL-12 production and priming of T cell responses. However, whether HIV-infected DC are still able to be activated by Vγ9Vδ2 T cells is unknown. To clarify this issue, we cultured activated Vγ9Vδ2 T cells

with HIV infected monocyte derived DC (MDC). After 5 days we evaluated MDC phenotype, and Vγ9Vδ2 T cells activation and proliferation. We found that HIV-infected MDC are not able to up-regulate CD86 molecules when co-cultured with activated Vγ9Vδ2 T cells, compared with not infected MDC. Moreover, we observed that, independently from HIV infection, activated Vγ9Vδ2 T cells induced CCR5 up-regulation, and CCR7 and HLA-DR down-modulation. We also observed that Vγ9Vδ2 T cells were not able to proliferate in response to HIV-infected MDC, although an up-regulation of CD69 was observed. Furthermore, upon phosphoantigens stimulation, Vγ9Vδ2 T cells proliferation was inhibited when co-culture with HIV-infected MDC. These data indicate that HIV infection could alter DC-Vγ9Vδ2 T cells crosstalk, inhibiting on one hand the presentation and migration capacities of DC, and, on the other hand, rendering Vγ9Vδ2 T cells unable to respond to antigen stimulation.

P1.05.16
Type 2 Innate Lymphoid cells (ILC2) reside in the human skin and contribute to atopic dermatitis

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Type 2 innate lymphoid cells (nuocytes, NHC) are a recently characterized RORγt independent subset of innate lymphoid cells (ILC) that require transcription factors RORα and GATA3 for their development. Their response to epithelial cytokines and production of IL-13 and IL-5 has raised the possibility of their role in atopic diseases. Here we show that ILC2s reside in human skin, but are enriched within lesional skin of atopic dermatitis and infiltrate the skin after allergen challenge. They associate with the production of type 2 cytokines in vivo in human skin and they migrate and produce high levels of type 2 cytokines in response to IL-33 in vitro. These data suggest that human type 2 ILCs may play a role in the pathogenesis of atopic dermatitis.

P1.05.17
The role of gamma-delta T lymphocytes in the switch of Ig isotypes in B-1 cells under the influence of TI-2 antigens

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B-1 cells respond to T-independent antigens type 2 (TI-2 Ag) by formation of IgM antibodies/total Ig. At the same time in the small intestine B-1 cells may switch from IgM to IgA. Mechanism of the switch in the absence of T help is unknown. Now the role of gamma-delta T cells in the switch of Ig isotypes in B-1 cells under the influence of TI-2 Ags was studied.
CBA mice were used. B-1 cells were isolated from peritoneal cavity, and intraepithelial gamma-delta T cells from small intestine. The purity of cell populations was determined by cytometry. Alpha (1→3) dextran (Dex) was used as TI-2 Ag.
B-1 and gamma-delta T cells (10:1) were cultured in the round-bottom 96-well plates in RPMI 1640 medium with 10% of FCS and all necessary supplements in CO2-incubator for 4 days with or without Dex. The number of IgM- and IgA-forming cells were determined by ELISPOT method.
It was found that: 1) the addition of Dex to peritoneal B-1 cells induced some increase in the numbers of IgM and IgA antibody- and Ig-forming cells (AFC and IFC, respectively; 2) the addition of gamma-delta T cells resulted in the decrease of the IgM-AFC and IgM-IFC number, and 3) the addition of gamma-delta T cells increased the quantities of IgA-AFC and IFC. The data obtained point to the possible role of intraepithelial gamma-delta T cells in the isotype switch in B-1 cells in response to TI-2 Ag.

P1.05.18
Innate protective responses of B1 cells dependent on activation-induced cytidine deaminase (AID) and interleukin-13

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We describe a similarity between two early immune responses: protection from bacterial pneumonia, and initiation of contact sensitivity (CS). *AID*$^{-/-}$ (activation induced cytidine deaminase), *Jα18*$^{-/-}$ (iNKT cell), *xid* (B1 B cell), and *IL-13*$^{-/-}$ mice had impaired early pathogen clearance, similar to defective early initiation in CS, except that CS-initiation depends on IL-4. Transfer of sorted wild type peritoneal B1 cells reconstituted protection in *AID*$^{-/-}$ and *xid* mice, but not in iNKT cell deficients. This suggested that, early bacterial clearance was dependent on AID, IL-13 and IgM antibodies produced by activated normal B1 cells, and that NKT cells also are required in both pneumonia and CS.
T15 idiotype, anti-phosphorylcholine (PC) IgM, previously claimed protective in pneumoccal sepsis, were abundant but actually unprotective as "natural" T15^{+} IgM in the serum of *AID*$^{-/-}$ mice, against *S. pneumoniae* lower airway infection. Higher affinity IgM derived of activated normal B1 cells appeared superior in resolution of pneumonia compared to abundant but less potential affinity, and actually ineffective T15^{+} IgM in *AID*$^{-/-}$ mice.

P1.06 Macrophages

P1.06.01
T cell derived IL-3 enhances the pro- and anti-inflammatory properties of human macrophages dependent of the activation status

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Macrophages play a critical role in regulating the local immune response, contributing to both the initiation and resolution of inflammatory processes. Depending on their activation mode, macrophages secrete a broad repertoire of pro- or anti-inflammatory cytokines and are able to interact and activate bystander immune cells. Conversely, macrophages activation is dependent on the cytokines produced by other leukocytes. One example is T cell derived IFN-γ driving classical macrophage activation. In addition, T cells are able to secrete IL-3 upon T cell receptor engagement. Here we demonstrate that activated human macrophages up-regulate the IL-3 receptor. Furthermore IL-3 treatment induces specific changes in the macrophage phenotype and differentially enhances its function depending on the pre-treatment activation status. In classically activated macrophages IL-3 promoted an inflammasome response measured by elevated IL-1β levels and increased the expression of the co-stimulatory molecule CD86. In contrast, non-activated resting macrophages displayed signs of alternative activation upon IL-3 treatment demonstrated by increased surface expression of CD206 and CD163 and high IL-10 production. Interestingly, IL-3 also enhanced the secretion of IL-12p40 which was more pronounced in resting and alternatively activated macrophages. In conclusion, we demonstrate that T cell derived IL-3 is an important regulator of macrophage function and that this regulation is highly dependent on the preexisting activation status of the macrophage. Overall, these findings suggest a role for IL-3 being part of a positive feedback loop from T cells to macrophages regulating the course of adaptive immune responses.

P1.06.02
Dissociation of innate immune responses in microglia infected with Listeria monocytogenes limits activation and neuronal damage

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Microglia are innate immune cells of the brain and here we present that they are the cellular targets of Listeria in murine hippocampal cultures. Using murine cell-lines, we report that microglia handle Listeria infection differently than other well-known macrophages. Infection of microglia with Listeria dissociates two gene expression programs involved in innate immunity, while both transcriptional responses are linked in macrophages and regulated by the bacterial virulence factor, LLO. In microglia, Listeria induces the TNF regulated early innate immune genes but represses the late innate immune genes grouped in two clusters, microbial degradation and IFN responses. ActA gene seems responsible for both gene transcriptional responses with participation of LLO in repressing IFN genes. As a consequence of this transcriptional strategy, microglia phagosomes transform into innate immunity platforms deficient in IFN-regulated microbicidal mechanisms responsible for Listeria destruction in macrophages. Second, the dissociation of macrophage innate immunity produces high levels of TNF-α and MCP-1 pro-inflammatory factors and certain neuronal destruction, but to a lesser extent than in macrophages. Finally, analysis of Listeria infected primary hypocampal cultures confirmed the high levels of TNF-α and MCP-1, the low listericidal potency and a limited induction of inflammation to preserve neuron integrity.

P1.06.03
Copper nanoparticles inhibit LPS-mediated nitric oxide and IL-12p40 production in macrophages

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In biomedicine, nanoparticles have been utilized in different applications such as drug delivery, image contrast agents and diagnostic purposes. Depending on their unique physicochemical properties some nanoparticles can stimulate the immune system triggering strong pro-inflammatory reactions. The use of copper nanoparticles is relatively new; therefore there is an increasing interest in thorough characterization of their impact on the immune system. CuNP have shown to induce one of the most potent acute inflammatory reactions among several types of nanoparticles. Recently, in a pulmonary infection model, it was demonstrated that mice, which had been previously exposed to CuNP, presented an impaired bacterial clearance. These effects were due to a reduction in macrophage and neutrophil function; however, the precise molecular mechanism underlying these effects is still unknown. Here, we described the effects of CuNP in the functioning of thioglycollate-elicited mouse peritoneal macrophages in response to lipopolysaccharide (LPS). Transmission electron microscopy (TEM) data showed that CuNP are rapidly internalized by macrophages. The challenge with CuNP inhibited LPS-mediated nitric oxide (NO) production in a dose dependent manner. The reduction of NO generation was related with a diminished expression of inducible nitric oxide synthase (iNOS) upon CuNP treatment. In addition, CuNP significantly reduced the expression of IL-12p40 but not IL-6 in response to LPS. These results showed that CuNP reduce the expression of key inflammatory mediators that are crucial to mount an appropriated immune response against bacterial infection.
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P1.06.04
Impact of acute and chronic stress on innate immune system in elderly

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Catastrophic events like hip fractures or certain inflammatory diseases like Type 2 Diabetes Mellitus (T2DM) are quite common amongst elderly subjects. Those inflammatory conditions are usually associated with a deregulated immune response.
In this study, we aim to compare the impact of inflammatory stress on the innate immune response. We define 3 different stages of inflammatory stress: acute stress (at the moment of a hip fracture: T_0), short-term chronic stress (6 months after the hip fracture: T_{6m}) and long-term chronic stress (T2DM).
From whole blood, we evaluated the capacity of neutrophils and monocytes to phagocyte. After e-coli stimulation, we also tested their ability to produce reactive oxygen species (ROS). We also determined the fate of monocytes in differentiating in MI or MII under various stimulations.
Our findings suggest that neutrophils and monocytes exposed to acute or long-term chronic stress show less capacity of phagocytosis when compared to neutrophils and monocytes of healthy subjects. Furthermore, ROS production is overall reduced in the three stress categories in comparison with the control group. MI and MII differentiations are different in these stress situations.
We conclude that acute and both short- and long-term chronic stresses alter the functionality of the innate immune system.

P1.06.05
Modulation by lipoxin of tumor-associated macrophages profile

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In tumor microenvironment, pro-inflammatory macrophages (M1) acquire anti-inflammatory and pro-tumor characteristics. Those tumor-associated macrophages (TAM) exhibit a M2-like profile, with low cytotoxic properties, as an impairment of NO and ROS production. Lipoxins (LX) are lipid mediators with anti-inflammatory and pro-resolution activities in mononuclear cells, but its effects on TAM are not well investigated. In this study, we demonstrated the effects of ATL-1, a synthetic analog of 15-epi-lipoxin A4, on TAM activity profile.
Human macrophages were differentiated in TAM after incubation with the conditioned medium of MV3, a human melanoma lineage cell. The treatment with ATL-1 (10nM) does not alter TAM viability, as demonstrated by Trypan Blue exclusion and MTT assays, but decrease CD200 and CD206 expression, both characteristic markers of M2 macrophages. In parallel, ATL-1 (1-100nM) stimulates TAM to produce NO, by increasing iNOS/arginase ratio, an also triggers ROS production, which is inhibited in the presence of DPI, an NADPH oxidase inhibitor. These results suggested that lipoxin may restore the antitumoral properties of TAM, activating iNOS and NADPH oxidase system. The alterations in TAM profile and the recovery of their cytotoxic properties could control tumor progression trough promotion of tumor cell apoptosis. Accordingly, ATL-1 (10nM) reverts TAM anti-apoptotic effect on MV3 and also inhibits endothelial cell tubulogenesis propitiated by TAM, a crucial step in angiogenesis process.
Our results suggest that lipoxin down-modulates the tumor progression stimulated by TAM, inducing the shift from M2 to M1 profile, leading to tumor cell apoptosis.
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P1.06.06
The tyrosine kinase Abl is a component of macrophage podosomes and is required for podosome formation and macrophage migration

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Tyrosine kinases of the Abl family regulate cytoskeleton dynamics in different cell types. We found that in murine bone-marrow derived (BMDM) macrophages, the tyrosine kinase Abl is associated with the integrin-bound Src-family kinases (SFKs) Hck and Fgr and expression and kinase activity of both Hck/Fgr and Abl are indispensable for macrophage migration. Abl-silenced BMDM displayed a marked alteration in two-dimentional cell migration (in vitro wound healing assays) and trans-endothelial migration. Additionally, inhibition of Abl kinase activity an/or silencing of Abl expression resulted in reduction of podosome formation in both BMDM and monocyte-derived macrophages. Consistently with a cross-talk between SFKs and Abl in the regulation of actin dynamics, both the single Fgr and the double Fgr/Hck deficiency reduced podosome formation in BMDM. Overexpression of Fgr or a constitutively active form of Abl in COS-7 cells resulted in increased tyrosine phosphorylation of Vav proteins and Sos-1, two guanine nucletotide exchange factors (GEFs) for Rac. Additionally, deficiency of Fgr/Hck in BMDM resulted in reduced phosphorylation of Vav and Sos-1. Silencing of Sos-1 in BMDM resulted in profound alterations of the cytoskeleton whose impact on cell migration and podosome formation are under investigation. Considering that Abl regulates cytoskeleton dynamics leading to invadopodia formation in cancer cells and our studies implicates Abl in macrophage podosome formation and migratory ability, we propose that Abl targeting may represent a double-edged sword, acting simultaneously on tumor cells and cancer-related inflammation.

P1.06.07
Oncogene-induced senescence in histiocytes: role of BRAFV600E in the pathogenesis of Erdheim-Chester disease

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Erdheim-Chester disease (ECD) is a rare form of non-Langerhans cell histiocytosis of yet unknown etiology, characterized by infiltration of lipid-laden CD68+CD1a- histiocytes. It is still under debate whether ECD is a clonal/neoplastic disease or the expression of a deregulated immune/inflammatory response. Recently, the presence of the oncogenic BRAFV600E mutation has been described in patients with ECD: so far, the largest published report disclosed this mutation in lesions from 19/37 studied patients by means of pyrosequencing. BRAFV600 has also been implicated in oncogene-induced senescence (OIS), a mechanism that prevents cellular proliferation by activating the p16Ink4a oncosuppressor.
In this study we found the BRAFV600E mutation in ECD lesions from 19/23 patients from our cohort by means of pyrosequencing. Furthermore, when coupling pyrosequencing with a minor allele amplification technique, we were able to demonstrate the mutation in the lesions of all the 23 studied patients, in the peripheral blood monocytes obtained from 15/15 ECD patients and from 0/15 controls. We further investigated the possible association of the BRAFV600E mutation with cellular proliferation or OIS in ECD biopsies: indeed, mutated histiocytes expressed senescence markers including p16Ink4a, p21 and β-Gal, whereas they were negative for the proliferation marker Ki67.
Taken altogether, our data provide further evidence that a constitutively activated RAS/RAF pathway have a role in ECD pathogenesis, also by conferring a senescence phenotype to mutated histiocytes in the lesions. Although further investigations are needed, the detection of BRAFV600E in peripheral blood monocytes may help to further support the diagnosis of ECD.

P1.06.08
Jacalin drives macrophage polarization toward an anti-tumor M1-like phenotype

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Tumor-associated macrophages (TAMs) have an ambiguous and complex role in the carcinogenic process, since these cells can be polarized into different phenotypes by the tumor microenvironment. As the interactions between tumor cells and TAMs involve several players, a better understanding of the function and regulation of TAMs is crucial to drive their differentiation into cells with an anti-tumor phenotype. In this study, we investigated the modulation of macrophage tumoricidal activities by jacalin. The lectin bound to macrophage surface through its carbohydrate recognition domains, and induced cytokine production by these cells. Jacalin stimulation increased the expression of TNF-α, IL-1β and MIP-1α mRNA. As measured by ELISA, higher levels of TNF, IL-6, IL-12, IL-1β and IL-10 were detected in the supernatant from jacalin-stimulated macrophages. Given that pro-inflammatory cytokines were predominantly produced by macrophages in response to stimulation with jacalin, we next investigated whether NF-κB signaling is activated by the lectin. We found that jacalin was able to activate the transcription factor. The results obtained indicate that the lectin polarize macrophages toward the pro-inflammatory, anti-tumor M1-like phenotype. Therefore, we studied the tumoricidal activities of jacalin-stimulated macrophages. Incubation of HT-29 or MCF-7 cells with supernatants from macrophages stimulated with jacalin resulted in a reduction of cell viability, as assessed by MTT assays. Furthermore, flow cytometric analysis showed that the reduction of cell viability was due to apoptosis of tumor cells. Taken together, these results indicate that jacalin, through its ability to exert a pro-inflammatory activity, can direct macrophages to an anti-tumor phenotype.
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P1.06.09
Modulation of NKG2D and NKG2A ligands expression during monocyte differentiation

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Natural killer cells and macrophages are the major effectors of the innate immune system. Crosstalk between these two populations is still poorly understood although it is likely to have strong implications in the development of both innate and adaptive immune response. NK cells express a plethora of activating and inhibitory receptors which bind different ligands on target cells. HLA-E, a non classical MHC class I molecule, binds the inhibitory CD94/NKG2A receptor thus protecting cells from the NK killing. The expression and function of HLA-E require peptides derived from the leader sequence of HLA class I molecules. However, HLA-E molecules can also bind self or pathogen-derived peptides, whose effect on the NK response is still controversial. We show here that the expression of the HLA-E molecules is strongly up-regulated during differentiation of monocytes to macrophages along with other molecules involved in HLA class I antigen processing and presentation such as ERAP1 and ERp57 whereas, the expression of classical HLA class I molecules remains unaffected. These findings strengthen the hypothesis that HLA-E exploits the same machinery as the classical HLA-class I molecules to present self proteins. Ligands (MIC-A/B, ULBPs) for the activating NKG2D receptors are also modulated during monocyte differentiation. The influence of this strong modulation on the crosstalk between NK cells and macrophages is under investigation.

P1.06.10
Decoy receptor 3 enhances tumor progression via induction of tumor-associated macrophages

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Tumor-associated macrophages (TAMs) are the major component of tumor infiltrating leukocytes. TAMs are heterogeneous with distinct phenotypes influenced by the microenvironment surrounding tumor tissues. Decoy receptor 3 (DcR3), a member of the tumor necrosis factor receptor superfamily, is over-expressed in tumor cells and is capable of modulating host immunity as either a neutralizing decoy receptor or an effector molecule. Up-regulation of DcR3 has been observed to correlate with a poor prognosis in various cancers. However, the mechanisms of DcR3-mediated tumor-promoting effect remain unclear. We previously demonstrated that DcR3 modulates macrophage activation toward an M2-like phenotype in vitro, and that DcR3 down-regulates MHC class II (MHC-II) expression in TAMs via epigenetic control. To investigate whether DcR3 promotes tumor growth, CT26-DcR3 stable transfectants were established. Compared with the vector control clone, DcR3-transfectants grew faster and attracted TAMs infiltration. We further generated CD68 promoter-driven DcR3 transgenic (Tg) mice to investigate tumor growth in vivo. Compared with wild type mice, macrophages isolated from DcR3-Tg mice displayed higher levels of IL-10, IL-1ra, Ym1, and arginase activity, while the expression of IL-12, TNF-α, IL-6, NO, and MHC-II was downregulated. Significantly enhanced tumor growth and spreading were observed in DcR3-Tg mice, and the enhanced tumor growth was abolished by arginase inhibitor nor-NOHA and histone deacetylase inhibitor sodium valproate. These results indicate that induction of TAMs is an important mechanism for DcR3-mediated tumor progression. Our findings also suggest that targeting DcR3 might help in the development of novel treatment strategies for tumors with high DcR3 expression.

P1.06.11
PTPN22 modulates macrophage polarization and susceptibility to dextran sulfate sodium-induced colitis

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PTPN22, a protein tyrosine phosphatase expressed mainly in hematopoietic cells, has been linked to many autoimmune diseases. A C-to-T single nucleotide polymorphism (SNP) at position 1858 of human PTPN22 cDNA increases the risk of rheumatoid arthritis but reduces the risk of inflammatory bowel disease. However, the function of PTPN22 and how this SNP influences the risk of autoimmune diseases are poorly understood. We found that PTPN22 was located at cytoplasm and nucleus of primary macrophages. Cytoplasmic PTPN22 suppressed M1 macrophage polarization, whereas nuclear PTPN22 promoted M2 polarization. PTPN22-deficient mice developed severe colitis induced by dextran sulfate sodium and their intestinal macrophages expressed higher levels of M1 genes but lower levels of M2 genes. Furthermore, the minor T allele of the C1858T SNP was associated with attenuated expression of inflammatory cytokines in human M1 macrophages due to heightened expression of PTPN22. This T allele-associated aberrant expression of PTPN22 was partly attributed to an auto-inhibition mechanism, in which PTPN22 suppresses its own expression in M1 but not M2 macrophages. Our data not only demonstrate a critical role of PTPN22 in regulating macrophage polarization but also provide a molecular explanation for the protective effect of the C1858T SNP in inflammatory bowel disease.

P1.06.12
Triggering receptor expressed on myeloid cells-1 regulates macrophage polarization in an obstruction-induced nephritis mouse model

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Chronic kidney disease (CKD) is an emerging worldwide public health problem. Inflammatory cell infiltration and activation during the early stages in the injured kidneys is a common pathologic feature of CKD. Here we demonstrate that TREM-1, an important inflammatory regulator, is up-regulated in renal tissues collected from mouse obstruction-induced nephritis. TREM-1 is crucial for modulating macrophage polarization, and plays a pivotal role in mediating tubular injury and interstitial collagen deposition in obstructive nephritis. Nephritis lysates triggered a TREM-1-dependent M1 polarization ex vivo, consistent with the observation that GM-CSF-derived M1 macrophages express higher levels of TREM-1 in comparison with M-CSF-derived M2 cells. Moreover, agonistic TREM-1 crosslink significantly strengthened the inductions of iNOS and GM-CSF in M1 cells. These observations were validated by a strong clinical correlation between infiltrating TREM-1 expressing/iNOS-positive macrophages and renal injury in human obstructive nephropathy, suggesting that TREM-1 may be a potential diagnostic and therapeutic target in human kidney disease.

P1.06.13
Polarized activation phenotypes of spontaneously differentiated human monocyte-derived macrophages: role of dexamethasone

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Macrophage polarization has been observed in vivo under physiological and pathological conditions, and may represent an attractive target for pharmacological modulation. Given the lack of gold standard protocols for classic (M1) and alternative (M2) activation in vitro, we polarized human macrophages using different protocols and characterized resulting phenotypes and investigated their modulation by dexamethasone. Human monocytes were isolated from healthy donors by 2-step gradient centrifugation, and macrophages were obtained by spontaneously differentiating monocytes in RPMI 1640 with 10% FCS for 7 days. Macrophages stained positively for intracellular CD68, whereas cell surface CD68 was detected in only 10% of cells, indicating a low basal activation state. When resting macrophages were incubated with LPS/IFN-γ (M1) for 4 h, the percentage of cells expressing the M1 surface markers CD68 and CCR2 did not change. After prolonged stimulation (48 h), the number of CD68- but not CCR2-positive cells doubled, while expression of M2 markers CD206, CD163 and CX3CR1 was down-regulated compared with resting macrophages. In contrast, none of these markers were affected by alternative (M2) polarization with IL-4/IL-13 for 48 h, whereas CD206 was up-regulated after 7 days. Q-PCR phenotyping after M1 polarization for 6-48 h showed increased TNF-α, IL-1β, COX-2, IL-10, VEGF, and decreased CD206 and COX-1 transcript levels. Overnight pretreatment with 10 nM dexamethasone enhanced CD163 surface expression in resting and M2-polarized macrophages while reversing M1-induced CD163 down-regulation. We conclude that the phenotypic characterization of polarized macrophages is required and provides a basis for pharmacological macrophage targeting.

P1.06.14
Influence of the phagocytizing mononuclear system (PMS) stimulation on liver and kidney regeneration

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Study objective: To study the potential of liver and kidney reparative growth by means of PMS activity modulation.
The experiment was carried out on 35 outbred albino male mice with the use of modern equipment and methods of testing: morphologic, histological, morphometric and flow cytometry. The animals were treated in accordance with the Directive 86/609/EEC.
The intensification of regeneration in both organs under investigation was demonstrated both in case of local and toxic lesion in experimental models of partial hepatectomy, nephrectomy, and toxic effect of tetraclormetane under impact of immunomodulator 3-Aminophthalhydrazide. These results are clearly explained by the ability of activated macrophages to accelerate proliferation, migration and homing of hemopoietic stem cells as in response to PMS stimulation by 3-Aminophthalhydrazide the number of hemopoietic stem cells (phenotype $CD45^{low}CD117+CD38+$) increased in bone marrow, blood and liver. Multiplication of the number of this type of cells in liver can be due to both their proliferation in the organ itself and their migration from bone marrow. The results of the experiment can be of significance in substantiating new approaches in the treatment of hepatitis and renal insufficiency of various etiologies.
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P1.06.15
Modulation of macrophage function by growth hormone

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Studies have shown that macrophage activity can be modulated by neuropeptides such as the growth hormone (GH). Herein, studies were performed to determine the immunomodulatory effect of GH on macrophages. Peritoneal macrophages from GH-treated mice with 20 and 200 µg/kg for 7 days showed alterations on Mac-1, VLA-5 and VLA-6 integrins surface expression and an increase on extracellular matrix deposition. They also showed lower adherence to fibronectin (FN) and to laminin (LN), and augmented transwell cell migration toward these substrates. Besides, the peritoneal macrophages from GH-treated mice presented higher porcentage of phagocytosis and also higher phagocytic capacity than cells from control animals. To elucidate the pattern of GH action, freshly isolated peritoneal macrophages were treated *in vitro* with 20 and 200 ng/mL of GH at different times. The GH-treated macrophage cultures showed alterations in cell morphology as well as on surface expression of Mac-1 and VLA-6 integrins. Increased FN and LN deposition and decreased cell adhesion to LN substrate were also observed. Moreover, the *in vitro* GH treatment influenced the macrophage transwell migration in two distinct ways: after 6 hours there was an increase in migrating cells whereas after 12 hours there was a decrease in cell migration. Differently from the *in vivo* treatment, *in vitro* GH treatment was not able to induce the macrophage phagocytic activity. These results show that macrophage activity can be modulated directly and/or indirectly by GH and reinforce the literature data which indicate the GH as a macrophage activating-factor in the immune response.

P1.06.16
The overexpression of glucocorticoid-induced leucine zipper (GILZ) protein in macrophages enhance lifetime in mouse septic shock model

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According to the literature, the Glucocorticoid-induced leucine zipper (GILZ) protein exerts immunoregulatory activities on macrophages. For instance, the over-expression of GILZ in macrophages decreases their secretions of inflammatory cytokines upon LPS-exposure. We thus hypothesized that GILZ could contribute to the endotoxin tolerance (ET) process that comes in macrophages after prolong stimulations with endotoxins and limits inflammation.
To address this issue, we first evaluated the expression of GILZ in human monocytes which were stimulated to induced ET (tolerant-monocytes, pre-exposure to LPS for 24hrs followed by 2hrs of stimulation) and inflammatory-monocytes (a single stimulation of 2hrs). Naïve unstimulated monocytes served as controls. We observed a statistically significant decrease of GILZ expression in inflammatory-monocytes compared with their naïve counterparts. Conversely, the ET process significantly enhances GILZ expression in macrophages. High GILZ levels were significantly correlated with IL-10 production and regulatory phenotype in ET-monocytes. To explore in vivo the contribution of GILZ in macrophage functions, transgenic mice were generated with constitutive and restricted over-expression of GILZ in macrophages (CD68-GILZ transgenic mice). We then confirmed that CD68-GILZ mice have a significant prolonged lifetime in septic shock models where inflammation is responsible for death.
Our data suggest that GILZ regulates the functions of macrophages during bacterial infections and that an over-expression of GILZ protects from septic shock. We currently use an unbiased wide screening approach on peritoneal macrophages isolated from CD68-GILZ mice and their controls after LPS stimulation to link functions/pathways involved in GILZ effects. Overall, these results will improve our comprehension on macrophage regulation.

P1.06.17
CD200R-dependent regulation of monocytes is subset- and tissue specific

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Monocyte/macrophage regulation is crucial to avoid the potentially detrimental effects of inflammation. The CD200-CD200R pathway has been implicated to play an important role in controlling excessive myeloid cell activation (Hoek et al 2000; Snelgrove et al., 2008). Recent studies have revealed a role for CD200R expression on alternatively activated macrophage subsets (Koning et al, 2009), yet no studies have described the expression of CD200R controlling monocyte inflammation in murine models. Our in vivo data generated from FACS studies on bone marrow and PBMCs in WT C57BL/6 mice suggests CD200R is a pathway up-regulated during monocyte maturation, during the change from Ly6Chi to Ly6Clow expression ($P<0.001$) and could potentially serve as a natural slow-down mechanism for classical Ly6Chi monocyte inflammatory activity. Complementary in vitro studies using bone marrow derived macrophages, studying mRNA expression assessed by RT-PCR, have revealed the expression of CD200R pertaining to TLR stimuli after 6 hours stimulation with TLR ligands FSL-1, POLY(I:C),LPS and R848. Here we show for the first time that stimulation of TLR3, the only MyD88-independent TLR, does not down-regulate CD200R expression (P=ns), contrary to TLR2/6 ($P<0.05$), TLR4 ($P<0.01$) and TLR7 ($P<0.05$). This finding goes in accordance with previous findings describing the MyD88-dependent down-regulation of CD200R during TLR4 stimulation (Mukhopadhyay et al., 2010).

P1.06.18
Study of aminoacidic sequences of vitamin D binding proteins involved in macrophage activation

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Vitamin D binding protein-derived macrophage activating factor (DBP-MAF) is a powerful activator of macrophages. Polymorphisms of the gene coding for vitamin D receptor (VDR) were associated with differential responses to DBP-MAF in human monocytes. Here we examined the relationships between these vitamin D binding proteins, i.e. DBP-MAF and VDR. VDR and two isoforms of DBP share 20% identity of aminoacid (aa) sequence in the tract coded for by exons 1, 2, 3 ,4, 5, i.e. aa 1-197. An higher degree of identity (40%) was observed in the tract coded for by exons 6, 7, 8, i.e. aa 217-330. The aa in position 445 was of particular interest; VDR and isoforms 1 and 3 of the DBP show a histidine in this position, whereas the isoform 2 shows an arginine. The observation that the VDR and isoforms 1 and 3 of the DBP have the same aa, at variance with the isoform 2, suggests that this variation is implicated in the evolutionary divergence in the genes coding for these vitamin D binding proteins. In fact, isoform 2 has a lysine in position 420 at variance with isoforms 1 and 3 that have a threonine that in turn is glycosylated so that DBP-MAF can be produced from isoforms 1 and 3 but not from isoform 2. These results are consistent with the concept that all the component of the vitamin D axis, i.e. vitamin D, DBP, DBP-MAF, and VDR participate in the regulation of the immune system through macrophage activation.

P1.06.19
Characterization of the murine CD163 receptor

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CD163 is a scavenger receptor exclusively expressed in monocytes and macrophages. Since CD163+ macrophages are found in the healing phase of acute inflammation and anti-inflammatory factors increase CD163 expression, CD163 is thought to be involved in resolution of inflammation. However, little is known regarding the murine CD163 (mCD163). The aim of this study was to analyze structure, expression and regulation of the murine CD163.
Extracellular domain of mCD163 was cloned in order to produce rabbit polyclonal antibody (LFR1-Ab). Regulation of CD163 expression in freshly isolated murine bone marrow cells (BMC), bone marrow-derived macrophages as well as the CD163 distribution in various organs were assessed using fluorescence microscopy and Western Blot.
LFR1-Ab specifically recognized a 170kDa protein in lysates of murine BMC and also has shown cross-reactivity with human CD163 (130kDa). The differences in molecular weight were due to strong glycosylation of mCD163. High expression of CD163 was detected in freshly isolated BMC. This was lost during in vitro culture. Stimulation of BMC with dexamethasone (DEX), but not IL-4, IL-6 or LPS and IFNγ increased CD163 expression. Similar but weaker effect was observed when bone marrow-derived macrophages were stimulated with DEX. In vivo analysis of CD163 distribution in mouse revealed the presence of CD163+ cells in all tested organs (spleen, liver, lung, intestine). However i.p. injection of DEX enhanced expression of CD163 only in the lung. CD163 is considered as a M2 marker, however we detected an increase of CD163-positive subpopulation in several experimental diseases (infection with S. aureus, L. major).

P1.06.20
Effects of biodegradable polylactic acid fibers and dexamethasone on classical and alternative activation pathways in macrophages

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Fully biodegradable medical devices are under development for various applications, and addition of drug-eluting properties extends their usefulness e.g. in conditions complicated with inflammatory and fibrotic reactions. On the other hand, biodegradable material itself may trigger inflammatory responses. In the present study we investigated the effects of pure and drug-eluting biodegradable fiber on macrophage activation.
PLA96/4 (polylactide of 96% L-lactide and 4% D-lactide) fiber was found to induce a low-level inflammatory reaction in macrophages which was attenuated by dexamethasone as detected by an antibody array. Also, dexamethasone and dexamethasone-releasing PLA96/4 fibers were found to inhibit the production of IL-8, TNFα, VEGF and MCP-1 in activated macrophages.
To further study the effect of PLA96/4 fiber on macrophages, markers typical for “classical” (M1) and “alternative” (M2) macrophage activation were investigated. Macrophages were cultured on PLA96/4 fibers and exposed to LPS or IL-4+IL-13 to induce classical or alternative activation, respectively. The PLA fiber had a minor inhibitory effect on LPS-induced production of classical inflammatory mediators IL-12 and nitric oxide, and no effect on IL-6. On the other hand, PLA fiber clearly attenuated IL-4 and IL-13 induced expression of alternative activation markers arginase-1, Fizz1, Ym-1 and IL-10.
In the present study, we investigated the effects of polylactic acid on macrophage responses. PLA96/4 was found to suppress alternative type activation while only minor effects were found on classical type activation. The results are implicated in the biocompatibility of biodegradable medical devises and can be utilized in the development of improved drug-eluting materials and devises.

P1.06.21
Involvement of T-cell-Ig-mucin-3 (Tim-3) in macrophage polarization

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T-cell-Ig-mucin-3 (Tim-3) is widely involved in the negative regulation of adaptive immunity, and its dysregulation on $CD4^+$T and $CD8^+$T cells is associated with autoimmune diseases, chronic virus infection or tumor. However, its roles in innate immunity remain unclear. We previously reported that Tim-3 promotes macrophage homeostasis by negatively regulating TLRs response (J.Immunol.2013), indicating that Tim-3 might also negatively regulate innate immunity. Here we further identified a dysregulated downregulation of Tim-3 on lamina propria macrophage in mice with ulcerative colitis (UC), which is related to a M1 shift of macrophage as marked by increased pro-inflammatory cytokines expression including IL-12, IL-6, TNF-α, and IL-1β. Blockade of Tim-3 pathway in vivo using Tim-3 antibody or soluble protein lead to enhanced the shift of M1 macrophage and exacerbated inflammation in UC. On the other hand, in mice with colonic tumor, lamina propria macrophage showed increased Tim-3 expression and increased M2-related anti-inflammatory cytokines including IL-10, TGF-β expression by macrophage. These data indicated that Tim-3 expression is closely related to the polarization of macrophage and its dysregulation significantly affects diseases progression. When the underlying mechanisms were investigated, our data showed that Tim-3 down regulation leads to increased NOS2 expression and decreased Arg-1 and Dectin-1 expression in macrophage and changed micro RNA profiles including increased mi-155, miR-27a expression. The intracellular pathways of Tim-3 mediated microphage polarization were also investigated. A better understanding of this pathway might shed new light on the mechanisms of Tim-3 regulated innate immunity.

P1.06.22
Thymic cortical dendritic macrophages (TCDMs); professional scavengers of apoptotic thymocytes in the human thymus

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[Aim] We previously reported the presence of the special type of macrphages showing features intermediate between macrophages and dendritic cells (DCs) in the the human thymus and named them “thymic cortical dendritic macrophages” (TCDMs) (Immunobiology

213: 837-847, 2008). Because TCDMs are so similar to DCs that they are often misinterpreted as “cortical DCs”. Here we demonstrate the distinctive macrophage-nature of TCDMs. [Materials and Methods] Frozen and paraffin sections obtained from the normal human thymus tissues were examined immunohistochemically and electron microscopically. [Results] TCDMs were identified as fascin+ large extremely dendritic-shaped cells evenly scattered throughout the cortex. TCDMs were positive for fascin, CD209, CD11c, CD68, CD163, and HAM-56, and negative for HLA-DR, CD83, CD86, CD208. Moreover, double immunostaining for fascin and CD34 indicated that approximately 50% of TCDMs intimately adhered to capillaries. At ultrastructural level, TCDMs possess wide extremely electron-lucent cytoplasm containing numerous apoptotic dense bodies, and exhibited vigorous phagocytic activity to apoptotic thymocytes. They frequently found to be attached to the basal lamina of capillary with cell-body or cytoplasmic projection. In contrast to TCDMs, DCs were distributed exclusively in the medulla. DCs were positive for fascin, HLA-DR, CD83, CD86, and CD208, and negative for CD11c, CD68, CD163, HAM-56. They did not exhibit any affinity to capillary. DCs possessed extremely irregularly shaped nuclei, numerous membrane invaginations, and did scarcely contain apoptotic dense bodies. We also found that TCDMs are useful diagnostic markers of thymoma. [Conclusion] TCDMs are not DCs but professional scavenger macrophages of apoptotic thymocytes.

P1.06.23
Tissue macrophages arise from fetal liver monocytes during late embryonic development

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Macrophages (MPh) and dendritic cells (DCs) play a central role in tissue homeostasis and immunity and are thought to derive from a common adult bone marrow progenitor. However, we recently showed that microglia derive exclusively from yolk sac (YS)-derived primitive myeloid progenitors (Ginhoux et al, Science, 2010) and Langerhans cells, the DC population of the epidermis, present a dual embryonic origin and mainly derived from fetal liver (FL) monocytes (Hoeffel et al, JEM, 2012).
Accumulating evidences suggests that other macrophage populations are also independent of adult bone marrow hematopoiesis. Thus, we investigated the contribution of YS and FL hematopoiesis in adult macrophage development.
Fate mapping analysis shows that the first wave of YS-derived macrophages is only maintained lifelong in brain and epidermis. We describe here the wave of FL monocytes that colonize every tissue during late embryonic development. Gene array analysis shows a close proximity between these monocytes and tissue macrophages. Using a gene tagging system based on the S100A4 protein expression by FL monocytes, we observed their differentiation in macrophages in vivo at steady state. Tissue macrophages depletion in utero induces FL monocytes differentiation and is CSF-1R dependent. We then identified committed progenitors in the fetal liver able to regenerate tissue macrophages after in utero adoptive transfer. Our data highlight the relative importance of early and late embryonic myeloid development in the formation of key elements of the adult immune system.

P1.06.24
Fighting chronic inflammation - CD64-specific elimination of M1 macrophages

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Macrophages are key players of the innate immunity and versatile cells that can adapt to microenvironmental signals resulting in distinct polarization states with altered phenotype and function. The dynamic ratio of M1 and M2 polarized macrophages is critical for the appropriate development and resolution of inflammation. In chronic diseases, this normal progression is arrested in the M1 dominant phase. Here we show the selective elimination of M1 macrophages by CD64-targeted immunotoxins both in vitro and in vivo in a transgenic mouse model of chronic cutaneous inflammation. We corroborated this using a skin biopsy from a patient with atopic dermatitis. We also show that both populations show phenotypic plasticity and that after conversion into either type, only M1 polarization results in sensitivity towards the CD64-directed immunotoxin. Targeting CD64 on the surface of M1 macrophages will facilitate the development of novel intervention strategies for most chronic inflammatory diseases.

P1.06.25
The functional fate of human monocytes after engagement of various pattern recognition receptors

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After priming with the human commensal *Candida albicans* or the fungal cell wall component β-glucan, monocytes respond with an increased cytokine production upon restimulation, a phenomenon termed "trained immunity". Because the vast majority of commensal microorganisms are of the bacterial or viral phyla, we sought to determine whether similar monocyte reprogramming could be induced by recognition of bacterial or viral ligands by pattern-recognition receptors. Monocytes were functionally programmed for either an enhanced (*training*) or a decreased (*tolerance*) cytokine production, depending on the type of ligand they encountered. The training effect required p38 and JNK mediated MAPK-signalling, with specific signalling patterns directing the functional fate of the cell. The long-term effects on the function of monocytes were mediated by epigenetic events, with both histone methylation and acetylation inhibitors blocking the training effects. In conclusion, our experiments identify the ability of monocytes to acquire adaptive characteristics after prior activation with a wide variety of microbial ligands. Trained immunity and tolerance are two distinct functional programs induced by the specific microbial ligands engaging the monocytes.

P1.06.26
Intracellular chloride channel protein CLIC1 regulates macrophage functions via modulation of phagosomal acidification

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Intracellular chloride channel protein 1 (CLIC1) is a 241 amino acid protein of the glutathione S transferase fold family with redox and pH dependent membrane association and chloride ion channel activity. Whilst the proteins of the 6 member CLIC family are highly evolutionarily conserved in metazoa, indicating an important role, little is known about their biology.
CLIC1 was first cloned on the basis of increased expression in activated macrophages. We therefore examined its subcellular localisation in murine peritoneal macrophages by immunofluorescence confocal microscopy. In resting cells, CLIC1 is observed in punctate cytoplasmic structures that do not colocalise with markers for endosomes or secretory vesicles. However, when these macrophages phagocytose serum opsonized zymosan, CLIC1 translocates onto the phagosomal membrane. Macrophages from CLIC1-/- mice display defective phagosome acidification as determined by imaging live cells phagocytosing zymosan tagged with the pH sensitive fluorophore Oregon Green. This altered phagosomal acidification was not accompanied by a detectable impairment in

phagosomal-lysosomal fusion. However, consistent with a defect in acidification, CLIC1-/- macrophages also displayed impaired phagosomal proteolytic capacity and reduced reactive oxygen species production. Further, CLIC1-/- mice were protected from development of serum transfer induced K/BxN arthritis.
These data all point to an important role for CLIC1 in regulating macrophage function via its ion channel activity and suggest it may be a suitable target for the development of anti-inflammatory drugs.

P1.06.27
Glucocorticoids and IL-4 synergize in M2 polarization of macrophages

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Macrophages play an important although ambivalent role in many inflammatory conditions. This is due to the fact that they can polarize into at least two different subtypes. Classically activated M1 macrophages are induced upon IFNγ and LPS stimulation, pro-inflammatory in nature and responsible for pathogen removal. In contrast, M2 macrophages are induced by Th2 cytokines such as IL-4 and IL-13, glucocorticoids (GCs) or TGFβ, have anti-inflammatory properties and play a role during parasite infections, cancer, tolerance and tissue repair. The balance between both subtypes is believed to strongly impact the outcome of inflammatory responses. In this study we set out to characterize M2 polarization induced in murine bone marrow derived macrophages (BMDMs) upon treatment with a combination of IL-4 and the synthetic GC dexamethasone (Dex). Each reagent caused distinct alterations in macrophage morphology and adherence. Flow cytometry and expression analysis by quantitative RT-PCR revealed that IL-4 and Dex acted synergistically in the induction M2 markers such as CD136 and CD206 while M1 markers including TNFα and CD86 were down regulated. The phagocytotic activity of macrophages was enhanced by Dex but IL-4 failed to further increase this property. Collectively, our study suggests that the combined treatment of macrophages with IL-4 and Dex is a highly efficient strategy to achieve alternative macrophage polarization which could become instrumental in treating inflammatory diseases in the future.

P1.06.28
Intestinal epithelial cell-derived Semaphorin 7A negatively regulates development of colitis via αvβ1 integrin

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The intestinal immune system is constantly challenged by commensal bacteria, therefore it must maintain quiescence via several regulatory mechanisms. Although intestinal macrophages have been implicated in repression of excessive inflammation, it remains unclear how their functions are regulated during inflammation. Here, we report that Sema7A, a glycosylphosphatidylinositol-anchored semaphorin expressed in intestinal epithelial cells (IECs), induces IL-10 production by intestinal macrophages to regulate intestinal inflammation. Sema7A-deficient mice showed severe signs of dextran sodium sulfate (DSS)-induced colitis due to reduced intestinal IL-10-levels. We further identified $CX3CR1^{+}MHCII^{int}F4/80^{hi}CD11b^{hi}$ macrophages as the main producers of IL-10 via αvβ1 integrin in response to Sema7A. Notably, Sema7A was predominantly expressed on the basolateral side of IECs, and its expression pattern was responsible for protective effects against DSS-induced colitis and IL-10-production by macrophages during interactions between IECs and macrophages. Furthermore, we determined that the administration of recombinant Sema7A proteins ameliorated the severity of colitis and these effects were diminished by IL-10 blocking antibodies. Therefore, our findings not only indicate that Sema7A plays crucial roles in suppressing intestinal inflammation through αvβ1 integrin, but also provides a novel mode of IL-10 induction via interactions between IECs and macrophages.

P1.06.29
SHP-1 contributes to the CD40 signaling reciprocity and controls Leishmania major infection

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CD40, a costimulatory molecule expressed on macrophages can induce counteractive immune responses depending on the intensity of CD40 crosslinking. A strong CD40 stimulation induces preferential phosphorylation of p38MAPK and pro-inflammatory cytokine IL-12 production whereas a weaker CD40 stimulation induces preferential phosphorylation of ERK-1/2 and anti-inflammatory cytokine IL-10 production. In the experimental infection with Leishmania major, CD40-induced p38MAPK activation and IL-12 expression are suppressed whereas ERK-1/2 activation and IL-10 expression are enhanced. We concluded that the observed reciprocity was due to differential phosphorylation of these two MAPKs and we propose that this observed reciprocity can be function of a phosphatase that dephosphorylates these MAPKs differentially. Leishmania infection activates a phosphotyrosine phosphatase SHP-1, which we propose to possibly play a role in MAPK dephosphorylation and thereby influences the reciprocity in CD40 signaling. Although CD40 plays important roles in L. major infection, whether SHP-1 influences CD40 signaling and affects L. major infection or vice versa have never been examined.
In this report, we show that SHP-1 does contribute to the CD40 signaling reciprocity by differential p38MAPK and ERK-1/2 dephosphorylation. In L. major infection, SHP-1 phosphorylation and activity are increased causing reciprocal down-regulation of CD40-induced p38MAPK phosphorylation. SHP-1 overexpression renders the resistant C57BL/6 mice susceptible whereas its inhibition protects susceptible BALB/c mice to L. major infection. Thus, we demonstrate for the first time that SHP-1 contributes to the CD40 signaling reciprocity. These properties are exploited by the parasite to design the SHP-1-targeted immune evasion strategy ensuring their survival in a mammalian host.

P1.06.30
The effects of silica nanoparticles in macrophage cells

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Silica nanoparticles, which are applicable in many industrial fields, have been reported to induce cellular changes such as cytotoxicity in various cells and fibrosis in lungs. Because the immune system is the primary targeting organ reacting to internalized exogenous nanoparticles, we tried to figure out the immunostimulatory effect of silica nanoparticles in macrophages using differently sized silica nanoparticles. At non-toxic concentration, the intracellular Ca++ level has increased immediately after exposure to 15 nm particles, not to larger particles. ROS generation was detected significantly in response to 15 nm particles. However, all three different sizes of silica nanoparticles induced IL-8 production. 15 nm silica nanoparticles are more stimulatory than larger particles in cytotoxicity, intracellular Ca++ increase and ROS generation. But IL-8 production was induced to same levels with 50 or 100 nm particles. Therefore, IL-8 production induced by silica nanoparticles may be dependent on other mechanisms rather than intracellular Ca++ increase and ROS generation.

P1.06.31
Alternatively Activated Macrophages - Cause or Consequence in Allergic Airway inflammation?

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IL-4 and IL-13 have numerous functions in allergic diseases, including the induction of Th2 and IgE synthesis, mucus hypersecretion, airway inflammation and airway hyperreactivity. In addition, IL-4 and IL-13 induce alternative activation of macrophages through IL-4 receptor alpha (IL-4Rα). Markers of alternatively activated macrophages (AAM) are upregulated in the lungs of asthmatic patients and mice. AAM are thought to contribute to the pathogenesis of allergic asthma

by virtue of their decreased NO production and increased production of proline and polyamines, important in the synthesis of connective tissues.
However, no studies have shown clear evidence that these macrophages play a causative role in the disease. Therefore, we aimed to define the role of AAM in the pathogenesis of allergic asthma.
The IL-4Rα gene is genetically abrogated in macrophages in LysMcreIL-4Rα-/lox mice, which therefore have impaired activation of AAM. Responses of LysMcreIL-4Rα-/lox mice were examined in ovalbumin (OVA) - and house dust mite - induced allergic asthma. Although expression of markers of AAM such as Ym-1, arginase and FIZZ-1 were decreased in macrophages of LysMcreIL-4Rα-/lox mice, airway hyperreactivity, Th2 responses, mucus hypersecretion, eosinophil infiltration and collagen deposition were not significantly reduced in a OVA- and house dust mite-induced allergic asthma.
In conclusion, we find no evidence to suggest that AAMs are detrimental in allergic asthma in mice, suggesting that effector functions of other cell types are more important in the development of airway pathology. The presence of AAMs in allergic asthma may be merely associative - a consequence of elevated TH2 responses.

P1.06.32
MyD88 and Stat3 signaling are fundamental to tumor associated macrophage function

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Macrophage infiltration of solid tumors correlates with poor prognosis for cancer patients. However, the tumor associated macrophage (TAM) phenotype appears complex and does not emulate the current in vitro paradigms of macrophage activation. TAMs from a variety of solid tumors from distinct organ types display a highly similar gene expression profile, suggesting a common phenotype regardless of tumor type or location. We have also collected gene expression data from TAMs impaired in IL-1 and TLR signaling (Myd88-/-) and observed a MyD88-depedent regulation of multiple secreted proteins involved in Stat3 signaling (IL-6, IL-10, G-CSF) and T cell functional differentiation (IL-23p19, IL-12p40, IL-12p35). TAMs can be further divided into inflammation resolving (R-) and non-resolving NR-TAMs. While IL1/TLR signaling affects both populations, the anti-inflammatory phenotype of Stat3 expressing R-TAMs is specifically regulated by posttranscriptional suppression of cytokine production by Tristetraprolin in a MyD88-independent fashion. Accordingly, deletion of Tristetraprolin leads to the accumulation of NR-TAMs and reduced tumor growth. We therefore suggest that the transition of NR- to tumor-supporting R-TAMs is regulated by Tristetraprolin in a Stat3-dependent manner.
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P1.06.33
SHP-1/Src protein complex differentially regulates IL-12 and IL-23 cytokines in TLR4-activated signaling pathways in human macrophages

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Dysregulated production of IL-12 family cytokines namely IL-12 and IL-23 may result in chronic inflammatory conditions such as rheumatoid arthritis and Crohn's disease. In spite of their critical role in inflammation and autoimmune diseases, the regulation of IL-12 and IL-23 production downstream of LPS/TLR4 is not well understood. Tyrosine phosphorylation and is one of the first events observed following LPS activation of TLR4. Recently Src protein tyrosine kinase (PTK) and SHP1 protein tyrosine phosphatase (PTP) have been implicated in the regulation of cytokine production in LPS-stimulated murine macrophages. However, the involvement of SHP-1/Src complex in LPS stimulated IL-12 and IL-23 production is not known. We hypothesized that SHP-1 and Src form a protein complex required for positive regulation of IL-12 and IL-23 production in LPS-stimulated human monocyte-derived macrophages (MDMs). Inhibition of SHP-1 function with sodium stibogluconate, resulted in decreased secretion of IL-12 and IL-23 in a dose-dependent manner in normal human monocytes and MDMs as determined by ELISA and mRNA analysis by PCR and SHP-1 silencing RNA. The inhibition of Src using SU6656 resulted in enhanced production of IL-12 and IL-23 in a dose-dependent manner as determined by ELISA , mRNA analysis and c-Src silencing RNA. Moreover, SHP-1 and Src were shown to form a protein complex by Immunofluorescence and following their immunoprecipitation and inhibition of SHP-1 prevented activation of Src (pSrc). Our results show for the first time that SHP-1 serves as a positive regulator of IL-12 whereas Src kinase negatively regulates IL-23 production in LPS-stimulated human macrophages.

P1.06.34
DMXAA, a vascular disruptive agent, is a potent inhibitor of prostaglandin synthesis

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5,6-dimethylxantheone-4-acetic acid, DMXAA has been developed as a vascular disruptive agent for use in cancer therapy. The anti-cancer property of DMXAA was attributed to its induction of TNF-a, type-I IFN and reactive oxygen species. The as same as cytokines and ROS, prostaglandins have been known to be involved in progression of cancer, and NSAIDs are known to suppress incidence of some types of cancer. In this study, we show that DMXAA is a potent inhibitor of prostaglandin synthesis.
Macrophages are efficient producer of prostaglandins (PGs). LPS-activated murine macrophages (such as peritoneal macrophages, bone marrow-derived macrophages and macrophage cell lines) produced higher levels of PGE2. PGE2 production from macrophages was significantly suppressed by treatment with DMXAA. DMXAA suppressed production of other type of PG such as PGD2 and TXA2, and also suppressed PGE2 production from LPS-activated human PBMCs and IL-1-activated HUVEC. Levels of COX-2 and PTGES expression and phosphorylation of cytosolic PLA2 were not affected by DMXAA treatment. In addition, DMXAA did not inhibit COX activity, different from NSAIDs such as indomethacin. These results suggest that DMXAA suppress PG production via non-canonical mechanisms.
In vivo tumor models, treatment with DMXAA in E.G7 (murine lymphoma cells)-bearing mice delayed progression of tumor growth. In addition, ptges-deficient mice, whose macrophages do not produce COX-2-dependent PGE2, inoculated with E.G7 cells have delayed tumor growth, suggesting that PGE2 biosynthesis mediates tumor growth in vivo. Now, we are investigating the mechanisms of suppressive effect of DMXAA on PGE2 production.

P1.06.35
Myeloid cell expressed proprotein convertase Furin attenuates inflammation

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Proprotein convertase Furin converts proteolytically immature proproteins into functional end products. Previously we have shown that Furin is a key regulator of T cell mediated peripheral immune tolerance and that it is upregulated upon the activation of monocytes. The aim of this study is to assess the role of Furin in innate immunity using a mouse model with a conditional deletion for Furin in myeloid cells (LysMcre-furf/f).
LysMcre-furf/f mice remain healthy during the first year of life. Flow cytometry analysis of the immune cells populations in WT and KO mice did not reveal marked differences in the lymphocyte, macrophage and neutrophil populations. To address the consequence of Furin deficiency in myeloid cells in vivo, mice were

intraperitoneally injected with LPS and survival and serum cytokines were monitored. LPS injection resulted in significantly higher mortality of KO mice, and elevated levels of TNFa and IL-6 in serum. In contrast, IL10 cytokine levels were markedly lower. Furthermore, the in vitro experiments with peritoneal macrophages demonstrated higher production of pro-inflammatory cytokines and diminished upregulation of IL-10 upon TLR activation. TGF-β1 is a reported target for Furin as well as a downregulator of activation NFKb-p65 transcription factor. Furin deficient myeloid cells were observed to produce significantly lower levels of bioactive TGF-β1 and show hyper-activation of NFKb-p65 upon LPS stimulus.
We conclude that deletion of Furin in myeloid cells results in a proinflammatory immune phenotype, which is due at least in part to the upregulation of NFKb-p65 as result of a deficiency in TGF-β1 production.

P1.06.36
The marine natural product Honaucin A inhibits innate inflammation

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Inflammation is a hallmark of numerous diseases and represents an enormous public health and financial burden. Current drugs do not always adequately treat inflammation and often have undesirable side effects. Marine natural products represent a unique and promising source of novel therapeutic molecules. Many of these compounds exhibit a natural diversity and complexity that make them attractive from a drug screening approach as compared to synthetic libraries. In addition, possible evolutionary molecular modeling of marine natural products may have resulted in compounds with greater relevance in inflammatory pathways. The marine natural product honaucin A was isolated from the cyanobacterium *Leptolyngbya crossbyana* found overgrowing a Hawaiian coral reef. Composed of (S)-3-hydroxy-γ-butyrolactone and 4-chlorocrotonic acid connected via an ester linkage, this small molecule inhibited LPS-induced nitric oxide production in RAW 264.7 macrophages with an IC_{50} of ~4 μM. This activity was accompanied by a decrease in the production of the pro-inflammatory cytokines IL-1β, TNF-α, and IL-6. Treatment of RAW 264.7 cells with honaucin A did not result in phosphorylation of eIF2α indicating that this stress pathway was not activated, nor did the compound inhibit mitochondrial respiration in T47D breast cancer cells. In an *in vivo* ear edema model, the compound significantly inhibited swelling caused by the application of the irritant phorbol 12-myristate 13-acetate relative to a vehicle-only control. Honaucin A's potent biological activity and its relatively simple preparation by synthetic means make it a promising lead in the pursuit of new inflammatory interventions.

P1.06.37
Differential effect of cyanobacterium *Anabaena* sp. lipopolysaccharide (LPS) on rat brain microglia-derived proinflammatory and anti-inflammatory cytokines and chemokines

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Cosmopolitan Gram-negative cyanobacteria may contaminate freshwater by releasing toxins, such as lipopolysaccharide (LPS) thus affecting human health. We recently reported that cyanobacterial lipopolysaccharide (LPS) elicited release of superoxide anion, thromboxane B_2, and matrix metalloproteinase-9 by rat microglia (BMG) *in vitro* (The Toxicologist CD 132 (S-1), 2013). We hypothesized that freshwater cyanobacterium *Anabaena* sp. LPS (AnaLPS) would also activate both classical and alternative activation of BMG *in vitro* and concomitant release of cytokines and chemokines. AnaLPS was prepared by hot phenol/water extraction. *E. coli* LPS (EcLPS) 026:B6 from Difco Lab, Detroit, MI was used as a positive control in these experiments. BMG were isolated from neonatal rats, and treated *in vitro* with either AnaLPS or EcLPS in a concentration-dependent manner for 18 hours at 35.9 °C. Cytokines and chemokines were determined using a Milliplex® MAP rat cytokine/chemokine multiplex immunoassay. Results were the following: EcLPS and AnaLPS stimulated statistically significant and concentration-dependent release of (a) Pro-inflammatory cytokines: IL-6>IL-1β>TNF-α; (b) Pro-inflammatory chemokines: MIP-2/CXCL2>MIP-1α/CCL3>MCP-1/CXCL2; and the (c) Anti-inflammatory cytokine: IL-10, at > than 1ng/mL and 10^4ng/mL, respectively. We conclude that after a 18 hour *in vitro* stimulation AnaLPS stimulated both "classical" and alternative" activation of rat brain microglia, but was considerably *less* potent than EcLPS in stimulating release of the pro-inflammatory cytokines and chemokines, and the anti-inflammatory cytokine IL-10. Continued investigation of the mechanism responsible for the differential response observed with AnaLPS on BMG is currently ongoing in our laboratory. Support by Midwestern University and the University of Hawaii at Manoa is gratefully acknowledged.

P1.06.38
Pulmonary surfactant protein A strengthens IL-4-mediated proliferation and alternative activation of rat alveolar macrophages

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It is well established that IL-4 induces alternative activation of macrophages involving intracellular activation of STAT6. Moreover, it has recently been shown that this cytokine has a proliferative effect on macrophages. However, little is known about the effect of pulmonary surfactant protein A (SP-A), the most abundant immunomodulatory protein in the alveolar fluid, on IL-4-induced effects on alveolar macrophages (aMφ). This study was undertaken to determine whether human SP-A interferes with IL-4 immune actions on aMφ. To accomplish this, purified rat aMφ were stimulated with IL-4 in the presence and absence of SP-A. We measured arginase activity, STAT6 phosphorylation, and aMφ proliferation. We found that SP-A increased IL-4-induced arginase activity and STAT6 phosphorylation. We also found that the synergistic effect of SP-A on IL-4-mediated signaling involved PKC activation since inhibition of PKC abolished SP-A-induced reinforcement of IL-4 signaling effects. In addition, we observed that SP-A augmented IL-4-induced aMφ proliferation. We conclude that SP-A strengthens IL-4 effects on aMφ,. suggesting that SP-A might play a key role in Th2-related pathologies by enhancing IL-4 effects on aMφ.

P1.06.39
Characterization of the innate immune response in human macrophages by Mycobacterium bovis BCG

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Differential protection is attained against TB depending on the *Mycobacterium bovis* BCG substrain used, age and population in trial. Little information exists regarding innate immune response among BCG vaccine substrain infection of human macrophages. Innate immune response characterization of human macrophage infection with different *Mycobacterium bovis* BCG substrains would expand knowledge on protective efficacy. Macrophage cell line THP-I was infected with BCG substrains: Moreau, Danish, Mexico, Tice and Phipps. Live mycobacterium was determinated by intracellular ATP, Macrophage, nitric oxide production and cytokine profile at 0, 24 and 72h were also assessed. The number of bacteria of the BCG substrains Mexico, Moreau and Tice increased more than double at

24h of infection and declined by 72 h while BCG Phipps and Danish substrains grown slowly. Nitric oxide production at 72h by Moreau and Tice BCG infected macrophages increased 100% and 500% respectively as intracellular BCG number decrement was observed. On the other hand the BCG Phipps substrain showed able to induce higher levels of TNF, IL-12 and IL-10 at 72h production post-stimulus, suggesting a Th1 immune response and associated with a protective response in human macrophages.

P1.06.40
Regulation of macrophage complement receptor immunoglobulin (CRIg) expression by cytokines

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Complement receptor immunoglobulin (CRIg) differs in structure, tissue expression and in biological activity to the classical complement receptors, CR3 and CR4. The receptor is expressed in subpopulations of macrophages and promotes the rapid phagocytosis of bacteria and has been shown to be essential in controlling bacterial infection and preventing bacterial dissemination and overwhelming pathogenesis. Apart from its role in innate immunity, CRIg has been demonstrated to regulate T cell function in an immunosuppressive manner. However, little is known about the regulation of its expression. We examined the effects of cytokines which have pro-inflammatory, anti-inflammatory and regulatory functions. The cytokines were found to cause changes to the expression of CRIg on human monocyte derived macrophages, in a manner not akin to the expected functional roles of these cytokines. The effects were seen over a concentration range at which the cytokines cause other effects on the immune system. Changes in expression were evident at both CRIg mRNA and protein level. IFNγ, TNFα, IL-4, IL-13 and TGFβ caused a decreased expression, while IL-1β, IL-6, IL-10, LT, M-CSF and GM-CSF increased CRIg expression in macrophages. In comparison the expression of CR3 and CR4 was differently regulated by these cytokines. Dendritic cells developed in culture from monocytes under the influence of IL-4 and GM-CSF also expressed CRIg and cytokines regulated the expression of CRIg on these cells. The data show that cytokines regulate the expression of CRIg in macrophages, highlighting another point of control of innate immunity and adaptive immunity by cytokines.

P1.06.41
Ubiquitin-like protein MNSFβ covalently binds to Bcl-G and enhances lipopolysaccharide (LPS)/interferon γ (IFNγ)-induced apoptosis in macrophages

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MNSFβ is a ubiquitously expressed member of the ubiquitin-like family that has been involved in various biological functions. Previous studies have demonstrated that MNSFβ covalently binds to intracellular pro-apoptotic protein Bcl-G and regulates the ERK-MAPK cascade in mouse macrophage cell line, Raw264.7. In this study, we demonstrate that MNSFβ promotes lipopolysaccharide (LPS)/interferon γ (IFNγ)-induced apoptosis of Raw264.7 macrophages. In Raw264.7 cells treated with MNSFβ small interfering RNA (siRNA), LPS/IFNγ- or NO donor S-nitrosoglutathione (GSNO)- induced apoptosis was inhibited. SiRNA-mediated knockdown of MNSFβ did not affect iNOS expression in LPS/IFNγ-stimulated Raw264.7 cells. Conversely, co-transfection with MNSFβ and Bcl-G greatly enhanced LPS/IFNγ- induced apoptosis in Raw264.7 cells, accompanied with an increased expression of p53 and a decreased Cox-2 activity. Unlike co-transfection with wild-type MNSFβ, co-transfection of a mutant MNSFβ (G74A) and Bcl-G did not result in an enhancement of LPS/IFNγ- induced apoptosis. Co-overexpression of MNSFβ and Bcl-G reduced GSNO-induced ERK1/2 phosphorylation. Furthermore, EMSA experiments revealed that MNSFβ down-regulates ERK/AP-1 signaling cascade leading to Cox-2 activation. We also observed that MNSFβ•Bcl-G promotes LPS/IFNγ-induced apoptosis of mouse peritoneal macrophages, together with a decrease in Cox-2 expression. Taken together, our data indicate an apoptosis enhancing effect of MNSFβ•Bcl-G is due in part to the down-regulation of Cox-2 activation in macrophages.

P1.06.42
T CD8+ suppressor lymphocyte-derived exosomes carrying miRNA150 impair macrophage ability to induce humoral immune response in mice

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Introduction. Macrophages possess the ability to antigen phagocytosis, processing and presentation inducing adaptive immunity and act as effector cells in contact hypersensitivity, which in mice could be regulated by T CD8+ suppressor lymphocytes producing T suppressor factor (TsF) consisting of antigen-specific exosomes carrying miRNA150. TsF affects macrophages mediating cellular reaction. Macrophage activity can also be observed in development of humoral response to corpuscular antigen (SRBC). Therefore, our studies were aimed to determine if TsF influences the macrophage function in induction of B lymphocyte response.
Materials and methods. Thioglycollate-induced peritoneal macrophages were incubated with exosomes from either trinitrophenol-specific TsF (TNP-TsF) or negative factor (NF), fed with TNP-labelled sheep erythrocytes (TNP-SRBC) and transferred intraperitoneally into naive recipients. Seven days later sera and spleens were collected separately from each recipient and humoral response was assessed by plaque forming assay (PFA) and direct haemagglutination assay (HA) determining titers of anti-TNP-SRBC IgM and IgG antibodies, in comparison to control group of recipients of untreated macrophages fed with TNP-SRBC.
Results. The treatment of macrophages with TNP-TsF exosomes significantly reduced the number of plaque forming cells in PFA (by 34%) and changed IgM/IgG titers ratio (2/8, compared with control 0,5/9) while NF exosomes did not affect estimated parameters.
Conclusion. The impairment of macrophage activity by TNP-TsF exosomes resulted in inhibited maturation of humoral response expressed as lower number of antibody-producing B cells and decreased immunoglobulin class switching. Present study demonstrated the potential mechanism of regulation of B-cell mediated immunity via macrophages affected by antigen-specific exosomes carrying miRNA.

P1.06.43
The role of MAP kinase phosphatase-2 (MKP-2) in macrophage development and gene expression

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Mitogen-activated protein kinase phosphatase-2 (MKP-2) is a type 1 nuclear dual specific phosphatase (DUSP4) and an important immune regulator. It specifically dephosphorylates the MAP kinases ERK and JNK to influence pro- and anti-inflammatory cytokine production. MKP-2 has recently been shown to play a significant role in controlling *Leishmania mexicana* and *Toxoplasma gondii* infections (Al-Mutairi et al., 2010 and Woods et al., in press) primarily by influencing macrophage activity. However, information on the effect of MKP-2 deletion at the molecular level on macrophage development and function is limited. Consequently, we conducted a comparative genome-wide microarray analysis on MKP-$2^{-/-}$ and MKP-$2^{+/+}$ macrophages following innate (LPS), classical (IFN-γ) and alternative (IL-4) activation. As demonstrated previously, and associated with a role for MKP-2 in antimicrobial activity, arginase-1 expression was up-regulated in MKP-$2^{-/-}$ compared with MKP-$2^{+/+}$ macrophages. Surprisingly, and in contrast, we found that the alternative activation

markers YM1 (Chi3l3) and Fizz1/Relm-α (Retnla) were significantly reduced in MKP-2$^{-/-}$ macrophages when compared with their wild-type counterparts. As both YM-1 and Relm-α have been implicated to play a major role in extracellular matrix disposition this suggests a significant role for MKP-2 in wound healing. In addition, our microarray data revealed that the expression of two genes related to macrophage differentiation and proliferation, monocyte to macrophage associated differentiation (MMD) and colony stimulating factor-2 (CSF-2) were dependent on MKP-2. MAPK inhibition also elucidated a mechanism for MKP-2 dependent gene expression. Collectively, our findings establish that MKP-2 is an essential regulator of macrophage development and function.

P1.06.44
Differential regulation of innate immunity in the lungs of smokers and non-smokers

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Recent studies have shown that a smoking increases the risk for autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. The mechanism underlying the risk increase is not known, but we hypothesized that tobacco smoke exposure could modulate the innate immune response, both systemically and in lungs. This was investigated by a comprehensive analysis of innate immune response, including TLR signaling and inflammasome activation in peripheral blood monocytes and alveolar macrophages collected through bronchoalveolar lavage (BAL) from healthy smokers and non-smokers.
Our results indicate that key components for a functional inflammasome response, including NLRP3 and pro-IL-1β are upregulated in alveolar macrophages from healthy smokers, compared to non-smokers. In contrast, in vitro stimulation with known inflammasome activators (e.g. ATP) induced a lower secretion of IL-1β in BAL-cells and peripheral blood monocytes from smokers. These results indicate a role of inflammasome activation in the regulation of smoking induced pathology, and its role in the development of autoimmune diseases will be further investigated.
Transcriptional analysis of selected TLR-dependent genes indicates that smoke exposure induces a specific expression profile, including downregulation of TNF, in light to moderate smokers. These results give further insight into the regulation of the immune response in the airways by tobacco smoke exposure, and will be follow by in-dept analysis of chromatin remodeling and transcriptional regulation of central pathways in the innate immune response.

P1.06.45
Notch Signaling Regulates Expression of Mcl-1 and Apoptosis in PPD-treated Macrophages

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Macrophages are cellular targets for infection by pathogens. The fate of infected macrophages plays a key role in determining the outcome of the host immune response. Apoptosis of macrophages is a protective host defense that eliminates pathogens and infected cells. In this study, we investigated the involvement of Notch signaling in regulating apoptosis in macrophages treated with tuberculin purified protein derivative (PPD). Murine bone-marrow-derived macrophages (BMM) treated with PPD or infected with *Mycobacterium bovis* BCG induced upregulation of Notch1. This upregulation correlated well with the upregulation of the anti-apoptotic gene *Mcl-1* both at the transcriptional and translational levels. Decreased levels of Notch1 and Mcl-1 were observed in BMM treated with PPD when a gamma secretase inhibitor (GSI), which inhibits the processing of Notch receptors, was used. Moreover, silencing Notch1 in cell line RAW264.7 decreased Mcl-1 protein expression, suggesting that Notch1 is critical for Mcl-1 expression in macrophages. A significant increase of apoptotic cells was observed upon treatment of BMM with PPD in the presence of GSI compared to the vehicle-control treated cells. Furthermore, overexpression of truncated Notch1 or dominant negative MAML (DN-MAML) in human monocytic leukemia cell line THP-1 affected the level of Mcl-1. Finally, analysis of the *mcl-1* promoter in human and mouse revealed a conserved potential CSL/RBP-J[[Unsupported Character - Symbol Font ]] binding site. The association of Notch1 with the *mcl-1* promoter was confirmed by chromatin immunoprecipitation. Taken together, these results indicate that Notch1 inhibits apoptosis of macrophages stimulated with PPD by directly controlling the promoter of *mcl-1*.

P1.06.46
Evaluation of the potency of CD137-activated macrophages in inhibiting tumor cell growth

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Macrophages are hematopoietic cells which play a pivotal role in immune defense. Macrophages can phagocytose pathogens and even entire cells. Also, by releasing toxic compounds including reactive oxygen intermediates or by expressing apoptosis-inducing cytokines such as tumor necrosis factor (TNF) or TRAIL, macrophages can kill bacteria and tumor cells. Macrophages express the ligand for CD137 on their cell surface which induces their activation, via reverse signaling. In this study, we aim to investigate whether CD137 ligand signaling can enhance the ability of macrophages to interfere with the growth and /or viability of cancer cells. Peripheral human monocytes were cultured for up to 10 days. Macrophage colony-stimulating factor (M-CSF) was added to differentiate the monocytes into macrophages which were then treated with recombinant immobilized CD137-Fc protein overnight before being co-cultured with MCF-7, a human breast cancer cell line, or with HeLa, a human cervical cancer cell line. Macrophages that had been pre-treated with immobilized CD137-Fc protein significantly reduced the proliferation of MCF-7 and HeLa cells, and caused a higher percentage of MCF-7 and HeLa cells with a reduced DNA content, which would be consistent with an increased incidence of apoptosis. Also, higher levels of TNF, M-CSF and IL-6 were present in the supernatants of these macrophage tumor cell co-cultures. Thus, our study shows that activation of macrophages by recombinant CD137 protein which induces reverse CD137 ligand signaling enables macrophages to suppress proliferation of cancer cells in vitro.

P1.06.47
Statins mediate protection against Mycobacterium tuberculosis infection by enhancing phagosomal maturation and autophagy

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Statins are effective pharmacological inhibitors of the HMG-CoA reductase, a rate-limiting enzyme for cholesterol biosynthesis, and have been shown to reduce bacteremia in statin-users.
We aimed to investigate the effect of statins on M. tuberculosis infected monocytes/macrophages isolated from statin-treated patients and uncovered the possible biological mechanisms in macrophages and mice.
Peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages (MDMs) were isolated from patients with familial hypercholesterolemia (FH) on statin therapy. Cells were then infected with Mtb and their bacterial burden determined in comparison to cells from healthy controls. In addition, mice were treated with statins prior to aerosol infection with Mtb and disease progression in infected mice was monitored in comparison to sham-treated mice.
PBMCs and macrophages from FH patients displayed reduced M. tuberculosis burdens compared to cells from healthy non-statin users.

Statin treatment also reduced bacterial growth in both Mycobacterium-infected PBMCs isolated from healthy human subjects and murine macrophages. Statin treatment reduced mycobacterial burden in lungs and improved pathology as compared to sham-treated infected mice. Mechanistically, statins counteracted Mycobacterium-induced inhibition of phagosomal maturation and increased host-protective autophagy of infected macrophages.
Human patients on statin therapy show increased protection against Mtb infection. In addition, statin treatment led to increased protection against Mtb in infected mice as shown by reduced bacterial burdens and dissemination of bacteria to peripheral organs. Murine bone marrow-derived macrophages (BMDMs) displayed reduced mycobacterium loads following statin treatment. We found that statins inhibited bacterial growth by enhancing phagosomal maturation and autophagy in infected BMDMs.

P1.06.48
Metalloproteinase-9 production by monocytes from cutaneous leishmaniasis patients

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Cutaneous leishmaniasis caused by Leishmania braziliensis is characterized by strong cell infiltrate and inflammatory response. Circulating monocytes are a heterogeneous population subdivided into classical monocytes (CD14 + CD16), intermediate monocytes (CD14 + CD16 +) and non-classical monocytes (CD14-CD16 +), and are known to migrate to inflammatory sites and secrete cytokines. TNF can mediate the pathology of CL through various mechanisms including, induction of nitric oxide (NO), increased cytotoxicity and expression of matrix metalloproteinases (MMPs). MMP-9 is a zinc-dependent enzyme that degrades type IV collagen, a component of basal membrane, and MMP-9 effect is controlled by TIMP-1 (tissue inhibitor of metalloproteinase 1). Although the factors that induce the breakdown of the basal membrane leading to the development of ulcer are unknown, it is likely that MMP-9 contributes to tissue injury in the CL. Mononuclear cells from CL patients were obtained for ex-vivo labeling of sub-populations of monocytes and MMP-9, and the frequency determined by flow cytometry. Our results showed that monocytes were major cells producing MMP-9 and all three sub-populations of monocytes from patients with early CL and CL more expressed MMP-9, when compared with those in healthy individuals. In patients with CL non-classical monocytes producer was the main source of MMP-9. Elevated levels of MMP-9 and lower levels of TIMP-1 were found in PBMC cultures from patients with cutaneous leishmaniasis. We also found that TNF induces MMP-9 production in these individuals. In conclusion, high levels of TNF in patients with cutaneous leishmaniasis contribute to MMP-9 production by monocytes.

P1.06.49
The importance of experimental milieu in regulation of macrophage activation

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Background: Macrophages are important source of cytokines and other compounds, which recruit additional cells to sites of infection or tissue injury. Importantly, their functions might be directly controlled by the composition of milieu in their immediate vicinity. Therefore, the analysis of their function under different in vitro as well as “simulated” in vivo condition is of particular interest.
Materials and methods: Mice peritoneal macrophages were stimulated with different activators (e.g. LPS, IFN-γ, TNF-α and interleukins or their combination) under different experimental conditions (in vitro and “simulated” in vivo conditions). Consequently, the physiological functions of macrophages were assessed using different luminometric, spectrophotometric, molecular, and immunohistochemical methods.
Results: Our data demonstrate that there exists crucial difference in activation of macrophages under classical in vitro and “simulated” in vivo conditions. These abnormalities are accompanied by significant changes in inflammatory response of macrophages as well as in activation of intracellular signaling pathways.
Conclusions: The activation of mice peritoneal macrophages is dependent on the composition of milieu in their immediate vicinity and the “classically used” in vitro systems seems to be not suitable for correct analysis of their functions.

P1.06.50
The interaction of monocytes from Sjögren Syndrome patients with human salivary gland epithelial cells promotes an inflammatory microenvironment modulated by VIP

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Suppressant phagocytosis by macrophages is central for tissue homeostasis maintenance. Sjögren syndrome (pSS) is an autoimmune disease characterized by progressive oral and ocular dryness with high impact in healthcare. Aberrant expression of inflammatory mediators in glandular epithelium and epithelial cell apoptosis were proposed as early events in pSS pathogenesis. Vasoactive Intestinal Peptide (VIP) is a pleiotropic, secretory and vasodilator neuropeptide with immunomodulatory effects through VPAC1/VPAC2 receptors on immune cells.
Our goal was to study the role of VIP / VPAC system in the interaction of monocytes from pSS patients with a human salivary gland epithelial cell line.
Monocytes were isolated from peripheral blood of women with pSS (Vitali C et al. 2002) (n:34) and age-matched healthy women as the control group (n:16). Mononuclear cells were analyzed by flow cytometry and RT-qPCR for phagocytosis, cytokines, chemokines and VIP receptor expression before or after coculture with a human salivary gland cell line (HSG).
pSS monocytes showed an inflammatory profile with an increased expression of VPAC2 (absent in healthy volunteers) and IL-12 expression after coculture prevented by VIP. A decreased phagocytosis of apoptotic cells was observed by pSS monocytes (pSS 23,93%±4,42 vs control 11,61%±1,52 $p<0,05$) . During coculture pSS cells promoted the expression of inflammatory (IL-6, TLR3, TLR4) and migration markers (MCP-1) on epithelial cells without inducing apoptosis markers. VIP reduced TLR3 expression.
We conclude that the interaction of pSS monocytes with gland ephitelium promotes an inflammatory microenvironment partially modulated by VIP.
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P1.06.51
Contribution of microRNAs to human macrophage polarization processes

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Macrophages are essential components of the innate immune system and stand as the first line of defense of the organism against pathogens and virus. Macrophages can be schematically described as classically activated (M1) or alternatively activated (M2). We sought to define the microRNA (miRNA) expression profiles of human polarized macrophages and to characterize macrophage polarization-relevant miRNA targets.
MiRNA profiles from human resting (M0) and polarized macrophages (M1 and M2) were obtained with microfluidic miRNA arrays. 74 miRNAs were significantly differentially expressed among macrophage phenotypes. Hierarchical cluster analysis of these 74 miRNAs showed that M0, M1 and M2 could be discriminated by the differential expression of these miRNAs. For instance hsa-miR-135b

was strongly upregulated in M1 whereas hsa-miR-193b and hsa-miR-424 were upregulated in M2. The modulation of these miRNAs was further validated by RT-PCR in independent biological replicates. Moreover, biological pathways analysis showed that hsa-miR-135b is highly predicted to impact on the IL-4 signaling pathway, while hsa-miR-193b and hsa-miR-424 may modulate pathways associated with recognition of bacteria and virus by pattern recognition receptors (hsa-miR-193b and hsa-miR-424) and IL-15 production (hsa-miR-424). Finally, using 3'UTR luciferase assays, we validated the M2-related transcription factors c-MYC, STAT6 and KLF4 as targets of M1-associated hsa-miR-135b. In conclusion we show that macrophage phenotypes can be discriminated by the differential expression of a panel of miRNAs. The pathways impacted by some of the top-regulated miRNAs indicate a potential profound effect of these miRNAs on macrophage polarization processes and functions.

P1.06.52
Effective macrophage expression strategy

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Differentiation of bone marrow progenitors into macrophages requires macrophage colony-stimulating factor (Csf1) which signals via its receptor Csf1r. Expression of Csf1r within the haemopoeitic system is highest in monocytes and macrophages. MacGreen mice were created previously by placing EGFP expression under the control of the Csf1r proximal promoter and included Fms Intronic Regulatory Element (FIRE) located in the first intron. These mice have consistent expression of EGFP in the same locations as the endogenous gene. FIRE is remarkably conserved across mammalian species, including marsupials, with greater than 90% nucleotide homology between mouse and human, higher than any exons of the gene. Our laboratory has utilised both the murine Csf1r promoter and FIRE sequences in a lentiviral system to drive macrophage specific expression of EGFP in mouse, rat, sheep, pig and human cells. *In vitro* transduction of rat bone marrow cells resulted in EGFP expression only in macrophages when cells were differentiated with Csf1. This work has also resulted in the production of MacGreen sheep with the generation of rats currently underway. The broad species cross reactivity of this lentivirus makes it an attractive vehicle for gene therapy as macrophages have been implicated in numerous diseases including arthritis, atherosclerosis, lysosomal storage diseases and malignant tumours. Lentiviral vectors have already been shown to promote efficient antigen expression and presentation both *in vivo* and *in vitro* highlighting the potential use of our Csf1r-EGFP lentivirus as a vaccine that targets a wide range of cancers and infectious diseases.

P1.06.53
Altered Expression of CD200/CD200R1 Immunoinhibitory Proteins is Associated with the Dysregulated Microglial Response to Ischemic Stroke Injury in Aged Mice

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Objective: The role of CD200-CD200R1 signaling in the healthy brain is important in maintaining microglia in an inactive, resting state. Recent studies investigating the imbalance of CD200-CD200R1 signaling in models of neurodegenerative disease support the hypothesis that these neuronal-glial interactions may also be important in the aged brain. The purpose of this study was to investigate the microglial response to ischemic injury in the aged brain.
Methods: Young (8 wks) and aged (20 mos) male C57Bl/6 mice were subject to sham surgery or 90min right middle cerebral artery occlusion and sacrificed at 72hrs post-reperfusion. The stroke hemisphere, spleen, and blood were harvested in separate cohorts for immunohistochemistry, Western blot analysis, and flow cytometry.
Results: Aging males had significantly smaller infarcts than young males. A significant decrease in CD200 protein expression was seen in aged brains at baseline compared to young, and further decreased after stroke in both aged and young brains. While CD200R1+ microglia significantly increased in all stroke animals, aged mice had significantly less peripheral leukocyte infiltration. The expression level of activation markers (CD80, MHCII) was significantly higher in aged microglia compared to young after stroke.
Conclusions: The aged mouse brain shows a marked imbalance in CD200-CD200R1 signaling compared to the young brain at baseline. Despite similar imbalances in expression after stroke, surprisingly, the inflammatory response appears to be more robust in the young. In summary, we observed that aged mice had higher baseline activation, leading to a muted response to injury.

P1.06.54
Age-and Location-Related Changes in the Immunoregulatory Status of Microglia

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Objective: Microglia display regional heterogeneity in the adult CNS which may contribute to region-specific responses to injury. Despite their importance to many CNS diseases, many of which manifest in the elderly population, how microglia change with aging is less understood. We examined region-specific changes in brain and spinal microglia immunophenotype across the lifespan.
Methods: Naïve young (8 wks) and aged (20 mos) male C57Bl/6 mice were sacrificed and perfused with PBS prior to brain and spinal cord harvest. Samples were processed for immunohistochemistry and into single cell suspensions for analysis by flow cytometry.
Results: The percentage and mean fluorescence intensity of microglia expressing the activation markers CD40, CD68, CD80, MHCII, CD200R1 were significantly upregulated in the aged CNS. Intracellular cytokine staining revealed age- and region-specific changes in TNF, IL1β, and IFNɣ expression. The percentage of phagocytic microglia was higher in the young brain compared to the aged brain, while spinal microglia showed low activity across the lifespan.
Conclusions: We found an age-related increase in expression of various myeloid activation markers and cytokine production that displayed regional heterogeneity. These changes were associated with an increased shift in scatter properties suggesting more dystrophic, senescent microglia and fewer ramified, resting populations in aged, as confirmed by immunohistochemistry. Aged microglia appeared dysfunctional based on phagocytic potential and enhanced pro-inflammatory cytokine production. These findings are consistent with the existence of diverse immunological phenotypes among microglia in the mouse CNS and add to our understanding of the compartmentalized functions performed by microglia in the aged CNS.

P1.06.55
Modulation of FcgammaRs expression in macrophages derived from THP-1 cells (THP-1-MΦ) polarized to inflammatory, wound-healing and regulatory phenotypes

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Macrophages can display a high degree of phenotypic heterogeneity, depending on their anatomical location, and also as a result of activation in response to microenvironmental factors. Characterization of these diverse activation states is important because of its relevance for the induction of an effective immune response and because inappropriate activation can have pathogenic effects.
We sought to establish a cell-line model in which to study and characterize the phenotypic changes of human macrophages associated with distinct types of activation. We used THP-1 promonocytic cells, and differentiated them to a macrophage-like phenotype (THP-1-MΦ) with PMA. THP-1-MΦ cells were exposed to different cytokines, to be polarized into three subpopulations of macrophages: INFγ for inflammatory macrophages, IL-4 for wound-healing macrophages and IL-10 for regulatory macrophages. The polarization phenotype was corroborated by the expression of the surface markers CD80, CD206 and CD163 respectively. To assess the effect of polarization on FcγR expression and function, we evaluated the expression of CD64 (FcγRI) and CD32 (FcγRII), as well

as phagocytosis of IgG-coated sheep erythrocytes. INFγ polarized macrophages showed a 2.7-fold increase in CD64 expression, but a reduction of 50% in phagocytic capacity. In contrast, IL-10 treatment increased 1.7-fold the membrane expression of CD32, and three times the phagocytic capacity as compared with control cells. In IL-4 treated macrophages we observed a decrease of 30% in phagocytosis, and no statistically significant changes in CD32 and CD64. This model can be used to study phenotypic changes and plasticity in macrophage-like cells.

P1.06.56
Non-classical circulating monocytes are reduced in patients with systemic lupus erythematous (SLE) and they show regulatory functions on classical monocytes in healthy controls

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Monocytes modulate inflammatory processes; recognize, remove, and present auto-antigens from apoptotic cells (AC), influencing the activity of SLE, innate and adaptive responses. Three subpopulations are recognized: classical, non-classical and intermediate. Patients with active SLE had normal monocyte counts with a reduction in the proportion of non-classical ones. Monocytes from patients with active SLE also showed reduced expression of HLA-DR, CD14 and CD16, decreased binding and phagocytosis of AC and did not up-regulate CD80 after its interaction with AC. We developed a co-cultures model to study interactions between classical and non-classical monocytes on the differentiation of classic monocytes and their interaction with autologous T lymphocytes in the presence of apoptotic Jurkat cells. Monocytes were electromagnetically sorted as non-classical and classical ones and reconstituted in different proportions. Co-cultures were made in the presence or the absence of AC evaluating the influence of non-classical monocytes and AC in the process of monocyte differentiation. AC induced up regulation of CD80 and HLA-DR in classical monocytes; however, the presence of non-classical ones prevented these increase. Co-cultures of classical and non-classical monocytes, independent of the presence of AC, showed decreased T cell proliferation and low numbers of IFN-γ+ proliferating cells. Although the presence of AC and non-classical phagocytes, affected cytokine accumulation; there was not a clear association between decreased T cell proliferation and monocyte cytokines. The evidence suggests that non-classical monocytes play a role as regulatory cells suppressing auto-reactive T lymphocytes in SLE.
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P1.06.57
Isolation of mouse alveolar macrophage using mixed primary culture from lungs

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Alveolar macrophages are phagocytes that present a key role on the immune response against a variety of pulmonary infectious diseases. However to study this population ex vivo in high purity manner, are necessary expensive and/or trick techniques and several animals to achieve a reasonable number of cells. Recently, Kitani and colleagues (2010) adapted a very classic and broad accepted protocol - used to isolate macrophages from brain (microglia) - to isolate liver macrophages (Kupffer cells). Surprisingly, this protocol presented similar efficacy compared to the former one. Following this rational we thought if it would work on lungs to isolate the resident macrophages.
To address that, lungs from BALB/c mice were excised and homogenized. The suspension was centrifuged at 450g for 10 minutes and pellet was resuspended with culture medium and plated on culture flask mantained at 37°C, 5% of CO2 in a humidified incubator. The medium was replace every 3 days and the day 12-14 were selected as the best time frame to recover macrophages. These macrophages grow above the concomitant formed fibroblast cell sheet and are easily detachable, through 30 minutes of orbital shaking at 120 RPM. Cell morphology observed by microscope reveals similarities with Kitani's results and flow cytometry analysis showed that more than 98,5 % was positive to macrophage markers. Further assays will verify the phagocyte ability of these cells and the mediators released by potent stimuli incubation.
We showed that mixed primary culture technique isolate alveolar macrophages efficiently, comparable to microglia and Kupffer cells isolation procedure.

P1.06.58
Mac-1 controls the pro-inflammatory phenotype of type 1 macrophages

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Macrophages perform dichotomous tasks; they are mediators of inflammation yet are capable of suppressing immune responses and mediate tissue homeostasis. The mechanisms by which macrophages shift their phenotype is unknown. We hypothesized that accumulation of macrophages during inflammation induces a shift from a pro-inflammatory to an anti-inflammatory phenotype, aimed at dampening the immune response. Pro-inflammatory type-1 (mϕ-1) but not anti-inflammatory type-2 macrophages were highly sensitive to homotypic cell-cell contact. Increased cell-cell contact led to mϕ-1 producing lower levels of TNF, IL-1 and IL-6, while the IL-10 production was increased upon stimulation with TLR ligands or *M. tuberculosis*. To confirm this association *in vivo* a wound healing model was performed in mice. At day 4 and 6 of wound healing an increased amount of $F480^+$ macrophages was found compared to day 2. Fitting with our *in vitro* data, macrophages isolated from the wound at day 4 and 6 produced less TNF, IL-1 and IL-6 after TLR stimulation, compared to day 2. Further *in vitro* data showed that this cytokine shift depended on Mac-1 mediated cell-cell contact, signaling via p38 and the activation of COX2. Low IL-10 and high TNFα levels in high density cells were associated with an up regulation of SOCS3. Concluding, Mac-1 activation during macrophage differentiation serves as a natural inhibitory mechanism designed to dampen chronic inflammation. This places signaling via Mac-1 at a center stage in the regulation of macrophage behavior in a wide range of diseases including autoimmune diseases as well as cancer, elucidating novel therapeutic avenues.

P1.06.59
The immunomodulatory effect of propolis and chlorhexidine on cytokine production by human monocytes

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Propolis (P) is a resinous product made by bees from several parts of plants, showing plenty of biological and pharmacological properties, with a great importance in food and in the pharmaceutical industry. Periodontal disease may occur due to an imbalance in the oral microbiota, whose agents can be transmitted. Chlorhexidine (CHX) is an oral hygiene product typically prescribed to periodontal disease patients, but its use is controversial due to side effects. This work aimed to investigate propolis, chlorhexidine and the association P/CHX on pro- and anti-inflammatory cytokine production (TNF-α and IL-10) respectively. Human monocytes were obtained from healthy donors (n=10) and incubated with propolis (0.2, 1.0), chlorhexidine (1.2, 6.0) and P/CHX (0.2/1.2, 1.0/6.0 µg/mL) or control cells for 18h. Cell viability was assessed by the MTT assay, and cytokine

production was determined by ELISA. Significant differences were determined by analysis of variance (ANOVA), followed by Dunnett's test ($p<0.05$). Treatment with P, CHX and P/CHX had no effect in monocytes viability. CHX has stimulated TNF-α production in both concentrations; on the other hand, the association P/CHX increased significantly IL-10 production. One may conclude that this association showed anti-inflammatory effects, what indicates its use in the the treatment of periodontal diseases, with no side effects.

P1.06.60
Polyporus alveolaris extract (PAE) have the ability to stimulate macrophages against tumor with unique manner

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To find the new anti-tumor reagents from natural solutions, we examined a series of plant extracts and mushroom extracts to activate the macrophage induced anti-tumor activities without over production of inflammatory monokines. We used RAW264.7 cells as effector cells (murine macrophage cell line), and we also used EL-4 or P815 (murine tumor cell line) as target cells. More than thousands of screening, finally, we found PAE from Hachinosutake (*Polyporus alveolaris*; this is a kind of the mushroom) by heated water extraction which has much possibility. The growth suppression activity against tumor cell by PAE stimulated RAW264.7 was the level that was about almost same as the LPS which was the classical well known as macrophage activator. Interestingly, anti-tumor activity of RAW264.7 by LPS stimulation used for a control disappeared under Polymyxin B coexistence, whereas the effect of PAE was not almost affected by Polymyxin B. Besides, we measured TNF-α production, nitric oxide and phosphorylation patterns of the signal in the cell. As the result, PAE stimulated RAW264.7 produced TNF-α and nitric oxide dose dependently without receiving most of the influence of Polymyxin B. We also analyzed the signal in the cell to study their production mechanism and confirmed phosphorylation of ERK, JNK and P38. As the result, all MAP kinase was phosphorylated in chronological order unlike the LPS by PAE. We are now focused on a difference of PAE receptor and TLRs on macrophage activation.

P1.06.61
Realtime analysis of monocyte migration in 3D synovial micromass tissue cultures

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In the synovial tissue of arthritic joints, monocyte (Mo) migration appears to be in close contact to fibroblast-like synovial cells (FLS).
Here we analyzed Mo migration under inflammatory conditions in regard to cell-cell interactions with FLS.
Human FLS were prepared from synovial tissues following joint arthroplasty. CD14+ Mo were isolated from peripheral blood. Cells were labeled with fluorescent membrane dyes and cultured in spherical extra- cellular matrix micromasses. For stimulation 10 ng/ml of tumor necrosis factor (TNF) was added. Cell migration was monitored by real-time confocal microscopy and subdivided into three phases of cell movement. Phase I (day 1-3) was characterized by the formation of the synovial lining layer. Mo in close contact with FLS appeared sessile. On average 20% of Mo displayed a mobile and seeking behavior. During phase II (day 3-7) already >95% of Mo were in contact with FLS. The majority of Mo remained sessile whereas a fraction of Mo moved with an impressive maximum speed of up to 15 mcm/min. In addition the formation of Mo cell clusters was observed. Rapid Mo migration ceased during phase III (day 7-14). TNF i) increased the frequency and size of Mo cell clusters during phase II two and ii) prolonged the mobility of Mo into phase III. This 3D synovial tissue culture system allows to monitor and analyzed subtle migration patterns of Mo in relation to the FLS lining. Ongoing experiments address molecular mechanism(s) of Mo – FLS interaction and will try to identify potential therapeutic targets

P1.06.62
The sulfation level of glycosaminoglycans in monocytes and macrophages

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Sulfation of glycosaminoglycans of components of extracellular matrix and cell surface is an important regulatory mechanism in inflammatory immune response. All sulfotransferases use 3´-phosphoadenosine 5´-phosphosulfate (PAPS) as a common sulfate donor that is produced by PAPS synthase.
In order to detect the sulfation level of monocytes/macrophages we established a CD44-hyaluronan binding assay. Monocytes were isolated from human peripheral blood of healthy volunteers. Macrophages were cultivated from monocytes at 37 °C in the presence of 100 U/ml granulocytes/macrophages - colony stimulating factor. CD44 is a cell-surface receptor that binds via heparan sulfate side chains hyaluronan, a glycosaminoglycan that is released from immune cells at inflammatory sites. Using flow cytometry approaches, the expression of CD44 was followed by antibodies against CD44, while the ability of CD44 to bind hyaluronan was assessed with FITC-labelled hyaluronan.
The additional use of a Blyscan™ dye binding assay was used to detect the sulfation level of glycosaminoglycans on the whole cell surface. To reduce stimulation of the cells by adherence, teflon-coated cell culture dishes were used. In order to simulate pro-inflammatory conditions monocytes were stimulated with TNFα.
TNFα increased the expression of CD44 on cells similar to macrophages, but shows no significant alterations of CD44 expression in monocytes. TNFα has no significant influence concerning hyaluronan binding on monocytes. The inhibitor of sulfation reactions chlorate diminished the binding of hyaluronan in a concentration-dependent manner in both TNFα- stimulated and non-stimulated cells.
This assay is a convenient tool to investigate effect on sulfation after the treatment with anti-inflammatory agents.

P1.06.63
IRF8 and IRF3 cooperatively regulate rapid interferon-β induction in human blood monocytes

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Type I IFN production differs in magnitude and kinetics between different cell types. Robust and rapid induction of interferon-β (IFN-β) after pathogenic stimulation is a key property that is exclusive to human blood monocytes. This disparity raised the possibility that distinct myeloid-specific transcription factor(s) may be involved in the rapid induction of IFN-β in monocytes compared with non-myeloid cell types. We found that IFN-β was produced rapidly in primary human monocytes as a result of cooperation between the myeloid-specific transcription factor IRF8 and the ubiquitous transcription factor IRF3. We provide evidence that IRF8 constitutively binds to the IFN-β promoter region, and knockdown of IRF8 in monocytes abrogated IFN-β transcription. We uncovered a requirement for IRF3, a master regulator of IFN-β production, as a previously unidentified interaction partner of IRF8. We produced a range of deletion constructs of IRF3 and IRF8 mutants, and, using co-transfection and co-immunoprecipitation, mapped the protein-protein interacting regions of IRF3 and IRF8, and found that their interaction was independent of the DNA-binding domain (DBD) and the IRF association domain (IAD) of IRF8 and IRF3, respectively. Through these and other experiments, we have been able to demonstrate that IRF8 directly synergizes with IRF3 in monocytes to facilitate faster IFN-β transcription after pathogenic stimulation.

P1.06.64
Resolving inflammation: M2 macrophages contribute to the resolution of postoperative ileus

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Background: Postoperative ileus (POI) is a common complication after abdominal surgery which is mediated by a severe inflammation of the intestinal muscularis externa (ME). Monocytes, infiltrating already 3h after surgery and present up to 7 days within the ME have been shown to be the dominant immunocyte population. This long persistence indicates a contribution in the resolution of POI. Here, we characterized the infiltrating monocytes and analysed their properties in resolving POI.
Material & Methods: POI was induced by intestinal manipulation of the small bowel in mice. Expression of typical M2 macrophage markers Arginase-1, IL-10, mannose receptor 1, and Ym1 was investigated by quantitative PCR and arginase assay in ME tissue. Cytokine and surface receptor expression was analysed by flow cytometry. Additionally, FACS-separated resident and infiltrating macrophages were also characterized by gene expression analysis.
Results: Expression and secretion of Arginase 1 was significantly increased in ME tissues with a peak at 24 hours after intestinal manipulation (750-fold upregulation). $F4/80^{+}$ $Ly6C^{+}$ macrophages were identified as the main incoming cell population (70% of immunocytes) with high expression of M2 macrophage marker Arginase-1 as well as anti-inflammatory cytokine IL-10. Further classification of the alternative activated macrophages revealed a myeloid derived suppressor cell (MDSC) phenotype demonstrated by $CD11b^{high}$ $GR1^{low}$ surface expression by flow cytometric analysis.
Conclusions: $F4/80^{+}$ $CD11b^{high}$ $GR1^{low}$ macrophages are the dominant infiltrating immunocyte population in POI, showing a MDSC phenotype. Our data indicate that these cells can facilitate active resolution of POI by providing an anti-inflammatory milieu within the postoperative ME.

P1.06.65
CX3CR1+ monocytes support P. gingivalis survival

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Two major subsets of monocytes play an important role in the innate immune response. Murine LY6C-high monocytes, termed inflammatory monocytes, are rapidly recruited to sites of infection, whereas Ly6C-negative, CX3CR1-high monocytes, termed patrolling monocytes, contribute to tissue homeostasis. *Porphyromonas gingivalis* is a gram-negative anaerobic bacterium known to be a major pathogen in periodontal disease. By manipulating the innate immune response**,** *P.gingivails* persists within a highly inflamed environment that leads to dysbiosis and eventually soft tissue and bone destruction. Surprisingly, we found that monocyte depletion using clodronate liposomes resulted in improved clearance of *P. gingivalis* following subcutaneous infection. In contrast, bacterial survival in CCR2-/- mice (characterized by defective recruitment of Ly6C-high monocytes) was enhanced, suggesting that Ly6C-high monocytes are required to clear *P. gingivalis*. Bacterial clearance was improved in CX3CR1-/- mice consistent with the effect of monocyte depletion using clodronate liposomes, suggesting that *P. gingivalis* persistence in the host depends on this monocyte subpopulation. We further demonstrated that monocyte TLR2 expression is linked to bacterial survival. Collectively, our results imply that while LY6C-high monocytes provide protection against P. gingivalis, CX3CR1-high monocytes, in a TLR2 dependent manner, support bacterial survival and ability to cause tissue destruction.

P1.06.66
Identification of RORC as regulator of polarized inflammation

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The signaling pathways that cause innate immune cells to dampen adaptive immunity and to promote tumor development remain incompletely understood. We have identified a new subset of retinoid-related orphan receptor gamma (ROR gamma) expressing myeloid cells that arise exclusively from processes of chronic inflammation. Our data indicate that ROR gamma is a negative regulator of M1 inflammation in vitro and in vivo. In particular, we observed that ROR gamma down-modulates expression of pro-inflammatory cytokines and cytotoxic functions of myeloid cells in response to TLR-agonists. Further, we observed that tumor growth (fibrosarcoma) in ROR gamma deficient mice is significantly inhibited. ROR gamma is known as a circadian regulator of glucose/lipid metabolism and insulin resistance and is a key transcription factor of Th17 cell development. Our findings link transcriptional regulators of energy metabolism to polarization of innate immunity, providing new approaches to target, cure and prevent chronic inflammatory diseases, including cancer, related to the metabolic disease cluster.

P1.06.67
Role of macrophages reaction in pathogenesis of duodenal ulcer bleeding

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Macrophages are considered as key regulator of inflammation and reparation which are tightly associated with hemostasis state. In this work we analyzed the role and mechanisms of macrophage dysfunction in patients with primary and recurrent duodenal ulcer bleeding.
Materials and methods. The morphological observation of marginal ulcer zone of 64 patients with sustained hemostasis (1^{st} group) and 28 patients with recurrent bleeding (2^{nd} group) was performed. The number and distribution of CD68 positive cells were assessed in different regions of duodenal mucosa, including villi, transitional and pericryptal zones. Additionally to this iNOS activity in peripheral blood monocytes was measured under basal conditions and after incubation with lipopolysaccharide (LPS), 5-hydroxytryptamine (5-HT) and epinephrine in NBT test.
Results. The high density of macrophages was observed in villi rather than pericryptal region of duodenal mucosa biopsy in 1^{st} group. Increased recruitment of macrophages in duodenal mucosa in transitional and perycryptal space in 2^{nd} group was associated with rising of basal activity of iNOS while monocytes response on LPS was restricted. Recurrent ulcer bleeding was associated with increase of macrophages number ($P = 0.012$). Moreover their accumulation around bottoms of crypts in biopsy of 2^{nd} group patients correlated with monocytes iNOS activity under basal conditions ($P = 0,001$), and after incubation with epinephrine ($P = 0.001$) or thrombine ($P = 0.02$), but not with 5-HT. Conclusion. Proinflammatory activation of monocytes and increase of macrophages recruitment into duodenal mucosa were associated with changes of sensitivity to humoral and coagulation factors.

P1.06.68
Effect of phytoestrogens on helper T cell and macrophage differentiation

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We previously reported that bisphenol A promoted Th2 differentiation (**Int Arch Allergy Immunol.** 132:240-247, 2003) and that prenatal BPA exposure is further effective compared to the exposure in

adulthood (**Environ Health Perspect** 116:514-9. 2008). In this paper, Th cell differentiation and M1 and M2 markers of macrophages from C57BL6 mice given soy bean-free (SF) or normal (N) chows for several generations were investigated. Splenocytes from SF mice produced less IFN-γ than those of N mice. Percentages of Th1 cells decreased in SF mice. In addition, BPA treatment before immunization suppressed differentiation of Th1 cells and IFN- γ production. These results indicate that component(s) of soy beans inhibit Th1 differentiation. BPA influences additively on the inhibitory effects of the soy bean component(s). Direct effects of bisphenol A or soy bean component(s) must not be responsible for the differentiation of Th2, macrophages differentiation was investigated. Peritoneal macrophages from SF mice expressed less M2 markers such as CD204 and CD206 than those of N mice. TLR4 expression was comparable in both SF and N. These results indicate that component(s) of soy beans might induce M2 differentiation.

P1.06.69
Immune-modifying particles inhibit inflammatory monocyte migration to the central nervous system and improve outcomes in experimental autoimmune encephalomyelitis

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Inflammatory monocytes play a significant role in the pathogenesis of most autoimmune diseases. Although the specificity of the immune response against self antigens is dictated and driven by T and/or B cells, the multifarious but non-specific factors secreted by monocyte-derived cells contributes significantly to the destruction of host tissue. Experimental autoimmune encephalomyelitis, the murine model of multiple sclerosis, is characterized by significant infiltration of inflammatory monocytes into the central nervous system (CNS). Here, they give rise to macrophage and dendritic cell populations that produce numerous factors including proinflammatory cytokines, reactive oxygen species and proteinases that contribute to neuronal damage and death. Furthermore, these cells process and present self-antigens to T cells, which further potentiates the inflammatory response by promoting epitope spreading. Thus, inflammatory monocytes have been identified as key targets for immunomodulatory therapies. However, there are few treatments available that specifically target these cells during disease. Here, we show that intravenous infusion of a novel therapeutic agent, highly negatively-charged “Immune-modifying particles” (IMP), from the onset of disease, significantly reduced inflammatory monocyte migration into the CNS by 70%, which was associated with significantly improved clinical outcomes. Furthermore, treatment of animals with IMP during remission prevented subsequent relapse. The anti-inflammatory effects of IMP resulted in the prevention of monocyte trafficking to the CNS and retention of these cells in the spleen, where they undergo apoptosis. Future studies will investigate the cytokines/chemokines involved in the redirection and retention of inflammatory monocytes in the spleen and their differentiation into M1 vs M2 effector cells.

P1.06.70
E.coli upregulates CD39 expression in RAW264.7 macrophages

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Adenosine is a purine nucleoside signaling molecule that elicits its physiological responses by binding to and activating 4 G protein-coupled transmembrane adenosine receptors. Extracellular adenosine accumulates following the release of ATP and ADP, which are metabolized to adenosine by a cascade of ectoenzymes on the cell surface. In the first step the extracellular hydrolysis of ATP and ADP to adenosine monophosphate (AMP) is catalyzed by the ectonucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1, CD39). AMP then metabolized to adenosine by 5’- ectonucleotidase (Ecto5’Ntase, CD73). Macrophages are pivotal in detecting bacteria and in initiating the host’s immune/inflammatory response. The regulation of CD39 in macrophages exposed to bacteria is poorly understood. To study this regulation we stimulated RAW264.7 murine macrophages with heat inactivated *E. coli* and specific Toll-like and NOD-like receptor agonists. *E. coli* increased CD39 mRNA and cell surface protein expression level. The selective TLR2 agonists Pam3CSK4 and FSL-1, TLR3 agonist poly(I:C), TLR4 agonist LPS, TLR5 agonist flagellin and TLR9 agonist ODN1826, but not the TLR7 agonist ssRNA40 or NOD agonists mimicked the effect of *E. coli* in upregulating CD39 expression. *E. coli* increased luciferase activity when the cells were transfected with a CD39 promoter luciferase construct. Thus, bacteria and their products can upregulate CD39 expression.

P1.06.71
Pulmonary GM-CSF regulates alveolar macrophage population size and function via a reciprocal feedback mechanism

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Pulmonary alveolar macrophages are critical to surfactant homeostasis and lung host defense. While multiple cytokines affect survival, proliferation, and functions, the mechanism(s) maintaining population size and functions of resident alveolar macrophages are poorly understood. Disruption of GM-CSF signaling by CSF2RA or CSF2RB mutations impairs alveolar macrophage functions including surfactant clearance and causes hereditary pulmonary alveolar proteinosis (hPAP) a lung disorder of surfactant accumulation. We evaluated Csf2rbKO mice as an experimental model of hPAP in humans and then conducted preclinical safety and efficacy studies evaluating intrapulmonary administration of functional macrophages (pulmonary macrophage transplantation or PMT) in mice. The key features of hPAP in children with recessive CSF2RA or CSF2RB mutations were faithfully reproduced in Csf2rbKO mice. PMT was well-tolerated and effectively resolved hPAP after only one administration. PMT synchronously normalized the increased GM-CSF observed and expanded the number of functional alveolar macrophages to levels similar to those of normal mice. PMT using Csf2rbKO-derived, lentiviral vector Csf2rb-corrected macrophages was equally effective as PMT with normal macrophages., M-CSF expression by alveolar macrophages was increased in Csf2rbKO mice and promoted their survival but not normal functions and was repressed by restoration of GM-CSF signaling. Results demonstrate that PMT is well-tolerated, efficacious and durable as therapy of hPAP in Csf2rbKO mice, and identified a novel, reciprocal feedback mechanism by which pulmonary GM-CSF regulates alveolar macrophage accumulation and functions. These results have therapeutic implications for hPAP and provide a novel experimental approach for studying macrophages in vivo.
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P1.06.72
Effect of CCL1 depletion on the tumoricidal monocyte generation in cultures of peripheral blood monocytes derived from patients with hepatocellular carcinoma (HCC)

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A majority of peripheral blood monocytes in advanced HCC patients has been classified as M2b monocytes, which are inhibitory on host antitumor immunities. In this study, we tried to convert macrophages (monocytes) from M2b macrophages (HCC patient monocytes) to M1 macrophages (tumoricidal macrophages) on the basis of CCL1 knockdown. CCL1 released from M2b macrophages has been characterized as an essential chemokine for the maintenance of their M2b macrophage properties. $CD14^+$ monocytes with M2b macrophage properties were isolated from peripheral blood of advanced HCC patients by Ficoll-Hypaque sedimentation and magnetic beads. $CD14^+$ monocytes (1×10^6 cells/ml) were treated with 6 μg/ml of CCL1 antisense oligodeoxynucleotides (ODN) for 48 hrs. These cells were, then, stimulated with CpG DNA (10 μg/ml, 24 hrs) for the M1 macrophage induction. Cells harvested were assayed

for their (1) tumoricidal activities against cultured HCC cells and (2) IL-12-producing capabilities by flow cytometry and immunochemical staining. In the results performed under the CpG DNA stimulation, advanced HCC patient peripheral blood monocytes were shown to be IL-12$^-$IL-10$^+$ monocytes without any tumoricidal activities. However, IL-12$^+$IL-10$^-$ monocytes were induced by CpG DNA in cultures of advanced HCC patient peripheral blood monocytes previously treated with CCL1 antisense ODN. Also, these IL-12$^+$IL-10$^-$ monocytes induced by CpG DNA from advanced HCC patient monocytes killed HCC cells that were syngeneic to the monocytes. These results indicate that, in advanced patients, macrophage-mediated host's antitumor immunities are controllable, in part, through the depletion of CCL1, an essential chemokine for the prolongation of M2b macrophages.

P1.06.73
Regulation of complement receptor immunoglobulin (CRIg) expression in human macrophages by protein kinase Cα

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Members of complement, Toll-like and scavenger receptors as well as C-type lectins are amongst the groups of genes that initially recognize opsonized-pathogen or pathogen-associated molecular patterns. Recently a new gene (VSIG4) has been added to this list and its product termed complement receptor Ig (CRIg) represents a novel complement receptor with properties distinct from those of classically known complement receptors. CRIg also inhibits the adaptive immune response; suppressing lymphocyte responses. We have been studying the mechanisms by which CRIg expression is regulated in inflammation. Results show that PKC activation depresses CRIg expression and PKC inhibition increase expression in human macrophages. We have now used PKCα specific shRNA to examine the role of this PKC isozyme in CRIg expression in human monocyte derived macrophages (MDM). The macrophages were nucleofected with plasmid containing shRNA for PKCα. The lack of PKCα in the cells was demonstrated by western blot. The specificity of the knock down was demonstrated by a normal expression of PKCζ in these cells and visa versa. PKCα-deficient MDM showed markedly increased CRIg mRNA and protein expression but decreased CR3 expression. This was associated with an increase in phagocytosis of complement-opsonised particles. The findings are conducive with arachidonic acid causing a decrease and dexamethasone an increase in CRIg expression/phagocytosis, in relation to stimulation and inhibition of PKC activation. The data showed that PKCα-deficient MDMs have significantly decreased TNF and IL-6 production. The findings suggest that PKCα promotes inflammation by decreasing CRIg expression but increasing CR3 expression and production of pyrogenic cytokines.

P1.06.74
Modulation of phagocytosis in Mononuclear Phagocyte Cells by IL-21: Involvement of Spleen Tyrosine Kinase (Syk)

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The biological significance of the IL-21/IL-21R system in human monocytes/macrophages is not well documented, and the expression of IL-21R is unclear and has been disputed. In this study, we showed that human monocyte-like THP-1 cells, GM-CSF monocyte-derived macrophage and M-CSF monocyte-derived macrophage expressed the two IL-21R components, CD132 (γc) and IL-21Rα, on their cell surface, as assessed by flow cytometry. Moreover, IL-21 was found to enhance FcR-mediated phagocytosis in all these cells. The ability of IL-21 to enhance FcR mediated phagocytosis was not associated with an increased expression of both IL-21R components at the cell surface, and IL-21 did not act in synergy with IL-15. IL-21 activated spleen tyrosine kinase (Syk), as evidenced by its ability to increase Syk phosphorylation. Using a pharmacological approach to inhibit Syk activity, and an antisense technique to downregulate Syk protein expression, we demonstrated the importance of Syk in IL-21-induced phagocytosis. We conclude that IL-21 possesses important biological effects in mononuclear phagocyte cells and that Syk is a novel molecular target of IL-21 that was previously unknown. Therefore, future development of therapeutic strategies targeting the IL-21/IL-21R system should consider that monocyte and macrophage cell physiology may be affected by this system.

P1.06.75
Characterization of central macrophages in Anemia of Inflammation (AI): African trypanosomiasis as a model system

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Background: Anemia of Inflammation (AI), or Anemia of Chronic Disease (ACD), is the most prominent form of anemia in hospitalized patients, affecting quality of life and resulting from an imbalance between erythrophagocytosis and erythropoiesis. Multiple mechanisms contribute to its pathogenesis, including iron restriction, direct erythropoietic suppression and shortened erythrocyte survival. A common parameter in AI is a persistent pro-inflammatory immune response of the host, whereby macrophages and their activation state play a key role. In the regulation of erythropoiesis, the enigmatic central macrophages are thought to be essential as part of erythroblastic islands.
Results: By scrutinizing African trypanosomiasis in mice as a model of infectious disease associated with AI, we could discriminate within the F4/80+ER-HR3+ macrophage population 4 distinct subsets (CD11b-/lowLy6C-/low, CD11b-/lowLy6C+, CD11b+Ly6C+Fo-SGL+ and CD11b+Ly6C-/low). Using an in vitro erythropoiesis assay we could establish that CD11b+Ly6C+Fo-SGL+ cells represent the best candidates to function as central macrophages, since these cells are able to aid in the maturation of erythroblasts. Furthermore, they express crucial adhesion molecules (VCAM-1, CD49d, CD169 and CD36) favoring erythroblastic island formation. Moreover, the gene expression levels of crucial iron-homeostasis-associated genes such as CD71, hmox-1, fpn-1 and fhc suggest that this population rather exports iron during infection in contrast to other myeloid cells. Interestingly, by comparing Trypanosome-infected wild-type and mice exhibiting different degrees of anemia, we observed that there was an inverse correlation between the CD11b+Ly6C+Fo-SGL+ population size and anemia development. Therefore, this population requires further investigation to unravel its exact role in AI development.

P1.06.76
The immunomodulatory activity of glycerophosphoinositols

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Glycerophosphoinositols are multifunctional and widespread phosphoinositide metabolites produced by phospholipase A_2IVα (PLA_2IVα). We proved that immune cells strictly regulate PLA_2IVα, providing a fine modulation of intracellular glycerophosphoinositols in response to pro-inflammatory agents such as lipopolysaccharide. The immunomodulatory activity of glycerophosphoinositols has been evaluated in an *in vitro* model of innate immunity/inflammation on human primary cells. Peripheral blood monocytes have been sequentially exposed in culture to CCL-2, LPS, TNF-α, IFN-γ, IL-10 and TGF-β in a period of 48 h, to mimic the microenvironmental changes characterizing the onset, the development and the eventual resolution of inflammation. Upon exposure to LPS, TNF-α and IFN-γ monocytes become activated in inflammatory direction, release pro-inflammatory cytokines and undergo pyroptosis.

Glycerophosphoinositol (GroPIns), added exogenously, attenuated the inflammatory phenotype preserving cellular viability, decreased both expression and release of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, and reduced the expression of cyclooxygenase 2 and $PLA_2IV\alpha$, both involved in the biosynthesis of eicosanoids. GroPIns mainly affected the onset of inflammation, corroborating its role in counteracting the pro-inflammatory signalling from LPS. Conversely, GroPIns increased the expression of two inhibitors of IL-1, the IL-1 receptor antagonist (IL-1Ra) and IL-1 receptor II (IL-1RII). The genes modulated by GroPIns are common transcription target of NFκB. Indeed, GroPIns, decreased the LPS-induced NFκB binding to κB-promoter and modulated the expression of the IκB-α gene, the canonical transcription target of NFκB. These data support the hypothesis of an anti-inflammatory function of GroPIns which is probably due to a modulation of NFκB transcriptional function.

P1.06.77
Salmonella autophagy

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happy ~Salmonella Typhi caused Typhoid fever, a systemic inflammatory response syndrome (SIRS) in humans but S. Typhimurium causes only gastroenteritis. When infecting humans, S. Typhi uses Vi capsule to evade host immune system and survive in macrophages to circulate in bloodstream. After proliferating in bone marrow, liver, and spleen, S. Typhi reenters into bloodstream, causing SIRS, and intestine, causing hemorrhage and perforation. S. Macrophages are the important reservoir for S. Typhi during the infection. The interaction of macrophages with S. Typhi and S. Typhmurium was studied. S. Typhi, rather than S. Typhimurium, induced less inflammation when infecting epithelial cells by virtue of Vi. But when infecting THP-1 macrophages in MOI=100, we found that within an hour S. Typhi with or without Vi caused more cell death than S. Typhimurium. It was confirned to be caspase-1-mediated pyroptosis. The IL-1β secretion induced by S. Typhi was also significantly higher than S. Typhimurium. We constructed a mutant library of S. Typhi to search early cell death related genes. We found that SPI-1 mutants induced less cell death as well as caspase-1 activation than the parent s

P1.06.78
Regulation of myogenesis by differentially activated macrophages during human skeletal muscle regeneration

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Macrophages exert either beneficial or deleterious effects on tissue repair, depending on their activation/polarization state. They are crucial for adult skeletal muscle repair, notably by acting on myogenic precursor cells. However, these interactions have not been fully characterized. Here, we explored both in vitro and in vivo, in human, the interactions of differentially activated MPs with muscle precursor cells (MPCs) during adult myogenesis and skeletal muscle regeneration. We showed in vitro that through the differential secretion of cytokines and growth factors, proinflammatory macrophages inhibited MPC fusion while anti-inflammatory macrophages strongly promoted MPC differentiation by increasing their commitment into differentiated myocytes and the formation of mature myotubes. Furthermore, the in vivo time course of expression of myogenic and macrophage markers was studied in regenerating human healthy muscle after damage. We observed that regenerating areas containing proliferating MPCs were preferentially associated with macrophages expressing pro-inflammatory markers. In the same muscle, regenerating areas containing differentiating myogenin positive MPCs were preferentially coupled to macrophages harbouring anti-inflammatory markers. These data demonstrate for the first time in human that macrophages sequentially orchestrate adult myogenesis during regeneration of damaged skeletal muscle. These results support the emerging concept that inflammation, through macrophage activation, controls stem cell fate and coordinates tissue repair.

P1.06.79
The role of a novel DEAD-box protein in LPS-induced NLRP3 inflammasome activation

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Innate immune response is the first host response when host encounters pathogens or senses danger signals released from damaged tissues. Recently, a novel system named the inflammasome, a multiple protein complex for caspase-1 activation, has been characterized. Activation of the inflammasome can lead to the cleavage of pro-inflammatory cytokines IL-1β and IL-18 into their active forms. It has showed that the inflammasome plays a critical role in host defense against bacterial and viral infection in vivo. Deregulation of inflammasome, however, is linked to many diseases, such as cancer, autoinflammatory diseases, and diabetes.
We recently identified a novel DEAD box-containing protein in our yeast two-hybrid screening for regulation of the NLRP3 complex. We thus continue to study the functional role of this DEAD-box containing protein in NLRP3 inflammasome activation and its molecular mechanism. Depletion of this novel DEAD box-containing protein in THP-1 macrophages and bone marrow derived macrophages (BMDMs) largely enhanced caspase-1 activity and IL-1β production solely upon LPS challenge, suggesting that this DEAD-box protein is involved in the regulation of NLRP3 inflammasome. We also found that this DEAD-box protein suppressed IL-1β maturation and caspase-1 activation after LPS/ATP challenge in J774A.1 macrophages and BMDMs. In addition, we demonstrated that this novel DEAD-box protein associated with NALP3/ASC in a co-immunoprecipitation assay. Together, our results strongly suggest that this novel DEAD-box containing protein negatively regulates NLRP3 inflammasome activation.

P1.06.80
Necrotic cells induce $CD14^-/CD68^+$ peritoneal exudate cells to proliferate and differentiate into macrophage-like cells

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In a previous study, we noticed that some ascitic cells isolated from melanoma patients survive, proliferate, and differentiate into **giant** phagocytes after the other cells died. Similar phenomena were observed in the primary cultures of ascitic cells collected from mice inoculated with H22 hepatoma cells in the peritoneal cavity, as well as in the primary cultures of mouse lung and liver cells. In the present study, the relevance between cell proliferation and dying cells, and the biological characteristics of the differentiated cells were studied. The results indicate that necrotic cells induce non-attached abdominal exudate cells to proliferate and differentiate into **giant** phagocytes; however, apoptotic cells had no such effect. Morphologic studies revealed that the large phagocytes were abundantly covered with spherical and elongated protrusions, laminations, and cell surface vesicles. In addition, numerous phagosomes, secondary lysosomes, and mitochondria were observed inside each cell. Moreover, necrotic cells enhance the expression of CD14, CD68, CD80, and CD86, and the differentiated cells expressed high levels of CD68 and CD86. Our results indicate that necrotic cells induce non-attached abdominal exudate cells to proliferate and differentiate, and the differentiated cells possess characteristics similar to macrophages.

P1.06.81
The Effect of Polyporus umbellatus(Chinese medicine)on LPS-induced Viability and Cytokine Expression in RAW264.7 cells

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Polyporus umbellatus is a medicinal fungus with mild taste and neutral property. The investigations of immunological action of *Polyporus umbellatus* focus much more on ingredients than the compound of *Polyporus umbellatus*. The mechanism researches on toxicity attenuation and efficacy potentiation effects of *Polyporus umbellatus* in recent years reveal that the interaction among the various ingredients from *Polyporus umbellatus* probably account for the bidirectional regulation of *Polyporus umbellatus* prescription on immunity. To investigate the immune regulatory function of *Polyporus umbellatus* by studying its impact on LPS-induced expression of cytokines (IL-1β, IL-6 and IL-10) in RAW264.7 cells, based on our previous studies. Results**:** In comparison to LPS group, LPS-induced expression of IL-1β, IL-6 and IL-10 in RAW264.7 cells was inhibited by *Polyporus umbellatus* in a dose-response manner. IL-10 mRNA was promoted by 0.05μg/mL *Polyporus umbellatus* while inhibited by 0.5 and 5μg/mL.*Polyporus umbellatus* is capable of attenuating stimulation of LPS on RAW264.7 cells by suppressing the expression of cytokines like IL-1β and IL-6 as well as elevating the anti-inflammatory cytokine IL-10. The correlation between the dose of Polyporus umbellatus and IL-10 expression indicates the bidirectional regulatory effect of *Polyporus umbellatus* on LPS-induced expression of cytokines in RAW264.7 cells.

P1.06.82
Suppression of PTRF inhibits TLR4 signaling: a novel regulator of sepsis

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Rationale: Sepsis and sepsis associated end-organ failure are devastating syndromes. Understanding the detailed molecular mechanisms of sepsis potentially uncovers novel targets for therapy. PTRF is a newly discovered protein which may carry divergent cellular functions.
Objectives: to dissect the role of a novel protein, PTRF, in TLR4 mediated sepsis using mouse models in vitro and in vivo.
Methods: Cecal ligation and puncture (CLP) was used as the sepsis model in vivo to determine mortality and end-organ damage. Macrophages were adopted as the cellular models in vitro to conduct mechanistic studies.
Measurements and results: PTRF +/- mice survived better and suffered less organ damage after CLP. Further, less nitric oxide production and iNOS biosynthesis were observed in plasma, macrophages and/or vital organs from PTRF +/- mice. Using our acute sepsis models induced by CLP, we found that iNOS-/- mice carried the similar survival benefits comparing with the PTRF +/- mice. Further, similar as in the in vivo models, PTRF deficiency also resulted in decreased iNOS expression and NO/ROS production in vitro. Mechanistically, LPS enhanced the co-localization and interaction between PTRF and TLR4 in the lipid rafts. Deletion of PTRF prohibited the formation of TLR4 /Myd88 complex after LPS. Consistently, lack of PTRF impaired the TLR4 downstream signaling represented by decreased p-JNK, p-ERK and p-p38, which are upstream signals of iNOS mRNA transcription.
Conclusion: PTRF is a crucial regulator on TLR4 signaling in the development of sepsis.

P1.07 Effector functions of phagocytes

P1.07.01
Diverse role of *Plasmodium falciparum* haemozoin towards anti-malarial innate response

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Malaria continues to kill hundreds of thousands of children, pregnant women and non-immune adults each year. Understanding the cellular and molecular mechanisms during onset of malaria infection still remains an elemental issue in immunology. During early stages of infection, cells involved in innate immunity play a predominant role in controlling or modulating immune responses. Of which, cells of the myeloid lineage and natural killer cells are of greater interest. The interaction of these host innate immune cells with the parasite results in secretion of cell signaling protein molecules and reactive oxygen species (ROS) leading to parasite death and clearance. We investigated the effect of parasite's food vacuole containing haemozoin produced by *Plasmodium falciparum* on peripheral blood mononuclear cells (PBMCs) and neutrophils. We co-cultured parasite's food vacuole containing haemozoin, merozoites with parasite's food vacuole containing haemozoin and merozoites devoid of parasite's food vacuole or haemozoin with PBMCs or neutrophils and measured ROS production over time by Chemiluminescence. Co-culture of opsonized merozoites with and without parasite's food vacuole containing haemozoin revealed a significant lower ROS levels generated by neutrophils compared to parasite's food vacuole containing haemozoin ($p<0.05$). With similar concentrations, the non-opsonized parasite's food vacuole containing haemozoin induced a swift ROS production than opsonized one. Taken together, our preliminary results indicate that immune antibodies may not be important in parasite's food vacuole or haemozoin uptake by neutrophils and subsequent generation of ROS.

P1.07.02
Modulation of Moraxella catarrhalis and nontypeable Haemophilus influenzae phagocytosis by neuropeptides NPY and CGRP

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Colonization of respiratory tract is the first step in the infectious process. Since mucosal surfaces of the upper respiratory tract are richly innervated, human-restricted respiratory pathogens such as Moraxella catarrhalis and nontypeable Haemophilus influenzae are potentially exposed to the action of released neuropeptides including neuropeptide Y (NPY), and calcitonin-gene related peptide (CGRP).
The study was designed to verify neuropeptide-mediated modulation of human neutrophils (PMNs) phagocytosis against studied bacteria by determination of two critical phagocytic steps: bacterial uptake and respiratory burst response.
The phagocytic uptake of antibody-opsonized and non-opsonized FITC-labeled bacteria was measured using FACs. Reactive oxygen species (ROS) formation by phagocytosing PMNs was measured using luminol-dependent chemiluminescence assay (CL).
We found the bimodal modulatory action of neuropeptides that was observed in distinct types of pathogen recognition (opsonic versus nonopsonic). The phagocytic uptake of both opsonized mucosal pathogens by PMNs was markedly improved in the presence of NPs. The uptake remained unchanged in the presence of nonopsonized bacteria. In the case of ROS production only CGRP significantly intensified the respiratory burst of PMNs triggered by phagocytosable nonopsonic M. catarrhalis. The observed stimulating effect of CGRP was not preserved for opsonized bacteria. The contradictory action of NPs on engulfment and ROS response seems to be determined both by the nature of pathogen recognition and the physiological status of PMN donor.

The results suggest that physiologic concentrations of CGRP and NPY are modulators of PMNs uptake and respiratory burst in response to M.catarrhalis and H.influenzae and may be valuable innate components of immunity.

P1.07.03
Understanding innate immune functions of peripheral blood monocytes

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Background: Monocytes play an important role in host immunity as precursors of tissue macrophages.Macrophages are involved in phagocytosis and killing of pathogens.The innate immune functions of monocytes prior to differentiation into tissue macrophages and the impact of HIV on these functions are not well described.We assessed phagocytic,oxidative burst and proteolytic functions of blood monocytes obtained from healthy,asymptomatic HIV-uninfected and HIV-infected adults.
Methods: We recruited 45 HIV-uninfected,34 chronically HIV-infected and 7 acutely HIV-infected individuals.We isolated peripheral blood mononuclear cells (PBMCs) and measured their phagocytic, oxidative burst and proteolytic capacity using fluorochrome-conjugated reporter beads and flow cytometry.For phagocytosis,silica beads conjugated with streptavidin-IgG and streptavidin-horseradish peroxidise (HRP) were used to assess internalisation via Fc and mannose receptors respectively.Oxidative burst and proteolysis were measured using reporter beads carrying a fluorogenic reporter and a calibration fluorochrome.When the beads are internalized by monocytes,they gain fluorescence intensity proportional to the degree of activity in the phagosomal compartment.
Results: There was a significant difference in internalisation of beads via the mannose-receptor between HIV-uninfected volunteers compared with acutely HIV-infected adults (72.3% vs. 50.1%; p=0.01), but not via the fc-receptor across all groups (HIV- 65.0% vs.Chronic HIV+ 61.5% vs. Acute HIV+ 62.9%; p>0.05).There was no significant difference in oxidative burst (0.82 vs. 0.76; p>0.05) and proteolysis (1.64 vs. 1.24; p>0.05) in HIV-uninfected volunteers compared to chronically HIV-infected adults.
Conclusion: This suggests that peripheral blood monocytes from acutely-HIV-infected adults have impaired mannose-receptor-mediated phagocytosis.This may adversely affect host defence against pathogens internalised via this route.

P1.07.04
Downregulation of dendritic cells functions by Neutrophil Extracellular Traps

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Neutrophils are key components of the innate response via several mechanisms, in particular the production of Neutrophil Extracellular Traps (NETs), chromatin fibers associated with granule and cytoplasmic proteins that play important roles in antimicrobial defenses but seem also involved in some auto-immune and inflammatory diseases.
Our aim was to investigate whether NETs could interact with dendritic cell (DC) maturation and modulate adaptive immunity.
We first developed a model to induce, isolate and characterize NETs from human blood neutrophils. Calcium ionophore A23187 was chosen to induce NETs and the restriction enzyme Alu I allowed the recovery of heterogeneous-sized fragments of NETs. The presence of dsDNA and proteins was shown and, using western-blotting, NET major proteins (lactoferrin, histone 3, citrullinated histone 3) could be quantified. Finally, these isolated-NET were active as they were able to kill bacteria.
The co-culture of isolated-NETs preparations with DC alone did not induce DC maturation as measured by phenotypic modifications and cytokine productions. By contrast, NETs induced a significant decrease of lipolysaccharide) LPS-induced expression of CD86, CD83, CD80, CD40 and HLA-DR, as well as TNF, IL12-p70, IL23, IL8, IL6 and IL-10 release. This downregulation of LPS-induced DC maturation led to a decrease of T lymphocyte proliferation (allogenic model) and is associated with a markedly reduction of IFN-gamma and IL17 while promoting IL5 and IL13.
Our data suggest that, in our model, the NETs produced during inflammation can participate to the regulation of DC activation and maturation in order to potentially limit excessive immune responses.

P1.07.05
Immunological prognosing of complications of locomotors trauma

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Immunological status is investigated at 382 patients with damages and diseases of the locomotors system (obverse skeleton, backbone, long bones and large joints). Changes of basic immunological parameters at the noncomplicated current after operation were characterized in the whole line of similar features. In early term after operation we discover attributes of inflammation reaction - came to light developed neutrophils leucocytosis, were observed dysimmunoglobulinaemia, dynamics acute phase reactants changed. By the end of month of supervision basic immunological parameters were normalized. The occurrence of complications was characterized by increase of leukocyte number, first of all, at the expense of population's lymphocytes and eosynophils and decrease of monocytic quantity, oppression of a phagocytic link, decrease of functional activity of immunocompetent crates. Inflammation reaction to operative intervention were characterized by the greater duration and less expressed changes of concentration of acute phase reactants of fibers in comparison with the not complicated current after operation period. Thus, using of immunological tests in an estimation of damages of a skeleton allows predicting complications and in timing to carry out correction of a condition of the patient and by that to avoid or, when it is impossible, to defer development of complication.

P1.07.06
Monocyte subsets and immunopathology in Leishmania braziliensis infection

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Cutaneous leishmaniasis (CL) is an inflammatory parasitic disease characterized by the presence of ulcerated lesion on the skin. Patients with CL due to Leishmania braziliensis produce high levels of TNF, cytokine that contribute to tissue damage and ulcer development. Mononuclear cell infiltrate are found in lesions of CL patients, with presence of T and B lymphocytes, and mononuclear phagocytes. Recently, three populations of monocytes have been described based on expression of CD14 and CD16: Classical (CD14+CD16-), intermediate (CD14+CD16+) and non-classical (CD14dimCD16+) monocytes. Here, peripheral blood mononuclear cells were obtained from healthy controls, CL patients in pre-ulcerative phase and CL patients with skin ulcer. Monocyte subsets characterization was performed by flow cytometry. We observed that the frequency of intermediate and non-classical monocyte populations was increased in individuals on pre-ulcerative phase and CL patients with skin ulcer. Expression of MHC class II was increased in intermediate monocytes, suggesting that these cells might better present antigen to T cells. Also, classical and intermediate monocytes produced more TNF than the non-classical ones in response to soluble Leishmania antigen and LPS. CCR2 is the ligand for CCL2, a chemokine known to play important role in immune response against Leishmania. The levels of CCL2 were increased in both, lesion and peripheral blood from CL patents, and CCR2 was more expressed in

classical and intermediate populations. Altogether, our data show differences among monocyte subsets in CL. While classical and intermediate monocytes produce more TNF, non-classical ones are more frequent in lesions of these patients.

P1.07.07
Modulation of Chitinases and CLPs during Macrophage Differentiation and Polarization

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Enzymatically active chitinases chitotriosidase (CHIT-1) and AMC-ase; as well as CLPs YKL-40, YKL-39 have specific patterns of association with immune response. Serum levels of chitotriosidase and YKL-40 mainly reflect the activation status of macrophages, and in particular lipid-laden macrophages. While chitotriosidase is a specific marker for lysosomal storage disorders, overexpression of YKL-40 was found in numerous of tumors, chronic and acute inflammations and during fibrosis progression. We compared the Chitinases variations during the differentiation and polarization of human macrophages. Gene expression analysis was investigated by real-time PCR from mRNA of human monocytes obtained from buffy coat of healthy volunteers, polarized to classically activated macrophages (or M1), whose prototypical activating stimuli are Interferon-gamma and lipopolysaccharide, and alternatively activated macrophages (or M2) obtained by interleukin-4 exposure. We found that CHIT-1 and YKL-40 were significantly modulated throughout monocyte-to-macrophage differentiation and polarization. In contrast, AMC-ase, YKL-39 showed slight modulation in the diverse stage of macrophages differentiation and/or polarization. In conclusion our results show that the expression of CHIT-1 AMC-ase, YKL-40 and YKL-39 is differentially regulated throughout monocyte-to-macrophage differentiation and polarization. Our finding suggests that whereas the immune function of AMC-ase and YKL-39 is restricted and selective, CHIT-1 and YKL-40 are protagonists in innate and acquired immunity.

P1.07.08
Analysis of temporally-resolved phagosome proteomes following uptake via key phagocytic receptors

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Macrophages operate at the forefront of innate immunity, and their discrimination of pathogenic versus "self" particles is critical for a number of responses including antigen presentation, efficient pathogen killing, and cytokine induction. Recent studies have investigated modulation of downstream effects dependent on activation of key receptors during phagocytosis, including IgG and TLR receptors. In this work, phagosome proteomes were tracked over three timepoints to elucidate developmental differences among phagosomes taken up by well-studied receptors. Polystyrene beads conjugated to ligands representing bacterial membrane and fungal cell wall components, immune opsonins, or apoptotic cell markers were inoculated to murine bone marrow-derived macrophages (BMMs) for 30 minutes, and phagosomes were isolated after 0, 30, or 150 minutes and processed for proteomic analysis. For accurate quantitation among conditions and across the timecourse, stable isotope labelling and use of a common pooled internal control was used as a reference for all measurements. Analysis of resulting high-resolution mass spectrometric data resulted in the identification of over 1800 proteins and quantification of over 1500 proteins. Gene ontology enrichment analysis indicates increased representation of functional groups over the three timepoints, correlating with accumulation or loss of protein groups during maturation. Additionally, a mixture of subtle and distinct differences was detected between certain ligand-phagosomes, indicative of different rates of maturation and degradative capacity. For example, decreased levels of Ripk1 and Rab-19 were detected in 30'/0 LPS-bead phagosomes. Together, data support the concept of phagosome autonomy, where phagosomal fate is dependent on triggering of specific receptors during phagocytosis.

P1.07.09
Effects of aging on phagocytic activity and nitric oxide production of peritoneal macrophages from elderly mice in response to *Candida albicans*

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Macrophages are an essential component of innate immunity, including their participation in the phagocytosis process and nitric oxide production. An altered function of these cells with aging may play a key role in the defense mechanisms, including infectious diseases. In this work, we use young (2-3 months old - control) and aged (18-20 months old) C57BL/6 mice to study, *in vitro*, the influence of aging on the fungicidal activity of the resident peritoneal macrophages by analyzing the phagocytosis and the production of nitric oxide in response to different proportions of dead (1 macrophage: 1 yeast, 1:2, 2:1) and viable (1 macrophage: 1 yeast, 1:5, 5:1) *Candida albicans*, for 30 and 120 minutes. Macrophages from elderly mice are able to phagocytose *C. albicans* similar to young mice, although the elderly cells can store more dead fungi (≥ 5 yeasts) when compared to the matched control. However, the production of nitric oxide by peritoneal macrophages in response to *C. albicans* was lower in aged mice compared with young mice. These data indicate that the senescent mice have the ability to store more dead fungi than young in order to kill the infectious agent, but fails, due to inhibition of the production of microbicidal substance, nitric oxide. Thus, these cells would serve as disseminator's vehicles, favoring the dispersion of the pathogen into the body and protecting the yeasts from other mechanisms of the innate immune response.

P1.07.10
Functional analyses and cytochemical staining of leucocytes from lumpsucker (*Cyclopterus lumpus* L.), including IgM$^+$ cells

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Lumpsucker, *Cyclopterus lumpus* L., is a species of marine fish belonging to the order Scopaeniformes. They are found in cold waters of the Artic and North Atlantic oceans. We have performed functional studies like phagocytosis and respiratory burst to evaluate its innate immune responses by use of flow cytometry. We found that the phagocytic capacity and ability of isolated leukocytes from head kidney, spleen and peripheral blood were very high and cells with different morphology phagocytized beads rapidly. Regarding killing/degrading mechanisms, both oxygen-dependent (respiratory burst and myeloperoxidase) and oxygen-independent mechanisms (enzymes) were proven. We have differentiated between ten different subtypes of leukocytes based on morphology and cytochemical staining and cell types like lymphocytes, monocytes/ macrophages, dendritic cells and polymorphonuclear granulocytes (basophil, eosinophil and neutrophil) were identified. Further, we have characterized a polyclonal antibody against IgM using flow cytometry, immunostaining and immunoblotting. To our knowledge, the present study represents the first analyses of lumpsucker immunity and also the first immunological study within the order Scopaeniformes.

P1.07.11
The differences in innate immune responses of Atlantic salmon, Atlantic cod and lumpsucker

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In comparative studies, the fish immune system can provide important knowledge and contribute to an increased understanding of the evolution of the vertebrate immune system. The teleosts are the

first group of vertebrates with both innate and adaptive immunity. This group is highly diverse concerning both the composition of the immune system and the responses to pathogens. We have studied the innate immune responses in salmon, cod and lumpsucker, representing three different phylogenetic orders. Leucocytes were isolated from peripheral blood, head kidney (primary and secondary lymphoid tissue in fish) and spleen (lymphoid organ in fish). The innate immune responses, phagocytosis and respiratory burst, were analysed by flow cytometry and immunofluorescence microscopy. Further, enzymes involved in microbial degradation were visualized by cytochemical staining. Interestingly, we found that the immune cells and responses upon pathogen challenge were highly different in these species.

P1.07.12
The effects of anabolic steroids on dendritic cells

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To date, there is limited information on the effects of Anabolic Androgenic Steroids (AAS) on antigen presentation function of dendritic cells (DC). Co-stimulatory molecules CD80 and CD86 are important during antigen presentation as they associate with CD28 on T cells which is important for T cell survival. Various factors including AAS may alter these processes, particularly at high levels. The objective of this study was to investigate the effects of a commonly used AAS, testosterone cypionate (TC), on DC antigen presentation over time *in vitro*. Ten healthy male university students (mean age = 22.9 ± 0.7SD) were recruited for this study. Blood samples from the participants were incubated with or without 3µM of TC and PMA for 4, 12 and 24 hours. Following incubation, the samples were fluorescently labelled with CD80, CD86, CD3, CD14, CD19, CD20, CD56 and CD11c to facilitate preferential identification of DCs from other cells. Flow cytometry analysis determined the expression of CD80/CD86 surface markers on stimulated and unstimulated DC. A repeated measures ANOVA identified significance where $p<0.05$.TC treated samples demonstrated a significant increase in CD80 expression over time for both stimulated and unstimulated DCs. CD86 expression in unstimulated samples was significantly decreased. However, TC treated samples had significantly higher expression of CD86 compared to controls at 12 and 24 hours post incubation. These studies have highlighted the effects of testosterone on DCs which may have important implications in individuals who administer different types of AAS for recreational purposes.

P1.07.13
Inflammation driven functional differentiation of human macrophages *in vitro*

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Monocytes/macrophages are key players in all phases of physiological and pathological inflammation. To understanding the regulation of macrophage functional differentiation during inflammation, we have designed an *in vitro* model of human inflammation that recapitulates the different phases of the reaction (recruitment, initiation, development, and resolution), based on human primary blood monocytes exposed in culture to sequential changes of microenvironmental conditions. All phases of the inflammatory response were profiled by transcriptomic analysis. Broad distinct clusters of genes were identified that are differentially regulated during the different phases of inflammation. The gene sets identified by GSEA revealed that the inflammatory phase was enriched in inflammatory pathways while the resolution phase comprised pathways related to metabolism and gene rearrangement. By comparing gene clusters differentially expressed in monocytes *vs*. M1 and *vs*. M2 macrophages extracted from a meta-database, it was shown that cells in the *in vitro* model resemble M1 during the inflammatory phase and M2 during resolution. A set of genes of the IL-1 family was validated for expression by real-time PCR and protein production by ELISA. In two experimental set-ups (representing bacterial- and viral-induced inflammation, respectively), IL-1 and IL-18 expression and production strongly correlated with inflammation, while their inhibitors IL-1Ra and IL-18BP were expressed and produced starting from the late phases of inflammation.
Thus, this *in vitro* kinetical model of human inflammation allows an accurate description of the development of the inflammatory reaction and can help in identifying regulatory mechanisms in physiological conditions and in pathological derangements.
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P1.07.14
Alveolar macrophage innate immune function in HIV-infected African adults

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Background: HIV-infected individuals are at high risk of lower respiratory tract infections (LRTIs). Alveolar macrophages (AM) and T cells are the predominant cell types in the alveoli. We have previously demonstrated that antigen-specific bronchoalveolar CD4+ T cell responses to common respiratory pathogens are impaired in HIV-infected adults. The effect of HIV on AM function is, however, still poorly understood.
Methods: We recruited 45 HIV-uninfected and 34 asymptomatic HIV-infected healthy Malawian adults. We obtained AM by bronchoscopy and bronchoalveolar lavage. The function of AM was measured using novel flow cytometry based bead assays. HIV-infected AM were detected using a novel flow cytometry-based FISH assay that involves probing target HIV mRNA using a pool of 48 fluorophore-labeled oligonucleotides.
Results: The phagocytic ability and oxidative burst function of AM was not significantly different between HIV-infected and HIV-uninfected adults (phagocytosis, 62.0% vs. 70.2%, $p>0.05$; Oxidative burst, 1.31 vs. 1.34, $p>0.05$). AM from HIV-infected adults had reduced proteoytic function than those from HIV-uninfected adults (1.25 vs. 1.52; $p=0.04$). We found that 1.7%[0.7-3.8] of AM were HIV infected and these infected cells were predominantly 'small' AM. Furthermore, we found that HIV-infected small AM had reduced phagocytic ability (62.4 vs 45.5%, $p=0.03$).
Conclusion: The findings demonstrate that proteolytic function is impaired in AM from HIV-infected adults and that a group of "small" AM dominate in harbouring HIV and have impaired phagocytic function. The findings suggest that some AM innate functions are impaired in HIV-infected individuals and this may in part render HIV-infected adults more susceptible to LRTIs.

P1.07.15
The influence of T4 phage preparations on the activation of human monocytes in vitro

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Bacteriophages (bacterial viruses) are increasingly considered as a means of treating antibiotic-resistant bacterial infections. A number of

studies have revealed that bacteriophages, apart from killing bacteria, can also directly interact with immune cells, including phagocytic cells. However, there are practically no data on the potential mechanisms that might underlie the interactions between phages and phagocytic cells. The main objective of the study was to evaluate the effects of T4 phage preparation on the expression of CD80, CD86, and CD40 on monocytes and their production of IL-6, and TNF-α, two important proinflammatory cytokines, in vitro. The study was performed on peripheral blood mononuclear cells (PBMCs) from healthy blood donors. The expression of CD80, CD86, and CD40 molecules was measured by flow cytometry on CD14+ cells after 20-h cultures in the presence or absence of bacteriophage preparations. Separate experiments were performed to determine the percentage of CD80+, CD86+, and CD40+ cells in unstimulated and LPS-stimulated monocyte populations. Flow cytometry was carried out on a FACS-Calibur (Becton Dickinson) using Cell Quest software. Although T4 phage lysate significantly increased the expression of CD80 and CD40 molecules on unstimulated monocytes, this effect was not observed in LPS-activated cells. T4 phage lysate increased also the synthesis of proinflammatory cytokines by unstimulated monocytes, but not by LPS-activated cells. This suggests that monocytes activated during bacterial infection should not respond to phage preparations which confirm our earlier data on safety of phage therapy.

P1.07.16
12/15-lipoxygenase mediates GC-induced anti-inflammatory mechanisms in monocytes

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Glucocorticoids (GC) are commonly used in the treatment of many chronic inflammatory diseases. Our previous studies have shown that GC treatment does not suppress monocyte functions but induces a distinct anti-inflammatory phenotype in these cells. Similarly the treatment of LPS-stimulated monocytes with GC leads to re-programming of the cells towards a specific population involved in resolution of inflammation. Microarray analysis has shown up-regulated expression of anti-inflammatory and pro-resolution 12/15-lipoxygenase (12/15-LOX) in GC- and LPS/GC-treated monocytes.
The aim of our studies was to determine lipoxygenase-dependent effects in GC-treated monocytes. Bone marrow-derived monocytes from wild-type (wt) and 12/15-LOX-/- mice were stimulated with GC and/or LPS. GC induced a much more pronounced reduction of adhesion to plastic surface in wt monocytes as compared to 12/15-LOX-/- cells. Conversely migration was enhanced in GC- and LPS/GC-treated monocytes isolated either from wt or 12/15-LOX-/- mice. GC induced protection from cell death as well as the inhibition of ROS production were much more pronounced in wt cells as compared to 12/15-LOX-/- monocytes. Phagocytosis of carboxylated-modified latex-beads (mimicking apoptotic cells) was increased in GC-treated monocytes. However, this effect was much weaker in GC-treated monocytes isolated from 12/15-LOX-/- mice. In contrast, no significant differences between wt and 12/15-LOX-/- monocytes were observed in phagocytosis of latex beads
Our results indicate that inhibition of ROS production, protection from cell death, migration and adhesion of GC-treated monocytes are at least partially mediated by 12/15-LOX.
Specific targeting of the 12/15-LOX-pathway may be a promising strategy to block undesirable inflammation with fewer side effects.

P1.07.17
Synthetic immunomodulatory peptides IDR-HH2, IDR-1002 and IDR-1018 regulate neutrophil functions

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Although antimicrobial peptides or host defense peptides were originally hypothesized to act as antimicrobial agents, they also have been shown to broadly modulate the immune responses. We recently developed a series of novel synthetic immunomodulatory peptides, termed innate defense regulators (IDRs), which are conceptually based on a natural host defense peptide, bovine bactenecin. We showed that IDR-1 and IDR-1002 protect the host against bacterial infections through the induction of chemokines. The objective of this study was to investigate the effects of the IDRs on various functions of human neutrophils. Here, we demonstrated that IDR-HH2, IDR-1002 and IDR-1018 modulated the expression of neutrophil adhesion and activation markers. Moreover, these IDRs enhanced neutrophil adhesion to endothelial cells in a β_2 integrin-dependent manner and induced neutrophil migration. The IDR also promoted chemokine production via MAPK activation, increased the release of the neutrophil-generated human α-defensins and LL-37, and augmented neutrophil-mediated killing of *Escherichia coli*. Notably, the IDRs significantly suppressed LPS-mediated neutrophil degranulation, the release of reactive oxygen species and the production of the inflammatory cytokines tumor necrosis factor-α and interleukin-10, consistent with their ability to dampen inflammation. Taken together, our findings indicate that immunomodulatory peptides IDR-HH2, IDR-1002 and IDR-1018 regulate a range of neutrophil functions, and provide novel evidence regarding the contribution of the IDR peptides to the innate immune response through the modulation of neutrophil functions. The results described here may aid in the development of IDRs as novel anti-infective and immunomodulatory agents.

P1.07.18
Role of Circadian Rhythm in cell metabolism and macrophage function

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Thermoregulation, wakefulness, feeding, sleep, and other vital functions are influenced by circadian rhythms, regulated by timing activation of positive and negative oscillator proteins. In murine models, the absence or deregulation of any of these proteins may lead to metabolic syndrome. It has also been shown that NK cell cytotoxic activity is higher during day-light and that macrophage pro-inflammatory cytokines production in response to LPS follows a circadian-dependent pattern. Here, we present some evidence that mitochondrial dynamics and glucose metabolism are dependent on circadian rhythm and try to relate this with macrophage anti-microbial activities.
Aim: To analyze the metabolic and functional behavior of macrophages at defined stages of a circadian cycle.
Methods: Peritoneal macrophages and J774 A.1 cells were circadian rhythm-synchronized by serum-depletion followed by high serum shock in order to analyze Per-2 and Rev-Erb gene expression (circadian clocks), mitochondrial dynamics, glycolysis rate and Glut-3 expression (as metabolic traits), as well as endocytosis of S. thyphimurium and bacterial survival (macrophage functional activity) at defined time points spanning a whole circadian cycle (24h) plus 12h.
Results and conclusion: Mitochondria shifted from fusion to fission forms in the first 20h post-synchronization and back to fusion over the next 12h. Glut-3 expression was lower in the first 12h post-synchronization as compared to the next 12h, whereas the glycolysis rate was higher in the first 4h post-synchronization thus indicating circadian-related metabolic changes. Endocytosis and bacterial killing by macrophages seem to be related with these metabolic traits.

P1.07.19
Respiratory Syncytial Virus Fusion protein stimulates neutrophil extracellular traps formation

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Respiratory Syncytial Virus (RSV)-induced acute bronchiolitis is the most prevalent disease in children under 2 years old, which causes a huge impact in hospitalizations and costs to the health system. RSV is a single stranded RNA virus, whose genome encodes up to 11

proteins. The Fusion (F) protein is present at the virion surface and mediates the virus-target cell fusion. RSV F protein activates TLR-4 receptor, inducing cytokine secretion. Neutrophils activated by a wide range of microorganisms form neutrophil extracellular traps (NETs), which are composed of decondensed DNA and antimicrobial proteins. Our hypothesis is that F protein activates TLR-4 on neutrophils, inducing NETs formation. Human neutrophils were stimulated with F protein (1µg/mL), LPS (100ng/mL), PMA (25nM) or medium alone for 3 h at 37°C with 5% CO_2. After that, cells were fixed with 4% paraformaldehyde and stained with Hoechst 33342. Images were taken in Olympus IX51 fluorescence microscope. Alternatively, NETs were quantified in culture supernatants using Quant-iT dsDNA kit. RSV F protein induced NETs, as visualized by fluorescence microscopy, similarly to LPS and PMA. F protein, LPS and PMA induced a 2-fold increase in NET release compared to control. We are currently investigating the effect of DNase on F protein-induced NET formation and the role of TLR-4 in this process. RSV F protein is able to stimulate NET formation, and the excess of NET production in the lungs of children with bronchiolitis caused by RSV may worse the pathology, since these DNA traps induce endothelial injury and impair lung function.

P1.07.20
An inactive chromatin configuration at the *IL-10* locus in human neutrophils

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The ability of human neutrophils to express IL-10, a key cytokine whose function is to limit and terminate the inflammatory responses by regulating the expression of pro- and anti-inflammatory mediators, is currently controversial in the literature. In our hands, in fact, stimulation of highly purified populations of human neutrophils with diverse pathogen-associated molecular patterns (PAMPs), including Toll-like receptors (TLR) and C-type lectin receptor (CLR) agonists, or damage-associated molecular patterns (DAMPs), including serum amyloid protein A (SAA), do not trigger any mRNA expression or production of IL-10, differently from autologous monocytes and from studies of other groups. To identify the molecular basis of IL-10 expression in human phagocytes, we evaluated the chromatin modification status at their IL-10 genomic locus. By chromatin immunoprecipitation (ChIP) assays, we analyzed posttranslational modifications of histones associated with genes that are active, repressed or poised for transcriptional activation, including H3K4me3, H4Ac, H3K27Ac and H3K4me1 marks. Differently from autologous IL-10-producing monocytes, none of the marks under evaluation was detected at the IL-10 locus of resting or activated neutrophils. By contrast, elevated H3K4me3, H4Ac, H3K4me1 and H3K27Ac levels were detected at syntenic regions of the IL-10 locus in mouse neutrophils. Altogether, data demonstrate that human neutrophils, differently from either monocytes or mouse neutrophils, cannot switch on the IL-10 gene because its locus is in an inactive state, likely reflecting a neutrophil-specific developmental outcome. Implicitly, data also definitively settle a currently unsolved issue on the capacity of human neutrophils to produce IL-10.

P1.07.21
Ouabain affects human monocyte activation and function

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Ouabain is a steroid that is capable of binding to Na^+-K^+-ATPase, inhibiting Na^+ and K^+ transport and triggering several cell signaling pathways. Despite the fact that the mechanisms of action and physiologic roles of ouabain are not well establish, some effects of ouabain in the immune system have been described, such as the inhibition of mitogen-induced proliferation of lymphocytes, the modulation of pro-inflammatory cytokines release by mononuclear cells and the decrease of CD14 expression by monocytes. However, its impact on monocyte activation and function has not been addressed so far. Thus, the aim of the present work was to examine if ouabain was able to modulate the expression of different surface molecules related to monocyte activation, as well as their endocytic activity and cytokine production. For that, mononuclear cells from healthy volunteers were separated by Ficoll-Hystopaque density gradient and cultured for 24 hours in the presence or absence of 10^{-7}M of ouabain, a concentration found in the plasma of patients treated with ouabain in clinical setups or in individuals under acute stress. Our results show that ouabain-treated monocytes expressed increased amounts of activation markers such as CD69, HLA-DR, CD80 and CD86. Moreover, ouabain induced an increase in the endocytosis of Dextran-FITC particles and in the production of IL-1, TNF-α and IL-10 by monocytes. Collectively, these results suggest that ouabain impacts monocyte activation and modulates monocyte functions, implying that this steroid could possibly act as an immunomodulator of these cells. This work was supported by CAPES, CNPq and FAPERJ.

P1.07.22
Test-system for observing heat shock protein 70kDa (Hsp70) effect on reactive oxygen species production by immune cells

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One of the most important aspects of the ROS biological activity is associated with the damaging effect on pathogens. But elevated ROS level can also lead to tissue damage. However, not all the mechanisms of ROS production regulation in cells are well investigated. We speculate that heat shock protein 70kDa (Hsp70) extracellular form may be one of the endogenous suppressors of the excessive ROS production by phagocytes.
The aim was to determine antioxidant potential of Hsp70.
Initially we have shown that 10 min heat stress treatment of the murine bone marrow cells with 60 min recovery at 37oC, prior to phorbol-12-myristate-13-acetate (PMA) activation, significantly reduced the intensity of the following ROS production compared with the control. Already after 5 min incubation in presence of Hsp70 we observed significant reduction of PMA-induced oxidative burst amplitude. The minimal concentration of Hsp70 required for the decrease of ROS production was strongly dependent from the incubation time and prolonged incubation decreased the effective Hsp70 concentration to 0.1 µg/ml. Strong dose-dependent effect of HSP70 was observed in the case of prolonged incubation. Besides effects on oxidative burst, Hsp70 reduces the level of spontaneous ROS production by the cells, which could be important in the process of aging regulation. In addition to the murine bone marrow model, the effect of HSP70 on oxidative burst was also demonstrated on human monocytes cell line THP-1. So, the antioxidant dose- and time-dependent effect of exogenously supplied Hsp70 on spontaneous and induced ROS production was shown using different cell models.

P1.08 Dendritic cell subsets

P1.08.01
Functional limitations of plasmacytoid dendritic cells limit type I interferon, T cell responses and virus control in early life

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Infant mortality from viral infection remains a major global health concern: viruses causing acute infections in immunologically mature hosts often follow a more severe course in early life, with prolonged or persistent viral replication. Similarly, the LCMV-WE strain causes acute self-limiting infection in adult mice but follows a protracted course in infant animals, in which LCMV-specific $CD8^+$ T cells fail to expand and control infection. By disrupting type I IFNs signaling in adult mice or providing IFN-α supplementation to infant mice, we show here that the impaired early life T cell responses and viral control result from limited early type I IFN responses. We postulated that pDCs, which have been identified as one major source of immediate-early IFN-I, may not exert adult-like function *in vivo* in the

early life microenvironment. We tested this hypothesis by studying pDC functions *in vivo* during LCMV infection and identified a coordinated downregulation of infant pDC maturation, activation and function: despite an adult-like *in vitro* activation capacity of infant pDCs, the expression of the E2-2 pDC master regulator (and of critical downstream antiviral genes such as MyD88, TLR7/TLR9, NF-kB, IRF7 and IRF8) is downregulated *in vivo* at baseline and during LCMV infection. This suggests that the limited T cell-mediated defense against early life viral infections is largely attributable to or regulated by infant pDC responses, and provides incentives for novel strategies to supplement or stimulate immediate-early IFN-α responses**.**

P1.08.02
The role of Dendritic Cells and Monocytes in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis

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Bidirectional communication between the adaptive and innate immune system is necessary for optimal immune function. Irregularities in innate immune cell function may affect cellular processes in the adaptive system and vice versa. In Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis (CFS/ME) a dysfunctional immune system has been described and this is characterised by reduced cytotoxic function in Natural Killer (NK) cells and CD8+T cells, altered neutrophil respiratory burst and equivocal levels in cytokine secretion. Dendritic cells (DCs) and macrophages are innate immune cells with highly versatile functions as they have a role in the development and function of almost all immune cells. Hence, the objective of this study was to determine the role of monocytes and DCs in CFS/ME patients. 20 CFS/ME patients (age=51.8±1.74 years) and 30 controls (age= 53.19±1.35years) were recruited for the study. Inclusion into the CFS/ME group was based on the Centre for Disease Prevention and Control (CDC 1994) criteria for CFS/ME. 10mL of whole blood was collected from all participants. Samples were stained with a cocktail of monoclonal antibodies containing HLA.DR, CD123, CD33, CD16 and CD11c. Samples were analysed on the flow cytometer to determine the levels of total monocytes, total DCs, myeloid DCs and lymphoplasmacytoid DCs. ANOVA was the statistical analysis used to analyse all data collected. Compared to the other immune cells, monocytes were significantly elevated in the CFS/ME patients in comparison to the controls. These results suggest further highlight the role of impaired immune function in the pathogenesis of CFS/ME.

P1.08.03
Lag-3 expression and role in human plasmacytoid dendritic cells biology

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In the last decade several studies provided evidence that pDC infiltrate several types of human solid tumors. However, the role of tumor-associated pDC remains controversial.
LAG-3 is a CD4-related, activation-induced cell surface molecule expressed by various cells of the human lymphoid compartments and binds to MHC class II with high affinity. Data presented here demonstrate that LAG-3 is expressed on the cell surface of a subset of mature human circulating pDC. Interestingly, *in vitro*, LAG-3+ pDC migrate toward melanoma cell lines and *in vivo* in the tumor invaded LN of melanoma patients; here they display an activated phenotype and express TRAIL molecule. Based on these observations, and on the evidence that pDC up-regulate the basal expression of LAG-3 upon TLR-9 triggering, we hypothesize a functional role of this molecule in the pDC biology. Accordingly, LAG-3 stimulation on pDC, through MHC-II interaction, leads to their activation. pDC co-cultured with target cells, expressing HLA-DR molecule, selectively release cytokines such as IFN-α, TNF-α and IL-6 but not IL-1β and TNF-β, which are only secreted upon stimulation by CpG-A. Notably, while IFN-α and TNF-α release is lower than CpG-A, IL-6 production is far higher. Based on our findings, LAG-3 is likely involved in modulating the final functional outcome of pDC activation resulting from microenvironmental stimuli. A better understanding of the LAG-3 direct role on human pDC, with regard to its contribution in modulating pDC immunogenic vs. tolerogenic response, could have significant therapeutic implications in the treatment of cancer.

P1.08.04
Distinct modulation of human myeloid and plasmacytoid dendritic cells by anandamide in multiple sclerosis

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The immunopathogenesis of multiple sclerosis (MS) has always been thought to be driven by chronically activated and autoreactive Th-1 and Th-17 cells. Recently, also dendritic cells (DC) are now believed to highly contribute to antigenic spread as well as to maturation of adaptive immunity, and have been linked with disease progression and exacerbation. Yet, the role of DC in MS pathogenesis remains poorly understood. Here we report that in MS myeloid dendritic cells (mDC) produced higher levels of IL-12 and IL-6, whereas plasmacytoid dendritic cells (pDC) accounted for lower levels of IFN-α compared to healthy donors. Given the recent involvement of the endocannabinoid system in MS and its role in modulating immune functions, we found that the main endocannabinoid anandamide (AEA) significantly inhibited cytokine production from healthy mDC and pDC, as well as their ability to induce Th-1 and Th-17 lineages. Moreover, we found that in MS only pDC were unresponsive to AEA-induced cytokine inhibition and that this specific cell subset expressed higher levels of AEA-hydrolyzing enzyme FAAH. Overall, our data disclose a distinct immunomodulatory effect of AEA in mDC and pDC from MS patients, which may reflect an alteration of the expression of FAAH, thus forming the basis for the rational design of new endocannabinoid-based immunotherapeutics targeting a specific cell subset.

P1.08.05
NF-kB/RelA subunit plays the critical roles on the functions of plasmacytoid dendritic cells to thymus

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Plasmacytoid dendritic cells (pDCs) are a subtype of dendritic cells and thought to play the important roles in immune systems. However the functions in lives remain to be resolved. Those cells are present in a lot of tissues in whole bodies, especially in bone marrows, spleens and thymi. pDCs are thought to be present originally in bone marrows and transfer to thymi. Nuclear factor kappa B (NF-kB)/RelA subunit is one member of NF-kB family consisting of five members; RelA, RelB, c-Rel, p50 and p52 and plays the critical roles in multiple loci and multiple mechanisms. Mice lacking RelA are embryonic lethal due to tumor necrosis factor (TNF) cytotoxicity and therefore cannot be analyzed. We generated mice deficient of RelA and TNF both to obtain live mice lacking RelA. We previously demonstrated a lot of phenotypes that those mice exhibited. One of the phenotypes is the immune deficiency. We also found that pDCs were absent in thymi, although those cells were present in bone marrows and spleens. It remains so far to be elucidated what roles pDCs play in thymi and how pDCs work in thymi for immune system of whole bodies, although the population of pDC is minor. We are analyzing mice lacking RelA and TNF focused on (1) the mechanismof transfer to thymi, (2) the functions in thymi, of pDCs.

P1.08.06
Diminished Memory T-cell Expansion due to Delayed Kinetics of Antigen Expression by Lentivectors

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Memory CD8+ T lymphocytes play a central role in protective immunity. In attempt to increase the frequencies of memory CD8+ T cells, repeated immunizations with viral vectors are regularly explored. Lentivectors have emerged as a powerful vaccine modality with relatively low pre-existing and anti-vector immunity, thus, thought to be ideal for boosting memory T cells. Nevertheless, we found that lentivectors elicited diminished secondary T-cell responses with inferior protective capacity than that observed after priming. This was not due to the presence of anti-vector immunity, as limited secondary responses were also observed following heterologous prime-boost immunizations. By dissecting the mechanisms involved in this process, we demonstrate that lentivectors trigger exceptionally slow kinetics of antigen expression, while optimal activation of lentivector-induced T cells relays on durable expression of the antigen. These qualities hamper secondary responses, since lentivector-encoded antigen is rapidly cleared by primary cytotoxic T cells that limit its presentation by dendritic cells. Indeed, blocking antigen clearance by cytotoxic T cells via FTY720 treatment, fully restored antigen presentation. Taken together, while low antigen expression is expected during secondary immunization with any vaccine vector, our results reveal that the intrinsic delayed expression kinetics of lentiviral-encoded antigen, further dampens secondary CD8+ T-cell expansion.

P1.08.07
Migratory behavior of dendritic cells during the development of lupus

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Analyses of lupus-prone congenic mice (NZWxNZB)F1 (BWF1) during the development of the disease have pointed out an important role of the spleen microenvironment since dendritic cells (DCs), that are essential for B and T cell function, accumulate in this tissue. We hypothesized that changes in DC biology may play a critical role in the pathogenesis of the disease. We found that most part of spleen DCs in lupic mice are of plasmacytoid origin and possess an overactivated phenotype. In this study we performed homing experiments demonstrating that lupic and pre-lupic DCs migrate preferentially to the spleen compared to DCs from control mice. In order to understand why pDC localize preferentially to the spleen in the course of the disease we evaluated the expression levels of chemokines and their receptors by macroarrays on DCs and stromal cells from spleens of lupic, prelupic and control mice. We observed significant differences in chemokine receptor pattern in DCs and stromal cells from lupic mice compared with control mice. Interestingly, we found that pDC of lupic mice do not express CCR9 receptor suggesting that these cells are unable to migrate to the thymus to promote central tolerance as it has been recently suggested. On the other hand, we are currently investigating whether DCs accumulation in spleen makes them resistant to apoptosis. In summary, our results show that the migratory behaviour of DCs from lupus prone mice BWF1 could contribute to the pathogenesis of lupus.
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P1.08.08
Differentially activated respiratory plasmacytoid and CD11b-high dendritic cells accumulate during sublethal Klebsiella pneumonia infection

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Klebsiella pneumoniae is a leading cause of severe hospital-acquired respiratory tract infections and death but little is known regarding the modulation of respiratory dendritic cell (DC) subsets. Plasmacytoid DC (pDC) are specialized type 1 interferon producing cells and considered to be classical mediators of antiviral immunity. By using multiparameter flow cytometry analysis of respiratory DC subsets during murine respiratory Klebsiella pneumonia infection we show that pDCs and CD11b^{hi} DC were markedly elevated in the post acute pneumonia phase when compared to mock-infected controls. pDC identification during bacterial pneumonia was confirmed by extended phenotyping for 120G8, mPDCA-1 and Siglec-H expression and by demonstration of high Interferon-alpha producing capacity after cell sorting. Cytokine expression analysis of ex vivo-sorted respiratory DC subpopulations revealed elevated Interferon-alpha in pDC, elevated IFN-gamma, IL-4 and IL-13 in CD103^{+} DC and high IL-19 and IL-12p35 in CD11b^{hi} DC subsets in comparison to monocytic DC indicating distinct functional roles. Antigen-specific naive CD4^{+} T cell stimulatory capacity of purified respiratory DC subsets was analysed in a model system with purified ovalbumin T cell receptor transgenic naive CD4^{+} responder T cells and respiratory DC subsets, pulsed with ovalbumin and matured with Klebsiella pneumoniae lysate. CD103^{+} DC and CD11b^{hi} DC subsets represented the most potent naive CD4^{+} T helper cell activators. These results provide novel insight into the differential activation of respiratory DC subsets during Klebsiella pneumonia infection. The detection of increased respiratory pDC numbers in bacterial pneumonia may indicate possible novel pDC functions with respect to lung repair and regeneration.

P1.08.09
Analysis of the characterization of bone marrow derived dendritic subsets

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Studies of dendritic cells are important to understand antigen processing and presentation in innate and acquired immunity. Several reports have discussed different procedures induced different types of dendritic cells. In vitro culture system, the factor to characterize each dendritic cell is a different types of cytokines those are added to immature dendritic cells. Bone marrow derived dendritic cells (BMDC) are useful experimental tool because those cells have relatively homogeneous property and can be collected a certain amount of the number. Using mice system, it has been indicated that BMDC induced with GM-CSF and IL-4 showed more mature phenotype than that induced with GM-CSF only or GM-CSF and IL-15. As the characters of each BMDCs are still unclear, we have induced three different types of BMDC subset with different cytokine conditions. BMDCs induced by GM-CSF, GM-CSF and IL-4 or GM-CSF and IL-15, were compared the proliferation capacities. BMDC induced by GM-CSF and IL-15 showed the most vigorous proliferation activity, whereas BMDC induced by GM-CSF and IL-4 showed the most mature type character. To confirm the antigen uptake capacity, we used different size of antigens. Moreover, susceptibility to pathogen infection was analyzed to evaluate the most useful antigen presenting cell. We concluded that BMDCs induced by GM-CSF and IL-4 are not the most useful antigen presenting cells, although the method to induce them is well known.

P1.08.10
Increased tubulointerstitial recruitment of human CD141^{hi} CLEC9A^{+} and CD1c^{+} myeloid dendritic cells in fibrotic kidney disease

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Dendritic cells (DC) play critical roles in immune-mediated kidney diseases. Little is known, however, about DC subsets in diseased human kidneys, with previous studies restricted to limited pathologies and using only immunohistochemistry. In this study, we developed novel protocols for extracting renal DC subsets from a wide range of human kidney diseases and characterised them quantitatively and phenotypically by 10-colour flow cytometry. We detected significantly elevated numbers of total DC (CD45^{+} lineage^{-} HLA-DR^{+}), CD11c^{+}CD141^{hi} and CD11c^{+}CD1c^{+} myeloid DC (mDC) subsets in diseased biopsies with interstitial fibrosis compared to diseased biopsies without fibrosis and healthy kidney tissue. In contrast, CD11c^{-}CD123^{hi} plasmacytoid DC numbers were significantly higher in the fibrotic group compared to healthy tissue only. Numbers of all DC subsets also correlated with loss of kidney function. CD141^{hi} DC expressed CLEC9A, whilst the majority of CD1c^{+} DC lacked expression of CD1a and DC-SIGN, suggesting these mDC subsets may be infiltrating tissue counterparts of CD141^{hi} and CD1c^{+} blood DC. Immunohistochemical analysis revealed CLEC9A^{+} and CD1c^{+} cells restricted to the renal tubulointerstitial compartment. Notably, DC expression of costimulatory and maturation molecule CD86 was significantly increased in both diseased cohorts compared to healthy tissue. Consistent with an inflammatory environment, significantly higher levels of chemokine IL-8 were detected in the dissociation supernatants of diseased biopsies with fibrosis than both non-fibrotic diseased biopsies and healthy tissue. Collectively, our data indicate that activated mDC subsets recruited into the tubulointerstitium may play a critical role in the development of interstitial fibrosis and thus, progression to chronic kidney disease.

P1.08.11
Human dendritic cell subpopulations show distinct behavior in their interaction with the pathogenic mould Aspergillus fumigatus

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Activities of dendritic cells (DCs) such as phagocytosis, expression of co-stimulatory molecules and cytokine release are required for the successful clearance of various pathogens. We investigated the interaction between different A. fumigatus morphotypes and subpopulations of human DCs (myeloid DCs (mDCs), plasmacytoid DCs (pDCs) and monocyte derived DCs (moDCs)).
MDCs, pDCs and monocytes were isolated from the peripheral blood of healthy volunteer donors using antibody bound magnetic MicroBeads. Monocytes were differentiated into moDCs over 6 days with IL-4 and GM-CSF. DCs were co-incubated with resting conidia or germlings and afterwards analyzed by time-lapse video microscopy, scanning electron microscopy, plating assays, flow cytometry, transwell assays and multiplex ELISA.
Co-incubation with fungal morphotypes did not result in pDC maturation and there was an absence of phagocytic activity and only limited cytokine secretion. The rare and random contacts of pDCs with fungal cells during live-imaging proved that pDCs do not recognize A. fumigatus. In contrast, mDCs and moDCs were able to recognize and to respond to fungal morphologies. Although, mDCs did not kill conidia or phagocytose fungal morphotypes as well as moDCs, they matured and secreted comparable amounts of cytokines; making them both potential T cell stimulators and recruiters of neutrophile granulocytes.
In conclusion, our in vitro data elucidate the distinct interaction between DC subpopulations and A. fumigatus, which indicate their different roles in the pathogenesis of invasive aspergillosis and may be important to decide on using moDCs or mDCs for future immunotherapy approaches.

P1.08.12
CCR2^{+} monocyte-derived antigen-presenting cells from the genital tract promote Th1 and Th17 immunity after HSV-2 infection

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Monocytes are a distinct cell subset that mediates innate and adaptive immunity in mucosal sites. However, the role of monocyte-derived APCs (MD-APCs) is not fully understood. Here we explored the *ex vivo* function of monocytes isolated from the genital tract after infection with herpes simplex virus 2 (HSV-2) which causes genital ulceration. The monocytes (CD11b^{+}Gr1^{+}MHC class II^{+}) exist in the vaginal tissue from Depo-Provera treated naïve female mice. This subset intrinsically acts as APCs by inducing antigen-specific T cell proliferation at a level comparable to that of conventional dendritic cells. Interestingly, the monocyte- subsets significantly accumulated in the genital tissues after HSV-2 infection and the recruited monocytes secreted inflammatory cytokines (i.e., IL-6 and TNF-α) and chemokines (i.e., MCP-1 and RANTES) upon recognizing the infection. The monocyte-derived APCs induced CD4^{+} T cells to generate numerous cytokines such as IFN-γ (Th1) and IL-17A (Th17) in an antigen-specific manner, as compared with other CD11b^{+} myeloid cells. The phenotype of MD-APCs was Ly6C^{high}, Ly6G^{low}; in addition, MD-APCs expressed co-stimulatory molecules such as CD40, CD80 and CD86. MD-APCs were located beneath the vaginal epithelium and migrated there in a CCR2-dependent, but CXCR2-independent manner. In summary, CD11b^{+}Gr1^{+}MHC class II^{+} monocytes obtained from the mouse genital tract performed as APCs to generate Th1 and Th17 CD4^{+} T cell responses in during HSV-2 infection.

P1.08.13
Interleukin-22 binding protein (IL-22BP) is constitutively expressed by a subset of conventional dendritic cells and is strongly induced by retinoic acid

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Interleukin-22 (IL-22) is mainly produced at barrier surfaces by T cells and innate lymphoid cells. By acting on its membrane receptor, IL-22 promotes antimicrobial immunity, inflammation and tissue repair of epithelial cells. These properties make IL-22 an important player in the maintenance of barrier surface integrity. However, dysregulated IL-22 actions lead to deleterious inflammation and is involved in diseases such as psoriasis, intestinal inflammation, and cancer. These two faces of IL-22 emphasize the importance of IL-22 regulation. Interestingly, IL-22 has a soluble inhibitory receptor, called IL-22BP, which acts as a crucial regulator of IL-22. We show both in rats and mice that, in the steady state, the main source of IL-22BP is a subset of conventional dendritic cells (DCs) in lymphoid and non-lymphoid tissues. IL-22BP expression was detected both at the mRNA and protein levels in rat splenic CD4+ DC. qPCR experiments revealed an even stronger expression by the same DC subset in both mesenteric lymph nodes and gut draining lymph. In mouse, high expression of IL-22BP was also specifically found in mouse CD103+ CD11b+ intestinal lamina propria DC. In humans, monocyte derived DC (MDDC) expressed IL-22BP whereas monocytes did not. IL-22BP expression was strongly induced in a retinoic acid (RA)-dependent manner in MDCC. Taken together, our results indicate that IL-22BP is constitutively produced by a subset of cDC and that RA positively regulates IL-22BP expression. Our data suggest that RA-producing intestinal DC may actively participate in the regulation of IL-22 in the gut by producing high levels of IL-22BP.

P1.08.14
Human 6-sulfo LacNAc$^+$/CD16$^+$ dendritic cells are distinct from Tie-2$^+$ monocytes

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6-sulfo LacNAc$^+$ dendritic cells (slanDCs) represent a distinct population of circulating and tissue pro-inflammatory DCs. Given their high CD16 and low CD14 expression by flow cytometry, slanDCs overlap with the heterogeneous population of the CD14^{low}CD16$^+$, non-classical monocytes, which also include the Tie2-expressing monocytes (also known as TEMs; Blood. 2007; 5276-85). TEMs have been shown to generate a perivascular cell population endowed with a prominent pro-angiogenic potential in a variety of human and mouse primary tumors, including human carcinomas. To clarify whether slanDCs and TEMs could eventually represent the same cell population, we analyzed if slanDCs express Tie-2 at both protein and mRNA level, in addition to other TEM markers. We found that even though circulating slanDCs, similarly to TEMs, stain positive for CD16, CD33, CD13, CD11b and CD11c, but negative for CCR2 and CD62L, they completely lack Tie-2, both at RNA and protein level, as assessed by means of flow cytometry, western blot and immunohistochemical analysis. Additional experiments proved that slanDCs are different from TEMs also for their behavior, since, at variance with TEMs, slanDCs are not perivascular in tissues and are not found in primary tumors. By contrast, slanDCs are selectively posed at the interface between epithelial cells and the nearby lymphoid compartments in mucosal lymphoid tissues, where, again, they result Tie2-negative. Taken together, these data demonstrate that human slanDCs are distinct from TEMs by a number of parameters.

P1.08.15
Distinct subsets of dendritic cells regulate the outcome of colonic inflammation

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Intestinal dendritic cells (DCs) are crucial in establishing tolerance towards food and commensals but, at the same time, they are fundamental in directing appropriate immune responses to pathogens. However, it is still not clear what are the roles of different DC subsets in regulating gut homeostasis in health and disease.
In order to get further insights into this, we have recently generated three transgenic mouse strains that allow us to ablate in vivo specifically different subsets of intestinal DCs (Siglec-H-, Clec9a-, Clec4a4-DTR strains). They allow us to study the roles of distinct intestinal DC subsets (SiglecH$^+$CD11c^{int} DCs, CD103$^+$CD11b$^-$ CD11c^{high} and CD103$^+$CD11b$^+$ CD11c^{high}) in acute DSS-mediated colitis. After a single round of 3% DSS treatment, a mild colitis was observed in wild type and in DT treated Siglec-H DTR mice suggesting that absence of pDCs does not increase the severity of intestinal inflammation. In contrast the DT injected Clec9A DTR mice, where CD103$^+$CD11b$^-$CD11c^{high} DCs are efficiently ablated showed much exasperated inflammation. This could be visualized by a more pronounced body weight lost, increased fecal blood as well as enhanced colonic infiltration of neutrophils and inflammatory Cx3CR1^{int} monocytes. On the other hand, the mice with CD103$^+$CD11b$^+$CD11c^{high} DC ablation did barely show any colitis signs under the same DSS regiment suggesting their pro-inflammatory nature. Our results strongly suggest a unique regulatory function of the CD103$^+$CD11b$^-$CD11c^{high} DC subset in controlling intestinal inflammation.
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P1.08.16
Analysis of the expression of the inhibitory receptor ILT4 on dendritic cells from systemic lupus erythematosus (SLE) patients

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Dendritic cells (DC) are a subpopulation of leukocytes specialized in the capture and process of antigens and its presentation to T lymphocytes. There are two major subsets of DCs, myeloid or classic DCs (mDC) and plasmacytoid DC (pDC). DCs have been recognized to play a pivotal role in the development and maintenance of immune tolerance. In this regard, the tolerogenic effect of DC seems to depend on different factors, as the presence of immune-regulatory cytokines, certain tissue factors, and the nature of the antigen as well as the receptors expressed on their cell surface. The regulatory ILT's/CD85 receptors are associated with a tolerogenic effect of mDCs and previous studies have indicated the important role of these receptors on the pathogenesis of autoimmune diseases. The inhibitory receptor ILT4/LILRB2 is expressed in myeloid cells and it has been related with the tolerogenic phenotype of DC. In this work, we analyzed the expression of ILT4 in circulating pDC and mDC by polychromatic flow cytometry. pDC and mDC were detected in the basis of the expression of linage markers, HLA-DR, CD11c, BDCA1 or BDCA4. We studied fourteen patients with SLE and ten healthy volunteers. We observed that ILT4 is expressed in high levels on both subpopulations of DC (media of positive cells- pDC: 77%; mDC: 76%) from healthy volunteers. In contrast, the expression of ILT4 was significantly diminished in both pDC`s and myeloid DC's (mDC's) from SLE patients. Our results suggest that ILT2 participates in the defective immune-regulation observed in patients with SLE.

P1.08.17
Human conventional CD1c+ Dendritic Cells produce IL-12 and induce potent CD8+ T cell priming

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BDCA-3+ dendritic cells are thought to be the human equivalents of murine CD8α+ DC, which cross-prime CD8+ T-cell responses and secrete high amounts of IL-12. We compared human myeloid DC expressing CD1c or BDCA-3 and plasmacytoid DC for cytokine production and surface receptor expression in peripheral blood and lymphoid tissues, and assessed their capacities to induce cytotoxic T cell responses.
We found that mDC1, but not other DC or monocyte subsets could secrete high amounts of IL-12p70. IL-12 production by mDC1 was tightly controlled, as it required combinational Toll-like receptor (TLR) stimulation and was fine-tuned by lymphocyte-derived stimuli. Conversely, mDC2 produced high levels of IFNlambda in response to TLR-3 stimulation alone. pDC secreted IFNalpha αvδ expressed lower levels of MHC class-I and -II, CD40 and CD86 than myeloid DC. Moreover, they poorly cross-presented soluble antigens to CD8+ T cells. In contrast, both mDC1 and mDC2 could cross-present efficiently, but required different combinations of TLR ligands. Nevertheless, all DC subsets induced secondary expansions of CD8+ memory T-cells upon licensing by CD4+ T-cells. Furthermore, following appropriate TLR stimulation and help by CD4+ T cells, all DC primed naive CD8+ T-cells. However, while mDC1 induced high levels of Granzyme B via IL-12, mDC2 and pDC induced preferentially Granzyme A.
Our results show that CD1c+ mDC1 are a relevant source of IL-12 for naïve T cell priming, and that specific pathogen-derived stimuli and CD4 help can instruct different DC subsets to induce cytotoxic T-cell responses.

P1.08.18

Tumor cell-derived Ag cross-presentation in human DC induced by NK cells: Mechanisms and specialization of the BDCA-3+ DC subset

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Dendritic cells (DC) cross-present antigen (Ag) to initiate T cell immunity against most infectious and tumors. A more detailed understanding of the mechanisms involved is thus a major challenge for immunologists.
Natural killer (NK) cells have emerged as key modulators of multiple DC functions. We showed that human NK cells also promote cross-presentation of tumor cell derived Ag by DC, independently of their cytotoxicity. Instead, we highlighted a critical role for IFN-γ and TNF-α production by NK cells in this cross-presentation function.
Furthermore, we studied the impact of IFN-γ and TNF-α in the regulation of Ag processing machinery, in particular Ag degradation which is known to impact on cross-presentation efficacy. It seems that IFN-γ and TNF-α do not act on endosomal degradation but may be involved in other key Ag processing steps.
Finally, recent issues have shown that human blood and lymph node BDCA-3+ DC are specialized for cell-associated Ag cross-presentation compared to CD11b+ DC. Thus we set up purification and *in vitro* generation protocols to obtain those myeloid DC subsets. After a better characterization of their extensive phenotype and TLR response, we observed a high Ag cross-presentation induced by NK cells specifically in the BDCA-3+ DC subset.
In conclusion, our findings point toward a novel role for NK cells bridging innate and adaptive immunity that could allow to better harness Ag specific cellular immunity in immunotherapy.
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P1.08.19

IL-21 promotes plasmacytoid dendritic cell-NK cell cooperation through the production of granzyme B

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Plasmacytoid dendritic cells (pDC) are at the interface of innate and adaptive immunity and regulate immune functions at multiple levels. Granzyme B (GrB) is one of the most abundant pDC transcripts, though the mechanisms of regulation and the biological role of this protein are largely unknown. Here we report that among the Th1 and Th17 cytokines investigated, IL-21 was the only one able to induce the expression and secretion of GrB. Conversely, TLR-induced maturation of pDC inhibited GrB expression through the production of type I IFN. IL-21 expression was strongly upregulated in systemic lupus erythematosus (LE), an autoimmune disease characterized by pDC and NK cell infiltration and epithelial cell damage. In LE, pDC co-localize in large extent with NK cells. In vitro, supernatants of IL-21-activated pDC promoted autologous keratinocyte killing by both resting and activated NK cells and this action was inhibited following GrB immunodepletion. These results outline the mechanisms of GrB regulation in pDC and propose a role for GrB in pDC and NK cell biological interaction.

P1.08.20

Intestinal Aldh1a2$^+$CD103$^+$CD8a$^+$ DCs preferentially induce Th1 cells but not Tregs

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Intestinal CD103$^+$ DCs express retinoic acid (RA)-producing enzyme Aldh1a2 and efficiently promote generation of Foxp3$^+$ Treg cells. These DCs promote expression of gut homing receptors, such as CCR9 and integrin $\alpha_4\beta_7$, on T cells via a RA-dependent mechanism, but are thought to be largely incapable of inducing Th1 cells. However, Th1 cells are abundant in the intestine and IL-12p40 promoter is highly activated in the small intestine under steady-state conditions. Here, we have further divided the CD103$^+$ DC population in the lamina propria (LP) into two different subsets based on CD11b and CD8α expression and found that one of these subsets may be responsible for inducing the Th1 response. Specifically, CD103$^+$CD11b$^-$CD8α$^+$ LP-DCs preferentially produce IL-12 (p35/p40) and IL-12p40 whereas CD103$^+$CD11b$^+$CD8α$^-$ LP-DCs secrete IL-23 (p19/p40) upon stimulation with TLR agonists. Consistent with this, CD103$^+$CD11b$^-$CD8α$^+$ LP-DCs preferentially induce Th1 cells whereas CD103$^+$CD11b$^+$CD8α$^-$ LP-DCs induce Th17 cells. Interestingly, CD103$^+$CD11b$^-$CD8α$^+$ LP-DCs have lower efficiency to induce Foxp3$^+$ Treg cells compared to CD103$^+$CD11b$^+$CD8α$^-$ LP-DCs although both populations imprint gut homing CCR9$^+$ T cells in vitro. Taken together, these findings suggest that Aldh1a2$^+$CD103$^+$CD11b$^-$ CD8α$^+$ LP-DCs play an important role in generation of gut-tropic Th1 cells.

P1.08.21

Independence of ICOS-L expression on IL-10 production by DC and its impact on T cell subsets in respiratory tract infection

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The association of ICOS-L expression and IL-10 production by dendritic cells (DCs) has been commonly found in many infectious disease models. DCs with high ICOSL expression and IL-10 production are reportedly more efficient at inducing regulatory T cells (Treg). To test whether IL-10 production and ICOS-L expression has a causal relationship, we examined ICOS-L expression by DC in IL-10 knockout (KO) mice following *Chlamydia muridarum* (Cm) lung infection. In addition, we also examined the development of T cell subsets, including Treg, Th17 and Th1 cell, in the condition of IL-10 deficiency and its relationship with ICOSL/ICOS signaling following infection. Surprisingly, we found that DCs from IL-10 KO mice exhibited significantly higher ICOS-L expression than WT DCs. Moreover, we found that the IL-10 KO mice showed lower Treg but higher Th17 and Th1 responses. Further analysis of Th17 and Th1 cells showed that only the Th17 response was dependent on ICOS signaling. Consistently, most of the Th17 cells were ICOS+, while most of the Th1 cells were ICOS- following Cm infection. Furthermore, neutralization of IL-17 in IL-10 KO mice significantly exacerbated lung infection. These results suggest that ICOSL expression on DCs is independent of IL-10. They further suggest that ICOS-L expression on DCs in the presence or absence of IL-10 co-stimulation may respectively promoting Treg or Th17 response, without having significant impact on Th1.

P1.09 Dendritic cell differentiation and function

P1.09.01
House dust mite-sensitized myeloid dendritic cells induce reduction of allergen-specific murine airway inflammation

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Allergies affect about 20 to 30% of the world population and its prevalence, as well as the severity of their symptoms, has been increasing in recent decades. *Blomia tropicalis* (Bt) has been implicated as one of the most important causative agents of allergic diseases in many latin-american countries, been related to asthma, atopic dermatitis and rhinitis. Dendritic cells (DCs) in a state of partial maturation have a tolerogenic profile, being able to induce immunological tolerance. In this work, the effect of intraperitoneally injected myeloid DCs that had been *in vitro* sensitized with a Bt extract (BtE) was evaluated in an experimental respiratory allergy model. In allergic models, allergen-specific T helper type 2 (Th2) cells produce key cytokines that regulate the synthesis of allergen-specific IgE and control tissue eosinophilic airway inflammation and airway remodeling. These parameters were evaluated in mice that were intraperitoneally immunized with aluminium hydroxide-adsorbed BtE and intranasally challenged with soluble BtE. The previous inoculation of BtE-sensitized DCs significantly reduced the total number of leukocytes and the number of eosinophils in bronchoalveolar lavage fluid and reduced the production of IgE serum. In addition, reduced lung inflammatory infiltrate was observed in animals that had been treated either with BtE-sensitized DCs or with ovalbumin-sensitized DCs. Those results indicate that the use of tolerogenic DCs should be further investigated as a possible therapeutic strategy for the control of allergen-triggered airway inflammation.

P1.09.02
E-cadherin contribution for Langerhans cell differentiation

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Human skin contains the following two distinct DC subsets: (i) Langerhans cells (LCs), expressing Langerin but not DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), are predominantly localized in the epidermis; and (ii) dermal DCs, expressing DC-SIGN but not Langerin, are observed mainly in the dermis. By focusing on LC localization in the epidermis, we have recently reported that E-cadherin expressed by epidermal keratinocytes (KCs) is crucial for differentiation of LCs induced from peripheral blood monocytes (PBMOs) [Eur. J. Immunol. 43:280-280, 2013]. Monocytes differentiated into LC-like cells in the presence of IL-4, GM-CSF, and TGF-β1 expressed not only Langerin but also DC-SIGN. Notably, co-culturing of these LC-like cells with KCs expressing E-cadherin or recombinant E-cadherin strongly decreased expression of DC-SIGN and further induced a phenotype similar to purified epidermal LCs. Moreover, pretreatment of LC-like cells with anti-E-cadherin-specific antibody completely abolished their Langerin expression, indicating the requirement of E-cadherin-E-cadherin interactions for the differentiation into Langerin+ cells. These findings suggest that E-cadherin expressed by KCs provide environmental cues that induce differentiation of LCs in the epidermis. Based on these findings, we examined whether we could induce similar type of LCs from CD34+ human hematopoietic progenitor cord blood cells by co-culturing KCs or recombinant E-cadherin and compared their phenotypic features and functions with PBMO-derived LCs. These results may provide the new analytical tools for investigating various dermal diseases such as atopic dermatitis and psoriasis vulgaris.

P1.09.03
Novel inducible immortalized dendritic cell line with entire properties of primary cells

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As the sentinels between innate and adaptive immunity, dendritic cells maintain the balance of pathogen defense and tolerance to self proteins. Studies with these professional antigen presenting cells are mainly limited by their low numbers in vivo and their difficult maintenance in vitro. During last years, the potential and the important role of dendritic cells in orchestrating the adaptive immune response were examined in more detail. Elaborate investigations of dendritic cells for immunotherapies need standardized, reproducible experimental conditions. In our study, we generated conditionally immortalized dendritic cells with classical dendritic cell properties and a stable long term phenotype. We differentiated bone marrow cells from transgenic mice expressing an inducible SV40 large T-antigen into dendritic cells. When immortalized by dexamethasone and doxycycline, these cells were stable in long-term culture. In the absence of both factors (de-induction), dendritic cells displayed properties of primary cells, characterized by expression of classical dendritic cell surface markers CD11c, CD11b, MHCII, CD40 and CD86. Furthermore, de-induced LPS-activated dendritic cells secreted IL-1β, IL-6, TNFα and IL-12. De-induced, Ovalbumin-loaded dendritic cells polarize $CD4^+$ T cells into Th1, Th17 and Th2 cells, indicating their correct antigen presenting property. Consistent with intratracheal application of Ovalbumin-loaded primary dendritic cells into mice, the application of de-induced dendritic cells resulted in recruitment of lymphocytes to the lungs. In summary, we successfully expanded dendritic cells using conditional immortalization. The generated dendritic cells demonstrate the characteristic immunophenotype of primary dendritic cells and will facilitate further studies on immunomodulatory properties of dendritic cells.

P1.09.04
Age-associated shift in rat dendritic cell T-helper polarizing capacity

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Almost all cellular components of innate and adaptive immunity undergo age-related remodeling. The findings on age-related changes in human and mouse dendritic cells (DCs) are conflicting, whereas there is no data on the influence of aging on rat DCs. In attempt to fill this gap, freshly isolated splenic conventional OX62+ DCs from 3- (young) and 26-month-old (aged) Albino Oxford rats were examined for subset composition, cell surface expression of activation markers (CD80, CD86 and CD40 and MHC II molecules) and endocytic capacity using flow cytometric analysis (FCA). In addition, splenic OX62+ DCs isolated from rats of both ages were cultured in the presence or in the absence of LPS. These cells were examined for the activation marker and TNF-α, IL-6, IL-12, IL-23, TGF-β1, IL-10 expression using FCA, and RT-PCR and ELISA, respectively. Moreover, the allostimulatory capacity of OX62+ DCs and allogeneic CD4+ T cell cytokine (IFN-γ, IL-4 and IL-17) production in MLR was quantified using FCA and ELISA, respectively. It was found that aging: i) in OX62+ DCs population leads to a shift in CD4+:CD4- cell ratio towards CD4- cells and ii) influences OX62+ DCs maturation capacity (judging by activation marker expression and efficiency of endocytosis) by affecting action of intrinsic (TNF-α and IL-10) and extrinsic regulatory factor expression. Furthermore, in LPS-matured OX62+ DCs from aged rats TNF-α, IL-12, IL-23 and IL-6 expression was increased, while IL-10 expression was diminished. Moreover, in MLR, OX62+ DCs from aged rats exhibited enhanced Th1/Th17 driving force and diminished allostimulatory capacity.
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P1.09.05
Junctional Adhesion Molecules and Dendritic Cell Migration

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Dendritic cells (DC) are well-established migratory professional antigen presenting cells (APC) with the capacity of transendothelial migration (TEM). However, the mechanism underlying DC TEM has not yet been elucidated. Among JAM family molecules (JAM1, 2, 3, 4, and L), JAM-Like (JAML) has been reported to be involved in neutrophil and monocyte TEM. In the present study, we found that JAML is also involved in the TEM of mouse bone-marrow derived dendritic cells (BMDC), but not in the TEM of human monocyte-derived DC (MODC). BMDC efficiently transmigrated in response to chemokines *in vitro* and *in vivo*. Treatment with anti-JAML antibody or JAML knock-down significantly reduced the TEM activity of BMDC in a dose-dependent manner. Furthermore, the analysis of BMDC binding to TNF-α-preactivated bEND.3 monolayer revealed that the interaction between the JAML of BMDC and the coxsackie and adenovirus receptor (CAR) of endothelial cells plays an important role in TEM of BMDC. These findings suggest that JAML and CAR play an important role in the TEM of BMDC. In contrast, human MoDC do not express JAML protein, but have normal TEM capacity. MoDC express only JAM1 among the JAM family. In the TEM assay using HUVEC (Human Umbilical Vascular Endothelial Cells) monolayer, anti-JAM1 antibody efficiently inhibited the TEM capacity of MoDC. Pre-treatment of HUVEC with anti-JAM1 antibody also reduced the TEM activity of MoDC. Our present findings suggest that the expressions of JAM family molecules and JAM-mediated TEM of DC are likely dependent on the DC subtypes and/or species.

P1.09.06
CD300 molecules as regulators of dendritic cell responses

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The human CD300 family consists of six immunoregulatory membrane molecules that are expressed by leucocytes of which four, CD300b, d, e and f, are restricted to cells of the myeloid/ dendritic cell (DC) lineage. Ligands for CD300a-f include lipids such as phosphatidylserine, phosphatidylcholine and cardiolipin. We and others have shown that CD300 molecules regulate a broad range of cellular activity including differentiation, viability, cytokine and chemokine secretion, phagocytosis and chemotaxis. In transfectants CD300 molecules can form heterodimers. Human peripheral blood monocyte and DC sub-populations have specific functions, and many of these are regulated by CD300 molecules. The potential to form different CD300 heterodimers, adds further potential to their role in modulating DC function. We used in house produced mAb and commercially available mAb to document CD300a-f expression on human blood DC populations in response to a variety of inflammatory stimuli. The CD300a and CD300a/c mAb bound all DC populations. mAb to CD300d,e&f did not bind to the $CD141^+$ and pDC populations and the CD300f mAbs also failed to bind to $CD1c^+$ DC. DC mRNA analysis validated individual CD300 molecule expression and potential heterodimers. Exposure to different activators (TLR ligands, Retinoic acid) altered the pattern of CD300a-f expression. The finding that not all CD300 molecules appear on the plasma membrane at all times adds further complexity to CD300 mediated regulation. Changing their membrane levels can dramatically change CD300 mAb targeted DC functions. The data emphasizes CD300 molecules as important players in balancing DC stimulatory and tolerogenic outcomes.

P1.09.07
Human monocyte-derived dendritic cells required direct contact with natural killer cells to induce a Th1 profile of response

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Natural killer (NK) cells can promote the maturation of dendritic cells (DCs), but their role in the differentiation of monocytes into DCs remains unclear. Here, we investigated if human NK cells, through direct contact or by their products, affect Mo-DCs differentiation *in vitro*. $CD14^+$ monocytes were purified from healthy donors' blood and co-cultured with isolated NK cells ($CD56^+CD3^-$) in the presence of IL-4 and GM-CSF, in a transwell® system or in direct contact. At day 5, TNF-α was added and 48h later Mo-DCs were harvested, phenotyped and co-cultured with CFSE-labeled allogeneic T lymphocytes. After additional 5 days lymphoproliferation was determined and cytokines in supernatants quantified by CBA (BD™). Mo-DCs differentiated in contact with NK cells (NK-Mo-DCs) up-regulated the expression of HLA-DR, CD80, CD86, CD83, CCR7 and CD11c, were more potent stimulators of lymphocyte proliferation and induced a Th1-like cytokine secretion profile (IL-2, IFN-γ and TNF-α) when compared to the control group. In contrast, Mo-DCs differentiated only in the presence of NK cells' products down-regulated the expression of the mentioned molecules, were less stimulatory of lymphocyte proliferation, and induced an increase in IL-10 production. In both situations, the effect was independent of the NK cell donor. These results suggest that NK cells, through direct cell contact or through their products, may have opposite effects on Mo-DCs differentiation, and particularly NK-Mo-DCs contact seems to be required to induce a polarized Th1-like profile. This phenomenon could have potential implications for immunotherapeutic approaches based on Mo-DCs. Supported by FAPESP # 2009/54599-5, 2011/05331-0 and 2012/23478-0.

P1.09.08
Cytokine production by human dendritic cells treated with Brazilian propolis

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Dendritic cells (DC) are professional antigen presenting cells (APCs) and play an essential role in the initiation and modulation of immune responses. DC may generate either immunity or tolerance by expressing costimulatory molecules and producing pro- or anti-inflammatory cytokines. Propolis is a resinous product, made by honey bees from various plant parts, and has attracted the attention of researchers because of its biological properties, highlighting its immunomodulatory effects. Thus, this work aimed to investigate propolis modulatory effect in vitro, evaluating TNF-α and IL-10 production by human DC. Human monocytes were obtained from healthy donors and incubated with IL-4 and GM-CSF for 7 days for DC differentiation. Then DC were incubated with different concentration of propolis (2.5, 5, 10, 20µg/mL) and LPS (5µg/mL) for 24 hours. A possible cytotoxicity was assessed by the MTT assay, and cytokine production was determined by ELISA. Significant differences were determined by analysis of variance (ANOVA), followed by Dunnett's test ($p<0.05$). Data showed that cell viability was not affected after incubation with propolis and LPS. Propolis treatment did not affect TNF-α production by DC stimulated with LPS. On the other hand, propolis increased IL-10 production at lower concentrations. One may conclude that propolis displayed an immunomodulatory action in cytokine production depending on concentration, exerting an anti-inflammatory action.

P1.09.09
Chitosan/plasmid CD40 DNA nanoparticles for gene delivery to Dendritic cells

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In this study we evaluated synthesis and characterization of Co-stimulatory plasmid of DC40 gene in complex with chitosan nanoparticles due to transfer to dendritic immune cells. Material and Methods: chitosan were depolymerized to 10 KD oligomers using NaNO2 and Ionotrapic gelation method were done for chitosan/plasmid CD40 nanocomples formation in N/P=3-6, pH=7.2. Evaluation of particle size, polydispersity index, and zeta potential of the nanoparticles were performed by zetasizer nano particle analyzer. Dendritic cells were drived form Balb/c mice bone marrow. After transfection, DC phenptype using flowcytometery and DC functions in MLR test were evaluated. Results: SEM microscopy showed the

particles were about 300 nm in size and spherical in shape. Agarose gel electroforesis showed more than 95% integration if CD40 DNA to chitosan nanoparticles and DNA protection. DC matuaration were enhanced in nanocomplex exposed DCs but CD40 level was not different comparing with LPS matured DCs. Lymphocyte responces in MLR was not different with DCs that were matured with LPS. Conclusion: The goal of this study was to show that nonviral gene transfection to DCs. While these method is a safe technology for transfer of genes to DCs but the selected gene as CD40 will increase in mature DCs compared to iDCs and provided nanocomplex can not exceed form this maturation. Use of targeting molecules on this complex and appropriate nonviral transfection of complex will provide a good approach for many immune responses applications.

P1.09.10
The tetraspanin CD37 promotes dendritic cell migration

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Tetraspanins are a family of integral membrane proteins that function by forming tetraspanin-enriched microdomains (TEMs), thus organizing the cell surface. TEM formation allows tetraspanins to function in critical processes including cell adhesion and migration. Immune system aberrations are observed in tetraspanin deficient mice. T cells and dendritic cells (DC) derived from CD37-/- mice display hyperproliferative and hyperstimulatory phenotypes, respectively. However, interestingly these mice are unable to generate an effective T cell response in vivo, following antigenic challenge, including irradiated tumors. Here we present evidence to support the hypothesis that a defective migratory capacity of CD37-/- DC may be the underlying basis of this inadequate response. Adoptively transferred CFSE labelled wild-type (WT) OT-I T cells are inefficiently primed in vivo in CD37-/- mice, indicative of poor antigen presenting capability within these mice. Immunisation of WT mice with OVA peptide pulsed CD37-/- bone marrow derived DC (BMDC) generates a defective T cell response, however CD37-/- mice elicit a sufficient T cell response when primed with OVA peptide pulsed WT BMDC. This suggests an inability of CD37-/- DC to migrate effectively to the LN to prime T cells. We demonstrate through a range of models, including two-photon confocal microscopy and competitive migration assays that CD37-/- DC migrate poorly. This migratory defect is not dependant on reduced integrin expression as CD37-/- BMDC display normal integrin levels. As tetraspanins can regulate integrin outside-in signalling and cytoskeletal rearrangement, we show that CD37-/- BMDC are impaired in their ability to spread and form actin protrusions.

P1.09.11
CD14 and NFAT mediate lipopolysaccharide-induced skin edema formation in mice

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Inflammation is a multistep process triggered when innate immune cells - for example, DCs - sense a pathogen or injured cell or tissue. Edema formation is one of the first steps in the inflammatory response; it is fundamental for the local accumulation of inflammatory mediators. Injection of LPS into the skin provides a model for studying the mechanisms of inflammation and edema formation. While it is known that innate immune recognition of LPS leads to activation of numerous transcriptional activators, including nuclear factor of activated T cells (NFAT) isoforms, the molecular pathways that lead to edema formation have not been determined. As PGE2 regulates many proinflammatory processes, including swelling and pain, and it is induced by LPS, we hypothesized that PGE2 mediates the local generation of edema following LPS exposure. Here, we show that tissue-resident DCs are the main source of PGE2 and the main controllers of tissue edema formation in a mouse model of LPS-induced inflammation. LPS exposure induced expression of microsomal PGE synthase-1 (mPGES-1), a key enzyme in PGE2 biosynthesis. mPGES-1 activation, PGE2 production, and edema formation required CD14 (a component of the LPS receptor) and NFAT. Therefore, tissue edema formation induced by LPS is DC and CD14/NFAT dependent. Moreover, DCs can regulate free antigen arrival at the draining lymph nodes by controlling edema formation and interstitial fluid pressure in the presence of LPS. We therefore suggest that the CD14/NFAT/mPGES-1 pathway represents a possible target for antiinflammatory therapies.

P1.09.12
Role of the Hypoxia-Inducible Factor-1alpha and Akt on hypoxia-induced migratory program in dendritic cells

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Dendritic cells (DCs) are the most potent antigen-presenting cells and, during their life cycle, are exposed to different oxygen tensions. One of the essential functions of DCs is to migrate from/into sites characterized by a low oxygen environment. Therefore, their migratory properties may be affected by such environment with important consequences in immune response regulation, inflammation and tumour microenvironment. In the present study, we show that short-term hypoxia positively activates a migratory program in human monocyte-derived DCs regardless of the applied chemotactic stimuli. To directly study the role of HIF-1alpha, the master regulator of hypoxia-induced adaptative responses, we inhibited HIF-1alpha expression by RNA interference in DCs and thereafter a chemotactic assay was performed. Of interest, hypoxia did not enhance cell migration in immature DC silenced for HIF-1alpha, when compared with the normoxic control. In contrast, chemotaxis was still increased in mature DCs silenced for HIF-1alpha exposed to hypoxia versus normoxia. In an attempt to explain this unexpected results we evaluated several pathways involved in cell migration and we observed that hypoxia significantly enhances the phosphorylation of Akt, a protein which is associated with DC migration and maturation. Indeed, hypoxia-induced cell migration was abolished by specific Akt pathway inhibitors, also in mature DCs silenced for HIF-1alpha, indicating that Akt could be an alternative pathway to be activated in hypoxic DCs. Our results may contribute to further understand the modality by which hypoxia may affect DC migration with important implications in the regulation of the immune response.

P1.09.13
Ceramide formation is involved in Lactobacillus acidophilus-induced IFN-β response in dendritic cell

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The sphingolipid ceramide is known to play a role in lipid raft fusion and receptor clustering in the plasma membrane (PM). Upon bacterial encounter, dendritic cells (DCs) endocytose the bacteria and initiate a bacteria-specific downstream signaling event. We hypothesized that conversion of sphingomyelin to ceramide by acid sphingomyelinase (ASMase) at the outer leaflet of the PM is a key event in endocytosis of gram-positive *Lactobacillus acidophilus* and the subsequent induction of IFN-β in DCs and, as the gram-negative *Escherichia coli* does not induce appreciable amounts of IFN-β, the ASMase activity would affect endocytosis and the ensuing cytokine response of *L. acidophilus* and *E. coli* differently. SMase or an inhibitor of ASMase and acid ceramidase, chlorpromazine (CPZ) was added to DCs prior to stimulation with either of the bacteria. Endocytosis of fluorescent bacteria +/- FITC-dextran was measured by flow cytometry and gene expression and cytokine response of IFN-β and IL-12 was measured by qPCR and ELISA, respectively. Addition of SMase increased the uptake of *L. acidophilus* and *L. acidophilus*-induced IL-12/IFN-β but showed no effect on the uptake of *E. coli* though decreasing IL-12 induced by *E. coli*. SMase also showed to down-regulate Pam_3CSK_4-induced macropinocytosis of both bacteria. Addition of CPZ increased actin-dependent uptake of dextran and increased *Il-12/Ifn-β*

expression induced by *L. acidophilus*, thus further substantiating the key role of ceramide and thus, phagocytosis, in *L. acidophilus*-induced IL-12/IFN-β.

P1.09.15
T300A variant of autophagy ATG16L1 gene is associated with decreased antigen sampling and processing by dendritic cells in paediatric Crohn's disease

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The single nucleotide polymorphism (SNP) T300A of the Crohn's disease-(CD) associated gene ATG16L1is responsible for decreased autophagy. This study aimed to investigate the effects of this SNP on the uptake and processing of antigens by dendritic cells (DC), as well as in the interaction between DC and intestinal epithelium, in paediatric CD patients. We enrolled children that homozygously carry the protective (wild type, n=7), the risk allele (risk, n=13) of ATG16L1, or were heterozygous (het, n=13). The peripheral blood monocyte-derived DC were analysed for phenotype, antigen sampling and processing by flow cytometry, while the capability of DC to form transepithelial protrusions was determined by confocal microscopy. DC generated from wild type patients showed higher bacterial sampling and antigen processing compared to risk patients. Additionally, after exposure to either bacterial particles, or to the antigen DQ-OVA, the wild type DC showed a significant increase in the expression of the HLA-DR and CD86 when compared to risk DC. Interestingly, also het patients showed an impairment in bacterial uptake and expression of activation marker when compared to the wild type. In the Caco2/DC co-colture, the formation of transepithelial protrusions were less numerous in risk DC compared to wild type and the antigen uptake was decreased. In conclusion, DC of paediatric CD patients carrying the T300A allele showed a marked impairment of antigen uptake and processing, and defective interactions between DC and intestinal epithelium. Collectively, our results suggest that an autophagy defect is associated with an impairment of intestinal innate immunity in paediatric CD.

P1.09.16
CD137 ligand generated dendritic cells are more potent than classical dendritic cells in inducing an antigen-specific T cell response

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Dendritic cells (DC) are professional antigen-presenting cells that are essential in regulating and orchestrating T cell-based immunity. This property has prompted intense research in the use of DC-based immunotherapy. Nevertheless, DC vaccination is still in its infancy and improvements in terms of DC preparation, antigen-loading and increasing their immunostimulatory capacity are required. Here, we describe a potent and novel type of DC generated by CD137-ligand reverse signaling into peripheral monocytes.
The ligand for CD137 (CD137L) is expressed on peripheral human monocytes and delivers a potent activating signal via reverse signaling. Treatment of monocytes with a recombinant CD137 protein that induces reverse signaling through CD137L reduces typical macrophage characteristics such as phagocytosis and CD14 expression. However, distinct DC characteristics including endocytosis, costimulatory molecule expression and the ability to stimulate proliferation of allogenic naïve T-cells are induced. CD137L-generated DC (CD137L-DC) can be further matured which leads to an increase in DC marker expression such as CD83, CD86 and HLA-DR. This in turn enables a stronger activation of allogeneic T-cells. We also tested the ability of CD137L-DC to initiate antigen-specific T-cell activation in an autologous setting. Using cytomegalovirus (CMV) pp65 peptides, we show that matured CD137L-DC are able to activate pp65-specific T-cells more potently than classical DC. More importantly, these T cells are able to induce a stronger antigen-specific killing of HLA-matched target cells as compared to T cells activated by classical DC. These in vitro data show that CD137L-DC are potent antigen-presenting cells and should be evaluated for human immunotherapy.

P1.09.17
Inflammation in the periphery and dendritic cell progenitors in the bone marrow

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Immune cells in inflamed peripheral tissues require constant replacement by cells from the bone marrow. CD11c+ cells (dendritic cells, DCs) cultured from bone marrow of mice with inflammation of the skin, airways or peritoneal cavity, have poor priming ability when transferred into naïve mice, and create reduced antigen-specific memory responses. Inflammation-associated prostaglandin E2 is involved directly or indirectly as the cyclooxygenase inhibitor, indomethacin, prevents the development from bone marrow of poorly priming DCs. Use of different culture conditions suggests that the developmental pathway of an early DC committed cell is altered by prostaglandin E2-associated tissue inflammation. To investigate DC development *in vivo*, bone marrow-ablated mice were engrafted with bone marrow cells from control mice and mice with skin or airways inflammation. Sixteen weeks after bone marrow cell transfer, immune responses largely dependent on DC function were poor in recipients engrafted with cells from mice with tissue inflammation. When an inflammatory antigen was painted onto skin, the inflammatory response was negligible. T lymphocytes from all chimeric mice had similar proliferative capabilities. An effect of prostaglandin E2 on very early progenitors, possibly haemopoietic stem cells, is suggested. Further, when pregnant mice were UV-irradiated (which induced skin inflammation), the bone marrow of the progeny contained altered DC progenitors and suggests an epigenetic effect of tissue inflammation on these cells. The effect of tissue inflammation on DC development may be an important homeostatic process. However, this effect is removed by use of drugs (eg steroids) that prevent prostanoid production.

P1.09.18
Immune dysregulation in sickle cell disease

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Acute painful vaso-occlusive crises and recurrent infections are frequent complications of patients with sickle cell disease (SCD). Chronic activation of monocytes producing pro-inflammatory cytokines may play a role in immune dysregulation, susceptibility to infection, activation of vascular endothelium, and vaso-occlusion in SCD. The pathophysiology of SCD may be influenced by activated and/or immature monocytes. We compared activated monocytes in SCD with normal monocytes in healthy persons, analyzing CD4, CD14, CD16, and HLA-DR antigen expression using flow cytometry and morphologic assessment of monocytes with Wright and alpha-naphthyl butyrate esterase (ANBE) stains. SCD patients showed absolute monocytosis with decreased CD4+, CD4+HLA-DR+ and CD4+CD14+ monocyte expression and increased CD14(dim)+CD16+ expression. Monocytes in SCD showed immature morphology, including a high N/C ratio, indented nuclei, decreased cytoplasmic vacuolation and ANBE activity. We also examined the function of dendritic cells in SCD. Peripheral blood dendritic cells (PBDC) were purified from patients with SCD and from healthy donors. Monocyte-derived DC (MoDC) from patients and donors were generated *in vitro* with granulocyte/macrophage colony stimulating factor (GMCSF) and interleukin-(IL-)-4. Compared to healthy donors, PBDC from SCD patients showed reduced expression of HLA-DR, CD80, CD86, and

CD40; reduced stimulatory capacity to mixed lymphocyte reactions; and reduced production of IL-12 in response to lipopolysaccharide and to CD40 ligand. Similar reductions were observed in MoDC from SCD patients. These results indicate that alterations in dendritic cell function are present in SCD patients. Alterations in both monocytes and dendritic cells in SCD may contribute to deficiencies in immune response and susceptibility to infection.

P1.09.19
Characterization of Monocyte-Derived Dendritic Cells able to induce an E6/E7-specific, HLA I-restricted, cytotoxic activity

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The adoptive transfer of cancer Ag-specific effector T cells in patients can result in tumor rejection, thereby illustrating the immune system potential for cancer therapy. Viruses involved in tumorigenesis are useful targets because viral proteins are not expressed in normal cells and their expression is required to maintain the malignant phenotype. Between oncoviruses, high risk human papillomavirus (HPV) has a well-characterized transforming propriety and it has been associated with squamous cell carcinoma of the ano-genital and oral tracts. Cutaneous genotypes are, instead, associated with some forms of non-melanoma skin cancer. Transforming ability of HPV is based on the function of E6 and E7 viral oncoproteins, which interact and inactivate pRB and p53 oncosuppressors, respectively. For these reasons vaccines targeting oncogenic E6 and E7 are ideal candidates to elicit strong immune responses without generating autoimmunity.
Here we report results obtained using a protocol based on human monocyte-derived dendritic cells (MDDC) and/or Langerhans cells (MDLC) incubated *in vitro* with necrotic keratinocytes expressing both E6 and E7 oncoproteins derived from mucosal (HPV16) or cutaneous (HPV38) genotypes. We observed the capability of MDDCs i) to uptake necrotic material from transformed keratinocytes; ii) to upregulate the expression of class I HLA and CD86 co-stimulatory molecule following uptake and iii) to specifically prime *in vitro* cytotoxic T lymphocytes against E6/E7-expressing keratinocytes.
We also provide preliminary evidences indicating the ability of necrotic keratinocytes to alter the microRNA profile in immature MDLC compared to prototipical stimuli as bacterial lipopolysaccharide.

P1.09.20
Inhibition of nitric oxide production and pro-inflammatory cytokines by several medicinal plants

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BACKGROUND: A number of medicinal plants have been used to treat various immunological diseases. Nitric oxide (NO) has an important regulatory role in the various types of inflammatory processes.
OBJECTIVE: To investigate the NO modulatory activity of the extracts of several medicinal plants native to Iran including Dracocephalum kotschyi, Linum persicum, Dionysia termeana, Salvia mirzayanii, Ferulago angulata and Euphorbia cheiradenia. Methods: The methanolic extracts of the plants were prepared and examined for their effects on the NO production by lipopolysaccharide-stimulated mouse macrophages. The level of TNF-α and IL-1β pro-inflammatory cytokines in the macrophage culture were detected using enzyme-linked immunosorbent assay.
RESULTS: All the extracts at concentration of 50 µg/ml demonstrated a significant decrease in NO production ($p<0.001$) after a 24-hour treatment. This inhibitory effect was also seen after 48 hours. Among the extracts, L. persicum was the strongest extract in reducing the NO production at 1 µg/ml after both 24 and 48-hours (nearly 100% inhibition, $p<0.001$). S. mirzayanii extract with 66.2 ± 8% inhibition at 50 µg/ml, showed the mildest effects in 48 hour culture. In cytokine release determination, the extract of L. persicum significantly inhibited both TNF-α and IL-1β cytokines production by stimulated macrophages ($p<0.001$). D. kotschyi, D. termeana and F. angulata decreased secretion of IL-1β from the cells.
CONCLUSION: These results indicate the presence of anti-inflammatory and macrophage inhibitory substances in these plants.

P1.09.21
Soluble TNFRp75 regulation of TNF-TNFRp55 dependent dendritic cell activation controls protective immunity against *Mycobacterium tuberculosis*

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Tumour necrosis factor alpha (TNF) signalling is critical for host protective immunity against *M. tuberculosis* infection. TNFRp55 and TNFRp75 both bind TNF however the role of particularly TNFRp75 dependent immune function has been poorly defined. This study demonstrates superior control of *M. tuberculosis* infection by $TNFRp75^{-/-}$ mice with increased survival and enhanced bacilli clearance. $TNFRp75^{-/-}$ mice demonstrated increased pulmonary recruitment of activated dendritic cells. Moreover, IL-12 dependent migration of dendritic cells to lung draining lymph nodes of infected $TNFRp75^{-/-}$ mice was significantly higher than WT counterparts and was associated with enhanced frequencies of activated *M. tuberculosis* specific IFNγ-expressing $CD4^+$ T-cells. Shedding of TNFRp75 in WT mice correlated with significantly reduced bioactive TNF and IL-12p40 expression during *M. tuberculosis* challenge. Indeed, neutralisation of TNFRp75 in infected WT dendritic cell cultures increased both bioactive TNF and IL-12p40 production to equivalent levels observed in $TNFRp75^{-/-}$ cultures, directly implicating TNFRp75 shedding with the regulation of dendritic cell activation. Further, TNF-TNFRp55 mediated dendritic cell activation as opposed to signalling through TNFRp75 is required for *M. tuberculosis* specific Th1 immune responses. Thus, TNFRp75 significantly down-modulates protective immune function, reducing host resistance and survival after aerosol *M. tuberculosis* infection. Targeting TNFRp75 may therefore be therapeutically beneficial for improvement of disease outcome.

P1.09.22
SHIP-1 Expression maybe involved in the endocytic functions of early bone marrow-derived dendritic cells but not myeloid derived suppressor cells

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Dendritic cells (DCs) are often targeted in vaccine studies and immunotherapies. Granulocyte/macrophage colony-stimulating factor (GM-CSF) can generate DCs from bone marrow (BM), although it is commonly used in conjunction with other cytokines such IL-4 to produce mature DC cultures. Herein, early GM-CSF-derived DCs from murine bone marrow were evaluated in new detail, including their endocytic capacity over time. The early GM-CSF derived DCs expressed lower levels of co-stimulatory molecules and had higher endocytic capacity for nano-(40nm) and microparticles (500nm) when compared to GM-CSF+IL4-derived DCs. Surprisingly, endocytic capacity for nanoparticles decreased steeply as they matured over time, whereas microparticle uptake was largely maintained. Further investigation revealed that early GM-CSF-derived DCs take up nanoparticles via caveolae-mediated endocytosis and microparticles via phagocytosis. Myeloid derived suppressor cells (MDSCs) are also generated from the bone marrow in the presence of GM-CSF. The pattern of uptake by MDSCs was completely different from early DCs, with evidence for the use of alternative uptake pathways. Consistent with uptake via caveole, abrogation of Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP-1) expression in early GM-CSF-derived DCs dramatically reduced uptake of nanoparticles but not microparticles, and enhanced TNF and CCL2 secretion and reduced TGFβ levels. These findings shed new light on the function of early GM-CSF-derived DCs and MDSC, and the potential of early DC cells as targets in therapeutic applications.

P1.09.23
Tofacitinib, a JAK inhibitor, induces tolerogenic phenotype in human dendritic cells

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Objective: Tofacitinib, a Janus Kinase (JAK) inhibitor has shown striking clinical benefits in rheumatoid arthritis. Although JAKs are known for their importance in lymphocyte development and function, their role in human dendritic cells (DCs) remains unclear. This study was undertaken to elucidate the role of JAKs in DCs using tofacitinib.
Methods: Human monocyte-derived DCs were generated and matured with lipopolysaccharide (LPS) in the presence of tofacitinib. Cytokine production and surface marker were assessed. After elimination of tofacitinib, allogeneic-mixed lymphocyte reaction was performed with CD4+CD45RA+ naive T cells.
Results: The presence of tofacitinib during maturation with LPS inhibited DC clustering and reduced expression of CD80 and CD86 but not HLA-DR. Production of type1 IFN, TNF-α, IL-6 and IL-1β reduced, but that of TGF-β was not affected. These effects did not depend on the cell toxicity. Pretreatment with cyclohexamide or blockade of type1 IFN-receptor resulted in suppression of CD80 and CD86. Among the transcription factors involved in regulation of co-stimulatory molecules, IRF7 was the only one that was suppressed. Co-culture of tofacitinib-treated DCs with naïve CD4+ T cells reduced T-cell proliferation and IFN-γ production. Additionally, tofacitinib markedly increased the expression levels of indoleamine 2, 3-dioxygenase (IDO).
Conclusions: Tofacitinib inhibits the positive loop of type-I IFN and the subsequent expression of co-stimulatory molecules with increased IDO, leading to attenuation of T-cell stimulatory capacity. Our results indicate that tofacitinib induces transformation to tolerogenic DC subsets. The results describe a novel mode of action for tofacitinib and the pivotal role of JAKs in DCs.

P1.09.24
Estrogen receptor signaling and X-chromosome complement both independently contribute to the enhanced TLR-7-mediated responses of woman plasmacytoid dendritic cells

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Human plasmacytoid dendritic cells (pDCs) play a major role in innate immunity through the production of type I IFNs after Toll-like receptor (TLR) engagement by pathogens. Woman pDCs exhibit enhanced TLR-7-mediated responses as compared to man, which may account for sex-based differences in autoimmune and infectious diseases. To further characterize the mechanisms underlying this sex-based difference in pDC innate functions, we investigated the respective contribution of X chromosome dosage versus sex hormones using a humanized mouse model in which male or female NOD-SCID-ß2m$^{-/-}$ mice were transplanted with human progenitor cells (HPCs) purified from either male or female donors. We showed that the frequency of IFN-α- and TNF-α-producing pDCs in response to TLR-7 ligands was enhanced in female mice as compared to male, suggesting that endogenous estrogens positively regulated TLR responses in pDCs in agreement with our recently published work (Seillet et al., Blood 2012 119:454). Indeed, using an in vitro model of Flt3L/IL-7-driven human pDC differentiation from HPCs, we demonstrated that blockade of ER-signaling during pDC development inhibited TLR-7 and TLR-9-mediated IFN-a production by human pDCs from either sex. Lastly, by comparing the innate functions of female or male human pDCs that develop in HIS mice, we observed that female pDCs have an enhanced TLR-7-mediated IFN-α response as compared to male ones, irrespective of the sex of the recipient mice in which they developed. Taken together, these results indicate that cell-intrinsic ER-signaling and X chromosome complement both independently contribute to the enhanced TLR-mediated responses of woman pDCs.

P1.09.25
Bovine lactoferrin induces CCL1 production in myeloid cells

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Lactoferrin (LF) is now recognized as a key element in mammalian immune system for its pivotal role in host defence against infection and excessive inflammation.
We previously demonstrated that bovine LF (bLF) exerts a potent anti-inflammatory activity by skewing monocyte differentiation into monocyte-derived dendritic cells (MD-DCs) with impaired capacity to undergo activation and to promote Th1 responses.
In this study we show that the exposure of both MD-DCs and their monocyte precursors to bLF induces CCL1 production, even though with differences in the protein amount. Interestingly, in freshly isolated monocytes CCL1 mRNA expression levels are significantly enhanced at 2 hours and more markedly at 4 hours after bLf-treatment, suggesting a direct role of bLF on CCL1 mRNA transcriptional activation. Furthermore, cells continue to produce this chemokine even upon medium replacement 18 hours post bLF-treatment indicating CCL1 production as a result of permanent changes induced in bLF-treated DC precursor cells.
The analysis of the role of TLR2 and TLR4, and their co-receptors CD14 and CD36, on bLF-mediated CCL1 production, indicates that blocking each of these receptors significantly influences soluble factor secretion with cell-specific differences.
Finally, CCL1 is not implicated in the bLF-mediated inhibition of MD-DCs activation since blocking its biological activity does not rescue their LPS-mediated up-modulation of CD83 and IL-12 production, as well as their ability to induce T cell proliferation.
Understanding the biological role of the bLF-induced CCL1 production in both monocytes and MD-DCs could provide further evidence for mechanisms underlying LF immunomodulatory activity.

P1.09.26
Baicalin from *Scutellaria baicalensis* impairs Th1 polarization through the inhibition of dendritic cell maturation

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Baicalin from *Scutellaria baicalensis* is a major flavonoid constituent found in the traditional Chinese medicinal herb Baikal skull cap. It has been widely used for the treatment of various diseases, such as pneumonia, diarrhea, and hepatitis. Recent studies have demonstrated that baicalin possesses a wide range of pharmacological and biological activities, including anti-inflammatory, anti-microbial, anti-oxidant, and anti-tumor properties. Specifically, its anti-inflammatory activity has been estimated in various animal models of acute and chronic inflammation; however, its effects on dendritic cells (DCs) maturation and immuno-stimulatory activities are still unknown. In this study, we attempted to determine whether baicalin could influence DCs surface molecule expression, antigen uptake capacity, cytokine production, and capacity to induce T-cell differentiation. Baicalin was shown to significantly suppress the expression of surface molecules CD80, CD86, major histocompatibility complex (MHC) class I, and MHC class II as well as the levels of interleukin-12 production in lipopolysaccharide stimulated DCs. Moreover, baicalin-treated DCs showed an impaired induction of the T helper type 1 immune response and a normal cell-mediated immune response. These findings provide important understanding of the immunopharmacological functions of baicalin and have ramifications for the development of therapeutic adjuvants for the treatment of DCs-related acute and chronic diseases.

P1.09.27
notch ligand delta-like 4-pretreated dendritic cells alleviate allergic airway responses by enhancing IL-10 production

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Dendritic cells (DCs), as professional antigen-presenting cells (APCs), induce T cell activation and promote T cell differentiation by antigen stimulation. It is known that Notch ligand delta-like 4 (Dll4) on APCs is associated with stimulation of a Th1-type response. However, the regulatory roles of Dll4 in the activation and function of DCs have not been clearly elucidated. In this study, we demonstrated that activation of Dll4-pretreated bone marrow-derived DCs via ovalbumin (OVA) stimulation expressed a high level of IL-10. In contrast, the proinflammatory cytokines decreased in Dll4-pretreated DCs with either LPS or OVA stimulation. Compared to fully mature DCs, lower levels of MHC class II CD40 and higher levels of CD80 and CD86 molecules were expressed on these DCs. Dll4 Notch signaling also enhanced Notch ligand mRNA expressions of Dll1, Dll4, and Jagged1 in DCs. Dll4-modified DCs exhibited a reduced capacity to stimulate the proliferation of OVA-specific $CD4^+$ T cells but strongly promoted large amounts of IL-10 production in these activated T cells. After adoptive transfer of OVA-pulsed plus Dll4-pretreated DCs in OVA-immunized mice, OVA challenge induced lower OVA-specific IgE and higher IgG_{2a} antibody production, lower eotaxin, KC, IL-5, and IL-13 release in bronchial alveolar lavage fluid, attenuated airway hyperresponsiveness, and promoted higher IL-10 and IFN-γ production in the spleen. Taken together, our findings shed light on a new role of Dll4 in the phenotype and function of DCs and provide a novel approach for manipulating Th cell-driven deleterious immune diseases.

P1.09.28
LAMP-2, a novel endocytic receptor on human monocytes derived dendritic cells (MDDC).

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Lysosome-associated membrane protein-2 (LAMP-2) is a type 2 membrane-protein that is commonly used as lysosome and late endosomal marker and with essential roles in chaperone mediated autophagy and antigen presentation. It is also present on the cell-surface although its role there is uncertain. We focus on the role of LAMP-2 as a receptor on freshly isolated monocytes and iDCs with possible implication in antigen presentation.
LAMP-2 was detected on the surface of immature MDDC both by FACS and confocal microscopy, its expression increased after maturation with IFN-γ LPS. The monoclonal anti-LAMP-2 antibody (H4B4) bound to LAMP-2 on the surface of MDDC and was rapidly and specifically internalized when compared to control antibodies in the presence of Fc blockade. Also the Fab fragment alone is internalized as well. Uptake was absent in MDDC from an individual with genetic LAMP-2 deficiency, Danon-disease. As expected, MDDC LAMP-2 and HLA-DR localized to partially overlapping compartments in iDC but there was no co-localization with HLA-DM. However, confocal microscopy showed that H4B4 transits into a HLA-DM positive compartment 1 hour after ligating LAMP-2 on the cell surface. Co-localization is no longer detectable after 3 hours. Finally, stimulation of immature MDDCs with H4B4 promote activation in the presence of IFN-γ indicated by up-regulation of CD80/CD83.
This data suggest that cell surface LAMP-2 can act as endocytic receptor for internalization of extracellular molecules without cross-linking. H4B4 mimics natural ligands and traffics to the MIIC co-incident with DC maturation evidenced by up-regulation of CD80/83.

P1.09.29
Role of muscarinic activation in regulation of human dendritic cells maturation process

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We demonstrated the presence of muscarínic receptors M_3, M_4 and M_5 receptors, and choline acetyltransferase and acetylcholinesterase enzymes in human dendritic cells (DC). Given that the maturation process of the DC has a central role in the immune response, we investigated the effect of cholinergic stimulation on the expression of maturation markers and the proinflammatory cytokines. DC were differentiated from positive selected $CD14^+$ cells isolated from peripheral blood mononuclear cells and cultured with IL-4 and GM-CSF. Then cells were treated with the cholinergic agonist carbachol (Carb,10^{-8}M) for 1h followed by additional 24h in the absence (immature (iDC)) or presence (mature (mDC)) of LPS (0.5mg/ml). We observed that Carb increased the expression of HLA-DR and CD86, as well as the production of IL-12 (Carb:575±10; baseline:374±15 pg/ml) and TNF-α (Carb:6733±1167, baseline: 3667±629) in mDC (n=3, $P \leq 0.01$). Both effects were reduced in the presence of the muscarinic antagonist atropine (10^{-7}M). Nitric oxide synthase (NOS) isoforms are involved in the signaling pathway of M3 and M5 receptors and their product, nitric oxide, is an inflammatory mediator and an immune response regulator. We have shown by Western blot that iDC as well as mDC expressed NOS1 and NOS3 and had similar basal release of NO_2^-(uM) (iDC:2.99±0.96; mDC:1.98±0.51). Carb treatment increased the levels of NO_2^- in iDC. We concluded that cholinergic stimulation regulates the expression of phenotypic markers of maturation and production of proinflammatory cytokines in human DC. iDC also respond to the cholinergic agonist, increasing their nitric oxide production, which could be a key factor in the maturation process.

P1.09.30
Growth phase-dependent immune response to *Staphylococcus aureus*

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Staphylococcus aureus cause a wide range of human diseases, varying from minor skin infections to bacteremia associated with high morbidity and mortality. The bacteria express and secrete diverse proteins, polysaccharides and toxins during different stages of growth. It is unknown how growth phases of bacteria affect the initiation of the adaptive immune response. In this study, we hypothesized that professional antigen-presenting dendritic cells (DCs) respond differently to *S. aureus* in exponential and stationary phase (EP and SP), and hence to different T-cell responses. Human monocyte-derived DCs were cultured with *S. aureus* NCTC-8325-4 in EP or SP and analyzed for maturation status, cytokine production, and T-cell polarizing capacity. EP and SP bacteria equally induced maturation of the DCs. However, EP bacteria induced a higher expression of cytokines, including IL-12p70 and IL-23. Cocultures of autologous naive CD4+ T-cells with DCs and EP *S. aureus* resulted consistently in higher development of Th1 cells compared to cocultures with SP. Analysis of autologous memory T-cells revealed the same findings as seen with naive T-cells. In addition, EP also was able to drive a higher Th17 and Th1/Th17 response, compared with the SP bacteria. Collectively, these results reveal a growth phase dependent immune response to *S. aureus*. The immune response against the SP is almost absent compared to the EP, which suggests that the immune response has evolved to fight only *S. aureus* when it is growing rapidly, and thus preventing the bacteria from spreading, while allowing the presence of resting bacteria.

P1.09.31
ESAT-6 and HspX improve the effectiveness of Bacille Calmette-Guérin to induce human Dendritic Cells-dependent T cells and Natural Killer cells activation

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The limited efficacy of BCG vaccination is partly due to a missing expression of critical immunogenic proteins. We analyzed whether early-secreted-antigenic-target-protein-6 (ESAT-6) and 16-kDa-heat-shock-protein (HspX) Mycobacterium tuberculosis (Mtb) antigens improve the ability of BCG to activate human DC.
Addition of single HspX or ESAT-6 to BCG-treated DC did not influence cytokine release, whereas addition of both the antigens significantly improved pro-inflammatory cytokine, but not IL-10 secretion by DC. Moreover, DC incubated with BCG in presence of both ESAT-6 and HspX were able to elicit IFN-γ release by co-cultured CD4+ lymphocytes or NK. The above-mentioned effects of the antigens were inhibited by IL-12-blocking antibodies. A specific TLR2-blocking antibody decreased IL-12 release by DC stimulated with BCG, ESAT-6 and HspX, as well as IFN-γ secretion by CD4+ lymphocytes co-cultured with these cells. Furthermore, HspX and ESAT-6 improved the capacity of BCG-treated DC to induce the expression of the memory phenotype marker CD45RO by naïve CD4+ T cells.
Our results indicate that ESAT-6 and HspX cooperation enhances the BCG ability to stimulate human DC, that become able to induce an effective Th1 and NK response through TLR2-dependent IL-12 secretion. Therefore ESAT-6 and HspX represent good candidates for improving the effectiveness of vaccination with BCG.

P1.09.32
Dendritic cell CCAAT/ enhancer binding protein delta modulates Th17/Treg responses in an IL-10 dependent manner

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CCAAT/enhancer binding protein delta (CEBPD), a bZIP-transcription factor, is an emerging regulator of innate immune responses and therefore might influence adaptive immune responses. However, it hasn't been examined in the context of central nervous system (CNS) inflammation and T-cell activation. This study aimed to determine the role of CEBPD in experimental autoimmune encephalomylitis (EAE) a T-cell mediated CNS autoimmune disease. CEBPD was upregulated at the protein and RNA level in peripherally derived DCs and astrocytes in the EAE affected CNS and animals lacking CEBPD had significantly less disease. Follow on BM chimeric experiments showed that it was CEBPD expression in peripherally derived immune cells that mediated differences in EAE severity. To identify cells other than DCs that expressed CEBPD throughout disease we developed an intracellular staining method to detect CEBPD protein expression. There was no CEBPD expression in peripherally derived CNS immune cells, other than in DCs. To detect functional changes in DC activity we examined Th-lymphocytes phenotype throughout the course of EAE and showed a significant reduction in Th17:Treg ratio when DC CEBPD was missing. The same was observed in an in vitro model of T-cell development directed by DCs that lacked CEBPD. These DCs also had significantly increased IL10 mRNA expression. Further, blocking IL-10 signaling completely compensated for the lack of CEBPD expression in DCs both in vitro and in vivo. These studies identify CEBPD as an important DC factor in regulating inflammatory response through directing T-lymphocyte development and promoting autoimmunity.

P1.09.33
Study of leukemic cell products and IL-1β as modulators of human dendritic cells

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Although dendritic cells (DCs) play an important role in controlling tumors as antigen-presenting cells, they might be modulated by tumor-derived products. It was shown that leukemic cell products down regulate DC differentiation through the induction of IL-1β. The aim of this study was to compare the influence of leukemic cell products and exogenous IL-1β on DC development and function. For this, K562 cell line was used as a model of chronic myeloid leukemia to obtain the supernatant (SN K562). Monocytes from healthy donors were separated and cultured, for 5 days, in the presence of IL-4 and GM-CSF and also SN K562 or with exogenous IL-1β. After these 5 days, some cells were cultured with TNF-α for a further 2 days. Flow cytometry analyses and ELISAs were performed after 5 days and 7 days of culture. Our results showed that, in the presence of SN K562, CD14, CD16, CD68 and CD80 expressions were higher than in the control; otherwise CD1a and CD83 expressions were lower. Similarly, IL-1β modulated all these molecules, with exception of CD16 and CD68 that were not altered by its addition. Furthermore, K562 cells did not produce IL-10, yet both the addition of the supernatant and exogenous IL-1β induced IL-10 secretion by monocytes stimulated to differentiation and activation into DCs. Finally, these results suggest that both leukemic cell products and IL-1β alter DC development, inducing a suppressive phenotype. Moreover, IL-1β induction seems to be an important mechanism used by leukemic cells to inhibit DCs.

P1.09.34
Evaluation of the effect of the 14kDa protein isolated from aged garlic extract on dendritic cells

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Garlic is used all over the world for treatment of different diseases. A wide range of biological activities of garlic has been verified in vitro and in vivo. One of major proteins of garlic which has been isolated and purified is the 14 kDa protein. This protein has been shown to have immunomodulatory effects. In this study, the effect of the 14 kDa protein isolated from aged garlic extract (AGE) was investigated on maturation and immunomodulatory activity of dendritic cells (DC). Proteins were purified from AGE by biochemical method; the semi-purified 14 kDa protein was run on gel filtration Sephadex G50 and its purity was checked by SDS-PAGE. DC were isolated from spleen of BALB/c mice by Nycodenz centrifugation and their adhesiveness to plastic dish. 14 kDa protein from AGE was added to overnight culture of DC medium and the expression percentage of CD40, CD86, and MHC-II was evaluated by flowcytometric analysis. Also, proliferation of T cells was measured by allogenic mixed lymphocyte reaction (MLR) test. The purified 14 kDa protein isolated from AGE increased the expression of CD40 molecule on DC, but it did not influence CD86 and MHCII molecules. Furthermore, no significant differences were noticed between DCs pulsed or unplused with 14 kDa protein on the MLR assay.

P1.09.35
IL-1 and IL-1 Receptor Antagonist Genes Polymorphism in ACPA Positive Rheumatoid Arthritis Patients

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Background & objectives: Rheumatoid arthritis (RA) is the most common systemic autoimmune disorder where environmental, hormonal and genetic factors contribute to the disease. In recent years, studies have shown that the genetic risk may include IL-1 and

IL-1receptor antagonist (IL-1Ra), cytokines which play a critical role in the pathogenesis of RA. There have also been a major development in the field of immunity focused on antibody reactivity to proteins modified by citrullination, highly specific marker of RA, which has redefined RA phenotypes, demonstrating major differences in genetic and environmental risk factors, and thus probably in molecular pathogenesis too, between RA patients with and without the presence of antibodies to citrullinated proteins (ACPA). ACPA-positive RA patients show a disease course considerably more severe than that of ACPA-negative patients. The aim of our study was to evaluate the association between a genetic IL-1 polymorphism with susceptibility and clinical aspects of RA.
Materials and methods: In this case-control study, blood samples from 100 Algerian RA patients and 127 matched healthy controls were used to study the IL-1RA and IL-1B polymorphism. ACPA was detected using an ELISA anti-CCP-3 generation test.
Results: There was no difference in the frequency of different alleles between patients and healthy controls. However, a positive correlation between IL-1RN*1/IL-1B-511T/IL-1B+3953C haplotype and ACPA production was detected (RA/ACPA+: 31.28 vs RA/ACPA-: 6.66, P= 0.011, OR= 4.693).
Conclusion: Our findings indicate that IL-1B and IL-1RA polymorphism was not a susceptibility marker in RA in our population, but it could be associated with ACPA+RA.

P1.09.36
CD40-ligand signal response diversity through dendritic cell state modification

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Immune cells potentially encounter multiple signals sequentially, each signal affecting their transcriptional state. To what extent their transcriptional state modifies the response to a following signal is not known. We used human primary dendritic cells, which by nature sequentially respond to innate and adaptive signals, to measure the effect of innate signals on the response to adaptive signals. First, we stimulated human blood dendritic cells with the innate signals LPS, Thymic Stromal Lymphopoeitin (TSLP) or medium as a control in order to generate three distinct transcriptional states in DCs. To uncouple the transcriptional effect of the state modifiers from the response to a subsequent signal, we stimulated or not the cells with the adaptive signal CD40L. Differential analysis of large-scale transcriptomic data showed that 89% of the genes modified by CD40L depended on the cell state. Each cell state primed the regulation of different factors by CD40L, among them IL6, TNFalpha and Cxcl10. We identified genes, such as TNFSF15 and CD70, specifically induced only by the combination of TSLP and CD40L, indicating that their regulation requires temporal integration of signals. Additionally, pathway analysis suggested that cell state determines biological and immune functions regulated by CD40L.
Therefore, differences in dendritic cell state translate into quantitative differences in the response to a subsequent adaptive signal.

P1.09.37
Dendritic cell reprogramming by endogenously produced lactic acid

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The demand for controlling T cell responses via ex vivo generated dendritic cell (DC) vaccines initiated a quest for reliable and feasible DC modulatory strategies that would facilitate cytotoxicity against tumors or tolerance in autoimmunity or allergy.
We studied endogenous mechanisms in developing monocyte derived DCs (MoDCs) that can induce inflammatory or suppressor programs during differentiation. Such mechanisms can lead to functional heterogeneity of the cells and can influence the efficiency of DC therapies. We characterized a powerful autocrine regulatory pathway in developing MoDCs that, in a cell concentration-dependent manner, strongly interferes with inflammatory DC differentiation.
MoDCs developing in sparse cultures have superior ability to produce inflammatory cytokines, to induce TH1 polarization and to migrate towards the lymphoid tissue chemokine CCL19. On the contrary, MoDCs originated from dense cultures produce IL-10 but no inflammatory cytokines upon activation. DCs from high-density cultures maintained more differentiation plasticity and can develop to osteoclasts. The cell culture density-dependent pathway was independent of PPARγ, a known endogenous regulator of MoDC differentiation. Instead, it acted through lactic acid, which accumulated in dense cultures and induced an early and long-lasting reprogramming of MoDC differentiation.
Our results may indicate a strongly context-dependent nature of the in vitro MoDC model system, due to DC functions being already polarized in early stages of differentiation by cell culture density, but these results also provide the possibility for efficient and remarkably simple way of DC manipulation.

P1.09.38
CD1c$^+$ DCs are superior at stimulating Th2 cytokine production upon grass pollen stimulation compared to circulating basophils and pDCs

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Background: Dendritic cells (DCs) are a heterogeneous group of antigen-presenting cells (APCs) and subpopulations have been identified. However, their role in allergic immune responses is not fully clarified. Additionally, basophils are suggested to be superior allergen-presenting cells in allergic responses against e.g papain in mice.
Objective: The present study aimed at elucidating the functional role of CD1c$^+$ myeloid DCs (mDCs) and CD123$^+$ plasmacytoid DCs (pDCs) in human allergic rhinitis and also to examine whether basophils operate as APCs in human allergic responses.
Method: PBMC was isolated from boosted grass pollen allergic donors. CD1c$^+$ and CD123$^+$ DCs, as well as monocyte-derived DCs (moDC) and basophils, were co-cultured with autologous CD4$^+$ effector memory T cells and pulsed with grass pollen (Phleum pratenase, Phl p). After 7 days, intracellular IL-4 and T cell proliferation were measured with flow cytometry and [^{3}H]-thymidine incorporation, respectively.
Results: Phl p stimulation of CD1c$^+$ DCs induced IL-4 producing T cells at similar levels as moDCs. Interestingly, the frequency of T cells producing IL-4 was higher upon co-culture with Phl p-pulsed CD1c$^+$ DCs as compared to pDCs as well as basophils, and Phl p-pulsed CD1c$^+$ DCs also stimulated a higher T cell proliferation. Further on, basophils induced neither IL-4 production nor T cell activation after Phl p stimulation.
Conclusion: Together, these results suggest that CD1c$^+$ DCs are superior, as compared to other primary APCs, at activating Th2 cells in grass pollen allergic individuals.

P1.09.39
Absence of Estrogen Receptor Alpha Reduces the Number and Function of Bone Marrow derived PDCA1+ cells in Lupus Prone Mice

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Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that affects women at a 9:1 ratio compared to men. Using a Lupus-prone mouse model, we have shown that estrogen receptor alpha (ERα) deficient mice have increased survival and less renal disease compared to wild type mice. We hypothesize that ERα deficiency improves SLE by reducing the innate immune system's response to Toll Like Receptor (TLR) Ligands. To study the innate

immune response our research focuses on the dendritic cell (DC). PDCA1+ DCs make up less than 5% of bone marrow derived myeloid dendritic cells (mDC) from B6 mice. Using the same mDC culture conditions, PDCA1+ cells accounted for 60% of mDCs from lupus-prone NZM 2410 mice. However, PDCA1+ DCs from NZM 2410 mice lacking ERα accounted for only 35% of mDCs. PDCA1 is a marker for plasmacytoid dendritic cells (pDCs), a major producer of type I interferon (IFN), which is a critical cytokine in the pathogenesis of SLE. Functionally, ERα deficient mDCs expressed lower levels of IFN response genes in response to TLR7 and 9 ligands compared to wild type cells. To further characterize these cells, a Flt3L driven pDC culture system was used to obtain pDCs. Using this culture system, ERα deficient bone marrow from NZM 2410, MRL/lpr, SLE1,3, and B6 mice had significantly decreased numbers of PDCA1+ cells. This data indicates that ERα plays a role in altering the number and function of PDCA1+ cells cultured under both mDC and pDC culture conditions.

P1.09.40
Endogenous production of activin A acts as negative regulator of NK-DC functional interactions

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The interaction of natural killer (NK) cells with dendritic cells (DC) is known to influence both innate and adaptive immune responses. Although it is known that NK cells and DC reciprocal activation occurs through the release of both soluble factors and membrane protein interactions, the characterization of the different molecules involved deserves further attention. Here we report that in NK-DC cocultures, among a set of 84 cytokines investigated, activin A was the second highest induced gene, with CXCL8 being the most upregulated one. Activin A is a member of the TGF-beta superfamily and was previously shown to possess both pro- and antiinflammatory activities. In NK-DC cocultures, the induction of activin A required cell contact and was dependent on the presence of proinflammatory cytokines (i.e. IFN-γ, TNF-α and GM-CSF) as well as on NK cell-mediated DC killing. In NK-DC cocultures, inhibition of acitivn A by follistatin, a natural inhibitory protein, resulted in the upregulation of proinflammatory cytokine release (i.e. IL-6, IL-8, TNF-α) and in the increase of DC maturation. In conclusion, our study suggests that the production of activin A during NK-DC interaction represents a relevant negative feedback mechanism that might function to prevent excessive immune activation.

P1.09.41
CD8α+ DCs can be induced in the absence of transcription factors Id2, Nfil3 and Batf3

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Dendritic cells (DCs) are heterogeneous cells in which distinct DC subsets, with specialized functionalities, are established through the expression of lineage-specific transcription factors. CD103+ and CD8α+ dendritic cells (DCs) are important for cross-presentation of antigens and for the induction of effector CD8+ T cell responses against pathogens. Development of these DC subsets is thought to require the transcription factors Irf8, Id2, Nfil3 and Batf3 although how this network is regulated is poorly defined. We have addressed the nature of the differentiation blocks observed in the absence of these factors and found that while all four factors are required for CD103+ DC development, only Irf8 is essential for CD8α+ DC. CD8α+ DCs can emerged in the absence of Id2, Nfil3 and Batf3 in short-term bone marrow reconstitution. Strikingly, these 'induced' CD8α+ DCs exhibit several hallmarks of classical CD8α+ DCs, found in steady state, including the expression of CD24, Tlr3, Xcr1, Clec9A and the capacity to cross-present soluble, cell-associated antigens and viral antigens even in the absence of Batf3. Collectively, these results uncover a previously undescribed pathway by which CD8α+ equivalent DCs, found immediately following transplantation, can bypass cues provided by the Id2-Nfil3-Batf3 pathway, but Irf8 was essential determinants for their differentiation.

P1.09.42
β2 adrenergic receptor-mediated IL12p70 suppression in LPS-activated dendritic cells is IL-10- and PKA-independent

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The β_2 adrenergic receptor (β_2AR) agonist inhibits IL12p70 and enhances IL10 in LPS-activated dendritic cells (LPS-DCs). We proposed to investigate the signaling pathways involved in the β2AR-mediated modulation of IL12p70 production by LPS-DCs. We used BMDCs from WT or IL10-/- mice. Immature DCs (iDCs) were treated with fenoterol (β_2AR agonist) and then activated with LPS for 18h. In some experiments, cells were pre-treated with H89 (PKA inhibitor) and then stimulated as mentioned above. The cytokine production was analyzed by ELISA. Following β_2AR signaling, we evaluated intracellular cAMP levels and PKA-induced CREB phosphorylation (pCREB) in iDCs. The pIBα was quantified by Western Blot and translocation of NFB (p65) was analyzed by immunofluorescence in LPS-DC treated with fenoterol. Fenoterol activated the canonical β_2AR signaling in iDC, leading to an increase in intracellular cAMP levels and to PKA-mediated pCREB. H89 did inhibit pCREB after β_2AR signaling in iDCs, but did not prevent β_2AR-mediated alterations in IL12p70 and IL10 production by LPS-DCs. Next, we asked if β_2AR-mediated increase in IL10 could be the reason by which IL12p70 production is inhibited in LPS-activated DCs. Just as observed in WT mice, β_2AR signaling inhibited LPS-induced IL-12p70 production in IL10-/- DCs. Finally, we verified if fenoterol impairs the activation of NFB(p65) in LPS-DC. We observed that β_2AR signaling induced a decrease of pIBα and nuclear translocation of NFB(p65) in LPS-DC. We concluded that in LPS-DCs, β2AR-mediated suppression of IL12p70 production is IL10-independent and also PKA-independent; however it is associated with inhibition of NFB translocation.

P1.09.43
Monocyte-derived dendritic cells pre-treated with TLR-3 and TLR-7 agonists express functional stability and Th17 polarization capacity in response to tumor conditioned media from colorectal cancer patients

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TLR ligands are widely used maturation agents for preparation of monocyte-derived dendritic cells (MoDCs). We assumed that MoDCs pre-treated with TLR agonists might gain resistance to the immunosuppressive effects of the tumor microenvironment. Therefore, MoDCs were pre-treated with TLR-3 (polyI:C) and TLR-7 (7-thia-8-oxoguanosine) agonists (TLR-pretreated MoDCs) for 4h, washed and stimulated overnight with tumor conditioned medium (TCM) from cultured colorectal cancer tumors.
Exposure of TLR-pretreated and corresponding control MoDCs to TCM resulted in the reduction of $CD86^+$ $HLA\text{-}DR^+$ cells. However, control MoDCs responded to TCM with up-regulation of these molecules, while the CD86 expression was unchanged in TLR-pretreated MoDCs. Consequently, TCM-treated control MoDCs, but not MoDCs treated with TLR agonists and TCM showed higher allostimulatory potential. In response to TCM, both cell groups showed up-regulation of IL-23, IL-6 and TNF-α. Control MoDCs and TLR-pretreated MoDCs secreted more IL-10 and IL-12p70, compared to TCM untreated cells, respectively. However, more than 50-fold increased production of IL-23 was detected in TLR-pretreated MoDCs

exposed to TCM and this finding correlated with several folds higher production of IL-17 in coculture with $CD4^+$T cells. Considering production of IL-10 and IL-6, the stimulatory effect of TCM was more potent in the group of control- than TLR-pretreated MoDCs. The opposite was observed for IL-12p70 and TNF-α.
These results suggest that pre-treatment with TLR-3 and TLR-7 agonists might be useful for preparation of MoDCs that could respond in the colorectal cancer microenvironment with a stabile function and potent Th17 polarizing capacity.

P1.09.44
Agonistic anti-4-1BB mAb promotes the maturation and activation of hypoxic dendritic cells

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Hypoxia resident in solid tumor and inflammatory tissues links closely with immune function. Maturation and activation of dendritic cells (DCs) are essential to drive immune response in hypoxic microenvironments. 4-1BB has been implicated involved in regulation of DC function, but its effects on hypoxic DCs remain unclear. In this study, we stimulated 4-1BB on murine hypoxic DCs using agonistic anti-4-1BB mAb and examined the phenotypic and functional changes of these DCs. We showed hypoxia had little influence on differentiation of DCs, but suppressed their maturation and activation via down-regulation of pro-inflammatory cytokines, MHC class II and co-stimulatory molecules. Compared with normoxic DCs, hypoxic DCs showed a slightly decreased expression of 4-1BB. Treatment of hypoxic DCs with anti-4-1BB mAb dramatically up-regulated the expression of pro-inflammatory cytokines including IL-6, IL-12 and TNF-α; the expression of matrix metalloproteinase 9 related to migration also increased in these DCs. Although anti-4-1BB mAb moderately up-regulated co-stimulatory molecules CD80, CD86 and MHC class II molecules on hypoxic DCs (remain lower compared to normoxic DCs), these DCs still showed limited ability to stimulate allogeneic $CD4^+$T cell proliferation compared with normoxic DCs. All together, we demonstrate that anti-4-1BB mAb partially restores the maturation and activation of hypoxic DCs by promoting their phenotypic maturation, inflammatory cytokines production and migration ability, thereby enhancing the immune function of DCs in hypoxic microenvironments.
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P1.09.45
The equivalents of human blood and spleen DC subtypes can be generated in vitro from human CD34+ stem cells in the presence of Flt3L and TPO

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Dendritic cells (DCs) are immune cells specialized to capture, process and present antigen to T cells in order to initiate an appropriate adaptive immune response. The study of mouse DC has revealed a heterogeneous population of cells that differ in their development, surface phenotype and function. These differences are being exploited in the targeting of immunotherapies to particular DC subsets with promising outcomes. Less is known about the DC heretorgeneity in the human system, due to their rarity in human blood, and the difficulty in accessing human lymphoid tissues. The study of human blood and spleen has shown the presence of two subsets of conventional DC including the CD1b/c+ and CD141+CLEC9A+ conventional DC (cDC) and a plasmacytoid DC (pDC) that is CD304+CD123+. Studies on these subpopulations have revealed phenotypic and functional differences that are similar to those described in the mouse. In this study, the three DC subsets have been generated in vitro from human CD34+ precursors in the presence of fms-like tyrosine kinase 3 ligand (Flt3L) and thrombopoietin (TPO). The DC subsets so generated, including the CD1b/c+ and CLEC9A+ cDCs and CD123+ pDCs, were largely similar to their blood and spleen counterparts with respect to surface phenotype, Toll-like receptor and transcription factor expression, capacity to stimulate T cells, cytokine secretion and cross presentation of antigens. This system may be utilized to study aspects of DC development and function, not possible in vivo.

P1.09.46
Stat6 in dendritic cells negatively regulate IL-10 production and enhance arthritis

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Stat6 is a transcription factor activated by JAK3 and is expressed on dendritic cells (DCs) in the synovium of rheumatoid arthritis patients in relation with disease activity. Previously, we reported that JAK3 negatively regulate IL-10 production in DCs. Stat6-/- DCs was evaluated the role of JAK3-Stat6 in IL-10 regulation in DCs and its involvement in inflammation. Development of Stat6-/- splenic DCs was comparable to that of wild-type (WT) mice. In fact, IL-10 production was increased in response to toll-like receptor ligands, while TNF-α and IL-6 were at comparable level with those of WT DCs, which was an identical phenotype with JAK3-/- DCs. Accordingly, LPS injection to Stat6-/- mouse resulted in increased serum IL-10 with TNF-α and IL-6 at comparable level with WT and immunostaining of the spleen revealed increased IL-10 positive cells. Genetic background did not alter the phenotype, firmly establishing that the overproduction of IL-10 by Stat6-/- DCs was a consistent phenomenon. To investigate the pathological role of Stat6 in DCs with arthritis, collagen antibody-induced arthritis (CAIA) was performed. Arthritis score was significantly suppressed and serum IL-10 concentration was increased in Stat6-/- mice and histopathological analysis revealed significantly suppressed joint destruction in Stat6-/- mice. Our results with Stat6-/- DCs confirmed that JAK3-Stat6 pathway negatively regulate IL-10 production in DCs and enhances inflammation. Therefore, the anti-inflammatory effect of the JAK inhibitor showing prominent effect on rheumatoid arthritis has the possibility of inducing IL-10 production by DCs.

P1.09.47
PU.1 regulates CCR7 expression in dendritic cells

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Dendritic cells (DCs) are professional antigen presenting cells that reside in peripheral tissue and survey the body for pathogens. Upon activation by contacting to antigens, DCs undergo a maturation process and migrate to lymphoid organs, where they prime naive T cells. CCR7 plays a key role in migration of DCs.
Several studies including ours have demonstrated that PU.1, which belongs to Ets family transcription factor, is indispensable for DC developments and functions. In this study, we investigated whether PU.1 regulates the expression of CCR7 in DCs.
PU.1 knockdown by small interfering RNA (siRNA) in bone marrow-derived DCs (BMDCs) decreased the expression of CCR7. PU.1 silencing also attenuated the CCR7 ligand-dependent migration. Reporter assays using luciferase constructs containing the CCR7 promoter revealed that multiple Ets motifs localized around transcription start site is involved in transcriptional activation. Furthermore, a chromatin immunoprecipitation assay using an anti-PU.1 antibody exhibited that PU.1 bound to the proximal region of the CCR7 promoter in BMDCs. Our results demonstrate that PU.1 transactivates the expression of CCR7 gene by interacting with its promoter. Importantly, in human monocyte derived DCs, PU.1 bound to the CCR7 promoter, suggesting that these mechanisms are conserved in both human and mouse.
Taken together, PU.1 plays a pivotal role in the DC migration by positively regulating the CCR7 expression.

P1.09.48
Specialized role of skin dendritic cell subsets in induction of antigen-specific cellular and humoral immunity following intradermal vaccination

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The efficacious delivery of antigens to antigen-presenting cells, in particular to dendritic cells (DCs), and their subsequent activation remains a significant challenge in the development of effective vaccines. In this study, we used polymeric dissolvable microneedle arrays (MNs) laden with antigen encapsulated nanoparticles to target murine skin DCs network and to investigate the capacity of different skin DC subsets to present antigen to antigen specific $CD4^+$ and $CD8^+$ T cells. We demonstrated that all dermal DC subsets could cross-present nano-encapsulated antigen but not as efficiently as Langerhans cells (LCs), that constituted the major subset capable of cross-presentation. We confirmed a critical role for LCs in the immunization process when depletion of LCs dramatically reduced the proliferation of antigen specific $CD8^+$ T cells *in vivo*. LCs were also necessary for IFN-γ production among antigen specific $CD4^+$ T cells, while Langerin- dermal DCs were required for the generation of antigen specific Th2 cells. We also investigated the role of LCs, Langerin+ and Langerin- dermal DCs for IgG1 and IgG2a antibody induction, confirming differential polarization of T helper cells by different skin DC subsets. Furthermore, we explored the contribution of skin DC subsets for the generation of antigen specific anti-tumor and anti-viral immune responses following MN immunization *in vivo* and found that depletion of LCs significantly reduced protective effects in both disease models. Therefore, we show that selective targeting of antigen to specific skin DC subsets, in particular to LCs through dissolvable MNs might provide a promising technology for improved vaccination efficacy.

P1.09.49
Unique features of sublingual mucosal dendritic cells after antigen application

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The sublingual mucosa (Slm) is utilized as the site for sublingual immunotherapy (SLIT) that induces tolerance against allergens. However, the involvement of oral mucosal DCs (OMDCs) in tolerance mechanisms has not been elucidated. Here we compared the distribution and dynamics of OMDCs between Slm and buccal mucosa (Bm) after topical antigen painting.
Based on the histological examination of CD207 and MHC class II expression, we evaluated three types of DCs; a) CD207+(MHCII+) epithelial LCs, b) MHCII+ submucosal (sm) DCs c) CD207+ smDCs after FITC painting. The distribution of resident LCs and smDCs was clearly less in the Slm, however, the number of MHCII+ smDCs was markedly increased at 6 h after FITC painting, suggesting newly recruitment of monocyte-derived DCs. At 24h, most DCs in the Slm were disappeared, whereas substantial numbers of DCs still existed in the Bm. OVA painting onto Slm showed similar but less and slower responses. The repeated antigen painting onto Slm induced a rapid exhaustion of resident OMDCs and recruitment of round-shaped CD11b+ DCs/macrophages. In a cedar pollen-induced pollinosis model, SLIT also induced exhaustion of resident DCs and appearance of CD11b+ DC/Mϕ. Our results suggest that these unique dynamics of sublingual OMDCs may contribute to tolerance induction of sublingual immunotherapy.

P1.10 Innate sensors

P1.10.01
TLR4 and DC-SIGN receptors recognized Mycobacterium scrofulaceum promoting semi-activated phenotype on Bone Marrow Dendritic Cells

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The incidence of Non-Tuberculous Mycobacteria (NTMs) infections in both immunocompetent or immunocompromised individuals has been increasing. NTMs infections have been reported to interfere with acquired protection induced by BCG vaccination while Dendritic Cells (DCs) plays an important role in immune responses to Mycobacterium complex pathogens but some of them partially stimulate the maturity of the DCs. There have been several reports about the activation in mice Bone Marrow Dendritic Cells (BMDCs) by *Mycobacterium avium*, but there are not previous reports about the stimulation of another NTMs by similar path until now. We show that *M. avium* and *M. scrofulaceum* have both comparable ability in the induction of a semi-mature phenotype of DCs, which was assessed by confocal microscopy, in where the cytoskeletal architecture of DCs presented spikes or pseudopodia very limited and smaller accompanied by a decrease in cell migration in both cases. We also found that TLR-4 and DC-SIGN have an important role in the phenotypic activation of BMDCs by *M. scrofulaceum*, in where PD-L2 expression was induced 5.3-fold when compared with controls. Regarding to cytokines, IL-10 presented a two-fold increase while IFN-γ, TNF-α and IL-12p40 showed a significant decrease, as well as CD40, CD80 and CD86 had low expression when compared to BCG stimulated DCs. This result reveals that *M. scrofulaceum* stimulated semi-mature BMDC and that response differs from *M. avium* or BCG.

P1.10.02
NOD2 signaling promotes the Treg/Th17 imbalance and contributes to type 1 diabetes pathogenesis

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Type 1 diabetes (T1D) is an autoimmune disease that precipitates when immunological tolerance to self tissues fails, resulting in the autoimmune destruction of pancreatic beta cells in genetically predisposed individuals. Despite extensive studies about the progression and effector mechanisms in the pathogenesis of T1D, little is known about the initial steps of the disease. In this regard, NOD-like receptors (NLRs), which are intracellular receptors responsible for the recognition of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs), appear as an interesting target in the context of this initial interaction between beta-cells and the innate immune response. Therefore, we investigated the role of NOD2 in the pathogenesis of T1D. For that, NOD2 deficient mice and their wild-type (C57BL/6) counterparts were inoculated intraperitoneally with streptozotocin (STZ/40mg/Kg) for 5 consecutive days and both clinical and immunological parameters were assessed. Our results demonstrate that NOD2 deficient mice developed a less severe hyperglycemia. Also, NOD2 deficiency caused a decrease of Th17 and TC17 cells without interfering on the Treg cell population in the PLNs. In parallel, we observed an increase in the immunomodulatory cytokines IL-4 and IL-10 in the pancreas of these mice. Together, these results suggest that the NOD2 receptor induces a Th17 proinflammatory phenotype and thus, possibly contributes to the pancreatic islet damage during T1D onset.

P1.10.03
Role of TREM1-DAP12 in renal inflammation during obstructive nephropathy

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Tubulo-interstitial injury is a common finding in the chronically diseased kidney and is characterized by ongoing inflammation and fibrosis leading to renal dysfunction and end-stage renal disease. Inflammatory response can be triggered by TREM-1 (triggering receptor expressed on myeloid cell) activation and requires association with transmembrane adapter molecule DAP12 (DNAX-associated protein 12) for cell signaling. TREM1-DAP12 pathway has a cross-talk with intracellular signaling pathways of several Toll-like receptors (TLRs) and is able to amplify TLR signaling. So far, several studies have shown that TLRs play a role in obstructive nephropathy but the contribution of TREM1-DAP12 herein is unknown. We investigated the contribution of TREM1-DAP12 in obstructive nephropathy by subjecting wild-type (WT), TREM1/3 double KO and DAP12 KO mice to murine unilateral ureter obstruction (UUO) model. We showed that in kidneys from WT mice, DAP12 and especially TREM1 mRNA levels were highly elevated upon UUO. Compared to WT mice, DAP12 KO mice displayed less renal MCP-1, KC and TGF-β levels and less influx of macrophages during progression of UUO, whereas TREM1/3 double KO mice displayed less renal MCP-1 level. Renal fibrosis developed similar in WT, TREM1/3 double KO and DAP12 KO mice. We conclude that DAP12, partly through TREM1/3, is involved in renal inflammation during progression of UUO.

P1.10.04

A shortcut mechanism of innate immune defense by a solitary PRR

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The accepted paradigm about an innate immune response is a multi-stepped process - from recognition of the invading microbe by pathogen-recognition receptors (PRRs) to signal transduction, to the production of antimicrobial effectors by the immune cells. Contrary to this belief, we found that a PRR can act singly via a direct shortcut process, bypassing multiple cascades of reactions. We showed that the extracellular hemoglobin (Hb) acts as a solitary frontline defense PRR, directly recognizing pathogens and eliciting powerful antimicrobial potencies. This innate immune response phenomenon is evolutionarily entrenched for 500 million years, from the limulus to humans. While the invading microbe cleaves the cell-free hemoglobin to extract an iron-rich meal for its survival and further invasion, the host exploits the intruding microbe's proteases and PAMPs to produce toxic reactive oxygen species (ROS) that effectively kills the pathogen. In this process, the hemoglobin structure-function is rapidly reprogrammed to expose multiple dual antimicrobial activities and its redox reactivity is subsequently suppressed by plasma antioxidants, thus protecting the host from ROS-induced cytotoxicity. Furthermore, we demonstrated that the monocytes efficiently import the redox active Hb via a (a) novel endocrine loop of CD163 receptor recycling and (b) paracrine communication with vascular endothelial cells.

P1.10.05

Orchestration of tissue repair by the humoral pattern recognition molecule PTX3: linking microbe and matrix recognition

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Innate immunity includes a cellular and a humoral arm. Long pentraxin 3 (PTX3) is a fluid phase pattern recognition molecule and a key component of the humoral arm of innate immunity. PTX3 is normally produced in response to microbial recognition or inflammatory signals by different immune or stromal cells. In the early phase of skin wound healing, Pentraxin 3 (PTX3)-deficiency was associated with increased fibrin deposition and persistence, and thicker clots. At later phases, increased collagen deposition was observed in *Ptx3*-deficient mice. A similar phenotype was observed after liver injury. *Ptx3*-deficient macrophages and fibroblasts showed defective pericellular fibrinolysis *in vitro*. PTX3 bound fibrinogen/fibrin and plasminogen at acidic pH. The second exon-encoded N-terminal domain of PTX3 recapitulated the interactions observed with the whole molecule. Thus, a prototypic component of humoral innate immunity, PTX3, plays a non-redundant role in the orchestration of tissue repair and remodeling. We suggest that matrix and microbial recognition are commune, ancestral features of the humoral arm of innate immunity.

P1.10.06

Dectin-1 exerts opposing roles in the activation of macrophages from resistant and susceptible mice to Paracoccidioides brasiliensis infection

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Dectin-1 is a non-classical, C-type lectin receptor which binds predominantly to β-glucans, carbohydrates on the outer layer of cell wall of several fungi, including *Paracoccidioides brasiliensis* (Pb). The mechanisms of innate immunity that govern the interaction of this fungal pathogen with dectin-1 receptors are still unknown. This study aimed to investigate the role of dectin-1 in the interaction between Pb yeast cells and macrophages from susceptible and resistant mice. The phagocytic and fungicidal activities as well as NO and cytokines production were determined in laminarin-treated and untreated B10. A and A/J macrophages infected or not with Pb yeasts. The mRNA expression of NOS2, ARG-1 and SOCS3 genes was assessed by RT-PCR. The presence of laminarin resulted in increased phagocytosis, fungicidal activity, and NO production by B10.A macrophages. In contrast, all these activities were decreased in laminarin-treated A/J macrophages. In addition, A/J macrophages showed a prominent production of TGF-β, IL-10, IL-6 and TNF-α, whereas B10.A cells presented increased levels of IL-12, and IL-10. Furthermore, laminarin-treated macrophages of B10.A mice up regulated NOS2 and SOCS3 mRNA expression whereas ARG1 mRNA was up regulated in A/J macrophages. Thus, Dectin-1 activation appears to induce a "M1-like" differentiation of B10.A macrophages but a "M2-like" behavior of A/J cells. This is in agreement with our previous results demonstrating that a prevalent pro-inflammatory innate immunity results in suppressed T cell immunity and susceptibility, whereas an initial tolerogenic innate response followed by a tightly regulated adaptive immunity leads to resistance to *P. brasiliensis* infection .

P1.10.07

Induction of antimicrobial peptides in primary cultures from diabetic foot ulcers with 1,25-dihydroxyvitamin D3 and L-isoleucine

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Background: It is estimated that more than 356 million people worldwide have diabetes mellitus type 2 (DM2) and between 15 to 25% of these patients will develop diabetic foot ulceration (DFU) during their lifetime. A diminished immune response is a major factor in the development of severe infection in DM2 patients, which allows pathogens colonize those wounds that frequently became resistant to antibiotics. Therefore, new approaches for drug development or adjuvant to improve the current therapy are urgently needed. Antimicrobial peptides (AMPs) such as defensin and cathelicidin are versatile molecules of easy induction that have a strongly antimicrobial activity.
Objective: The aim of this study was to determine the efficacy of 1,25 (OH)2 D3 and L-isoleucine to induce Human β-defensin (HBD-2) and cathelicidin (LL-37) in primary skin cell culture from DFU.
Methods: 15 biopsies from DFU grade 2 according to Wagner´s classification and 15 biopsies from healthy donors were cultured and stimulated with 1,25 (OH)2 D3 and L-isoleucine. Flow cytometry, real

time PCR and ELISA were performed to measure HBD-2 and LL-37 expression.
Results: Primary cell cultures from DFU stimulated in vitro with 1,25 (OH)2 D3 overexpressed DEFB4 and CAMP gene expression and preliminary results showed a tendency to increases of HBD-2 and LL-37 in the culture supernatants.
Conclusion: 1,25 (OH)2 D3 is an inductor of HBD-2 and LL-37 in keratinocyte's cultures, which suggests their potential therapeutic use on the treatment of diabetic foot ulcers.

P1.10.08
Deficient antiviral responses to influenza in primary bronchial epithelial cells of chronic obstructive pulmonary disease

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AIM: People with chronic obstructive pulmonary disease (COPD) are more susceptible to influenza infection leading to acute exacerbations with excess mortality. The mechanisms underlying this observation in COPD however are unknown. Our aim was to assess the antiviral responses in primary bronchial epithelial cells (pBECs) from COPD and healthy control subjects to influenza infection.
METHODS: Primary BECs from healthy non-smoking volunteers and subjects with COPD were infected with human influenza A/H3N2, A/H1N1, and a low pathogenic avian influenza A/H11N9 at MOI of 5. Influenza haemagglutinin (HA), RIG-I, interferon (IFN) -β, and IFN-λ1 protein was measured by western blotting. RIG-I-initiated signalling complex antiviral stress granule and IFN-β enhanceosome was measured by confocal microscopy. Viral replication was measured by plaque assay.
RESULTS: Influenza viruses replicated more efficiently in COPD pBECs. The viruses entered COPD pBECs more efficiently than healthy control cells, and this was dependent on PI3 kinase, which showed increased expression in COPD. COPD pBECs also showed impaired IFN responses to infection. RIG-I, the primary viral RNA sensor and initiator of IFN responses, was minimally expressed in COPD cells, leading to impaired formation of IFN signalling complex antiviral stress granule and IFN-β enhanceosome. This resulted in reduced IFN protein induction and correlated with enhanced viral replication in the COPD pBECs compared to healthy control cells.
CONCLUSION: COPD pBECs have increased susceptibility to influenza viral entry, and have inherent deficiencies in innate antiviral responses, which in combination lead to more severe infection.

P1.10.09
Zinc-Finger Antiviral Protein Mediates RIG-I-Like Receptor-Independent Antiviral Response to Murine Leukemia Virus

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Innate immunity is induced after the recognition of viral RNAs by pattern-recognition receptors (PRRs) and is the first line of the host defenses against a variety of RNA viruses. Although Toll-like receptor 7 (TLR7) recognizes the genomic RNA of extracellular murine leukemia virus (MLV) to mediate the antiviral response, the RNA-sensing PRR that recognizes the MLV in the cytosol is not fully understood. Here, we show that zinc-finger antiviral protein (ZAP) is a cytosolic viral RNA sensor, inducing the degradation of the MLV transcripts by the exosome, an RNA degradation system, on RNA granules. Although loss of the RIG-I-like receptors (RLRs), which recognize various RNA viruses in the cytosol and induce the type I interferon (IFN)-dependent antiviral response, does not alter the replication efficiency of MLV, loss of ZAP greatly enhances the replication efficiency of MLV. ZAP localizes to RNA granules, where the processing-body and stress-granule proteins assemble. The CCCH-type zinc-finger domains of ZAP, which are RNA-binding motifs, mediate its localization to the RNA granules. ZAP recruits the MLV transcripts and an exosome component to the RNA granules leading to degradation of MLV transcripts. Although ZAP was known as a regulator of RLR signaling in human cells, ZAP does not regulate the RLR-dependent production of type I IFN in mouse cells. Thus, ZAP is a novel member of the cytosolic viral RNA-sensing PRR family that induces elimination of RNA viruses independently of TLR and RLR family members.

P1.10.10
Potential role of caveolin-1 in regulation of immune cell activation

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Caveolae are small plasmatic membrane invaginations, located mostly on surface of endothelial cells and adipocytes. They form a special group of lipid rafts compound of cholesterol, sphingolipids, and caveolin molecules simultaneously. Their unique function includes signal transduction, therefore caveolae plays a vital role in the regulation of almost all cellular processes. Caveolae are rich in proteins called caveolins, which serves as their basic structural units. Currently, three genes are known encoding three isoforms of caveolin: caveolin-1, -2, and -3. While caveolin-1 and -2 are abundant especially in adipocytes, endothelial cells, fibroblasts and smooth muscle cells, caveolin-3 is expressed mainly in striated muscle cells. Although their function is studied for many years, their relationship to the development of both local and systemic inflammation and related diseases had not yet been elucidated. Therefore, this study focuses on examining regulation of caveolin expression in various cell types (particullary in the in vivo and ex vivo systems). Importantly, our data showed that the expression of caveolins significantly differ in individual cell types in isolated peritoneal inflammation induced in mice in vivo. While the endothelial cell expression of caveolin-1 increases, in macrophages isolated from the peritoneum was observed reversed trend. In murine aorta we also found out that caveolin-1 is preferentially expressed in vascular lumen endothelial cells. For other types of immune cells was observed different expression of caveolins, while all three isoforms was confirmed only in mice peritoneal macrophages.

P1.10.11
Unbiased screen to identify novel host factors involved in the innate immune recognition of Streptococcus pneumoniae

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The Gram-positive bacterium Streptococcus pneumoniae is the leading agent to cause pneumonia, which is the most common cause of death by an infectious disease worldwide. Pneumococci colonize the upper respiratory tract and lead to severe diseases like pneumonia, meningitis and sepsis. Several structures of invasive clinical strains of S. pneumoniae are known to trigger innate immune responses, e.g. pneumococcal lipoteichoic acid, which is sensed by TLR2, pneumolysin, recognized by TLR4, and others that are less well known. In the current study, we aim to identify new mechanisms of innate immune recognition of S. pneumoniae to better understand how this pathogen is eliciting an immune response in innate immune cells.
We use a genetic screening method that is based on a haploid human cell line isolated from a chronic myeloid leukemia patient. Due to its haploidy a pool of full gene knock-outs can be generated by random mutagenesis. We could show that these cells harbor phenotypic characteristics of myelo-monocytic cells, and respond to S.pneumoniae as well as TLR stimulation with the secretion of the inflammatory cytokine IL-8. Currently we are establishing a reporter cell line for IL-8 secretion following S.pneumoniae stimulation that could be used as readout for the genetic screen. By complementing

this screen with purified bacterial molecules and mutagenized bacterial strains, we expect that this approach will allow us to comprehend the complex interplay between bacterial molecules and host cells in an unbiased manner.

P1.10.12
C-type lectin receptor Mincle recognizes amphiphilic acylglucoside derived from *Helicobacter pylori*

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Mincle (Macrophage-inducible C-type lectin) is a C-type lectin receptor induced on macrophages by several stresses. We and other groups have shown that Mincle broadly recognizes a wide variety of ligands such as damaged cells, fungus, yeast and mycobacteria. In this research, we explored new pathogenic ligands and examined the function of Mincle in the defense mechanism against the pathogen.
We found that Mincle recognizes amphiphilic acylglucoside (AAG) derived from *Helicobacter pylori*. AAG activated macrophages to produce inflammatory cytokines such as TNF and MIP-2 (CXCL2), whereas these productions were impaired in macrophages derived from Mincle-deficient mice. Thus, Mincle is a receptor essential for recognition of AAG.
It is reported that AAG activates NKT cells in a CD1d-restricted fashion. We found that the production of IFNγ induced by AAG is suppressed in splenocytes from Mincle-deficient mice. This result suggests that signaling through Mincle leads to augmentation of the activation of NKT cells.
Given that IFNγ is known to prime macrophages, these results suggest that TCR on NKT cells and Mincle on myeloid cells may cooperatively contribute to augment immune responses in response to *H. pylori* through a positive feed-back loop.

P1.10.13
Natural IgG is not non-reactive: it plays a vital role in innate immune response

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The function of natural antibodies, which pre-exist in neonates and uninfected individuals, has remained a biological enigma since decades of their discovery. However, our findings reveal that natural IgG, which constitutes a majority of the serum natural antibodies, plays a major protective role in innate immunity. We describe through a variety of in vitro and ex vivo techniques, a novel shortcut mechanism wherein natural IgG collaborates with specific interaction partners to recognize a diverse array of pathogens, and elicits vital frontline innate immune defense through receptor-mediated phagocytosis. This process is non-specific to antigens. To support our preliminary findings, in vivo studies using knockout mice which lack natural IgG, showed that these mice were susceptible to infection unless reconstituted with natural IgG. Overall, our findings shed light on the fundamental mechanistic role of natural IgG during infection that alters the general perception of the existence and biological function of natural antibodies.

P1.10.14
Antibodies against dengue NS1 and prM proteins increase the sensitivity of dengue diagnosis and differentiate Japanese encephalitis infection

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Co-circulation of flaviviruses and sequential infections are common in developing and under-developing countries.Though cross-reactivity due to shared antigenic determinants among flavivirus subgroups have been observed commonly , cross-reactivity among different members of subgroups is rare.During the laboratory diagnosis of dengue infection using MAC-ELISA, it is necessary to rule out possible cross reactions of co-circulating flaviviruses such as Japanese encephalitis virus. In the present investigation, the usefulness of dengue virus NS1 and prM antibodies in diagnosing and differentiating dengue virus infection from Japanese encephalitis was assessed. The limitation for using NS1 specific IgM detection for dengue diagnosis increased beyond the acute phase of illness and detection of antibodies against dengue NS1 and prM proteins able to differentiate dengue from Japanese encephalitis infection in ELISA. The cross reactivity of IgM response in dengue diagnosis needs to be taken in to account especially in tropical and sub-tropical areas where there two or more flaviviruses co-circulate in one area and sequential infections are common.

P1.10.15
Cloning and characterization of cytosolic DNA in tumors

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We have evidence that cytosolic DNA accumulates in response to DNA damage and is also present in tumor cells. To characterize the cytosolic DNA, we developed different strategies to clone single-stranded and double-stranded cytosolic DNA. Detailed analysis of the cloned DNA showed that the cytosolic DNA is derived from genomic DNA. Around 40% of the cloned DNA fragments corresponded to intronic sequences. Endogenous retroelements were found in over half the cloned sequences. To confirm the cloned DNA is present in the cytosol, we established a fluorescent in situ hybridization (FISH) method. FISH analysis confirmed the cytosolic localization of the cloned DNA. Cytosolic DNA is recognized by different DNA sensors which induce type I interferon secretion and regulate NKG2D ligands expression. We provide evidence that cytosolic DNA is interacting with different components of the DNA sensor pathway. In summary, our data suggest that genomic DNA accumulation in the cytosol of tumor cells and may contribute to immune responses by activating DNA sensors.

P1.10.16
Expression and role of the long pentraxin PTX3 in lymphatic endothelial cells

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Long pentraxin PTX3 is a soluble pattern recognition molecule which plays complex non redundant roles in vivo, ranging from the organization of a hyaluronic acid-rich extracellular matrix to female fertility and innate immune response to certain pathogens. PTX3 is produced in response to microbial recognition or inflammatory signals by different immune or stromal cells, however gene profiling efforts unexpectedly revealed that PTX3 is constitutively expressed by lymphatic endothelial cells (LECs) both in man and in the mouse. Immunohistochemistry and confocal analysis confirmed the presence of PTX3 in human and murine normal tissues around lymphatic but not blood vessels. A morphometric analysis of lymphatic vessels was performed by whole mounting in wild type and *ptx3-/-* mice. Results indicated that length, volume and cell area of lymphatic vessels in colon submucosa were increased in *ptx3-/-* mice compared to wild type. *Ptx3-/-* mice showed a reduced accumulation of Evans Blue dye in the draining popliteal lymph node at early times (15-30 min.) after injection into the foot pad, while no differences were observed between wild type and *ptx3-/-* mice one hour after injection. Preliminary data in *ptx3-/-* mice also indicated a reduced ability of dendritic cells to travel to draining lymph nodes under inflammatory conditions. Taken together these data indicate that PTX3 plays a non-redundant role in shaping and functionality of lymphatic vessels.

P1.10.17
Inhibition of interferon α expression with small interference RNA increases adenoviruses-GFP transduction and transgene expression in Huh7 cells

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Introduction: Recombinant adenoviruses (rAd) are the mostcommonly vectors used in clinical trials of gene therapy. Systemic administration of rAd presents high tropism for liver. However, host´s immune response against rAd orchestrated by Interferons type 1 (α and β) limits the therapeutic gene expression and prevents new administrations. Aim: To evaluate the effect of IFNα inhibition by a small interfering RNA (siRNA) on rAd-GFP transduction and transgene expression in Huh7 cell line. Methods: Huh7 cells are cultured in DMEM, 5% FBS at 37 °C and 5%CO2 and then transfected with 70 nM of siRNA-IFNα. 6h later culture was exposed to 1.0 x 10^9 vp/ml of rAd-GFP for 24 hrs. Expression of IFNα1 and TNFα were determined by qRT-PCR. Cell transduction was analyzed by fluorescent analysis cell sorting (FACS) and qPCR. GFP expression was determined by western blot and qRT-PCR. Results: 70 nM of siRNA-IFNα1 inhibited 96% of IFNα1 gene expression ($p < 0.001$) and 65% of TNFα($p<0.05$) respect to control siRNA-irrelevant. Transduction and transgen expression were increased in cells treated with siRNA-IFNα1 compared to control. Conclusions: Inhibition of IFNα by siRNA-IFNα1 permits a higher transgene expression (GFP) indicating the crucial role of IFNα on adenoviruses elimination in transducted cells. This strategy could be useful in clinical trials directed to liver diseases, where adenoviruses are used as vectors for therapeutic genes; achieving an increased expression of these genes leading to better results in liver diseases resolution.

P1.10.18
ApoH an innate immune sensor and hunter that captures and scavenges infectious pathogens

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Apolipoprotein H (ApoH), or β2-glycoprotein I (50-kDa) circulates in blood at high concentrations (200 µg/mL). Apo-H is mainly produced in mammal's liver, but is also expressed, in a lesser extent, in other organs, including prostate, kidneys, small intestine, brain, and heart. ApoH is a pleitropic multifunctional protein, notably playing diverse physiological roles. The most well known is its role in autoimmune diseases, hemostasis, atherogenesis and angiogenesis, as well as the host defense against bacteria. Despite this, many physiological functions remain unknown. ApoH is composed of five similar domains. The fifth domain is lysin-rich containing an aromatic residue. ApoH binds negatively charged compounds and, in some unexplained cases, this activity induces anti-phospholipid antibodies. Peptides from the domain V exhibit antimicrobial activities. In addition, we have shown that ApoH is not able to capture commensal microorganisms. In contrast, it catches, with high affinity (~10-100 nM), a large variety of pernicious pathogens including: Gram+ and Gram- bacteria (ex. *Mycobacterium*, MRSA); parasites; enveloped or non-enveloped viruses (ex. Endogenous retroviruses, Hantaviruses, Rotaviruses, Dengue, HBV). ApoH capture these pathogens *via* certain forms of their conserved antigens, including: HBV-HbsAg, HIV-1/Env, HERV-K/ENV HERV-W/Env and Gag, LPS, Sbi, SFAS, IUT-A, PAP-G3, CNF1 from *E. coli* and CCRB and MECA from *Staphylococcus aureus* (and MRSA). ApoH induces pathogens removal through macrophages. But, in certain cases, to escape this response pathogens could use ApoH to enhance infection. ApoH is an innate immune sensor to scavenge microbes and modulate immune and inflammatory responses induced by pernicious pathogens.

P1.10.19
DAI (DLM-1/ZBP1) promotes lupus nephritis by activating calcium pathway

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DNA-dependent activator of IFN-regulatory factors, also referred to as DAI/DLM-1/ZBP1, functions as a cytoplasmic DNA sensor and activates innate immunity. We previously found that activated lymphocyte-derived apoptotic DNA (ALD-DNA) immunization could mediate pathological macrophage M2b polarization and lead to lupus nephritis. However, the specific DNA sensor(s) and mechanisms involved in the ALD-DNA-induced M2b polarization in SLE remain still unknown. Here, we reported that DAI expression was significantly increased both in lupus mice and in SLE patients, and play an important role in ALD-DNA-induced M2b polarization. By further exploration, we found that ALD-DNA notably induced DAI dimerization/oligomerization, subsequently activated downstream NF-κB and IRF3 by calcium pathway and consequently caused macrophage M2b polarization. More importantly, blockade of DAI or selective knockdown of DAI in macrophages could efficiently ameliorate SLE manifestations in mice by blunting M2b polarization and inflammation. So in lupus nephritis, DAI could function not only as a DNA sensor, but also as a regulator of ALD-DNA-induced macrophage M2b polarization. This study provided the possible mechanisms underlying ALD-DNA-induced macrophage M2b polarization, and made DAI as a potential therapeutic target for SLE and other macrophage mediated autoimmune disease.

P1.10.20
Functional Analyses of Recombinant Mouse hepcidin-1 in Cell Culture and Animal Model

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Hepcidin as innate immune element plays an important role in iron metabolism. Human hepcidin-25 and mouse hepcidin-1 are major regulators of *iron metabolism in human and mouse respectively.* Using baculovirus expression system, we have produced the recombinant mouse hepcidin-1. The expression yield of it was obtained about 25 µg/ ml when cell culture media were supplemented with protease inhibitor cocktail. Functional assays indicated that recombinant mouse hepcidin-1 and synthetic human hepcidin-25 have similar effect on ferroportin expression as a target molecule in J774A cell line and peritoneal macrophages. However, recombinant mouse hepcidin-1 has lower effect than synthetic human hepcidin-25 in reducing iron level in blood circulation ($p < 0.01$)

P1.11 Toll-like and pattern recognition receptors

P1.11.001
***Neuinflam9/Toxo1* regulates the innate immune response after nerve injury and susceptibility to autoimmune neuroinflammation in rat**

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The activation of CNS resident cells is a common theme in different neurological disorders exhibiting innate immune responses through activating toll-like receptors signaling pathways. To identify the genes predisposing CNS disorders through innate responses regulation, we performed a genome-wide linkage scan for expression of innate immune molecules after a standardized nerve injury in a rat F2 (BNxLEW.1N) intercross. We identified multiple expression

quantitative trait loci (eQTLs) responsible for the regulation of several toll-like receptors (TLRs) and interferon regulatory factors (IRFs). Among these were the previously identified *Neuinflam9* QTL on rat chromosome 10, which overlaps with *Toxo1* regulating resistance to *Toxoplasma gondii*, here found to also regulate the expression of *Tlr2* and *Irf7*. The influence of *Neuinflam9,* on the expression of these innate immune molecules after nerve injury was verified in two different BN.LEWc10 congenic lines (lineages B and E). The congenic BN.LEWc10-E also demonstrated resistance to experimental autoimmune encephalomyelitis strongly suggesting that the same gene(s) that regulated expression of innate immune molecules also regulates predisposition to autoimmune disease in the CNS. Identification of the gene(s) underlying these QTLs may reveal common pathways regulating innate immune activation in the CNS and identify new therapeutic targets for neuroinflammatory disorders.

P1.11.002

The Toll-IL-1R member Tir8 modulates post-transplant kidney ischemia/reperfusion injury by inhibiting resident leukocyte expansion

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Ischemia/reperfusion injury (IRI), occurring after transplantation, activates TLRs/IL-1R on inflammatory leukocytes amplifying adaptive immune response. In a mouse model of renal artery clamp deficiency of Tir8, a IL-1R/TLR negative regulator, aggravated post-ischemic acute renal failure in association with intrarenal leukocyte accumulation.
We investigated the role of TIR8 in modulating post-transplant IRI and inflammatory response in mouse model of syngeneic kidney transplant. Donor and recipient C57BL/6 mice were congenic for CD45 leukocyte antigen to allow discriminating between intragraft recipient and donor leukocytes. Wild-type (wt-group n=5) or $Tir8^{-/-}$ ($Tir8^{-/-}$-group, n=5) donor kidneys were exposed to 30 min cold ischemia before transplant. Transient impairment of graft function was found at 1day post-transplant in wt-group (BUN: 1day 53±19, 30day: 27±3mg/dl). At variance the $Tir8^{-/-}$-group showed irreversible severe graft dysfunction (BUN: 1day 95±23, 30day: 67±23 mg/dl, P<0.05 vs wt-group).
Both at 10 and 30 days post-transplant lower numbers (P<0.05) of intragraft leukocytes were found in the wt-group (20±9/field, 61±18/field, respectively) vs the $Tir8^{-/-}$-group (104±17/field, 117±24/field, respectively). At 10 days the majority of leukocytes were of donor origin both in the wt and the $Tir8^{-/-}$ groups (79±8%, 78±2%, respectively). At 30days, in the wt-group the majority of intragraft leukocytes were of recipient origin (63±6%) while 63±2% of intragraft leukocytes were of donor origin in the $Tir8^{-/-}$-group.
These results indicate that TIR8 modulates post-transplant IRI possibly by suppressing expansion and activation of resident leukocytes within the kidney graft.

P1.11.003

Hematopoietic but not endothelial cell MyD88 contributes to host defense during gram-negative pneumonia derived sepsis

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Introduction: MyD88 is the common adapter of most Toll-like receptors (TLRs). MyD88 deficiency results in impaired host defense during infection. Knowledge of the contribution of different cell types to MyD88-mediated antibacterial defense is limited. The aim of our study was to determine the role of myeloid and endothelial cell MyD88 during murine *Klebsiella* pneumonia and sepsis.
Methods: We used the Cre-lox system to generate mice deficient for MyD88 in myeloid (LysM-MyD88-/-) or endothelial cells (Tek-MyD88-/-). Mice were intranasally inoculated with *Klebsiella pneumoniae* and euthanized after 24 hours for analyses or followed in a survival experiment.
Results: Both LysM-MyD88-/- and Tek-MyD88-/- mice showed a markedly accelerated lethality accompanied by a strongly enhanced bacterial outgrowth and dissemination after induction of *Klebsiella* pneumonia. In vitro studies with primary cells from Tek-MyD88-/- mice revealed that these mice were not only MyD88 deficient in endothelial but also in hematopoietic cells: TNF-a secretion in whole blood stimulated *ex vivo* with *Klebsiella* LPS or heat killed *Klebsiella* was even more strongly reduced than in LysM-MyD88-/- mice, while alveolar and peritoneal macrophages from both groups were impaired in a similar manner. Therefore, we transferred bone marrow from control mice in irradiated Tek-MyD88-/- mice to generate mice with a specific deletion of MyD88 in endothelial cells; these mice demonstrated an antibacterial defense that was restored to the level of control mice, indicating that endothelial cell MyD88 is not important for protective immunity.
Conclusion: Hematopoietic but not endothelial cell MyD88 contributes to host defense during gram-negative pneumonia derived sepsis.

P1.11.004

Activation and regulation of Toll-like receptors by infestation with Rhipicephalus sanguineus ticks

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1. Purpose/Objective: We have shown that tick saliva of Rhipicephalus sanguineus can supress the function of macrophages, lymphocytes and modulate differentiation, maturation, migration and function of DCs stimulated with ligands for TLRs. We observed that saliva of tick induced a high expression of TLR2 on DCs surface in vitro. Our goal is to check if the saliva is also able to modulate the expression of TLRs on DCs in vivo, and investigated if MyD88 disturb tick-infestation success on mice.
2. Material and Methods: We examined the expression of TLRs on DCs from the spleen and lymph nodes on days 3 and 7 of twice tick-infested mice. Additionally C57Bl/6 and MyD88(-/-) mice were infested with ticks and the biological parameters of tick were evaluated.
3. Results: Using flow cytometry, we observed that the expression the TLR1, 2, 5, 6 on DCs cells was significantly increased in both days analysed (p<0.05, t test) when compared with the non-infested group. Regarding the analysis of the tick-infestation we observed that there was an increase in the number of ticks that fed and the egg mass weight on MyD88-/- mice (p<0.05) compared with C57Bl/6 mice. Other biological parameters of ticks are still being analyzed.
4. Conclusions: These results suggest the importance of the MyD88 for the success of the tick infestation and that the augmented expression of some TLRs on DCs can modulate the host immune response. Consequently, this study can contribute to new perspectives for the control of the ticks
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P1.11.005

Combined Action of Nucleic Acid-Sensing Toll-Like Receptors and TLR11/TLR12 heterodimers imparts host resistance to Toxoplasma gondii

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One third of the human population in the world is chronically infected with *Toxoplasma gondii.* While the majority of infected individuals are asymptomatic, toxoplasmosis is a major cause of congenital disease, abortion, and a life threatening opportunistic disease in immunocompromised individuals. Early activation of the innate immune system and cytokine production (i.e. IL-12 and IFN-) by myeloid cells is required for establishment of protective immunity to *T.*

gondii infection. ‘‘Triple-defective’’ (3d) mice carrying a mutation in UNC93B1, a chaperone for the endosomal Nucleic Acid-Sensing (NAS) Toll-like receptors TLR3, 7 and 9, are highly susceptible to *T. gondii* infection. However, none of the single or even the triple NAS-TLR-deficient animals recapitulated the 3d susceptible phenotype. Investigating this further, we found that while parasite RNA and DNA activate innate immune responses via the NAS-TLRs TLR7 and TLR9, TLR11 and TLR12 working as heterodimers are required for sensing and responding to *Toxoplasma* profilin. Consequently, the triple TLR7/TLR9/TLR11-deficient mice are highly susceptible to *T. gondii* infection, recapitulating the phenotype of 3d mice. Humans lack functional TLR11 and TLR12 genes. Consistently, human cells produce high levels of proinflammatory cytokines in response to parasite derived RNA and DNA, but not to *Toxoplasma* profilin, supporting a more critical role for NAS TLRs in human toxoplasmosis.

Role of TIR8/SIGIRR in regulating TLR and IL-1R like-dependent platelet activation

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TIR8, or single Ig IL-1R-related molecule, SIGIRR, is a member of the IL-1R like (ILR) family, which acts as negative regulator of TLR and ILR signalling, playing non-redundant roles in tuning inflammatory responses in different pathological conditions.
Platelets express functional TLRs, which are involved in sepsis-induced thrombocytopenia, binding of bacteria, neutrophil extracellular trap production and platelet activation via P-selectin and active GPaIIbIII upregulation. In addition, IL-1RI has been shown in platelets.
We investigated the expression of TIR8/SIGIRR and other ILR regulated by TIR8 in platelets, and defined the potential involvement of TIR8/SIGIRR in TLR- and ILR-dependent platelet activation. We confirmed IL-1RI expression and showed for the first time TIR8/SIGIRR, IL-18R and IL-33R expression in human and murine platelets and megakaryocytes. Functional studies showed that stimulation with TLR and ILR ligands (LPS, IL-1β and IL-18) induced upregulation of P-selectin and active GPIIbIIIa and calcium mobilization. Platelet TIR8/SIGIRR expression was downregulated upon LPS stimulation or in patients with Systemic Inflammatory Response Syndrome compared to healthy donors. Finally, functional studies showed that Tir8-deficiency in mice was associated to platelet hyperactivity in basal conditions and upon stimulation with LPS, IL-18 and IL-1β, as demonstrated by increased P-selectin and active GPaIIbIII upregulation, calcium mobilization and neutrophil/platelet aggregation.
Thus, TIR8/SIGIRR is a regulator of platelet activation in inflammatory conditions induced by TLR and ILR family ligands. The modulation of TIR8/SIGIRR expression in platelets in severe inflammatory conditions suggests that it might represent a novel diagnostic or therapeutic target in prothrombotic conditions associated to inflammatory diseases.

P1.11.007
Interaction of Bordetella pertussis filamentous hemagglutinin with human TLR2: identification of the TLR2-binding domain

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Filamentous hemagglutinin (FHA) is a major adhesion and virulence factor of Bordetella pertussis and also a main component of acellular pertussis vaccines. Interaction of FHA with different receptors on human epithelial and immune cells facilitates entrance and colonization of bacteria as well as immunomodulation of the host immune response. Three overlapping segments of the FHA gene were cloned in a prokaryotic expression vector and the recombinant proteins were purified and applied along with the native FHA protein to assess their potential Toll-like receptor (TLR) stimulatory effects and to localize the TLR binding region. TLR stimulation was monitored by applying HEK293-Blue cell lines cotransfected with TLR2, 4 or 5 and a NF-κB reporter gene. Culture supernatants were checked for secretion of the reporter gene product and IL-8 as indicators of TLR stimulation. Native FHA was found to strongly stimulate TLR2, but not TLR4 or TLR5 transfected cells. Among the recombinant FHA fragments only the fragment spanning amino acid residues 1544-1917 was able to stimulate TLR2 transfected cells. Interaction of FHA with TLR2 suggests its involvement in induction of the innate immune system against Bordetella pertussis. The TLR2-binding domain of FHA may contribute to immunoprotection against pertussis infection.

P1.11.008
S100A8 and S100A9 promote hyporesponsiveness of phagocytes in polytrauma and burn patients

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Background: Phagocytes of polytrauma and burn patients develop a hypo-responsive stage leading to secondary infections and mortality. In Gram-negative sepsis this hypoinflammation is a well-known phenomenon which is triggered by a process called endotoxin-tolerance. Endotoxin-tolerance is induced by prior exposure of endotoxins to Toll-like receptor 4 (TLR4). Since an initial microbial trigger is often missing in polytrauma and burn patients we hypothesized that this inflammatory process can additionally be modulated by endogenous activators of TLR4, like the alarmins S100A8 and S100A9.
Materials and methods: Tolerance was induced by pretreatment of phagocytes with low doses of LPS or S100A8/S100A9 prior to activation. Inflammatory response was quantified by determining cytokine release and validating corresponding mRNA-levels via RT-PCR. In vivo experiments were performed by determining survival rates of mice in a septic shock model. NF-κB activation was determined by western blot analysis. ChIP-assays were used to manifest epigenetic modifications of the TNFα-promoter.
Results: We observed highly elevated S100A8/S100A9 serum concentrations in the initial disease phase in polytrauma and burn patients which predict lethal outcome in the latter. Pre-activation of TLR4 by S100A8/S100A9 induces tolerance, accompanied by diminished secretion of proinflammatory cytokines in vitro and enhanced survival rates of mice during septic shock. NF-κB transcription factor analysis and TNFα-promoter studies revealed an increase of nuclear RelB and of methyltransferase G9a-dependent histone methylation in S100-tolerized phagocytes. Conclusions: S100A8/S100A9 induces endotoxin-tolerance in phagocytes via activation of TLR4 signaling pathways and epigenetic modification of gene expression, relevant for development of hypoinflammation in burn and polytrauma patients.

P1.11.009
Extended anti-cancer immunity by liposomes coencapsulating nucleic acid TLR-ligands and antigen

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Nucleic acid based TLR ligands are initiating strong innate immune activation. However, their potential use in clinic due to in vivo degradation by nucleases or rapid clearance by serum protein

adsorption is hampered. Encapsulation of these labile ligands within liposome not only increases their stability and protects them from digestion but also enhances their immunostimulatory breadth. In this study, C57/BL6 mice were twice injected i.p. with free or encapsulated pI:C, CpGODN or their combinations together with ovalbumin. Two weeks after booster injection (@d=28) and every month thereafter sera were collected for eight months and OVA-specific Ig subtypes were studied by ELISA. Th1-biased immunity was calculated for each group (CpG+OVA or pIC+CpG+OVA gave the highest IgG2c/IgG1 ratio). Next, animals were inoculated with 4x106 EG7 thymoma cells expressing OVA. Tumor sizes were recorded and plotted as mm3. While all naive mice had palpable tumors by 4-5 days post inoculation, no tumor development was observed in animals vaccinated either with pI:C or D35 or their combinations with OVA in liposomes even after three weeks of post EG7 inoculation. Next, splenocytes were incubated with OVA-peptide and IFNg-producing T-cells were determined by ELISPOT. Tumor-resident, Foxp3+ cells were analyzed by FACS. Both CpG+OVA and pIC+CpG+OVA treated animals had the highest IFNg+ CD8 T-cells and the lowest number of Foxp3+ Treg cells, indicating a strong tumor-specific cytotoxicity was established by these vaccine formulations leading to complete tumor eradication 8 month post-vaccination. In conclusion, liposomes coencapsulating ssDNA or dsRNA plus antigen are promising anti-cancer vaccine candidates.

P1.11.010
R848, a Toll-like receptor 7/8 agonist, a potential therapy for allergic rhinitis patients

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Objectives: There is a growing interest in the targeting of Toll-like receptors (TLRs) for the treatment of allergic diseases. Several new compounds are now undergoing preclinical and clinical evaluation. The study focus on TLRs7/8 ligand; resiquimod (R848) as an adjuvant to immunotherapy (IT) in AR patient and assess its effect as an immune-modulator on the pro-inflammatory and anti-inflammatory cytokines.
Material and Methods: Peripheral blood mononuclear cells (PBMCs) were obtained from 20 non atopic donors, 10 non IT atopic donors and 10 IT atopic donors. PBMCs were cultured in the absence and presence of date palm pollen allergen (Phoenix dactylifera; Pho d) and/ or R848. Interleukin (IL4), IL-10, IL-13 and interferon gamma (IFN-γ) were measured in the culture supernatants.
Results: R848 was able to significantly increase the anti-inflammatory response in atopic donors more than non atopic donors. IL-10 production was significantly reduced in R848 + Pho d stimulated cultures when compared to Pho d stimulated ones. Moreover, IFN-γ production was significantly increased in R484+ Pho d stimulated cultures when compared to Pho d stimulated ones. Nevertheless, the combination of both; R848 and Pho d provides inferior stimulus when compared to R848 alone in both atopic and non atopic donors.
Conclusion: Our data show that in vitro treatment of PBMCs with R484 hijacks the pro-inflammatory immune process triggered by TLRs7/8 to mediate anti-inflammatory response. This provides important insight into the efficacy and mode of action of TLRs7/8 ligands in AR and paves the way for their clinical application in humans.

P1.11.011
TLR4-induced activation of human monocyte cell line U937 by sickle patient's mmLDL. Possible experimental model for atherosclerosis initial phase

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We studied the role of TLR4 in human monocytic cell U937 activation responding to in vivo minimally modified LDL (mmLDL) from patients with sickle cell disease (HbSS, n=48) compared with control (Hb AA, n=51). Free hemoglobin, hemin and iron overload, due to chronic hemolysis in these patients, promote an environment that catalyzes the production of free radicals that cause oxidative stress in the circulation. In these patients, LDL suffer oxidative modification while they circulate in the plasma. In vitro activation of U937 cells was performed with mmLDL isolated from patients. Membrane expression of CD54, Mac-1 and TLR4 on U937 cells was characterized by flow citometry. Concentration and mRNA of IL-8, MCP-1 and TNF-alpha were determined by ELISA and RT-PCR. Our results suggest that incubation for 48 h with mmLDL increased the expression of TLR4 on U937 cells surface as well as the expression of mRNA and secretion of MCP-1, IL-8 and TNF-alpha. Pre-incubation with anti-TLR4 antibody induced a significant inhibition of these cytokines concentration, suggesting that the expression of TLR4 on U937 cells surface has an important role in monocytes activation by mmLDL in patients with HbSS. We suggest that oxidative stress in hemolytic anemias can promote a minimal oxidation of LDL in circulation that would be recognized as an endogenous activating ligand for TLR4 on monocytes. This mmLDL could induce cytokines secretion which interaction with their receptors on endothelial cell surface might generate an inflammatory vessel response similar to that observed during the initial phase of an atherosclerotic process.

P1.11.012
Fungal stimulated inflammatory response of gestation associated tissues

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Inflammatory mediators produced by gestation-associated tissues (amnion, choriodecidua and placenta) in response to multiple pathogen-associated molecular patterns (PAMPs) are implicated in infection-associated preterm labour. Little is known about the inflammatory response of these tissues to fungal stimuli. C-type lectin receptors (CLRs), including dectin-1, are a family of pattern recognition receptors (PRRs) involved in host recognition of fungal pathogens.
Expression of transcripts for antifungal PRRs by healthy term amnion, choriodecidua and placenta was examined by PCR. The response of each tissue (n = 4) to live and inactivated *C. albicans* (10^6cells/ml) and to the dectin-1 PAMPs (depleted zymosan, curdlan; both 100ug/ml) was determined by measuring IL-6, IL-8 and IL-1β using ELISAs. Biochemical inhibitors were used to elucidate signalling pathways.
All gestation-associated tissues expressed transcripts for dectin-1, dectin-2, MINCLE, DC-SIGN and mannose receptor. Live *C. albicans* induced significantly increased IL-6 production in placenta and choriodecidua , but not amnion; IL-8 production was only induced in placenta. Inactivated *Candida* had no significant effect in any tissue. Curdlan and depleted zymosan significantly increased IL-6 production in amnion, choriodecidua, and placenta, although significant IL-8 production only occurred in curdlan stimulated placenta and IL-1β production only occurred in curdlan stimulated placenta and choriodecidua. Inhibition of reactive oxygen species (ROS) production with butylated hydroxyanisole abrogated curdlan-induced IL-1β production.
The placenta, choriodecidua and amnion can mount an inflammatory response to fungal stimuli including ROS-dependent activation of the inflammasome by curdlan for IL-1β production in the placenta and choriodecidua.

P1.11.013

Triggering receptor expressed on myeloid cells (TREM)-1 and -2 in bronchoalveolar lavage fluid in pulmonary sarcoidosis

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Background and objective. Pulmonary Sarcoidosis (PS) is characterized by the formation of granulomas in lungs and has been associated with infection by microorganisms. Triggering receptor expressed on the surface of myeloid cells-1 (TREM-1) is over-expressed in response to infection while TREM-2 is involved in granuloma formation. We hypothesised that these receptors are over-expressed in PS and might be useful for diagnostic testing.
Methods. Cell surface TREM-1 and TREM-2 expression in cells obtained at bronchoalveolar lavage (BAL) was measured in individuals with sarcoidosis (n = 26) and compared to that seen in individuals with other interstitial lung diseases (ILD) (n=27).
Results. TREM-1 and TREM-2 expression was significantly increased in sarcoidosis compared to other ILDs: total number of TREM-1: p=0.0039 (23.81 vs. 13.50 cells/µl), TREM-2: p<0.0001 (32.81 vs. 7.76 cells/µl); percentage of TREM-1: p=0.0002 (41.30% vs.15.70%); TREM-2: p<0.0001 (34% vs. 9.60%) and mean fluorescence of TREM-1: p=0.0005 (5.43 vs. 1.96), TREM-2: p=0.0011 (6.85 vs. 2.77). Increase in both of these receptors seems to be typical for pulmonary sarcoidosis. In discriminating sarcoidosis from other ILDs, the specificity (96%) and sensitivity (72%) of the combination of TREM-1 and TREM-2 was high.
Conclusions. Increased TREM-1 and TREM-2 cell surface expression is observed in sarcoidosis. Evaluation of BAL cell expression of both of these receptors may serve as a diagnostic marker for sarcoidosis.

P1.11.014

Increased CD4 T cell survival during in vivo OVA-LPS presentation is partly inhibited by indomethacin and recovered by PGE2 analog

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Clonal activation, proliferation and differentiation into effector cells occur when CD4+ T lymphocytes recognize antigenic peptides presented by dendritic cells (DCs). DC maturation occurs through stimulation of Toll like receptors (TLRs), which increase their antigen presentation ability and affects the profile of soluble molecules. The expansion and differentiation of antigen-specific CD4+ T cells are followed by a contraction phase due to Activation-Induced Cell Death (AICD) or Activated T Cell Autonomous cell Death (ACAD) to reestablish homeostasis. Our group demonstrated that LPS stimulated-DCs protects T cells from AICD by preventing TcR/CD3-mediated FASL upregulation. The protective effect is partially mediated by PGE2. Our hypothesis is that antigen presentation in the context of infection impacts on FASL expression and survival of CD4 T cells, dependently on TLR-mediated release of PGE2. To approach our hypothesis, we used an in vivo model of adoptive cell transfer in which splenocytes from DO11.10 mice were labeled with CFSE and transferred to wild-type syngeneic BALB/c. These mice were inoculated with pOVA±LPS and pretreated with indomethacin and/or misoprostol. The addition of LPS during pOVA presentation increased specific CD4+ T cells activation and proliferation. Pretreatment of mice with indomethacin, an inhibitor of COX2, reduces the frequency of specific T cells by increasing FASL expression and apoptosis, but did not interfere with proliferation. Addition of misoprostol, which mimics PGE2, inhibits FASL and restores the survival of specific cells, suggesting that endogenous PGE2 produced in response to LPS modulates the number of specific T cells by controlling FASL expression.
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P1.11.015

Optimal Immune Response Is Mediated by CD8-IFN Gamma Production and Macrophages-iNOS Expression in trigeminal ganglia, after Interactions of Toll Like Receptors in Herpes Simplex Virus -1 (HSV-1) Infection

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HSV-1 cause cold sores, or even encephalitis. In this work, $TLR2^{-/-}$, $TLR9^{-/-}$, $TLR2/9^{-/-}$ and WT mice were intranasally infected with HSV-1, and were euthanized at the 5th day post infection (dpi) and then trigeminal ganglia (TG) and brain were collected. $TCD8^{+}$ IFN gamma cell producers (cytometry assay) were higher in TG of infected than in uninfected mice. Infected WT mice showed increase in the expression of TLR1, TLR2, TLR3, TLR6, TLR7 and TLR9 in TG (Real Time PCR), but not in brain, compared to control. The infected knockout mice had increased expression of TLR1, TLR3, TLR6, TLR7 and additionally of TLR2 (for $TLR9^{-/-}$) and of TLR9 (for $TLR2^{-/-}$) in TG, compared to non infected mice, but decreased expression when compared to WT mice. iNOS expression in TG of infected WT was higher than in TG of knockout mice, what not occurs with the expression in TG of $gp91^{phox}$ and $p22^{phox}$, although the three genes had higher expression in TG of WT and knockouts infected mice in comparison to non infected mice. In addition, intraperitoneal macrophages of WT mice produced more nitric oxide after exposition to HSV-1 than TLR knockout mice. $RAG^{-/-}$ mice were not capable to express cytokines, and $iNOS^{-/-}$ mice showed an excessive response to cytokines expression. Finally, in survival assay with WT, $CCL3^{-/-}$, $CD8^{-/-}$, $RAG^{-/-}$ and $iNOS^{-/-}$ mice, we showed the importance of the CD8 and iNOS, because $CD8^{-/-}$, $RAG^{-/-}$ and $iNOS^{-/-}$ infected mice had 100% of mortality, compared to 10% in WT infected mice.

P1.11.016

Monocyte-produced TLR2 and TLR4 but not prolactin are involved in both, late-onset autoimmune diabetes and type 2 diabetes development

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We aim to define a role of monocytic PRL in the induction of immune dysbalances leading to late-onset autoimmune diabetes (AD) and by detecting TLRs, key molecules of innate immunity, to identify differences that may reflect reaction of organism to bacterial and viral infection in AD, type 2 diabetes (T2D), non-diabetic patients (nonDM) and healthy individuals.
In total, 107 blood samples were collected for monocytes separation and subsequent detection of PRL, TLR2 and TLR4 mRNA by quantitative PCR and protein molecules by flow cytometry. The analyzed adult diabetic samples were as follows: T1D (19), LADA (4), T2D (9). As controls, 60 healthy subjects and 15 specimens from non-diabetic patients (nonDM) were used.
Compared to data gained from system autoimmune disorders, prolactin produced by monocytes does not seem to contribute to the ethiopathogenesis of late-onset organ-specific AD. The autoimmune condition is accompanied by lowering numbers of monocytes, particularly $CD14^{high}TLR2/4^{high}$ expressing cells (2 times less, P<0.0001), in comparison to physiological state, whereas $CD14^{low}TLR2/4^{low}$ counts in AD are increased (P=NS). This finding is surprising when taken into consideration corresponding gene expression at the mRNA level, which is lower in healthy subjects than in all diseased patients (in TLR2 31-41.4 times depending on diseased group, P<0.0001, and in TLR4 9-11 times depending on

diseased group, P<0.0001). Further, the onset after 35 years of age corresponded with 1.37 times higher counts of monocytes (P<0.05) and 1.47 times increased numbers of cells with $CD14^{high}TLR^{2;4high}$ receptor expression (P<0.05).
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P1.11.017
Roles of an innate immune regulator TBK1-Associated Protein in Endolysosomes (TAPE) in the RIG-I-like receptor and DNA sensor pathways

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RIG-I-like receptors (RLRs) and DNA sensors are key cytosolic sensors for recognizing viral nucleic acids to trigger antiviral immunity. The underlying mechanisms linking RLR- and DNA sensor-mediated viral recognition to antiviral immunity remain to be further explored. A previous work from the lab led to the discovery of a novel innate immune regulator called TAPE (TBK1-Associated Protein in Endolysosomes), also known as CC2D1A/Freud-1/Aki-1. TAPE is located in endolysosomes and links TLR3 and TLR4 to IFN-β activation. We further explored the potential roles of TAPE in the RLR and DNA sensing pathways. First, knockdown of TAPE impaired RIG-I- and MDA5-mediated IFN-β activation. In contrast, knockdown of TAPE failed to impair IPS-1/MAVS- and STING-mediated IFN-β promoter activation. TAPE was shown to associate with IPS-1/MAVS in a complex with RIG-I or MDA5 in mammalian cells. Genetic evidence showed that TAPE-deficient mouse embryonic fibroblasts (MEFs) were defective in RLR ligand-induced IRF3 phosphorylation and IFN-β activation. Also, TAPE knockdown or deficiency diminished cytokine induction and antiviral responses upon RNA virus infection. However, TAPE deficiency in MEFs failed to impair DNA-induced IFN-β activation. Together, our data support a crucial role for TAPE in linking RIG-I and MDA5 to type I IFN-mediated antiviral responses. Future work will determine in vivo role of TAPE in innate immunity using TAPE conditional knockout mice and explore the mechanistic mechanisms of how TAPE regulates the RLR pathways.

P1.11.018
Immunostimulatory activities of CpG-oligodeoxynucleotides in zebrafish

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CpG-oligodeoxynucleotides (CpG-ODN) are potent immune stimuli. Their activity is dependent on the context of their nucleotide sequences, for example CpG-ODN containing GACGTT motif are more potent in activation of murine cells than CpG-ODN with GTCGTT motif, whereas the GTCGTT motif containing CpG-ODN are more active in human and different domestic animals. Toll-like receptor (TLR) 9 is the cellular receptor for CpG-ODN in mammals, and TLR21 mediates their functions in chicken. The avian genomes are luck of *TLR9*, and mammalian genomes do not contain *TLR21*, whereas both genes are presence in fishes. CpG-ODN are investigated as vaccine adjuvant and ant-microbial agent for fishes but the biological function of fish TLR9 and TLR21 have not yet investigated.
In the present study, we comparatively investigated the expression, structural relationship and function of zebrafish TLR9 and TLR21 in an attempt to address the molecular basis of the immunostimulatory activities of CpG-ODN in fishes. Our results showed that both zebTLR9 and zebTLR21 have parallel expression profiles. They are expressed in the early stages during embryonic development, and are expressed in innate immunity related organs in adult fish, as well as expressed inside cells. In addition, both zebTLRs are functional. They are responding to CpG-ODN stimulation and cooperatively mediated the immunostimulatory activities of CpG-ODN in zebrafish.

P1.11.019
New molecular tools to investigate LPS recognition

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Toll-Like Receptor 4 (TLR4) is one of the most important receptors of innate immunity, since it is responsible for the sensing of Lipopolysaccharide (LPS) and thus of the presence of bacteria. It works in association with proteins MD-2, LBP and CD14. In our laboratory we develop small molecules based on saccharidic scaffolds that target proteins of the TLR4 pathway and act as antagonists. Their selectivity makes them hit compounds for the treatment of several pathologies related to TLR4 pathway dysfunctions, but they are also good candidates as tools to selectively modify a biochemical route with no need to genetic engineer the cells (the “chemical genetics” approach). In this communication we will show a library of molecules that were developed in our laboratory and their biological characterization to this day.
Visualizing molecular interactions in biological samples or *in vivo* would also be very useful in order to understand which processes take place during LPS sensing by the immune system. Fluorescence microscopy is an efficient method to visualize molecular interaction both *in vivo* and *in vitro*.
The immuno-fluorescent staining of a sample is achieved with the use of fluorescent-labelled antibodies. The major drawback of this method is the high price of antibody-fluorescent conjugates. Fluorescent small molecules that show a good specificity for a protein target could be used as substitutes. Also, since chemical synthesis of small molecules frequently is easier and cheaper than biological production of antibodies, the chemical approach to fluorescent staining could be a convenient alternative to antibody labeling.

P1.11.020
The Q705K genetic variant in *NLRP3* leads to inflammasome hyperactivation and contributes to invasive aspergillosis after stem cell transplantation

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The role of inflammasome stimulation, in particular NLRP3, in antifungal immunity has been the subject of many recent investigations in both mice and humans. Stimulation of the NLRP3 inflammasome has been demonstrated to occur in response to fungi, suggesting a crucial role in antifungal resistance. To understand the cellular and molecular immune pathways by which NLRP3 inflammasome activation might affect antifungal immunity, we have analyzed the association between genetic variants in the *NLRP3* gene and susceptibility to invasive aspergillosis in hematopoietic stem cell transplanted patients. We found that a genetic variant in *NLRP3* (rs25829419, Q705K) was associated with or tended to invasive aspergillosis when present in either donors, recipients or both. Moreover, this variant also led to poorer overall survival after transplantation. Functionally, the presence of the Q705K variant in human monocytes was correlated with an increased functional activity of the NLRP3 inflammasome, as revealed by increased levels of IL-1β and IL-18 upon stimulation with *Aspergillus fumigatus*. In conclusion, our results suggest that an hyperactivation of the NLRP3 inflammasome may be detrimental to antifungal immunity and therefore contribute to susceptibility to fungal infections in immunocompromised patients.

P1.11.021
The Role of TLRs in the Dogs Immunized with the Gentamicin-attenuated L. infantum Compared with Dogs Infected with Wild-type Parasites

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Introduction: *Leishmania* is an intracellular pathogen that invades host immune system. *Leishmania infantum* (*L. infantum*) is a cause of

canine visceral leishmaniasis (CVL). The outcome of the disease basically relies on the balance between Th1/Th2 immune responses. How does *Leishmania* regulate host innate immune system? Still it is unanswered. The Toll-like receptors (TLRs) play very important role during inflammatory process of various diseases. Identification of leishmanial antigens that modulate toll-like receptor signaling will certainly help in the development of future vaccine. In this study we defined the role of TLRs in the dogs immunized with the gentamicin-attenuated *L. infantum* compared with dogs infected with wild-type parasites.
Material and methods: Thirty dogs (10 dogs per group) were used in this study. The peripheral blood mononuclear cells (PBMCs) were collected from dogs vaccinated with the attenuated parasites and dogs infected with wild-type. DNA will be extracted and the expression of TLR9 will be detected using RT- PCR

P1.11.022
Unbalanced cytokine production in inducible nitric oxide synthase-deficient neonate mice upon in vitro stimulation with TLR agonists

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Background: It has been described several differences regarding neonatal immune responses in comparison to adults. Previous data suggest TLR agonists are less effective in neonate than in adult mice to direct immune responses toward Th1. The aim of this work was to evaluate whether nitric oxide production is involved in the modulation of cytokine production by newoborn cells upon TLR stimuli. Methods: Spleen cells of ten days old and adult C57BL/6 wild type or iNOS(-/-) mice were stimulated with Pam3Cys, Poly I:C, lipopolyssacharide or CpG-ODN (type B). Supernatant was collected after 24 hours and IL-6, TNF-alpha, IL-10 and CCL-2 levels were evaluated by cytometric bead array. Results: As observed in previous results with BALB/c cells, adult C57BL/6 spleen cells secreted higher levels of IL-6 after CpG stimuli, but lower with LPS. Also, neonatal cells showed an increased IL-10 production with all agonists used. Further, an elevated production of monocyte chemotactic protein-1 (MCP-1/CCL2) - a Th2-related chemokine - was observed in culture supernatant of neonatal cells, mainly those stimulated with poly I:C or CpG. When we analyzed the cytokine production by cells from newborn iNOS(-/-) mice, LPS increased IL-6 production comparing to the same cells with medium alone, however the basal production of other cytokines were elevated and did not change with any TLR agonist. Conclusion: Altogether, we verified that the absence of iNOS causes an unbalance in TLR-mediated cytokine production in newborn mice. These findings may contribute to our knowledge about immunomodulation in early life.
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P1.11.023
Myeloid-related protein (Mrp)-8/14 contributes to protective immunity in sepsis caused by Burkholderia pseudomallei and elevated levels correlate with poor outcome

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Introduction: Toll-like receptor-4 activating Mrp8/14 (S100A8/A9) complexes are known to function as endogenous danger proteins that promote lipopolysaccharide induced systemic inflammation; their precise function in sepsis however remains to be determined. Melioidosis, caused by the Gram-negative bacteria *Burkholderia (B.) pseudomallei* is an important cause of sepsis in SE-Asia and associated with mortality up to 40%.
Objective: By using a translational approach we aimed to characterize the expression and function of Mrp8/14 in sepsis caused by *B. pseudomallei*.
Methods: Mrp8/14 expression was determined in isolated whole blood leukocytes and plasma from patients with *B. pseudomallei* induced sepsis (n=34) and compared to healthy controls (n=33). Mrp8/14 function was investigated in wild-type and Mrp14 deficient mice (which also lack Mrp8) intra-nasally infected with *B. pseudomallei*. Mice were sacrificed post-infection to assess Mrp8/14 expression, bacterial loads, inflammation and pathology.
Results: *Mrp8* gene expression was markedly increased in all melioidosis patients corresponding with elevated Mrp8/14 plasma levels. In patients, elevated Mrp8/14 concentrations were strongly associated with mortality. In line, mice inoculated with *B. pseudomallei* showed strong increases in plasma, BALF, and pulmonary Mrp8/14 levels. Strikingly, upon infection Mrp14 deficient mice displayed decreased pulmonary bacterial loads but increased bacterial counts in the systemic compartment, which was partially reflected in differences in cytokine response and pathology.
Conclusion: Mrp8/14 levels are increased during sepsis caused by *B. pseudomallei* and correlate with mortality. Mrp8/14 complexes can contribute to protective immunity during sepsis, but their role is versatile as reflected in its compartment dependent function during experimental *B. pseudomallei* infection.

P1.11.024
Response of human monocyte-derived dendritic cells to the combined effect of TLR3 and TLR7 agonists

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Targeting Toll-like receptors (TLRs) by specific agonists seems to be a promising in stimulation of the immunogenicity of dendritic cells (DCs). Since the functional outcome upon the activation of TLRs may be different, the aim of our study was to evaluate how different concentrations of 7-thia-8-oxo-guanosine (7-TOG), a selective TLR7 agonist, affects functional and phenotypic properties of human monocyte-derived DCs (MoDCs) and if they could be modulated by co-ligation of TLR3. Immature MoDCs were treated with different concentrations of 7-TOG (25, 100 and 250 µmol/L) alone, or together with poly (I:C) (10 ng/mL), a selective TLR3 agonist. We showed that the highest concentration of 7-TOG stimulated the differentiation, maturation and allostimulatory capability of MoDCs, which were accompanied by induction of T helper (Th)1 and Th17 immune responses. Both Th responses were significantly enhanced by additional stimulation of MoDCs with poly (I:C). Intermediate concentration of 7-TOG up-regulated the expression of CD86 and increased production of IL-1β and IL-6 by MoDCs, followed by the stimulation of Th17 immune response. The treatment of MoDCs with the lowest concentration of 7-TOG down-regulated the expression of CD40 on MoDCs and potentiated the Th2 immune response. Additional treatment of MoDCs with poly (I:C) also stimulated both Th1 and Th17 responses, whereas Th2 response was not significantly modulated. In conclusion, our results show that 7-TOG influences phenotype and functions of MoDCs in a dose-dependent manner and suggests that signaling through TLR7 may be modified by the engagement of TLR3, resulting in a different outcome of immune response.

P1.11.025
Association of TLR4-D299G polymorphism with inflammation and overexpression of TLR4-CD14 in obese mexican patients

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The Aim: Associate the TLR4-D299G polymorphism with pro-inflammatory cytokines and the expression of TLR4-CD+14 in obese Mexicans.

Methods: We evaluated 210 individuals Mexican Mestizos from western Mexico, 105 normal weight subjects (N) and 105 obese patients (O). Both study groups were determined genotypic and allelic frequency of TLR4-D299G polymorphism, inflammatory profile (IL-1β, IL-6, IL-8, IL-10 and TNFα) by ELISA, the Morpho-Anthropometric profile with the anthropometric technique and the percentage of expression and fluorescence index (FI) of TLR4 and CD14 in monocytes of peripheral blood by flow cytometry.
Results: The presence of the polymorphism was 2% N subjects and 6% in O patients. The cytokines results were: IL-1β = 2.5 vs 2.0 pg/ml (p = NS), IL-6 = 28 vs 36 pg/ml (p = 0.030), IL-8 = 25 vs 27 pg/ml (NS), IL-10 = 6.8 vs 8.4 pg/ml (NS), TNFα = 15 vs 31 pg/ml (p = 0.000) N vs O respectively. The expression of TLR4 was 70%, FI 6.41, CD14 84%, FI 1.25 and the percentage of double positive was 59% in N and TLR4 expression 77%, FI 7.70, CD14 86%, FI 1.61 and the percentage of double positive was 66% in O, with p = 0.000 as statistical difference
Conclusion: The western O Mexican, have higher concentrations of pro-inflammatory cytokines (IL-6 and TNFα) and expression of TLR4 receptor and CD14 on peripheral blood monocytes, compared to N. However not found a direct association of the polymorphism with obesity.

P1.11.026
Potential role of tlr ligand in aethiopathogenesis of Tunisian endemic pemphigus foliaceus

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Pemphigus foliaceus (PF) is an autoimmune skin disease in which environmental factors are thought to participate. Recent studies suggest that microbial components use signaling molecules of the human Toll-like receptor (TLR) family to transduce signals in keratinocytes. The aim of our research was to investigate the expression of TLRs 2, 3 and 4 by keratinocytes of PF patients compared to normal keratinocytes in order to characterise the nature of the microbial factor involved in the etiopathology of PF. Biopsies obtained from 43 PF patients and 20 healthy controls were assessed by immunohistochemical analysis using specific polyclonal antibodies. The TLR2, TLR3 and TLR4 expression was significantly upregulated in PF epidermis. The significant increase of those TLRs simultaneously may merely reflect the complicated environmental conditions of rural women in the southern rural regions of Tunisia. Interestingly, we have found that the TLR4 diffuse expression was associated with the production of anti-desmoglein 1 Abs (p=0.037). This could be in line with a potential role of TLR ligand in aethiopathogenesis of Tunisian endemic PF. TLR over-expression in pemphigus skin indicates that TLRs are involved in the pathogenesis of pemphigus through stimulation by infectious or endogenous ligands.

P1.11.027
The C-type lectin receptors on dendritic cells participate in the recognition and modulatory effect of high molecular weight components of Ascaris suum extract

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Dendritic cells (DCs) play a key role in linking innate with adaptive immunity. The interaction of pathogens with DCs is mediated by distinct receptors as the C-type lectin (CLRs), which recognize glycan structures. We previously described that high molecular weight components (PI) from *Ascaris suum* extract down-modulate the antigen-presenting cells activities. The aim of this work was to analyze the involvement of CLRs in the modulatory effect of glycan structures in PI on DCs. The PI binding to CLRs on DCs and its internalization was analyzed by flow cytometry or confocal microscopy. Immature DCs were incubated at 4° or 37°C with PI-Alexa488 or BSA-Alexa488 for 30-45 minutes and analyzed. The binding and internalization of PI were also evaluated in DCs pre-incubated with mannan, anti-CLR (DC-SIGN, MR, MGL, DEC205) MoAbs, sucrose or EGTA. The costimulatory molecules expression and IL-10 and IL-12 secretions were studied in DCs incubated with LPS, LPS+PI, LPS+PI+mannan or LPS+PI+anti-CLRs for 18h. Higher fluorescence was observed in DCs incubated with PI-Alexa at 37°C compared with the incubation at 4°C. Inhibition of PI binding and internalization was verified in DCs previously incubated with mannan, anti-CLRs, sucrose or EGTA. The inhibitory effect of PI in the costimulatory molecules expression and cytokines secretion was blocked when DCs were incubated with mannan, anti-CLRs and LPS. The results indicate the participation of the CLRs in the PI recognition by DCs and then triggering the modulation activity of these glycan structures. Financial support: CNPq and FAPESP (2011/23735-0 and 2010/10393-1).

P1.11.028
Isolation and characterization of *N*-linked glycan structures from high molecular weight components of *Ascaris suum* extract

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C-type lectin receptors (CLRs) are part of a heterogeneous superfamily of soluble and transmembrane proteins. They are involved in the homeostasis of immune system as well as the recognition of carbohydrate structures in pathogens by immune cells as dendritic cells (DCs). We showed that high molecular weight components (PI) from *Ascaris suum* are able to modulate the DCs activity. Here, we analyzed the presence of *N*-linked glycan antigens in PI and its ability to suppress the DCs maturation. The content of *N*-linked glycan components in PI was obtained by PNGaseF treatment and analyzed by HPAEC-PAD and UV-MALDI-TOF. The down-modulatory effect of these glycan components obtained by affinity with ConA-Sepharose was analyzed in DCs incubated with LPS. The HPAEC-PAD results at neutral and acidic conditions revealed several peaks indicating the presence of *N*-linked oligosaccharide structures with high mannose contents in PI and sialic acid residues, respectively. The UV-MALDI-TOF also confirmed the presence of *N*-linked glycan components with high-mannose type sugars. The chromatography affinity with ConA showed high content of *N*-glycosylated components on PI. The flow cytometry results showed that the ConA-linked components inhibited the expression of costimulatory molecules on DCs induced by LPS compared with those observed on DCs incubated only with LPS. These results indicate that PI contains *N*-glycosylated structures and these components are able to down-modulate the DCs maturation. Financial support: CNPq and FAPESP (2011/23735-0 and 2010/10393-1)

P1.11.029
Involvement of the nucleic acid recognizing Toll-like receptors TLR7 and TLR9 in the pathogenesis of erosive arthritis

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Abundant release of endogenous nucleic acids can trigger autoimmune reactions via activation of Toll-like receptors (TLR) 7 and 9, potentially leading to autoimmune diseases. In Rheumatoid arthritis (RA), an inflammatory joint disease, the involvement of these TLRs is not clear yet. Interestingly, in rats with pristane-induced arthritis (PIA), disease can be transferred by T cells together with antigen-presenting-cells pre-activated with TLR7 or TLR9 agonists.

To analyze the role of TLR7 and TLR9 in the pathogenesis of arthritis, we induced disease in rats with the mineral oil pristane, and in C57Bl/6 mice by injection of KRN serum. Immunoregulatory oligodeoxynucleotide (ODN) sequences (IRS) antagonizing TLR7 or TLR9 were applied throughout the whole experiment. A non-inhibitory ODN was used as control. Disease severity was assessed clinically and histologically. Serum cytokine levels were measured by ELISA.
The TLR7 inhibitor and control ODN showed no effect. Antagonizing TLR9 reduced arthritis severity in PIA. IL-6 levels were diminished in TLR9-antagonized animals. These effects were only observed when applying the inhibitor before disease onset. Moreover, neither inhibitor affected arthritis severity in the serum transfer model, which is independent of adaptive immunity.
Inhibition of TLR9 reduced inflammation and bone erosion in PIA, but not in the KRN serum transfer model, reflecting the late phase of erosive arthritis.
Thus, TLR9 appears to be involved in the initiation of arthritis. Nucleic acid binding TLRs do not seem to be important in later stages of the disease. Antagonizing TLR9 in human RA may act beneficial in the early disease phase.

P1.11.030
Training modifies the innate immune response both in the airways and in blood in horses

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Lower airway diseases are common problems in sports and racing horses. In humans, exercise has been associated with upper respiratory tract infections due to down-regulated expression of Toll-like receptors (TLRs), costimulatory and antigen-presenting molecules on monocytes. The objectives of this study were 1) to examine the expression of TLRs in equine bronchial epithelial cells (EBEC) and blood monocytes in untrained and trained horses; 2) to stimulate EBEC and monocytes *in vitro* with TLR ligands, in order to mimic bacterial/viral infections; 3) to compare the cytokine production of EBEC and monocytes in untrained and trained horses. Bronchial biopsies were taken from 8 horses during lower airway endoscopy at rest and 24 hours after a standardized exercise test (SET). Bronchial epithelial cells were grown *in vitro* and activated with TLR ligands. Blood monocytes were collected at rest and after the SET. TLR1-TLR9 expression was evaluated via real-time PCR and cytokine production was measured via ELISA. TLR3 and TLR4 expression was modified by training. The expression of TLR2, TLR7 and TLR8 was modified only by strenuous exercise in trained horses. Training had local immuno-suppressive effects shown by a decreased production of TNF-alpha and IFN-beta in EBEC in response to TLR2 and TLR3 ligands. Training also caused a systemic pro-inflammatory response evidenced by increased production of TNF-alpha in monocytes in response to TLR2 and TLR4 ligands. These findings suggest that training and strenuous exercise in trained subjects may result in an increased susceptibility of the lower airway to infections associated with systemic inflammation.

P1.11.031
The novel TLR7 agonist GS-9620 induces a diverse set of interferon stimulated genes

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Efficient innate and adaptive immunity is necessary for initial containment and subsequent clearance of viral infections, including hepatitis viruses B (HBV) and C (HCV). Part of the initial anti-viral immune response is the interaction of single stranded viral RNA with Toll-like receptor 7 (TLR7) within the endolysosomal compartment leading to activation of plasmacytoid dendritic cells and B lymphocytes. We demonstrated that the novel TLR7-selective agonist GS-9620 induces IFN-α and other cytokines and chemokines in human PBMC *in vitro* cultures in a dose-dependent manner. Moreover, we showed that GS-9620-dependent activation networks include the upregulated expression of lymph node retention marker CD69 on lymphocytes such as B cells and T cells. Profiling the GS-9620-dependent induction of gene expression in PBMC on a whole-genome level using the Illumina bead chip revealed a gene signature consistent with an anti-viral response including the upregulation of interferon-stimulated genes (ISGs). For a select panel of ISGs (MX1, OAS1, ISG15, IFIT1 and IFIT2) and other genes (CCL8, CCL19, CD38, IL-1RN), the time- and concentration-dependent induction after the stimulation with GS-9620 was confirmed using quantitative PCR. In addition, we showed a similar pattern of TLR7 agonist-dependent ISG induction in *ex vivo* experiments using fresh human whole blood. In conclusion, the TLR7 agonist GS-9620 broadly activates anti-viral genes including ISGs. These observations confirm the antiviral potential of GS-9620 and may facilitate the identification of sensitive pharmacodynamic markers to inform the clinical development of GS-9620.

P1.11.032
NOD-like receptor activation stimulates osteoclasts

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Many type of cells, including osteoblasts express NOD2, a member of the family of Nod-like receptors (NLRs). Nod2 is a general sensor of peptidoglycan through the recognition of muramyl dipeptide (MDP), the minimal bioactive peptidoglycan motif common to all bacteria. The activation of NOD2 in osteoblasts can induce RANKL expression which can indirectly stimulate osteoclastogenesis.
Our purpose is to determine the direct effect of MDP on osteoclasts differentiation and activation.
Bone marrow cells from C57/BL6 and NOD2-/- mice were cultured for 3 days with MCSF (30ng/ml). Adherent cells (bone marrow macrophages - BMM) was cultured for 4 more days with MCSF (30ng/ml) and RANKL (5ng/ml) and/or bacterial peptidoglycan-derived muramyl dipeptide MDP (0,01; 0,1; 1,0 ug/ml). The cells was stained to identify tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoclast. The differentiation and activation markers were evaluated, 96h after MCSF/RANKL stimulation, by Real time PCR.
The expression of mRNA encoding Nod2 in murine BMM was increased (2~3 fold) after 24 hours MCSF/RANKL stimulation. NOD2-/- derived RANKL/MCSF-stimulated BMM presented less multinuclear TRAP+ cells (~334 cells/well) than WT derived BMM (~402 cell/well). MDP did not alter the number of multinuclear TRAP+ cells. Treatment of BMM with MCSF and RANKL for 96 hours resulted in increased mRNA expression of TRAP, cathepsin k, ATP6i and integrin beta3, as assessed by Real time PCR.
These results confirm that NOD2 activation is important to osteoclastogenesis, with increased transcription of genes important for osteoclasts progenitor cell differentiation, fusion and function.
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P1.11.033
Collapse of TLR7/TLR9 balance leads genetical background dependent phenotypes

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Nucleic acid sensing-TLRs (Toll-like receptors) recognize pathogen derived-nucleic acid and induce immune response. It is important for host defense, however, it is known that nucleic acid sensing-TLRs also recognize host-derived nucleic acid and induce inflammatory disease. To prevent autoinflammation, nucleic acid sensing-TLRs should be controlled by multiple stages of mechanisms.
Unc93B1 (Unc93 homolog B1) was found as an essential molecule for responsiveness of nucleic acid sensing-TLRs (Tabeta et al., 2006). This function is depend on its binding activity to nucleic acid sensing-TLRs and following trafficking from ER to endolysosomes (Kim et al., 2008). We found that Unc93B1 has another function, controlling responses of TLR7 and TLR9 reciprocally (Fukui et al., 2009).

TLR7/TLR9 balancing function of Unc93B1 depends on N-terminal of Unc93B1 and alanine mutant of 34th aspartic acid (D34A) induces TLR7 hyper-response and TLR9 hypo-response. Because of this collapse of TLR7/TLR9 balance, *Unc93B1*$^{D34A/D34A}$ mice (D34A mice) developed lethal inflammatory disease (Fukui et al., 2011). We generated several strains of D34A mice and found that their phenotypes were dependent on genetical background. For example, most all of C57BL/6 background D34A mice developed thrombocytopenia within 4 months old but no BALB/c background D34A mice developed.
We hypothesized that there are some modifier genes linking inflammation caused by collapse of TLR7/TLR9 balance to phenotypes, and started deeper analysis. In this presentation, we will show the difference of cell activation, contributions of cytokines, and pilot data of linkage analysis between these strains of D34A mice.

P1.11.034
Toll-like receptors 2, 4 and CD14 gene polymorphisms in Tunisian kidney transplantation

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Introduction: To investigate the distribution of TLR2, TLR4 and CD14 gene polymorphisms among Tunisian renal transplantation patients in relation to occurrence of acute rejection (AR) and chronic allograft nephropathy (CAN)
Methods: TLR2 (Arg753Gln), TLR4 (Asp299Gly) and CD14 (-159 C/T) were genotyped using DNA samples from 214 renal recipient by applying Restriction Fragment Length Polymorphism (RFLP) methodology. Fifty-nine patients had developed AR episodes and 24 were identified as CAN by biopsy scored according to the Banff criteria.
Results: The frequency of TLR2 G/G genotype was higher in AR patients comparing with non-AR ones (p=0.039; CI 95% OR=2.87 [1.05-4.37]). However, no differences were found in genotypes or alleles distribution according to CAN. There was no significant effect of any TLR4 or CD14 polymorphism on graft survival or acute renal rejection and CAN. Besides, no association was found between any TLR genotypes and the presence of HLA antibodies before and after transplantation. Single allele and genotype association analyses showed no significant association with the incidence of any posttransplant infections, including CMV (cytomegalovirus) infection.
Conclusion: Our present study suggests that that, set apart the immunological factors, the Arg753Gln polymorphism of TLR2 may increase the risk of acute renal allograft rejection in Tunisian patients. However, TLR4 and CD14 do not play a significant role in the pathogenesis of both AR and CAN after renal transplantation. Monitoring mRNA expression levels of TLR molecules should offer more useful information about the role of this system in acute and chronic kidney allograft injuries.

P1.11.035
Polymorphisms of Toll-like receptor 4 and CD14 genes in systemic lupus erythematosus and rheumatoid arthritis

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Introduction: Toll-like receptor 4 (TLR4) and its co-receptor CD14 play a major role in innate immunity by recognizing PAMPs and signal the activation of adaptive responses. These receptors can recognize endogenous ligands mainly auto-antigens. In addition, TLR4 (Asp299Gly) and CD14 (C/T -159) polymorphisms (SNPs) may modify qualitatively and/or quantitatively their expression. Therefore, they could be implied in autoimmune diseases and can influence both susceptibility and severity of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).
Patients and methods: TLR4 (Asp299Gly) and CD14 (C/T -159) SNPs were genotyped using polymerase chain reaction (PCR) in 127 SLE patients, 100 RA patients, and 114 healthy controls matched in age and gender.
Results: CD14*T allele was significantly more frequent in SLE patients (0.456) comparatively to controls (0.355), p=0.02 OR (95% CI)=1.53 [1.04-2.24]. In RA patients, the higher frequency of CD14*T allele (0.405) failed to reach significance, p=0.28. Investigation of the TLR4 (Asp299Gly) SNP showed no significant association neither with SLE nor with RA.
Analysis of these SNPs according to clinical and biological features showed a significant higher frequency of arthritis in SLE patients with CD14*T/T genotype (92%) comparatively to those with C/C and C/T genotypes (72.5%), p=0.04. Moreover, SLE patients carrying CD14*T/T / TLR4*A/A haplotype had significantly more arthritis (91.3%) than the rest of SLE group (73%), p=0,044 and confirmed by multivariable analysis after adjustment according to age and gender, p=0.01.
Conclusion: The CD14 (-159)*T allele seems to be associated with susceptibility to SLE and arthritis occurrence.

P1.11.036
D type CpG oligonucleotide loaded EG-7 exosomes act as strong vaccine adjuvant and anti-cancer agent

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Exosomes are naturally occurring nanovesicles secreted from several cells harboring different cargoes. Upon internalization, they can regulate cell physiology as well as mediate immune response. Immunostimulatory CpG ODNs are strong candidates for clinical use against cancer, infectious diseases and allergy. Their labile nature hampers in vivo performance. In this study, we aimed to explore the immuno-therapeutic potential of exosomes as ODN-delivery nanovehicles.
Exosomes from EG-7 (OVA expressing EL-4 cells) supernatants were purified and loaded with D-ODN. In vitro immunostimulatory activity of CpG ODN-loaded vesicles as evidenced by cytokine production by ELISA and surface marker upregulation by FACS was tested on mouse splenocytes. Then, C57/Bl6 mice were immunized with D-ODN loaded EG7-exosomes. Mice sera were analyzed for anti-OVA IgG subtype-responses. OVA immunized animals post 2 months booster immunization were seeded with EG-7 cells and tumor development were followed.
In vitro stimulation assays revealed that D-ODN encapsulation into exosomes significantly increased IL6, IL12, IFNg and IFNa production to that of free ODN. CD86 and MHC-II expressions were 2-4 fold higher for D-Exo compared to free D-ODN stimulations. D-ODN loaded exosomes induced Th1-biased anti-OVA response compared to exosome treated animals. Following thymoma induction in naive and OVA-immunized mice, data indicated that >85% of treated mice lacked tumors whereas all naive animals were positive for tumor. This trend was constant for 4wks post-inoculation.
Collectively, our data suggest that D-ODN encapsulation in exosomes improve immunostimulatory activity, provide better anti-OVA immunity thereby contribute effective tumor clearance in mice.

P1.11.037
CpG Oligodeoxynucleotide/cationic peptide nanorings as immunotherapeutic agents

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Synthetic CpG containing oligodeoxynucleotides (ODNs) mimic the immunostimulatory activity of bacterial DNA and induce a strong pro-inflammatory response via Toll-like receptor 9 (TLR9) signaling. To date, most of the human clinical trials involved one type of CpG ODN (known as K-type or B-type) whereas the therapeutic potential of the

G-quadruplex forming type I interferon inducing D-type ODN (or A-type) remained unexplored due to product aggregation that complicates the pharmaceutical manufacturing of this ODN type. Herein we show that a short K-type ODN and the HIV-derived cationic peptide Tat(47-57) condense to form monodisperse nuclease resistant nanorings that replicate the high type I interferon stimulating activity of D-type ODN in human blood. The nanorings provide nuclease resistance, enhance the cellular uptake and alter the subcellular distribution of the associated CpG ODN. Consistent with their IFNa inducing activity from plasmacytoid dendritic cells, the nanorings localize to early endosomes. In conclusion, these immunostimulatory nanorings are effective D-ODN surrogates and could prove to be of value as anti-viral or anti-cancer agents and vaccine adjuvants in the clinic.

P1.11.038

Mechanisms of MyD88- and TRIF-dependent downregulation of the glucocorticoid induced leucine zipper (GILZ) in macrophages - mRNA destabilization vs. microRNAs

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Toll-like receptor (TLR) activation induces the production of inflammatory mediators *via* NF-κB. Glucocorticoid-Induced Leucine Zipper (GILZ) is an anti-inflammatory mediator which interferes with NF-κB-mediated gene transcription in macrophages. Thus, we aimed to investigate GILZ regulation and function in primary human and murine macrophages upon TLR activation.
Treatment with TLR agonists signalling *via* MyD88, e.g. Pam_3CSK_4 (TLR1/2) or heat-killed *Staphylococcus aureus,* rapidly decreased GILZ mRNA and protein levels. In return, GILZ downregulation lead to enhanced phagocytic activity and increased induction of inflammatory mediators, as shown in GILZ knockout macrophages.
The TLR3 ligand polyinosinic:polycytidylic acid (Poly(I:C)), acting *via* the MyD88-independent TRIF pathway, displayed no ability to decrease GILZ mRNA levels. However, GILZ protein expression was reduced by Poly(I:C)-treatment, which was paralleled by sensitization towards TLR1/2- and TLR4-agonists.
MyD88-dependent GILZ downregulation involves mRNA-destabilization, whereas our data on MyD88-independent downregulation suggest translational inhibition. Herein, we report that MyD88-independent GILZ downregulation is associated with microRNA induction, as shown by dicer knockdown experiments and by using the RISC loading inhibitor ATA. A bioinformatics approach indicated miR-24 as a potential GILZ regulator. Moreover, we observed that miR-24 is induced in a MyD88-independent manner. Finally, luciferase reporter gene assays using constructs containing the 3'-untranslated region of GILZ suggested that miR-24 mediated GILZ protein abrogation by translational repression.
Conclusion: Our data indicate a dual regulation of GILZ upon TLR stimulation, which contributes to macrophage activation.

P1.11.039

Expression Analysis of Molecular Markers of Inflammation in Human Monocytic THP-1 cells Cultured with Single Walled Carbon Nanotubes Functionalized with Polyethylene Glycol (PEG-SWNT)

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One of the key advantages of carbon nanotubes in biomedical applications is that they can be easily internalized by cells, and therefore can act as delivery vehicles for therapeutical and diagnosis purposes. The clinical applications of using carbon nanotubes in medicine will depend on the outcomes of efficacy and Immunotoxicological studies, which will provide the necessary risk-to-benefit assessments for carbon-nanotube based materials.
The objective of the present study was to evaluate the in vitro inflammatory properties of single walled carbon nanotubes functionalized with polyethylene glycol (PEG-SWNT). Human monocytic cell line THP-1 was cultured with various concentrations of PEG-SWNT in different time points and the expression of several innate immunity receptor genes as molecular markers of Inflammation was analyzed by real time quantitative PCR (qPCR). In this study the expression of several pattern recognition receptors (PRRs) including TLR2, TLR4, CD14 and adaptor protein MyD88 which are the main sensors of Inflammation was analyzed in the PEG-SWNT treated THP-1 cells.
According to our results a significant up-regulation of TLR2, TLR4, CD14 and MyD88 transcripts was observed when THP-1 cells were treated with various concentrations of PEG-SWNT. However, down-regulation of the expression of molecular markers of Inflammation was observed when the cells were treated with high concentrations of PEG-SWNT which might suggest that higher concentrations of PEG-SWNT are toxic for the cells.
In conclusion the results presented in this study show that PEG-SWNT could trigger cellular inflammatory responses as shown by the expression analysis of genes involved in innate immunity and inflammation.

P1.11.040

Characterization of antibody responses to M13 phage vaccine: MyD88-dependent and T-cell independent IgG subclass responses

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M13 phage can be administered in PBS in mice to evoke a strong primary IgG response that is dependent on MyD88 signaling (BBRC 402: 19-22, 2010). Even a single immunization with 10^{11} pfu of phage induced a long-lasting antibody response.
M13 phage injected by the intraperitoneal and subcutaneous routes induced serum IgG subclass responses to M13 phage in C57BL/6 mice dominated by IgG2c, IgG2b and IgG3 between 1 and 2 weeks of post injection. After a secondary injection of M13 phage, all four subclasses including IgG1 were detected at the same level. The same IgG subclass profiles was observed in athymic (nu/nu) BALB/c mice except the Ab level which was at least 20-fold lower than that observed in BALBc and C57BL/6 mice, indicating T-cell independent IgG class swithing. We have preiviously reported that phage-specific IgG2c, IgG2b and IgG3 responses in TLR2-, TLR4-, TLR7- or TLR9-deficient mice, except the primary IgG1 level was significantly elevated only in TLR9-deficient mice. These data suggested that the IgG1 producing cells in the secondary response in wt mice was derived from a distinct B-cell repertoire or subpopulation involved in the primary response, and IgG1 response to M13 phage is regulated by TLR9. We are currently analyzing which sensor molecules of innate immunity are involved in the recognition of M13 phage. The ability of M13 phage to induce a strong IgG response suggests a possible application of peptide-displaying phage clones as vaccine carriers for induction of therapeutic antibodies.

P1.11.041

Card9 is essential for the CEACAM3 mediated *Moraxella catarrhalis* induced NF-kappaB activation in human granulocytes

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The chronic obstructive pulmonary disease (COPD) is often associated with pathological bacterial colonization of the lower airways which are normally sterile. Here *Moraxella catarrhalis* is of particular importance, colonizing the lower respiratory tract of up to 32% of COPD. The pathogenesis of COPD is characterized by neutrophilic inflammation. Although phagocytosis is considered as most important neutrophilic function, they have also been demonstrated to orchestrate immune responses via secretion of proinflammatory cytokines and chemokines, such as interleukin 8 or MIP-1α. By activating human granulocytes, both cytokines mediate the influx of neutrophils in the airways.

The pattern recognition receptor CEACAM3 (carcinoembryonic antigen-related cell adhesion molecule 3) is exclusively expressed on the surface of human granulocytes. It consists of a single extracellular Ig_V-like domain, a transmembrane domain as well as a cytoplasmic region containing an immunoreceptor tyrosine-based activation motif (ITAM). Binding of *M. catarrhalis* via its ubiquitous surface protein A1 (UspA1) to CEACAM3 has been shown to mediate phosphorylation of the two spaced tyrosine residues in the consensus sequence of the ITAM by Src family kinases followed by engulfment of the bacteria.
In this study we demonstrated for the first time, that the specific Moraxella UspA1-CEACAM3 interaction triggers an increased chemokine response of human neutrophils via the Syk/ CARD9 pathway and the Bcl10 signalosome leading to activation of canonical NF-κB. Our results offer new insight into the impact of specific pathogen-receptor interactions modulating neutrophilic cell functions.

P1.11.042
Immune complexes isolated from malaria patients activate TLR9 and promote caspase-1 activation

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High levels of pyrogenic cytokines such as IL-1β, TNF-α and IFN-γ produced during infection by Plasmodium exacerbate host immune responses causing various debilitating symptoms. Previous studies suggest that TLR9 is critical for the onset of this early inflammatory response in malaria. This project focuses on how parasite DNA is carried to the inner cellular compartment and activates TLR9. We observed the presence of high circulating levels of immune complexes (ICs) and parasite DNA during acute episodes of malaria. We also detected high levels of both IgM and IgG anti-dsDNA in individuals undergoing acute malaria episodes. Furthermore, ICs from malaria patients were found to contain primarily parasite derived DNA. Evaluating the immunostimulatory activity, we observed that ICs induced high levels of TNF-α and IL-10 as well as caspase-1 activation and IL-1β release by PBMCs and purified monocytes from healthy donors. When monocytes were primed with IFNγ, stimulation with ICs resulted in a switch of cytokine profile, similar to monocytes from malaria patients that produce high levels of IL-1β and TNF-α, but low IL-10 levels. Importantly, cytokine production by PBMCs stimulated with ICs was inhibited with the TLR7/TLR9 antagonist, but not the TLR4 antagonist. Hence, ICs are important shuttles for carrying parasite DNA to inner compartment of innate immune cells, resulting in activation of nucleic acid sensing TLR9 and signaling for inflammasome assembly. Altogether, our results support the hypothesis that ICs are important components of the systemic pro-inflammatory response and pathogenesis of malaria.
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P1.11.043
Identification of novel innate pattern recognition receptors for *mycobacterium tuberculosis*

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Recent studies have shown that immunoreceptor tyrosine-based activation motif (ITAM)-coupled receptors (ITAMRs) play important roles in innate immunity to certain microbes such as fungi. ITAMRs deliver signals through the ITAM-containing adaptors DAP12 or FcRγ, or through a cytoplasmic ITAM-like motif called hemITAM. We have previously shown that the adaptor protein CARD9 is essential for ITAMR-mediated innate immune responses. CARD9-deficient mice were shown to display severe susceptibility to *M. tuberculosis* (Mtb) infection. Although some C-type lectin-type ITAMRs, such as Mincle or Dectin-1, are suggested to participate in the recognition of Mtb, any single deficiency in these receptors does not severely affect anti-mycobacterial protection in mice, implicating other unknown ITAMRs involved in innate immunity to Mtb. To identify novel ITAMRs recognizing Mtb, we examined binding activity of a series of recombinantITAMR-Ig fusion proteins for *M. BCG* and Mtb, and found two novel ITAMRs, IgSFR2 and IgSFR7, that could bind to Mtb.IgSFR7 requires DAP12, whereas IgSFR2 uses both DAP12 and FcRγ for their signal transduction. Next, ligand search by using NFAT-GFP reporter cells that ectopically express these receptors revealed that a ligand of IgSFR2 is mycolic acid (MA), which is a major cell wall component of Mtb. MA-induced MCP-1 production was markedly reduced in peritoneal macrophage lacking IgSFR2. On the other hand, we found that IgSFR7 recognized at least two different components in organic solvent fractions of Mtb as ligands. We are now exploring roles of these receptors in innate immune response to Mtb.

P1.11.045
The humoral pattern recognition molecule PTX3 is a key component of innate immunity against urinary tract infection

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Introduction: Urinary tract infections (UTIs), which remain a major public health problem, are mainly caused by uropathogenic strains of Escherichia coli (UPEC). UTIs can progress to acute pyelonephritis that gives rise to severe morbidity. Cellular innate immune receptors and mediators play an important role in defence against UTIs but the role of humoral innate immunity has not been explored. The present study was designed to investigate the role in UTI of the soluble pattern recognition molecule pentraxin 3 (PTX3), a key component of the innate immune system.
Results: UTI of mice with UPEC was associated with increased levels of PTX3 in tissues and blood that correlated with the severity of the infection. PTX3-deficient mice showed a defective capacity to control UTI and increased tissue inflammation and damage. Urothelium is identified as a new source of PTX3 and both hematopoietic cell-derived and stromal cell-derived PTX3 were required for protection against UTI. PTX3 recognized UPEC and PTX3 opsonisation amplified UPEC phagocytosis by murine and human neutrophils. In patients with UTI, disease severity correlated with PTX3 levels in urine and serum. Finally, human counterpart of our animal data were supported by genetic analysis of UTI-prone patients where PTX3 polymorphisms were associated with susceptibility to acute pyelonephritis.
Conclusion: Our results suggest that PTX3 is the first pattern recognition molecule involved and conserved in innate resistance against UTI. Thus, the cellular and humoral arm of innate immunity exert complementary functions in mediating resistance against UTI.

P1.11.046
ER stress sensitizes TLR signaling and desensitizes LPS tolerance through IRE1α-mediated activation of GSK-3β

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Tolerance to endotoxin that is triggered by prior exposure to toll-like receptor (TLR) ligands, provides a mechanism with which to dampen inflammatory cytokines. The ER stress activates GSK-3 to regulate the expression of inflammatory cytokines. Here we found that ER stress induces stimulation of IRE1-mediated tyrosine phosphorylation and serine dephosphorylation of GSK-3, which synergistically activates TLR signaling for cytokine production and obliterates endotoxin tolerance. Furthermore, activated GSK-3 increases the abundance of nuclear coactivator, RIP140 which enhances proinflammatory cytokine production and decreases anti-inflammatory cytokine production. Our results identify an unsuspected critical role for IRE1-mediated GSK-3 activation in inflammation and endotoxin tolerance.

P1.11.047
Investigation of kinase and inflammatory pathway modulation by I2PP2A inhibition in Prostate Cancer Progression

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Background: Inflammation is highly implicated in prostate cancer progression. The toll-like (TLR) and Rig-like (RLR) receptor families are involved in the implementation of innate immune response. PP2A is a tumour suppressor protein, who's dysregulation in cancer promotes activation of proliferation signalling pathways. Peptide mimetics of Apolipoprotein E (apoE) have previously been shown to inhibit SET, a PP2A inhibitor, consequently increasing PP2A mediated phosphatase activity and modulating kinase activation in signalling pathways.
Aims: This study aims to investigate the role of a novel compound which modulates PP2A, and to determine its effects on innate immune receptors and downstream kinase signalling in prostate cancer cell lines while establishing the effects of inhibition of I2PP2A/SET binding to PP2A in prostate cancer cell lines.
Material & Methods: The prostate cell lines used were RWPE1 (immortalised normal), CWR22 (Androgen dependent), 22RV1 (Androgen Independent), DU145 (brain metastasis) and PC3 (bone metastasis). To explore the expression of innate immune receptors, the cell lines were treated with COG compounds for 24, 48 and 72 hours. TLR, RLR, PP2A and SET proteins were probed for by western blot and Immunoprecipitation. A Reverse Phase Protein Array was used to explore alteration in kinase activity.
Results & Ongoing Research: TLRs and RLRs are differentially expressed in prostate cancer cell lines, in addition to PP2A and SET. Toxicity assays revealed that COG compounds can inhibit cell proliferation in prostate cancer cell lines; accompanied by altered TLR expression. Ongoing studies explore the mechanism by which PP2A influences innate immune signalling in prostate cancer.

P1.11.048
Toll-like receptor (TLR)-4 and Hepatitis C Virus (HCV): a systematic review

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Background: Hepatitis C virus induces inflammatory signals, leading to hepatitis, hepatocellular carcinomas and lymphomas. TLR4 has some roles in pathogenesis of hepatitis C, but its mechanism is controversial. To review the available literature on the Importance of TLR4 in pathogenesis of hepatitis C a systematic review of all published articles with the special MeSH terms was done.
Methods: A computerized search strategy using PubMed with MeSH Terms (Hepatitis C, hepacivirus and toll like receptor 4 as the main headings) was done.
Results: The computerized searches between years 1960-2012 yielded 39 articles of which 32 were potentially appropriate for inclusion; 5 out of 32 were on TLR4 gene polymorphisms and 27 were about its functional aspects. In 66%, TLR4's role was evaluated in immune cells, 26% in non-immune cells and in 7% both immune and non-immune cells were assessed. TLR4 up-regulation, down regulation and signaling/downstream effects were reported in 8, 2 and 8 articles respectively. From 8 articles about TLR4 up-regulation, 5 (63%) showed over-expression in immune cells and 3 in non-immune cells. TLR4 down regulation were also observed in both immune and non-immune cells. In 15% (5/32) of articles it is believed that activation of TLR4 is irrelevant of the origin or viral dose.
Conclusion: This is the first review about the Importance of TLR4 in hepatitis C. It seems that TLR4 may play different roles in different cells in HCV hepatic infection. More cell specific studies are recommended.

P1.11.049
TLR-signaling induces Type I interferon responses in microglia and astrocytes and regulates leukocyte infiltration to the CNS

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Our goal is to understand the glial signaling that controls inflammatory responses in the central nervous system (CNS). This glial response can play both a detrimental and beneficial role. Toll like receptor (TLR) signaling is implicated in responses to pathogens or endogenous signals. They mediates immune response by inducing cytokines, including IFN-beta, a member of the type I IFN family. IFN-beta is a first-line therapeutic for multiple sclerosis. Type I IFNs signal through a common receptor, IFNAR. The aim of this study was to investigate the in vivo response of glial cells to CNS administration of TLR ligand, and to examine whether this response involves type I IFN signaling. Mice were administered TLR ligand/agonist by injection to the cisterna magna. Injection of ligand for TLR2 resulted in strong infiltration of leukocytes to the CNS. FACS sorted astrocytes expressed equivalent levels of TLR3 mRNA to microglia but lower levels of TLR2 or 4. Astrocytes were induced by TLR3 signaling to express IRF7, which regulates the induction of type I IFN. The induction of IFN-beta in CNS in response to TLR3 signaling was verified in IFN-beta reporter mice. TLR2, 3 and 4 signaling led to increased levels of mRNA for glial CXCL10. Together these results suggest the involvement of type I IFN signaling. However, unlike CXCL10 gene expression, that was dependent on IFNAR signaling, TLR2-induced leukocyte infiltration was not affected in IFNAR deficient mice. These studies point to a role for TLR signaling in the innate glial response that regulates CNS inflammation.

P1.11.050
A reticulon family protein Nogo is an active component of TLR-triggered innate immune responses in macrophages

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The reticulon family is a large group of membrane-associated proteins localized primarily to the endoplasmic reticulum of most cells. The reticulon family member 4, also known as Nogo, consists of three isoforms, Nogo-A, Nogo-B and Nogo-C. Nogo-A and C are uniquely found in the central nervous system and/or skeletal muscle, and Nogo-A was originally characterized as a regulator of axonal sprouting. Nogo-B is distributed in most tissues including immune cells. However, the function of Nogo-B in the immune system remains unclear. Interestingly, recent reports indicated that Nogo binds to

Paired immunoglobulin-like receptor B (PirB), which is one of the ITIM-harboring inhibitory molecules express on the surface of B cells and myeloid-lineage cells. Since we previously found an inhibitory role of PirB in Toll-like receptor (TLR)9 signaling of B cells, we have attempted to determine whether Nogo could be involved in regulation of macrophages, a key player of pathogen recognition via TLRs. Bone marrow-derived macrophages prepared from Nogo-A/B-deficient mice (kindly provided by Dr. Stephen M. Strittmatter, Yale Univ.) showed attenuated induction of inflammatory cytokines such as IL-6 following stimulation of TLR9, TLR2/1 and TLR2/6 than those of wild-type cells. Then, we found significant delays of IκB-α degradation and of phosphorylation of MAPKs in Nogo-A/B-deficient macrophages after TLR9 stimulation. These findings suggest that Nogo plays a crucial role in activation of TLR-mediated signaling in macrophages. We speculate that Nogo is an active component in TLR signaling, in which Nogo may regulate PirB or other signaling molecules involved in the TLR pathway.

P1.11.051
Analysis of the B cell responsiveness to the TLR9 agonist CPG in malaria semi-immune versus malaria naïve individuals

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In the context of Phase 1 clinical trials of the malaria vaccine candidate AMA1 we found that the novel vaccine adjuvant CPG, TLR9 agonist that activates memory B cells, enhanced the antibody and memory B cell response to AMA1 in malaria-naïve U.S. adults, but not in malaria-immune Malian adults, suggesting that chronic *P. falciparum* exposure leads to TLR9 refractoriness. To follow up on this clinical observation we are conducting a systematic ex vivo analysis in which we compare the phenotype and function of memory B cells obtained from malaria-experienced and naïve individuals. In response to CPG stimulation we measure B cell proliferation, immunoglobulin class switching, cytokine production, antibodies secretion and the expression of co- stimulatory molecules. Male and female at age of 18 or greater from malaria endemic village are equally enrolled. Adult from malaria free area are used as control. Our preliminary results showed that both naïve and memory B cells from malaria semi-immune individuals respond less to CPG stimulation compared to malaria naïve individuals. Also malaria semi-immune individuals have a greater expression of the costimulatory molecule CD86. The next step will consist of comparing the above variables among 30 infected individuals, 30 uninfected individuals from Mali and 30 naïve individuals from the USA.
The results from this study may highlight the importance of testing the efficacy of novel vaccine adjuvants in the target population and may also provide fundamental insights into how chronic *P. falciparum* exposure modulates the innate immune response.

P1.11.052
TLR9-mediated synergistic T-bet expression and its implication for antibody production

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Chromatin-Ig complexes stimulate autoreactive B cells by co-engaging B-cell antigen receptor (BCR) and TLR9. TLR9 is an endosomal Toll-like receptor which recognizes CpG-containing DNA fragments. Its role in the production of pathologic antibodies is well established but the mechanism by which BCR and TLR9 communicate still remains to be determined.
In this work we aimed to characterize the synergistic cross-talk between BCR and TLR9 and prove T-bet as a molecular meeting point that could be responsible for the production of the pathologic antibodies of autoimmune mice.
For this purpose we tested murine lymph node B cells for receptor cross-talk after either simultaneous or single stimulation by anti-IgM or CpG-ODN. Responses were checked for synergy at protein and mRNA levels and experiments for B cell activation and isotype switching were also carried out.
We discovered that the cross-talk between BCR and TLR9 was indeed synergistic; we found enhanced gene and protein expressions and improved proliferation with more cell divisions. Although we could not get synergy for the number of IgG2a-switched B cells nor for the secretion of IgG2a so far, we strongly believe that the synergistic cross-talk between BCR and TLR9 contribute to the development of autoimmune diseases and to the production of pathologic autoantibodies.
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P1.11.053
RIG-I dependent cross-priming of CD8+ T-cells

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Genetic and epigenetic plasticity allows tumor cells to evade immune-mediated elimination and renders single-targeted treatments inefficient. Targeting the cytosolic RNA-helicase RIG-I elicits a potent systemic anti-tumor immune-response and induces apoptosis in tumor cells. Therefore, RIG-I activation is a promising approach in the treatment of cancer. Yet, little is known about the molecular details of RIG-I dependent anti-tumor immune responses. Here we show that engagement of murine RIG-I induces potent peptide-specific CD8+ T-cell responses in vitro and in vivo, but does not support the differentiation of CD4+ T-cells into Th1, Th2 or Th17 cells. RIG-I ligation fosters cross-priming by up-regulation of co-stimulatory molecules, secretion of type-I IFNs and induction of NF-kappa-B dependent proinflammatory cytokines. Cross-primed CD8 T-cells respond with robust IFN-gamma secretion and differentiate into highly effective CTLs. Furthermore, vaccination with RIG-I agonists significantly delays subcutaneous tumor growth in a peptide specific manner. Thus, our data (i) uncover an important role for RIG-I in cross-priming of specific CD8+ cytotoxic T-cells thereby leading to the induction of specific adaptive anti-tumor immune responses and (ii) underscore the usefulness of RIG-I ligands for immunotherapy and vaccination.

P1.11.054
Some features of cellular immunity in patients with chronic spontaneous urticaria inducing reactivation of chronic EBV infection

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In the pathogenesis of chronic spontaneous urticaria (CSU) viral infections, including EBV infection often play the role of triggers. The study of EBV-induced diseases is currently an important health problem, due to the spread of the pathogen, its tropism for immune system cells, a variety of clinical forms and nonspecific treatment.
To study TLRs expression in patients with CSU as a result of immunotherapy by Immunovac-VP-4 and Kagocel. To assess TLRs expression in PBMC via flow cytometry performed with monoclonal antibodies (Caltag Laboratories, USA).
We observed 28 patients with CSU associated with reactivation of chronic EBV infection. CSU patients demonstrated increased as compared to control the levels of TLR2, 4 expression by 3-4 fold exceeding the normal values, while the TLR 3, 9 expression was increased by 2-fold.

Immunotherapy by Immunovac-VP-4 was found to enhance the expression of TLR3, 9. After Kagocel therapy the TLR2, 4 expression tended to decrease. TLR3, 9 levels demonstrated the tendency to increase and approached 42,1±3,2 and 33±3,8%, respectively after the treatment. In the group receiving standard treatment, the decrease in TLR4, 3 expression was observed.
Immunotherapy by Immunovac-VP-4 and Kagocel led to adjusting for TLR2, 4, and increased TLR3, 9 receptor expression to a more pronounced extent in the group receiving Immunovac-VP-4. Immunotherapy enhanced the TLR9 action, indicating the involvement of intracellular receptor mechanism. Inclusion of immunomodulators in the therapy of patients with CSU associated with reactivation of EBV infection promotes the activation of innate immunity, while increasing the effectiveness of therapy.

P1.11.055
Influence of the PAMPs of opportunistic microorganisms on the TLRs expression

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Currently expanded interest in exploring the possibility of regulation of innate immune mechanisms with bacterial antigens, bearing PAMPs of microorganisms, determining the signaling pathways of the immune response. Purpose: To determine the degree of expression of TLRs on spleen cells, lymphoid tissue associated with the respiratory system - BALT / NALT, the intestinal mucosa - GALT at different methods of introducing bacterial vaccine Immunovac-VP-4 (containing antigens of *S. aureus, P.vulgaris, E. colli, K. pneumonia*).
Estimate of the number of cells expressing TLRs, was performed by flow cytometry with monoclonal antibodies (Caltag Laboratories, USA). Mice were vaccinated with Immunovac-VP-4 2-fold at intervals of 3 days subcutaneously (0.2 mg), intranasal (0.5 mg) and oral (2 mg).
The same vaccine Immunovac-VP-4 causes different signaling pathways of cell activation at different methods of administration: subcutaneously increased expression of TLR4, TLR9, TLR2, which causes further cell differentiation by Th-1 and, to some extent, Th-2 ways, with non-invasive methods for the expression of TLR4 and TLR9 involves the further development of immunity by Th-1 path accordingly induction of synthesis of IFN-γ, inhibits the synthesis of IL-4, and further the development of atopy.
Thus, it can be assumed that the different degree of sensitization with different routes of administration of the same drug is predetermined at the stage of interaction of the ligand with the TLR. Subcutaneous immunization Immunovac induced expression of all 3 TLRs, whereas non-invasive methods caused a significant increase in TLR4 and TLR9 in the absence of the dynamics of TLR2.

P1.11.056
Myeloid differentiation protein 2 as the target of curcumin analog in the inhibition of endotoxin LPS-induced TLR-4 activity

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Curcumin is the key constituent in the traditional herb Curcuma Longa, and has been used in diet and traditional medicine for a long time in China. Anti-inflammatory activity and inhibition of LPS signaling are some of its many activities. In spite of the favorable anti-inflammatory property of curcumin, low bioavailability limits the development of curcumin as a potential therapeutic agent. Our previous studies have demonstrated a series of non-steroidal mono-carbonyl analogues of curcumin possessing enhanced stability and improved pharmacokinetic profiles, as well as anti-inflammatory activity in vitro. Among these curcumin analogues, compound L48H37 showed strong inhibitory effects on LPS-induced TNF-α and IL-6 release and inflammatory gene expression in mouse macrophages. Further, we show that L48H37 binds to the myeloid differentiation protein 2 (MD-2), which is the LPS-binding component of the endotoxin surface receptor complex MD-2/TLR-4. Fluorescence emission of MD-2 decreases with an absorbance maximum shift after the addition of L48H37, indicating the interaction of L48H37 and MD-2. In addition, the presence of L48H37 significantly inhibited binding of MD-2 to the immobilized LPS in a concentration-dependent manner, suggesting that the binding site for L48H37 overlaps with the binding site for LPS. This results in the inhibition of MyD88-dependent and -independent signaling pathways of LPS signaling through TLR-4. Finally, the pre-treatment with L48H37 suppressed pro-inflammatory cytokine production in plasma and liver, attenuated lung histopathology, and significantly reduced mortality in endotoxemic mice. Taken together, these findings may reveal important implications of MD-2 specific inhibitors for treatment of inflammatory diseases caused by bacterial infection.

P1.11.057
Immunomodulatory effects of P-MAPA on TLR2, ROS and nitric oxide of blood mononuclear cells from canine with visceral leishmaniasis

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Canine visceral leishmaniasis (CVL) is caused by a protozoan *Leishmania chagasi* is an important zoonotic disease. The parasite in dogs suppress cellular immune response, and the therapeutic arsenal against LVC is limited, the new immunomodulator aggregate fosfolinoleato-palmitoleate magnesium and ammonium protein (P-MAPA) improves immunocompetence when the immune system is impaired, but their dependence on toll like receptors (TLRs) and the mechanisms involved in immune response are still unclear. To investigate the in vitro action of P-MAPA, mononuclear cells were isolated from peripheral blood from healthy dogs and dogs infected with *Leishmania* spp and cultured in the presence of P-MAPA concentrations of 0, 50, 100 and 200 µg/mL in a
humid environment at 37°C with 5% CO2 for 24h. The in vitro observation revealed that the P-MAPA significantly increased the expression of TLR2, ROS and nitric oxide in mononuclear cells from healthy dogs and *Leishmania* spp. infected dogs. These findings suggest that P-MAPA has potential as a therapeutic drug in the treatment of canine visceral leishmaniasis.

P1.11.058
Dendritic cells differentiated using GM-CSF/IL-4 or FLT3L respond differently to TLR ligand maturation signals, affecting the quality of the primed CD8+ T-cell response

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Priming of CD8+ T-cells is highly influenced by the nature of the antigen-presenting DC. Engagement of pattern recognition receptors (PRRs) expressed by DCs induces cytokine production, expression of costimulatory molecules and alters antigen processing and presentation pathways, which collectively shape the priming, expansion and polarization of the adaptive immune response. Despite the widespread use of PRR ligands as adjuvants for vaccination, the mode of action of many of these molecules, either alone or in combination, and their influence on the priming of CD8+ T-cell responses remains largely unknown. We employed the accelerated (acDC) protocol as a simple in vitro model of T cell priming, in order to i) compare the function of DCs differentiated using either GM-CSF/IL-4 or FLT3L, and ii) investigate which combinations of adjuvants (namely TLR ligands) are responsible for the induction of Melan-A-specific CD8+ T-cell responses of the highest quality. Initially, we observed that CD8+ T-cells primed using FLT3L-differentiated DCs (FL-DCs) displayed significantly higher levels of polyfunctionality compared to GM-CSF/IL-4-treated DCs (GM-DCs).

Interestingly, FL-DCs and GM-DCs also responded differently to certain TLR ligands tested. FL-DCs stimulated with TLR8 ligand ssRNA40 primed CD8+ T-cells displaying significantly higher polyfunctionality and cytotoxic potential compared to GM-DCs treated with the same adjuvant. These results suggest that FLT3L and GM-CSF/IL-4 may generate different subsets of DCs, displaying different patterns and/or levels of TLR expression. These findings have important implications for the selection of adjuvants most effective at inducing functional CD8+ T cell responses on vaccination.

P1.11.059
Synergy in cytokine production induced by combinatorial TLR activation

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Toll-like receptors (TLRs) are one of the best-characterized members of pattern recognition receptors (PRRs), which recognize specific pathogen-associated molecular patterns (PAMPs) from invading pathogens. Studies in this area have mainly focused attention on single TLRs recognizing a single PAMP. However, each invading pathogen carries with it multiple PAMPs, and at any one time, a host may encounter different types of pathogens each with multiple PAMPs. Therefore, it is crucial for the host to integrate these infection signals in order to mount the most appropriate and energetically favorable immune response. Previous studies in our lab have demonstrated that combinatorial TLR activation by multiple PAMP stimulation of macrophages can induce synergistic levels of cytokine protein production. It was noted that the synergy effect is subject to both the order and time interval of multiple PAMP stimulations, indicating a crosstalk among TLR signaling pathways, which boosts the immune signaling response. However, the mechanism underlying synergistic cytokine production upon combinatorial TLR activation is poorly understood. Here, we show how the TLRs crosstalk at multiple levels and identify the potential factor(s) that induce synergy in cytokine production. Unraveling the mechanism of TLR crosstalk will shed light on the complicated network of TLR signaling, thus contributing to infection control in pathogen invasion.

P1.11.060
The cationic lipid diC14-amidine and bacterial lipopolysaccharides both activate Toll-like receptor 4 pathways via different binding regions of TLR4

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DiC14-amidine is a cationic lipid which induces pro-inflammatory cytokine secretion in immune cells upon interaction with the Toll-like receptor 4 (TLR4)/Myeloid Differentiation factor-2 (MD-2) membrane bound-complex, the natural sensor of bacterial lipopolysaccharides (LPS) [1, 2].
The aim of the present work is to characterize the interaction between diC14-amidine and the TLR4/MD2 receptor complex. Taking advantage of the species-dependent activity of TLR4 agonists [3], we compared the TLR4 agonist activity of diC14-amidine in four different species in order to map domains in TLR4 and MD2 that are important for diC14-amidine TLR4-agonist activity. We demonstrate that, while LPS is an agonist in all species, diC14-amidine is a full agonist for human, mouse and cat receptors, but a poor agonist for horse. Using chimeric constructs made from human and horse TLR4 and single mutants, we identify two regions in the human TLR4 that modulates the agonist activity of diC14-amidine. Interestingly, these regions in TLR4 are different from the previously identified bacterial lipopolysaccharides binding domains [4].
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P1.11.061
Dectin-1 induces efficient T cell immunity and prominent expansion of CD8+ IL-17+ cells in a pulmonary model of fungal infection

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Dectin-1 is a C-type lectin receptor which binds to β1,3-glucan present in fungal pathogens and is required for fungal resistance in mice and humans. It promotes phagocytosis and produces cytokines in collaborative signaling with TLRs. However, in the paracoccidioidomycosis the mechanisms that govern interaction between hosts and the fungus Paracoccidioides brasiliensis have been scarcely studied. Objectives: The aim of our study was to characterize the role played by Dectin-1 in pulmonary paracoccidioidomycosis. Methodology: Wild-type (WT) and Dectin-1-KO mice were used. Measurement of nitric oxide and cytokines after macrophage infection with P.brasiliensis were performed. In vivo, after intra-tracheal infection, we analyzed fungal burdens, pulmonary cytokines, histopathological analysis and assessment of leukocyte subpopulations in lung inflammatory exudates. Results: We verified that Dectin-1-KO macrophages infected with P. brasiliensis presented increased fungal loads associated with impaired synthesis of NO and IL-6 associated with increased production of IL-10 and MCP-1. In vivo, Dectin-1-KO mice produced diminished levels of Th1, Th2 and Th17 cytokines. In addition, Dectin-1-KO mice developed an impaired immune response, evidenced by poorly activated CD8+ T cells to the lungs and impaired Th17 immune response. These events led to increased fungal loads in the lungs of Dectin-1KO mice and allowed a marked dissemination of the fungus to other organs. As consequence, Dectin-1KO mice were unable to control fungal growth and presented a decreased survival time. Conclusions: Our findings demonstrate that Dectin-1 receptor is important to the activation of fungicidal mechanisms and to the induction of the innate and adaptive immunity against P. brasiliensis.

P1.11.062
MSK1 and 2 inhibit LPS induced prostaglandin production via an IL-10 feedback loop

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Prostaglandin production is catalysed by cox-2. We demonstrate here that MSK1/2 can exert control on the induction of cox-2 mRNA by TLR agonists. In the initial phase of cox-2 induction MSK1/2 knockout macrophages confirm a role for MSK in the positive regulation of transcription. However at later time points both LPS induced prostaglandin and cox-2 protein levels were increased in MSK1/2 knockout. Further analysis found that while MSKs promoted cox-2 transcription, following longer LPS stimulation MSKs also promoted degradation of cox-2 mRNA. This was found to be the result of an IL-10 feedback mechanism with endogenously produced IL-10 promoting cox-2 degradation. The ability of IL-10 to do this was dependent on the mRNA binding protein TTP through a p38/MK2-mediated mechanism. As MSKs regulate IL-10 production in response to LPS, MSK1/2 knockout results in reduced IL-10 secretion and therefore reduced feedback from IL-10 on cox-2 mRNA stability. Following LPS stimulation, this increased mRNA stability correlated to an elevated induction of both of cox-2 protein and prostaglandin secretion in MSK1/2 knockout macrophages relative to wild type cells. This was not restricted to isolated macrophages, as a similar effect of MSK1/2 knockout was seen on plasma PGE(2) levels following intra-peritoneal injection of LPS.

P1.11.063
Functions of endogenous TLR4 ligands

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We have previously proposed that Toll-like receptor (TLR) 4 recognizes not only lipopolysaccharide (LPS) derived from pathogens but also some bioactive peptides of host origin such as S100A8 and SAA3, both of which we showed facilitate lung metastasis. Here we report that a synthetic SAA3 peptide bound MD-2 directly and stimulated cell migration in a CD14-independent manner. In contrast to LPS that induces both MyD88 and TRIF-dependent signaling, SAA3 promoted only the MyD88 pathway with little endocytosis of TLR4. In pre-metastatic lungs, pulmonary recruitment of CD11b+Gr1+ cells precedes the actual tumor cell arrival. We found that the SAA3 peptide induced their recruitment in an MD-2-dependent fashion. Moreover, we have discovered two mechanisms that allow tumor cell entry into the lungs. First, SAA3 directly promotes lung permeability. Tumor burden induced segmental vascular permeability regions in the pre-metastatic lungs with a significant up-regulation of CCL2, which in turn stimulated SAA3 expression. Tumor-mediated lung permeability and metastasis were abrogated in CCR2 knockout mice. Second, S100A8 induced expression of not only SAA3 in the pre-metastatic lungs but also that of the membrane-bound ephrin-A1 in primary tumors. TGFβ in the primary tumors activated ADAM12, which in turn shed the ephrin-A1. The released soluble ephrin-A1 then stimulated its receptor EphA1 and A2 in lung endothelial cells in an endocrine manner to deteriorate vascular barrier. In conclusion, we show that an endogenous peptide ligand(s) for TLR4 exists in mammals and, by binding MD-2, facilitates extravasation of leukocytes and tumor cells in the pre-metastatic lungs.

P1.11.064
Similarities and differences of innate immune responses elicited by smooth and rough LPS

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The lipopolysaccharide is the major component of Gram-negative bacteria outer membrane. LPS comprises three covalently linked regions: the lipid A, the rough core oligosaccharide, and the O-antigenic side chain determining serotype specificity. Wild-type LPS (sLPS) contains the O-antigenic side chain and is referred to as smooth. Rough LPS (rLPS) does not contain the O-side chain. Most wt bacteria and especially wt Enterobacteriaceae express prevalently the sLPS form. The two sLPS and rLPS forms are used almost indistinctly to study the effects on innate immune cells. Nevertheless, there is evidence that their mechanism of action may be different. Here we have identified additional differences in the signaling capacity of the two LPS species in the mouse. We have found that rLPS, diversely from sLPS, is capable of activating in dendritic cells (DCs) the Ca2+/calcineurin and NFAT pathway in a CD14-independent manner, moreover it is also capable per se of activating the inflammasome and eliciting IL-1 secretion independent of the presence of additional stimuli required instead for sLPS. The ability of rLPS of activating the inflammasome in vitro has as a direct consequence a higher efficiency of rLPS-exposed DCs in activating natural killer (NK) cells compared to sLPS-exposed DCs. However, diversely from possible predictions, we found that the different efficiencies of the two LPS species in eliciting innate responses are almost nullified in vivo. Therefore, sLPS and rLPS induce nearly similar in vivo innate responses but with different mechanisms of signaling.

P1.11.065
Multi-step regulation of STAT1 phosphorylation in response to double-stranded RNA

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Pattern recognition receptors which sense non-self RNA trigger to produce type I IFN. Type I IFN binds to its cognate receptor, IFNAR, resulting in the activation of STAT1. Therefore, it has long been thought that dsRNA-introduced STAT1 phosphorylation is through transactivation of type I IFN signaling. This study asked whether the phosphorylation of STAT1 is absolutely type I IFN-dependent. A synthetic dsRNA polyinosinic-polycytidylic acid (polyI:C) induces STAT1 phosphorylation in A549 human lung epithelial cells. We found the polyI:C induced STAT1 phosphorylation in a predominantly IFN-dependent manner. However, we also found that polyI:C was able to stimulate STAT1 phosphorylation in type I IFN receptor-deficient U5A cells, suggesting this phosphorylation is type I IFN-independent. Retinoic acid-inducible gene-I (RIG-I) was involved in the initial STAT1 phosphorylation in response to polyI:C; however, little contribution of RIG-I was observed in the late STAT1 phosphorylation. Taken together, our results showed comprehensive regulation in which dsRNA induces STAT1 phosphorylation, indicating importance of STAT1 with such a tight regulation in innate immune system.

P1.11.066
Hepatic TDO2-derived L-kynurenine controls acute inflammatory responses by engaging the Aryl hydrocarbon Receptor

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Tryptophan catabolism along the kynurenine pathway is an important mechanism of immune tolerance by induction of regulatory T cells (Treg). Recently, the amino acid L-kynurenine, a byproduct of tryptophan catabolism, was found to activate the Aryl hydrocarbon receptor (AhR) in CD4+ T cells, which effect contributes to the induction of adaptive Treg cells. However, the role of tryptophan catabolism in regulating innate immune responses has been unclear. Here we demonstrate that an initial exposure to lipopolysaccharide (LPS) induces a rapid increase in endogenous kynurenine in wild-type as well as indoleamine 2,3 dioxygenases (IDO1 or IDO2)-deficient mice. In contrast, LPS fails to promote protective kynurenine/AhR-dependent responses in mice lacking hepatic tryptophan 2,3 dioxygenase (TDO2), which is an early component of the acute phase protein response in inflammation. These results suggest a critical role of TDO2 in the kynurenine-driven AhR repression of early, LPS-responsive inflammatory genes. Increased levels of proinflammatory cytokines were detected in sera from TDO2-deficient mice as compared to WT mice or mice lacking IDO1 or IDO2, with the recruitment of migratory neutrophils responsible for multiorgan infiltration. However, the administration of exogenous kynurenine reverted the effect of TDO2 deficiency in the response of AhR-competent mice to LPS. In summary, our data suggest that, upon LPS exposure, TDO2-dependent production of L-kynurenine activates AhR to limit inflammation and systemic host damage. These findings also suggest new therapeutic options in controlling early inflammatory gene expression in endotoxemia.

P1.11.067
Expression of TLR2, 3, 9, MYD88 and TRIF in Kawasaki patients before and after IVIG therapy

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Kawasaki disease (KD) is an acute childhood vacuities with inflammation of medium-sized arteries throughout the body, particularly of the coronary arteries. Toll-like receptors are type 1 transmembrane protein which play a critical role in recognition of microbial antigen and use NF-KB signaling pathway to induce massive number of inflammatory genes. This study was designed the role of TLR2, 3, 9, MyD88 and TRIF in immunological pathogenesis of KD.

31 patients with KD and 20 age-matched febrile children due to pneumonia plus 20 healthy children were studied. Real time PCR was used to evaluate the expression of TLR2, 3, 9, MyD88 and TRIF in peripheral blood mononuclear cells.
There were no significant differences in expression levels of TLR3 and TLR9 in comparison normal group.TLR2 gene transcript level was significantly elevated in KD in comparison to normal subjects ($P<0.05$). There were a significant differences in transcript level of MyD88 and TRIF ($P=0.03$). TLR2, 3 and 9 gene transcripts have been down regulated in peripheral blood of KD following IVIG therapy compared with expression levels of TLRs before IVIG therapy in acute febrile state. Following IVIG therapy MyD88 gene transcripts were down regulated in KD patients.
Aberrant activation of TLR2 or TLR3 (cause up-regulation of MyD88&TRIF) might be one of the initiating factors of immune aberrance in KD. IVIG causes general down-regulation and inhibition of TLR pathways which suggest as a new mechanism of anti inflammatory effect of IVIG.

P1.11.068
CpG ODN ligation of TLR-9 stimulates IFN-α and antinuclear antibody production in SLE patients

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As there is no direct *in vivo* data yet for TLR-9 mediated mechanism of antinuclear antibody and IFN-α production in SLE, the level of TLR-9 expression on peripheral blood mononuclear cells in 35 SLE patients and 35 healthy controls was analyzed. The effect of TLR-9 ligation in IFN-α production was also investigated. PBMCs were stained with PE-conjugated mAb against TLR-9 and the expression of mRNA TLR-9 was determined. Antinuclear antibody titer was also determined in serum. Ultimately, the IFN-α concentration was measured in supernatants of PBMCs following CpG stimulation. Based on achieved data, the percentage of TLR-9-positive PBMCs and the TLR-9 mRNA expression were significantly higher in patients. There was a significant positive correlation between ANA titer and percentage of TLR-9 positive PBMCs. Also IFN-α concentration in patients significantly increased in response to CpG stimulation. In this study we provide evidence that TLR-9 plays an important role in SLE pathogenesis.

P1.11.069
Genetic polymorphisms in host innate immune sensor genes and the risk of Nasopharyngeal carcinoma in North Africa

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Nasopharyngeal carcinoma (NPC) is an Epstein-Barr virus-associated malignancy with an unusual racial and geographical distribution. The interaction between host pattern-recognition receptors and viruses profoundly influences infection dynamics. Therefore, we studied the association between single nucleotide polymorphisms (SNPs) in selected genes involved in host innate immunity (including families of toll-like receptors, C-type lectin receptors, and RIG-I-like receptors) and the risk of NPC. In total, 26 SNPs in five pattern-recognition genes (CD209, DDX58, MBL2, TLR3 and TLR9) were genotyped in 492 North African NPC cases and 373 frequency-matched controls. The most significant association adopting a dominant model was observed for a functional SNP in TLR3. The analysis showed also that SNPs within CD209 and DDX58 are associated with NPC risk. Moreover, an 18% increased risk per allele was observed for the five most significantly associated SNPs. Our results suggest that genetic variation in pattern-recognition genes is associated with the risk of NPC. These preliminary findings require replication in larger studies.

P1.11.070
The self-perpetuating mechanism of antiphospholipid antibody production depends on TLR7 activation and endosomal superoxide generation

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Objective. Antiphospholipid antibodies (aPLs) are associated with thrombosis and recurrent abortions during autoimmune pathologies. To elucidate the underlying signaling pathways leading to autoantibody secretion we analyzed the interaction between plasmacytoid dendritic cells (pDCs) and different B cell populations with special focus on the role of TLR7 and endosomal superoxide production.
Methods. We cultured aPL stimulated human pDCs together with allogeneic B cells. Upregulation of costimulatory molecules, plasma cell differentiation and memory B cell activation were determined by flow cytometry. aPL secretion was measured using appropriate ELISAs. Furthermore we immunized different transgenic mouse strains with aPL and CFA. APL titer was determined after 6 weeks in blood obtained from tail veins.
Results. We demonstrated that aPL induced upregulation of costimulatory molecules on pDCs depend on endosomal superoxide generation and TLR7 activation. Cocultured with B cells these stimulated pDCs are able to induce plasma cell differentiation and the proliferation of memory B cells secreting aPL antibodies. These *in vitro* data could be confirmed by immunizing transgenic mice with a human aPL. While wiltype mice and TLR4 deficient mice produce murine aPL, no aPL production could be detected in $TLR7^{-/-}$ or $Gp91phox^{-/-}$ mice. The latter do not express NADPH-oxidase2 and therefore are unable to generate endosomal superoxide.
Conclusion. We could demonstrate that aPL activated pDCs have the potential to induce aPL secretion by preformed memory B cells. Thereby TLR7 activation and endosomal ROS generation play a pivotal role in this self-perpetuating mechanism of autoantibody production.

P1.11.071
Dual regulation of osteopontin production by TLR stimulation in dendritic cells

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Osteopontin (OPN), a cytokine produced, among others, by dendritic cells (DC), is involved in inflammation and defence against pathogens. Here we report that the activation of the MyD88 pathway by TLR2, TLR5 and TLR7/8 agonists, or IL-1β, induces high levels of OPN in human DC. Conversely, LPS and Poly I:C, two TLR3 and TLR4 agonists that engage the TRIF pathway, were ineffective. TLR2 agonists were the strongest OPN inducers, and OPN production was highly stimulated by TLR2-triggering bacteria (*S. aureus*), but not by TLR4-triggering *E. coli*. Co-stimulation experiments revealed that TLR3 and TLR4 agonists, beyond being inactive by themselves, sharply limited TLR2-dependent OPN production, by activating a TRIF-dependent inhibition of the MyD88-dependent OPN production. MyD88 silencing impaired TLR2-dependent OPN induction, whereas TRIF pathway blockage by chloroquine, dynasore or TRIF knockdown prevented the TLR3/4 agonist-mediated inhibition, which was independent from the endogenous production of type I IFN, IL-29, IL-10 or TGF-β. LPS and Poly I:C inhibitory activity was associated with the release of a >10 kDa protein factor(s). We also demonstrated that the higher OPN levels produced by *S. aureus*-treated DC, compared to *E. coli*-treated DC, were responsible for a markedly increased

production of IL-17 by $CD4^+$ T cells. These results highlight the biological relevance of the differential OPN induction by TLR2 and TLR4 agonists, and emphasize the importance of TLR cross-talk in OPN induction. This implies that OPN regulation by TLR signaling is critical in shaping inflammatory responses, and may modulate IL-17 production in response to pathogens.

P1.11.072
Toll-like receptor genetic polymorphism influences susceptibility and severity to tuberculosis in the Indian Population

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Variations within TLR genes may, in part impair the ability of certain individuals to respond to the TLR ligands thus altering the susceptibility to inflammatory diseases. We investigated an association of candidate SNPs in TLR9, TLR4 and TLR2 genes with pulmonary tuberculosis. The data revealed significantly reduced frequency of TLR9 (-1237C) in patients versus controls (12% vs. 23%, p<0.0001, OR=0.45) and significantly increased frequency of *TLR4* Asp299Gly mutation in the patient group (17% vs. 8.8%, $\chi 2$= 10.7, p = 0.001, OR=2.1). Further, the homozygous mutant allele (TLR9-1237CC) was absent in patients with severe PTB infection group vs. healthy controls (5.5% vs. 0%). The TLR4 896GG mutant occurred only in patients with high bacillary load (3+) and those with far advanced lung disease. Similarly, the mutant 1196T was significantly pronounced in the homozygous state (TT) in these patients. These results suggest a possible association of TLR9 T-1237C polymorphism with protection to *M.tb* infection, while TLR4 substitutions at residues 299 and 399 are associated with susceptibility to pulmonary TB, particularly the most severe disease. TLR4 C1196T and TLR9 (T-1486C) mutations on the other hand occurred with comparable frequencies among patients and healthy controls(12.6% Vs 9% and 42% vs. 40% respectively). TLR2 polymorphism C2180T occurred at a frequency of 50% in both healthy controls as well as PTB patients, while the TLR2 (2408G/A) mutation was completely absent in the North Indian population. These results suggest that TLR2 gene does not predispose individuals to acquire tuberculosis, in this population.

P1.11.073
Association study between Toll-like receptor 4 single-nucleotide polymorphisms and bipolar disorder

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Signs of inflammation and immune dysfunctions have repeatedly been reported in bipolar disorders (BD). Moreover, early-life insults of infectious origin and reactivation of expression of the Human Endogenous Retrovirus type W have also been associated. In this context, immunogenetic background may modulate the effect of immune inflammatory responses and is thus of particular interest in this field. In fact, innate immune response molecules such as Toll-like receptor 4 (TLR4) have already been implicated in neuroinflammation and genetic variants associated with susceptibility to several inflammatory disorders. Therefore, we hypothesized that TLR4 single-nucleotide polymorphisms (SNPs) may influence susceptibility to BD. A case-control study was conducted involving 572 patients with BD type I or II and 152 healthy controls. Among BD patients, 231 were classified as early-onset, defined by an age at onset before the age of 21. Genotyping of six SNPs of TLR4 gene was performed using TaqMan®. We found an association between rs1927914 ($p < 0,05$) and rs11536891 ($p < 0,01$) and early-onset BD, influenced by the season of birth as it was only observed among non-winter born individuals. Our preliminary data suggest the existence of immunogenetic patterns of susceptibility and the importance of gene-environment interactions in BD.

P1.11.074
Schistosome-activated B cell surface toll-like receptor-4 expression correlates with CD23 and IgM expression

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Activation of adaptive immunity depends on the preceding innate immune recognition and signaling, with specific responses varying according to the involved receptors. B cell activation through toll-like receptors triggers production of pro-inflammatory cytokines and chemokines, and expression of co-stimulatory molecules by antigen presenting cells (APCs). These molecules are also important in responses to lipopolysaccharide signaling through toll-like receptor-4 (TLR4), and together with the microbial antigens presented by APCs they activate the CD4 T cells that initiate most adaptive immune responses. B cell role in resistance to *Schistosoma mansoni* reinfection has been associated with CD23 expression. We investigated B cell TLR4 in relation to CD23 expression during *S. mansoni* infections. Whole blood cells from 36 infected adult males were stained using fluorochrome-conjugated antibodies, and analyzed on a FACS Calibur (BD). B cell surface expression of CD23, TLR4, CD69 and IgM was determined using Flowjo (v. 7.5) and statistically analyzed using GraphPad Prism v.5. B cell TLR4 expression (%) was strongly associated with CD69 (p<0.0001), IgM (p<0.0004) and CD23 (p=0.0285) expression, but not MFI (p>0.05). Over 98% peripheral B cells expressed IgM but with lower MFI than TLR4 (p=0.0205). We found no association between the expression of any of these molecules with *S. mansoni* egg counts in stool. TLR4 expression was not different between B cells from praziquantel-naïve and severally-treated persons (p=0.6083), but the latter had lower TLR4 MFI (p=0.0392). TLR4 may be important in B cell recognition of schistosome antigens and the resultant CD23-mediated anti-schistosome responses involved in protection from re-infection.

P1.11.075
Toll-like receptor -2, -4 and -9 genetic variants in Mexican patients with amoebic liver abscess

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Toll-Like Receptors (TLRs) recognize *Entamoeba histolytica* molecular patterns generating a series of intracellular signaling that leads to the modulation of the innate immune system. Single nucleotide polymorphisms (SNPs) in TLR genes may influence their activity. We studied associations of TLRs SNPs with susceptibility to develop amoebic liver abscess (ALA) by *Entamoeba histolytica* in Mexican individuals from the State of Sonora and from Mexico City (DF). We typed by allelic exclusion the SNPs R677W and R753Q of TLR2, D299G and T399I of TLR4, and -1237T/C and 2848G/A of TLR9. We compared SNPs frequencies of ALA patients (Sonora n=32, DF n=32) with control individuals (Sonora n=58, DF n=21). The genotype TLR9 2848G/A in ALA patients from DF showed a statistically (p=0.008) increased frequency (63.3%) when compared with controls (25.0%), with an OR of 5.18 (95% CI 1.27-22.37). We will increase sample size in order to find out if the TLR9 2848G/A can be considered as a marker of susceptibility to ALA in population from the center of Mexico. Work partially supported by PAPIIT IN206408, IN206405, PAPIME 200105 and SEP-CONACYT 79220.

P1.11.076

The role of Damage Associated Molecular Patterns (DAMPs) in the genetic susceptibility towards cigarette smoke induced neutrophilic airway inflammation

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Damage Associated Molecular Patterns (DAMPs) that are released from necrotic epithelial cells upon exposure to cigarette smoke (CS) have been proposed to contribute to neutrophilic lung inflammation in Chronic Obstructive Pulmonary Disease (COPD). COPD is a progressive lung disease characterized by sustained neutrophilic airway inflammation caused by chronic exposure to noxious gasses and particles like cigarette smoke. Only 20% of smoking individuals develop COPD, indicating a role for genetic susceptibility. We hypothesize that the profile of DAMPs released upon CS-exposure contributes to the susceptibility to develop neutrophilic airway inflammation.
30 Inbred mouse strains were exposed to CS or air (control) in a 5-day exposure model. Subsequently, neutrophilic airway inflammation and the DAMP profile (e.g. HMGB1, HSP70, dsDNA, mtDNA) in the BAL fluid were determined in all mice by standard morphology, ELISA and qPCR. Haplotype Association Mapping (HAM), using 4 million SNPs, was performed to identify susceptibility genes for DAMP (dsDNA) release after CS exposure.
Linear regression analysis showed a positive correlation between neutrophilia and most, but not all, DAMPs. In a stepwise forward regression analysis dsDNA (p=0.013) appeared to be the best predictor for neutrophilic airway inflammation. HAM analysis of dsDNA revealed several susceptibility genes contributing to DAMP release after CS exposure including genes that are functionally mapped to cell-death pathways.
In conclusion, mice susceptible for neutrophilic airway inflammation after CS-exposure also show increased DAMP release which is associated with genes involved in the regulation of cell-death pathways.

P1.11.077

Friends of our enemy are our enemy: Bacteria modulate cancerous behavior of prostate cancer cell lines via TLR signaling

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Background and aim: Toll like receptors (TLR) trigger inflammatory responses upon microbial sensing. Inflammation has long been associated with cancer development. The aim of the present study was to investigate impact of Gram-negative bacterial lipololysaccharide (LPS) and Gram-positive bacterial lipoteichoic acid (LTA) on cancerous behavior of prostate cancer cell lines. **Materials and methods:** Expression of TLR1-10, CD14 and MyD88 transcripts and protein expression of TLR2 and 4 was investigated by RT-PCR, flowcytometry and Western blotting, respectively. Experiments were set up to assess effects of LPS and LTA at different concentrations and times on cell proliferation, invasion, adhesion and cytokine production. **Results:** Prostate cancer cell lines differentially expressed TLR1-10, MyD88 and CD14. LNCap and DU145 failed to express TLR2 and TLR4 genes, respectively. Flowcytometeric analysis showed that none of prostate cell lines express TLR2 and TLR4 on their surface. Interestingly, prostate cell lines lacking the surface expression of TLR2 or 4 had positive expression pattern in Western blot indicating intracellular expression of these receptors. LPS and LTA treatment of DU145, significantly increased cell proliferation regardless of lacking cognate receptor for LPS. Similarly, LTA treatment increased proliferative response of LNCap. Prostate cancer cell lines differentially produced pro-inflammatory cytokines in response to LPS or LTA treatment. While invasive capacity of PC3 or DU145 was not affected by aforesaid treatments, LPS caused increased invasiveness of LNCap. Stimulation of PC3 with LPS and LTA was associated with increased cell adhesion. **Conclusion:** Bacterial-induced pro-inflammatory milieu may shape the cancerous behavior of prostate malignancies via TLR signaling.

P1.11.078

Using recombinant lactococci constructs as a molecular tool to dissect the immunomodulating capacity of surface piliation in *Lactobacillus rhamnosus* GG

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Lactobacillus rhamnosus GG, supported by its well-documented clinical benefits, is one of the most widely used probiotics in the food and health-supplement industries. Despite this, numerous aspects of its molecular mechanisms and limitations as a probiotic remain unknown and thus are still to be explored and dissected. Given that *L. rhamnosus* GG is one of only a few such strains exhibiting surface piliation (called SpaCBA), we sought to examine whether this particular type of cell-surface appendage has a discernible immunomodulating capacity and can stimulate responses in human immune-related cells. To do this, we recombinantly engineered *Lactococcus lactis* to produce native (and pilin-deleted) SpaCBA pili that were assembled in a structurally authentic form and anchored to the cell surface, and which had still retained mucus-binding functionality. As a molecular tool, we used these recombinant lactococcal constructs to demonstrate that SpaCBA pili can contribute to the activation of Toll-like receptor 2-dependent signaling in a HEK-TLR2 reporter cell line as well as to the modulation of inflammatory cytokine production in human monocyte-derived dendritic cells. Based on these results, we offer the possibility that the recombinant-expressed and surface-anchored SpaCBA pilus, given its projected functioning in the gut environment, is a novel microbe-associated molecular pattern (MAMP)-like modulator of innate immunity. With this study, we have been able to further add mechanistic details into the molecular immunogenicity of the SpaCBA pilus, which will help lead to a clearer understanding of its role in the multifactorialness of *L. rhamnosus* GG health-related functioning in the human gut.

P1.11.079

LPR mice with TIR8 deficiency are prone to develop B-cell lymphomas

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The association among autoimmunity, chronic inflammation and malignancy has been described and confirmed by epidemiological studies. Notwithstanding these achievements, the molecular mechanisms underlying this association are still unknown. Several evidences suggest that patients suffering from Systemic Lupus Erythematosus, Rheumatoid Arthritis and Sjogren's Syndrome are prone to develop B-cell Non-Hodgkin's Lymphomas. We investigated the role of Tir8/Sigirr gene, already known to be associated with autoimmunity, in the development of lymphoma. Indeed, the ability to dampen signaling from IL-1R and TLR family members confers TIR8/SIGIRR the ability to act as regulator of inflammation, cancer-related inflammation and autoimmunity. In this study we describe the occurrence of B-cell lymphoma in B6lpr/lpr and B6lpr/lpr/Tir8−/− mice. Both strains developed Diffuse Large B-Cell Lymphoma (DLBCL) during their late age, but in B6lpr/lpr/Tir8−/− mice DLBCL occurred earlier (10-12 months vs 15-18 months) and were more aggressive, with significantly higher mortality (100% vs 22%, respectively in 15 months old B6lpr/lpr/Tir8−/− and B6 lpr/lpr mice). Histopathologic

analysis of spleen and lymph nodes of B6lpr/lpr/Tir8 mice documented clear-cut DLBCL areas arising within a context of atypical lymphoproliferative disorder; these results were corroborated by both molecular analysis and transplantation experiments. Clonal rearrangement was present in both strains. However, only recipients of spleen or lymph node cell suspensions collected from B6lpr/lpr/Tir8−/− mice developed DLBCL. These observations unveil a role of TIR8 in the occurrence and development of DLBCL, suggesting its potential role in targeted therapy. Moreover, the B6lpr/lpr/Tir8−/− mouse could be a model to establish and evaluate studies of novel therapeutic protocols in DLBCL.

P1.11.080
Vav proteins mediate Syk-coupled C-type lectin receptor-induced innate immunity

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Fungal infections are a major cause of morbidity and mortality worldwide - especially in immunocompromised individuals. Yet, anti-fungal immune defense mechanisms are poorly understood. C-type lectin receptors (CLRs) expressed by myeloid cells recognize structures present on fungi and other pathogens as well as endogenous danger molecules. They are crucial for host protection and immune homeostasis. Following ligand binding, CLRs activate via their cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAM) or by coupling to ITAM-containing signaling chains distinct intracellular signaling cascades that mediate the expression of specific cytokines. Besides the essential role of the tyrosine kinase Syk and the adaptor protein Card9 not much is known about the molecular mechanism involved in CLR triggered inflammatory responses. Here we demonstrate that specific Vav isoforms are differentially involved in cytokine responses to Dectin-1, Dectin-2, or Mincle stimulation in dendritic cells. Moreover, mice lacking certain isoforms of the Vav protein family are severely impaired in anti-fungal host defense. Thus, our results define distinct Vav isoforms as critical signaling molecules for CLR induced immune responses.

P1.11.081
Cigarette smoke modulates the oral epithelial cell innate immunity

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The gingival epithelium is becoming known as a regulator of the oral innate immune responses to a variety of insults such as bacteria and chemicals, including those chemicals found in cigarette smoke. Epithelial cells play an important role in the tissue innate immunity by specifically expressing certain receptors, including toll-like receptors (TLRs) that are involved in host immune response. Oral epithelial cells are most cells targeted by tobacco smoke. This may lead to innate immunity dysregulation. The purpose of this study was to investigate the effects of cigarette smoke on expression/production of Toll-like receptors (TLRs), antimicrobial peptides, and proinflammatory cytokine expression in primary human gingival epithelial cells. Following exposure to whole cigarette smoke there was an increase of TLR2, TLR4 and TLR6 expression. Cigarette smoke led to ERK1/2, p38 and JNK phosphorylation in conjunction with nuclear factor-κB (NFκB) translocation into the nucleus. TLR expression following cigarette smoke exposure was down regulated by the use of ERK1/2, p38, JNK MAP kinases, and NFκB inhibitors, suggesting the involvement of these signaling pathways in the cellular response against cigarette smoke. Cigarette smoke also promoted HBD2, HBD3, IL-1β, and IL-6 expression through the ERK1/2 and NFκB pathways. Interestingly, the modulation of TLR, HBD, and cytokine expression was maintained long after the gingival epithelial cells were exposed to smoke. By promoting TLR, HBDs, and proinflammatory cytokine expression, cigarette smoke may contribute to innate immunity dysregulation, which may have a negative effect on human health. (Funded by the NSERC, Canada, Fonds Emile-Beaulieu, U. Laval).

P1.11.082
The role of NF-κB oscillation in inflammatory responses to Salmonella infection

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Macrophages play an important role in the innate immune response to Salmonella infection. Macrophages detect Salmonella through Toll-like receptor 4 (TLR4), which binds to lipopolysaccharide (LPS) on the Salmonella membrane, and subsequently initiates inflammatory responses via the activation of nuclear factor-κB (NF-κB). Activated NF-κB translocates to the nucleus where it triggers the transcription of inflammatory genes. NF-κB is subsequently inactivated and shuttles back to the cytoplasm. Under a continuous LPS stimulation, NF-κB is activated again and reenters the nucleus to initiate further transcription. This suggests that inflammatory cytokine expression may be maintained by the repeated activation of NF-κB oscillating between the cytoplasm and the nucleus in response to LPS.
This study has investigated if there is a correlation between the pattern of NF-κB oscillation and TNFα expression by time-lapse microscopy using RAW264.7 cells fluorescent reporters. NF-κB oscillation was quantified as the ratio of the nuclear fluorescent intensity to the cytosolic one. The results showed that the first peak of the ratio became higher in a LPS dose-dependent manner. In addition, the number of the peaks increased with increasing LPS concentration. A strong correlation between the height of the first peak and TNFα expression could be seen while there was no correlation between the number of the peaks and TNFα expression.

P1.11.083
Characterization of SSc5D, a molecule of the Scavenger Receptor Cysteine-Rich Superfamily, as an innate immune receptor

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SSc5D is a member of the Scavenger Receptor Cysteine-Rich (SRCR) superfamily. This group of proteins is characterized by the presence of one or more SRCR domains, which are thought to mediate protein-protein interactions within the immune system and/or to serve as pathogen pattern recognition receptors. However, the overall function of the SRCR family is still poorly understood. Unraveling the biological and functional properties of SSc5D may thus contribute to clarification of the role of this group of highly conserved proteins. The cellular expression of SSc5D was analyzed in different types of cell by quantitative PCR and immunohistochemical staining of various tissues such as uterus, placenta, spleen, kidney, and others. Our results show that SSc5D is differentially expressed in myeloblastic, monocytic and lymphocytic cell lines, and also detected in some tissue-specific cell lines. By immunohistochemical staining, high levels of SSc5D expression in placenta were detected, most noticeably in syncytiotrophoblast cells, which can suggest a possible role in fetal immunity. In addition, we also observed high expression of SSc5D in the gastrointestinal tract, which is in agreement with the possible role of SSc5D as an innate immune receptor. Overall, our results indicate that SSc5D may act as a molecular bridge in defensive interactions established between immune cells and pathogens.
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P1.11.084
Essential role of IRF3 in microbiota-contributed Tslp induction and protection against intestinal inflammation

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The large intestinal epithelial cells and immune cells are exposed to a variety of pathogen associated molecular patterns (PAMPs) derived from commensal microbiota. Although recent studies suggest that constitutive activation of these cells through pattern recognition receptors (PPRs), such as Toll-like receptors (TLRs) or RIG-like receptors (RLRs), are required to keep homeostasis rather than inflammation of large intestine, the underlying gene regulatory mechanisms are not well understood. Here we demonstrated that IFN regulatory factor (IRF)3 had a critical role in the suppression of dextran sulfate sodium induced colitis. IRF3-deficient mice exhibited lethal defects in both inflammatory and recovery phases of colitis with decreased expression of thymic stromal lymphopoietin (TSLP), a cytokine known to be essential for protection of the large intestine. We further provided evidence that DNA and RNA of the large intestinal contents are critical for Tslp gene induction via IRF3 activation by cytosolic nucleic acid receptors. We also found that Tslp promoter contains ISRE sequence to which IRF3 binds and the cooperation between IRF3 and p65 synergistically induces the activation of Tslp promoter. This newly identified intestinal gene regulatory mechanism, wherein IRF3 activated by microbiota-derived nucleic acids plays a critical role in intestinal homeostasis, may have clinical implication in the treatment of colonic inflammatory disorders.

P1.11.085
Investigating the deregulation of Toll-like receptors, pro-inflammatory cytokines and cationic peptides (Human Beta Defensins) in normal and colon cancer tissue

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Cancer is a multi-factorial disorder with genetic and environmental components. Furthermore, evidence for the role of the natural immune system in the protection from tumor development was supported by studies involving immune-compromised patients. This control anti-tumor immune response may involve Toll-like receptors (TLRs). The TLRs are known as the principal innate immunity receptor in humans and as key components of the pathogen recognition process that mediates inflammatory responses gene. For this reason, TLRs are currently at the centre of both basic research and drug development. The aim of this study is to investigate the association of different TLRs (TLR2, TLR4, and TLR6), pro-inflammatory cytokines (IL-6, IL-1 and TNF) and Human Beta Defencins (HBD-1,HBD-2,HBD-3 and HBD-4) with colon cancer . To reach our goal we used quantitative real time PCR and immuno-histochemistry procedures to investigate the expression of these genes in normal (n = 40) and colon cancer (n = 40) Saudian patients. Polymorphism and mutation by exon sequencing in the TLRs and HBDs genes were also analyzed. Our results show that TLRs and HBDs levels decrease in colon tumor compared to normal tissues but IL-6, IL-1 and TNF alpha expression increase in tumors tissues. Down expression of the TLRs and HBDs in colon tissues is correlated to multiple mutations in the specific exons of each TLR and HBDs. Since these TLRs and HBDs are playing active role in the innate immunity, their dysregulation/gene mutation may hamper colon cell function shifting to overgrowth and cancer initiation/development

P1.11.086
Investigating the expression of Toll-like receptor-2,4 and 6 in normal and ovarian cancer tissue

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Ovarian cancer is estimated to be as one of the top ten cancers in Saudi Arabia. In the year 2007 the ovarian cancer accounted for 3.1% of all newly diagnosed cases among Saudi females (4,773). Genetic predisposition to ovarian carcinoma is one of the major risk factors especially in young patients. Gene mutation that may lead to ovarian cancer involves important genes including those of innate immunity causing dysregulation of defense system. Such genetic dysregulation may involve on the Toll-like recepror (TLR) genes, specifically (TLR2, TLR4 & TLR6), because these are highly expressed by one key cell involved in ovarian cancer: epithelial cell. The aim of this study is to investigate the association of different TLRs (TLR2, TLR4, and TLR6) with ovarian cancer by studying the level of gene expression of TLRs. To reach our goal we used quantitative real time PCR and immuno-histochemistry procedures to investigate the expression of TLRs in normal (n = 10) and ovarian cancer (n = 10) Saudian females. Polymorphism and mutation by exon sequencing in the TLRs genes were also analyzed. Our results show that TLR2, TLR4 and TLR6 levels decrease in ovarian tumor compared to normal tissues. Down expression of the TLR2, TLR4 and TLR6 in ovarian tissues is correlated to multiple mutations in the specific exons of each TLR. Since these TLRs are playing active role in the innate immunity, their dysregulation/gene mutation may hamper ovarian epithelial cell function shifting to overgrowth and cancer initiation/development.

P1.11.087
Proteolytic cleavage of human Toll-like receptor 3 by lysosomal proteases is required for receptor activation

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Toll-like receptors (TLRs) are a group of pattern-recognition receptor that plays an important role in innate immune response against bacterial and viral infections. Among them, intracellular TLRs are localized in endolysosomal compartment and recognize foreign nucleic acids from a variety of pathogens. Recent studies have shown that TLR9 proteolytically processed in the ectodomain by multiple lysosomal cysteine proteases, such as cathepsins and asparagine endopeptidase (AEP), and this processing is required for receptor activation. Similarly TLR3 is also processed in endolysosomal compartment. However, the main proteases involved in TLR3 proteolytic processing are not fully understood. To investigate which proteases are involved in proteolytic cleavage of TLR3, we examined the expression of cathepsins, AEP and TLR3 in THP-1 cells and PMA-differentiated macrophages by western blot using anti-cathepsins, anti-AEP as well as anti-TLR3 antibodies. In addition, the effect of inhibitors of cathepsins and AEP on cleavage of TLR3 was investigated. The expression of AEP and cleaved TLR3 C-term fragment was significantly increased in macrophages compared to THP-1 cells, but the expression level of cathepsins was decreased in macrophages. Intriguingly, the molecular size of cleaved TLR3 C-term fragment in macrophages was slightly smaller than that in THP-1 cells. Furthermore, cleavage of TLR3 was inhibited when both inhibitors were treated. Therefore our results suggest that lysosomal cysteine proteases, such as cathepsins and AEP, are required for proteolytic processing of TLR3 in THP-1 cells and macrophages.

P1.11.088
TLR7 signalling in macrophages activates IRF3 and is dependent on the adaptor TRAM

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Toll-like Receptors (TLRs) and their related signalling pathways are crucially important in the initiation of an immune response against many pathogens and are thus the focus of intense study to fully elucidate their regulation. TLR7 is an endosomal TLR that has evolved to sense viral single-stranded RNA (ssRNA). It therefore plays a vital role in the response to ssRNA viruses such as human rhinovirus and influenza against which, there are currently no treatments or vaccines with long term efficacy available. A more complete understanding of TLR7's signalling axis would therefore be advantageous in the designing of therapeutic agents targeting this important pathway. Previous studies have shown that the TIR-domain containing adaptor TRAM plays a role in TLR4 signalling by recruiting TRIF to TLR4 at the endosomal membrane to initiate IRF3 dependent type-1 interferon production. As TRAM, TLR4 and TLR7 can all localise to endosomal compartments, we investigated whether TRAM plays a role in transducing TLR7 dependent signalling also. Results from murine macrophages indicate that although TRAM plays no role in TLR7 mediated TNFα secretion, TRAM is required for maximal TLR7 mediated RANTES (CCL5) production. This process was transcriptionally regulated and was IRF3 dependent but NF-κB independent. RNA interference of human TRAM in macrophages also showed a requirement for TRAM in human rhinovirus induced RANTES, IFNβ and CXCL10 production. TRAM is therefore required for maximal TLR7 mediated, IRF3 dependent cytokine production in both murine and human macrophages.

P1.11.089
Inverse Correlation between Histamine Receptor Expression and Toll-like Receptor Activation in Peripheral Blood Mononuclear Cells from Inflammatory Bowel Disease Patients.

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Histamine is a key mediator in both immediate type hypersensitivity reaction and chronic inflammatory responses. Toll-like receptors (TLR) play crucial role in innate immune response because of their ability to recognize PAMPs (pathogen associated molecular patterns). The aim of the project was to assess the effects of histamine on PBMCs, stimulated with TLR-ligands, from patients with inflammatory bowel disease (IBD). Fourteen patients diagnosed with IBD (7 with Crohn's disease and 7 with ulcerative colitis) and 6 healthy volunteers were included in the study. After reverse transcription, expression of genes which encode Toll-like receptors and histamine receptors were examined using REAL-TIME PCR. PBMCs were stimulated with various TLR ligands in the presence or absence of histamine. Cytokine levels were measured in supernatants after 24 hours (IL-12, TNF-alpha, IP-10). A significant reduction in the expression of genes encoding the Toll-like receptors (TLR-1, TLR-2, TLR-4, TLR-6, TLR-9) in UC and CD patients were found in comparison to healthy volunteers. The expression of histamine receptors (H1R, H2R, H4R) were significantly lover (especially in CD group) than in the control group. In PBMCs stimulated with TLR ligands, the level of proinflammatory cytokines was higher for patients with CD and UC in comparison to healthy volunteers and also secretion of cytokines was higher in patients with CD than in patients with UC. After addition of histamine, the level of proinflammatory cytokines decreased in each group. In conclusion, patients with IBD display dysregulated expression of histamine receptors associated with altered TLR responses to microbial ligands.

P1.11.090
Plasmacytoid dendritic cells play a key role in tumor progression in LPS-stimulated lung tumor-bearing mice

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The anti-tumor activity of LPS was first described by Dr. William Coley. However its role in lung cancer remains unclear. The aim of our study was to elucidate the dose-dependent effects of LPS (0.1-10μg/mouse) in a mouse model of B16-F10-induced metastatic lung cancer. Lung tumor growth increased at 3 and 7 days after the administration of low dose of LPS (0.1μg/mouse) compared to control mice. This was associated with an influx of plasmacytoid dendritic cells (pDCs), regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSC) and CD8+ Tregs. In contrast, high dose LPS (10μg/mouse) reduced lung tumor burden and was associated with a greater influx of pDCs, and a stronger Th1- and Th-17 polarization. Depletion of pDCs during low dose LPS administration resulted in a decreased lung tumor burden. Depletion of pDCs during high dose of LPS treatment resulted in an increased tumor burden. The dichotomy in LPS effects was due to the phenotype of pDCs, which were immuno-suppressive after the low dose of LPS, and Th1- and Tc-polarizing cells after the high dose LPS. Adoptive transfer of T cells into nude mice demonstrated that CD8+ T cells were responsible for pDC recruitment following low dose LPS administration, whereas CD4+ T cells were required for pDC influx after the high dose of LPS. In conclusion, our data suggest differential effects of low dose versus high dose LPS on pDC phenotype and tumor progression or regression in the lungs of mice.

P1.11.091
Pam3CSK4, a TLR2 agonist, induces in vitro osteoclastogenesis RANKL-independently and resorbs calvarial and alveolar bone in vivo

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Oral bacteria are a common cause of periodontitis, in which irreversible breakdown of alveolar bone may ultimately lead to tooth loss. It is well established that bacteria interact with host cells by binding to corresponding Toll-like receptors (TLRs). Among these, LPS, a component of gram-negative bacteria and a ligand for TLR4, is the most clearly defined molecule as a potent inducer of osteoclastogenesis and bone resorption. On the other hand, the fact that TLR2, in addition to TLR4, is prominently expressed in both precursors and differentiated osteoclasts, raises the possibility that components of gram-positive bacteria are involved in periodontitis pathogenesis. To clarify the functional role of TLR2 agonist in osteoclastogenesis, we stimulated osteoclast precursors with synthetic tri-acylated lipopeptide, Pam3CSK4. Furthermore, we examined whether Pam3CSK4 and Streptococcus mutans (S. mutans) 109c, a gram-positive oral bacterium, caused bone resorption in vivo. The results showed that; i) Pam3CSK4 induced osteoclastogenesis in both RAW264.7 cells and mouse bone marrow precursor cells, without adding RANKL exogenously; whereas, LPS (Re mutant), a specific ligand for TLR4, did not, ii) Quantitative RT-PCR analysis showed that Pam3CSK4 up-regulated mature osteoclast markers, but not RANK or RANKL, iii) Both Pam3CSK4 and S. mutans 109c injected into murine calvarial periosteum and rat palatal bone were able to cause bone resorption within 7 and 9 days, respectively, in vivo. Together these results provide important findings that components of gram-positive bacteria, as well as those of gram-negative bacteria including LPS, may be a periodontal pathogen which causes alveolar bone destruction.

P1.11.092
TLR3 recognizes single-stranded RNA with incomplete stem structures

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Toll-like receptor (TLR) 3 recognizes dsRNA in the endosomes and induces type I interferons and proinflammatory cytokines via the adaptor protein, TICAM-1/TRIF. TLR3 serves as a sensor for viral infection and sterile tissue necrosis, but little is known about the essential structural elements of virus- or host-derived RNAs that activate TLR3 in infection/inflammatory states. Here we demonstrate that poliovirus (PV)-derived ssRNA segments bearing dsRNA regions with internal/bulge loops are potent TLR3 agonists. Functional ssRNAs were resistant to degradation and activated mouse/human TLR3, leading to the production of type I interferons and proinflammatory cytokines. Like polyinosinic:polycytidylic acid, PV-ssRNA was internalized into cells via raftlin-mediated endocytosis and colocalized with TLR3. Interaction of PV-ssRNA with the N- and C-terminal dsRNA binding sites in the TLR3 ectodomain was required for TLR3 activation, which was inhibited by RNase III treatment. RNA uptake and the TLR3 RNA-sensing system require an appropriate topology of multiple RNA duplexes for its specific recognition and signaling. Hence, TLR3 is a sensor of extracellular viral/host RNA with stable stem structures derived from infection or inflammation-damaged cells. The expansive recognition ability of TLR3 identified here will support a broad range of physiological functions of TLR3 recently reported, such that TLR3 signaling is involved in nuclear reprogramming and intrinsic retrovirus reactivation.

P1.11.093
Studies on the full-length human NOD-like receptor family CARD domain containing 5 (NLRC5) protein

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The NOD-like receptor family CARD domain containing 5 (NLRC5) is the largest member of the NLR protein family containing an unusually high number of leucine-rich repeats (LRRs) and an untypical CARD (caspase activation and recruitment domain) effector domain. NLRC5 is an intensively studied receptor with various functions, including the capability to transactivate MHC class I and to regulate various signal transduction pathways. As a consequence of the lack of crystal structures of full-length NLRs including NLRC5, the low number of template structures for homologous modeling and the unique domain architecture of NLRC5, only few structural data are available for NLRC5.
We aimed to analyze the sequences of NLRC5 and some NLRC5 related proteins and to build a homologous model for the full-length human NLRC5. Sequence alignments resulted in the recognition of consensus patterns within the studied LRR sequences and two typical types of consensus LRR sequences were identified in the RI and NLRC5 proteins. Investigation of LRR sequences helped to identify LRRs in NLRC5 and to predict their number and position within the protein. We have constructed a homologous model for both the monomeric and a homo-heptameric full-length human NLRC5 protein having open conformations for the monomers. To determine the molecular weight of NLRC5, we expressed flag-tagged NLRC5 in cell cultures and gel-filtration of cell lysates followed by immune-detection was utilized to determine the physiological molecular size of the protein in various circumstances and to correlate the findings with the models.
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P1.11.094
Activation of innate immunity through the CARD9 pathway is involved in severe influenza pneumonia

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Background: Influenza virus (IFV) infection is a frequent cause of severe pneumonia and encephalopathy, especially in infants and the elderly. It is supposed that excessive activation of innate immune responses to IFV infection is responsible for such severe pathologies. In this study, we focused on the function of the adaptor molecule CARD9, which is essential for NF-kB signaling pathway activation through RIG-I or ITAM-coupled receptors, and analyzed the role for innate immune activation through the CARD9 pathway in defense and pathogenesis in IFV infection.
Methods: C57BL6(WT) mice and *Card9*$^{-/-}$ mice were infected intratracheally with IFV (strain A/PR/8/34), and were analyzed for daily survival, and production of inflammatory cytokines/chemokines, viral titers and histology in lung tissues. IFV was brought into contact with various macrophages(MFs) and dendritic cells(DCs) derived from WT mice or *Card9*$^{-/-}$ mice *in vitro*, and production of inflammatory cytokines was measured by ELISA.
Results: *Card9*$^{-/-}$ mice showed improved survival rate, and decrease in the production of inflammatory cytokines in BAL fluid and the inflammatory cell infiltration in lungs was observed, but viral titers in the lungs did not change compared with WT mice. Inflammatory cytokine production by MFs stimulated with IFV remained unchanged, but that by conventional DCs or plasmacytoid DCs was decreased in *Card9*$^{-/-}$ mice.
Conclusions: Activation of innate immunity through the CARD9 pathway in DCs is involved in the excessive inflammation in influenza virus-infected lungs. Thus, inhibition of the CARD9 pathway would be a promising therapeutic target for treatment of severe influenza pneumonia.

P1.11.095
TLR3 as an Exacerbating Factor for Radiation-Induced Gastrointestinal Syndrome

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High-dose ionizing radiation induces a fatal disorder of the digestive tract called gastrointestinal syndrome. We show that TLR3 is critically involved in gastrointestinal syndrome. Tlr3-/- mice showed significantly milder symptoms of gastrointestinal syndrome, including mortality, diarrhea and weight loss, than did Tlr3+/+ mice. Tlr3-/- mice showed markedly reduced radiation-induced crypt cell death and avoided villous destruction owing to re-epithelization from surviving crypts. The TLR3 signaling components TRIF and RIP1, but not IRF3 and type I interferons, were involved in radiation-induced crypt cell death. Germ-free mice showed no changes in radiation-induced crypt cell death, indicating that endogenous molecules were responsible for TLR3 activation. We detected leakage of self-RNAs from dying crypt cells following irradiation. Furthermore, large (>200 nucleotides) RNAs derived from irradiated small intestine induced crypt cell death via TLR3. Thus, TLR3 exacerbates gastrointestinal syndrome via induction of extensive crypt cell death through the recognition of self-RNAs released from dying cells.

P1.11.096
AGE-BSA induces self- and cross-tolerance in the monocytic Mono Mac 6 cell line

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The induction of tolerance is an important negative feedback mechanism to control activated innate immune cells and thereby prevent excessive inflammation. The phenomenon of self- and cross-tolerance induction has been described so far for a variety of ligands and their corresponding pattern recocgnition receptors. We were interested to assess the capability of different ligands of the Receptor for Advanced Glycation Endproducts (RAGE) to induce tolerance. An initial screening of the monocytic cell line MonoMac-6 using a variety of immune receptor agonists revealed a tolerance induction, beside others, by the RAGE ligand advanced glycation endproduct-modified bovine serum albumin (AGE-BSA), measurable by a diminished TNF-alpha secretion in response to a second stimulation. The effect was specific for AGE-modified BSA, as unmodified BSA did not mediate the effect. In contrast to AGE-BSA, the RAGE ligand HMGB-1, neither in its chemokine nor in its cytokine redox conformation, lead to an induction of tolerance. The tolerance induction was not accompanied by a decrease, but rather with a slight increase of receptor expression as assessed by quantitative RT-PCR. To exclude the possibility, that induction of apoptosis or modulation of the cell cycle might be the reason for the altered response, we assessed both using flow cytometry and found no agonist-induced changes. Interestingly, while LPS was capable to induce tolerance in minute amounts (0,1ng/ml), higher levels of AGE-BSA (≥10µg/ml) were necessary. In conclusion, we have evidence for an AGE-BSA-mediated tolerance induction in monocytic cells, but further work is necessary to decipher the underlying pathways involved.

P1.11.097
Role of the *TLR7, 8 and 9* polymorphisms in the susceptibility to HIV-1 infection in southern Brazilian patients

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This study investigates the role of the*TLR7/8/9* genes in susceptibility to HIV-1 infection through the analyses of *TLR7* rs179008, *TLR8* rs3764880, *TLR9* rs5743836 and rs352140 polymorphisms. The frequency of the variant alleles was analyzed in 366 HIV-1 infected patients and 415 healthy individuals from the southernmost state of Brazil. The promoter variant from *TLR9* was genotyped using a BI-PASA technique and all others were analyzed by PCR-RFLP. Chi-square test was used to compare allelic and genotypic frequencies between patients and controls. All analyses were conducted controlling by gender and ethnic origin. Among the Euro-derived individuals, statistically significant differences were observed for genotypic and allelic frequencies of the *TLR9* promoter variant rs5743836 between patients and controls (P=0.028 and P=0.011, respectively). The CC genotype was observed at higher frequency in patients when compared to controls (P= 0.0012, OR 1.86 95% CI: 1.28-2.71) and conferred susceptibility. However, among the Afro-derived individuals, the TC genotype was less represented in patients when compared with controls (P =0.014, OR: 0.51 95% CI 0.30-0.87) and conferred protection. No statistical significant differences were observed for all other polymorphisms or for haplotypic analyses. This study reveals a fundamental role of the *TLR9* rs5743836 polymorphism against HIV-1 infection, the CC genotype conferring susceptibility among Euro-derived individuals while TC genotype conferring protection among Afro-derived individuals. Also, we observed the importance of the genetic background in analyses of susceptibility to infectious agents, pointing to the need of studies in different human admixed populations.

P1.11.098
TLR9 enhances bacterial clearance and limits lung consolidation in murine MRSA pneumonia

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BACKGROUND:
Methicillin-resistant Staphylococcus aureus (MRSA) is an emerging pathogen in pneumonia, associated with severe lung damage. Tissue injury causes release of Damage Associated Molecular Patterns (DAMPs), which may perpetuate inflammation. Host DNA has been implicated as a DAMP that activates inflammation through Toll-like receptor (TLR)9.
OBJECTIVE:
To evaluate the role of TLR9 in MRSA pneumonia.
METHODS:
Wild-type (WT) and TLR9 knockout (KO) mice were infected intranasally with MRSA (5E7CFU) and euthanized at 6,24,48 or 72 hours for analyses.
RESULTS:
MRSA pneumonia was associated with profound release of cell-free DNA in the airways, as reflected by a 4-log increase in nucleosome levels in bronchoalveolar lavage fluid (BALF) from 24 hours after infection onward. In BALF, TLR9KO mice showed reduced TNFα and IL-6 levels (P<0.005 and P<0.05 resp.vs WT mice) at 6 hours and reduced bacterial clearance at 6 and 24 hours post infection (both P<0.005 vs WT mice). Furthermore, TLR9KO mice had a greater influx of neutrophils in BALF at 24 and 48 hours (P<0.05 and P<0.005 resp. vs WT mice). Finally, lung consolidation was significantly increased after 24 and 48 hours in TLR9KO mice.
CONCLUSION:
TLR9 has pro-inflammatory effects during MRSA pneumonia in mice associated with enhanced bacterial clearance and limitation of lung consolidation.

P1.11.099
A TLR9/TRIF pathway induces tolerance via TGF-β and tryptophan catabolism in plasmacytoid dendritic cells

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Cytosine-phosphate-guanosine (CpG)-rich oligodeoxynucleotides (ODNs) stimulate Toll-like receptor 9 (TLR9)-dependent signaling and activate the innate and acquired responses of the immune system. The immunostimulatory effects of CpG-ODN are being evaluated in clinical trials as a novel therapeutic approach for treatment of human diseases. Here we show that pre-treatment in vitro of plasmacytoid dendritic cells with a low-dose (1 µg/ml) of a CpG-ODN phosphorothioate (CpG 1826) induced, as expected, immunostimulatory effects in vivo. However, pre-treatment of the same cells with a higher concentration of the ODN (10 µg/ml) induced tolerogenic effects, which allowed us to unveil a previously undescribed role for the adaptor TRIF and TRAF6 proteins in TLR9 signaling. The low-dose CpG stimulation activated the TLR9/MyD88-dependent NF-κB pathway and the production of the pro-inflammatory cytokine IL-23, whereas the high-dose CpG stimulation triggered the TLR9/TRIF pathway, leading to activation of the noncanonical NF-κB pathway, production of the anti-inflammatory cytokine TGF-β, and subsequent induction of the immunosuppressive pathway of tryptophan catabolism. Thus, opposite in vivo outcomes of TLR9 stimulation by a CpG-ODN phosphorothioate (CpG 1826) can occur as a function of dosage. Our findings may be relevant not only for an increased understanding of the complexity of TLR signaling and for an optimal exploitation of CpG-ODNs as immune adjuvants, but also for the development of nonconventional forms of CpG-based immunotherapy in human allergy and autoimmunity.

P1.11.100

Mannan-binding lectin inhibits *Candida albicans*-induced cellular responses of human THP-1 cells through toll-like receptor 2 and toll-like receptor 4

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Background: *Candida albicans* (*C. albicans*), the most common human fungal pathogen, can cause fatal systemic infections under certain circumstances. Mannan-binding lectin (MBL) ,a member of the collectin family in the C-type lectin superfamily, is an important serum component associated with innate immunity. Toll-like receptors (TLRs) are expressed extensively, and have been shown to be involved in *C. albicans*-induced cellular responses. We first examined whether MBL modulated heat-killed (HK) *C. albicans*-induced cellular responses in the monocytoid cell line, THP-1 cells. We then investigated the possible mechanisms of its inhibitory effect.
Results: Enzyme-linked immunosorbent assay (ELISA) and reverse transcriptasepolymerase chain reaction (RT-PCR) analysis showed that MBL at higher concentrations (10-20 μg/ml) significantly attenuated *C. albicans*-induced proinflammatory cytokine (e.g., TNF-α) and chemokine (e.g., IL-8) production from THP-1 cells at both protein and mRNA levels. Electrophoretic mobility shift assay (EMSA) and Western blot (WB) analysis showed that MBL could inhibit *C. albicans*-induced NF-κB DNA binding and its translocation in THP-1 cells. Furthermore, MBL could directly bind to *C. albicans* in a Ca^{2+}-dependent manner and decrease TLR2 and TLR4 expressions in *C. albicans*-induced THP-1 cells.
Conclusion: All these data suggest that MBL could affect cytokine expression by modifying *C. albicans*-/TLR-signaling pathways. This study supports an important role for MBL on the regulation of *C. albicans*-induced cellular responses.

P1.11.101
Rab7 GTPase negatively regulate inflammatory cytokine and type I interferon expression in LPS-stimulated macrophages via GTP-binding dependent manner

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Toll-like receptors (TLRs) play a critical role in innate immunity against invading pathogens. Rab (Ras related in brain) proteins are small guanosine triphosphatases (GTPases) that are involved in signal transduction by regulating the membrane trafficking of several receptors. The relationship between Rabs and TLR signaling remains largely unknown. In this study, we found that Rab7 can inhibit lipopolysaccharide (LPS)-induced expression of interleukin (IL)-6, IL-1β, interferon (IFN)-β and interferon-inducible protein 10 (IP-10), and suppress LPS-induced activation of mitogen-activated protein kinases (MAPKs), nuclear factor κB (NF-κB), and IFN regulatory factor 3 (IRF3) signaling pathways in RAW264.7 macrophages. However, GTP-binding deficient mutant of Rab7 (Rab7T22N) failed to inhibit TLR4-triggered expression of pro-inflammatory cytokines and IFN-β and activation of MAPKs, NF-κB and IRF3. Moreover, knockdown of endogenous Rab7 expression increased LPS-induced proinflammatory cytokines, IFN-β as well as TLR4 expression in RAW264.7 macrophages. Therefore, we demonstrate that Rab7 can negatively regulate TLR4-triggered pro-inflammatory cytokine and IFN-β expression in RAW264.7 cells in a GTP-binding-dependent manner. Our results indicate that Rab7 negatively regulate LPS/TLR4 signaling by regulating the expression of TLR4.

P1.11.102
Both Toll-like receptor-2 and -4 contribute to signalling of Burkholderia pseudomallei's lipopolysaccharide

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Introduction The Gram-negative bacterium *B.pseudomallei* causes melioidosis and is a recognized biothreat agent. While enterobacterial lipopolysaccharide (LPS) signals via MD2-Toll-like receptor (TLR)-4, we have previously shown that TLR2 impairs host defence during pulmonary *B.pseudomallei* infection while TLR4 only has a limited impact. We now investigate the role of TLRs in *B.pseudomallei*-LPS induced inflammation.
Methods Purity of isolated *B.pseudomalle*i-LPS was determined with Silver-staining, Coomassie-blue and polymyxin-B. HEK-cells transfected with TLR2/CD14 or TLR4/CD14/MD2, human whole blood treated with anti-TLR2 or anti-TLR4 and both whole blood and peritoneal macrophages of wild-type (WT), TLR2 knock-out (KO), TLR4 KO and TLR2/4 double KO's were stimulated with purified *B.pseudomallei*-LPS. Additionally, WT and selected TLR KO mice were inoculated intranasally with *B.pseudomallei*-LPS.
Results Purified LPS of *B.pseudomallei* activated only HEK-TLR2 during short stimulation and both HEK-TLR2 and HEK-TLR4 cells during overnight stimulation. In human whole blood, an additive effect of TLR2 on TLR4 mediated signalling induced by *B.pseudomallei*-LPS was observed. Murine peritoneal macrophages recognized *B.pseudomallei*-LPS solely through TLR4. Both TLR4 KO and TLR2x4 double KO's, but not TLR2 KO's, displayed diminished cytokine responses and neutrophilic influx upon inoculation with *B.pseudomallei*-LPS when compared to WT mice.
Conclusion While TLR4 is the main TLR for *in vivo* signalling of *B.pseudomallei*-LPS, TLR2 recognition seems to play an additional role. Altogether these results highlight important differences between the specificity of human or murine models in terms of TLR4-MD2 and TLR2 recognition that may have important consequences for *B.pseudomallei*-LPS sensing and subsequent susceptibility to melioidosis.

P1.11.103
Toll-like receptor-5 contributes to protective immunity in Gram-negative sepsis caused by Burkholderia pseudomallei, independent of flagella presence

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Introduction: *Burkholderia pseudomallei* is the causative agent of melioidosis, an important cause of sepsis in south-east Asia and a recognized biothreat agent. Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns and initiate the immune response. TLR5, which is highly upregulated in patients with melioidosis, recognizes bacterial flagellin. We here studied the role of TLR5 and its interaction with flagellin in experimental melioidosis.
Methods: Human embryonic kidney (HEK)-TLR5 cells, murine alveolar macrophage (MH-S), murine lung epithelial cells (MLE-15) cells and bone-marrow-derived-macrophages (BMDM) derived from wild-type (WT) C57BL/6 and TLR5 deficient ($TLR5^{-/-}$) mice were incubated with wild-type *B. pseudomallei 1026*b, flagellin-deficient *B. pseudomallei MM36* or purified *Burkholderia* flagellin. Additionally, WT and $TLR5^{-/-}$ mice were intranasally challenged with *B.pseudomallei 1026b* or *MM36*. Mice were sacrificed at different time-points post-infection to assess bacterial loads, inflammation and pathology.

Results: *B.pseudomallei MM36* was less potent than *1026b* in eliciting an inflammatory response in different cells *in vitro*. *B.pseudomallei* flagellin was confirmed to signal through TLR5. $TLR5^{-/-}$ mice challenged with *B.pseudomallei 1026b* were more susceptible to infection when compared to WT mice as demonstrated by higher systemic bacterial loads and increased organ injury. Strikingly, this effect was not dependent on the presence of flagella: $TLR5^{-/-}$ mice showed a similarly impaired antibacterial defense after infection with either *B.pseudomallei 102b* or *MM36*.
Conclusion: The presence of TLR5 contributes to protective immunity during experimental melioidosis. Surprisingly, this effect seems to be unrelated to the presence of *B. pseudomallei* flagellin, suggesting a role for yet to be discovered endogenous TLR5 ligands.

P1.11.104
A comparison of Pattern Recognition Receptor requirement for human and horse strains of Streptococcus pneumoniae

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Streptococcus pneumoniae is an important respiratory pathogen of humans and horses. Pneumolysin and autolysin are major virulence factors of *S. pneumoniae* and damage host tissue through direct effects and/or the ability to induce activation of pattern recognition receptors (PRRs) including Toll-like receptors (TLRs). In the horse functional pneumolysin and autolysin are absent due to a chromosome deletion, yet respiratory disease still occurs in these animals. How then do the horse strains of *S. pneumonia* cause disease?
The response of horse or human TLR4 to pneumolysin was characterised using an in vitro transient transfection model. Pneumolysin is an agonist at human, mouse and horse TLR4. Disruption of pneumolysin binding using TLR4 antagonists revealed that the pneumolysin interaction occurs at the same binding pocket as the TLR4 agonist lipopolysaccharide (LPS). Molecular techniques, including fluorescent single molecule imaging, will be used to further investigate the nature of the interaction. Pneumolysin interaction with the TLR4/LPS binding pocket suggests that it may activate TLR4 in a similar manner to LPS.
This study will be expanded to compare how horse, human and mutant pneumolysin- and autolysin strains interaction with PRRs using in vitro macrophage cell culture studies and mouse infection models.

P1.11.105
Molecular mechanisms regulating the synergism between IL-32γ and NOD ligands for the induction of adhesion molecules, cytokines and chemokines of eosinophils in allergic inflammation

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Background: Key intracytosolic pattern recognition receptors in innate immune responses for bacterial infections are nucleotide binding oligomerization domain (NOD) like receptors (NLR). IL-32 is novel pro-inflammatory cytokine that are associated with viral and bacterial infections, and allergic asthma. We elucidated the synergistic effect of IL-32γ and NOD-mediated the activation of human eosinophils, the principal effector cells for allergic inflammation and the underlying mechanisms.
Methodology: Expression of adhesion molecules and chemokines was quantitated by flow cytometry and multiplex assay, respectively. Phosphorylation of signaling molecules and caspase-1 activity was analysed by Western blot.
Results: IL-32γ was more capable in activating eosinophils than its isotype variant IL-32α, and the internalization of IL-32γ is dispensable for this effect. IL-32γ together with NOD1 ligand iE-DAP or NOD2 ligand MDP exhibit significant up-regulation of cell surface expression of CD18 and intercellular adhesion molecule-1 on eosinophils, and the adherence of eosinophils on dermal fibroblasts/bronchial epithelial cells. Moreover, IL-32γ exhibit synergistic effect with iE-DAP and MDP on the induction of allergic inflammation-related IL-1β, chemokines CXCL8, CCL3 and CCL4, and IL-1β, IL-6, CXCL8, TNF-α, CCL3 and CCL4, respectively (all $p<0.05$). Synergism between IL-32γ and NOD depended on the activation of intracellular caspase 1, ERK, p38 MAPK and NF-κB in eosinophils.
Conclusion: The above study provides immunological mechanisms by which bacterial infection-mediated activation of NOD1,2 together with novel pro-inflammatory IL-32 in synergizing allergic inflammation via the activation of eosinophils. This work was supported by the Research Grant Committee General Research Fund, Hong Kong (Project ref. no. CUHK 476411)

P1.11.106
Up-regualtion of HuR was critical for TLR9 signaling induced repression of tumor suppressor miR-7 in human lung cancer cells

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Accumulating evidence showed that miRNAs, a major class of gene expression regulators, played critical roles in regulating the biological effects of TLR9 signaling pathway on various cells. And TLR9 signaling could alter biological character of lung cancer cells including promoting the proliferation and enhancing the metastatic potential of tumor cells. However, the underlying mechanism regulation on the expression of TLR9 signaling-associated miRNAs in lung cancer cells remains largely unknown. Our recent work showed that TLR9 signaling could enhance the progression of human lung cancer cells through repressing miR-7 expression. Here, we further reported that TLR9 signaling could induce the expression of human antigen R (HuR) in human lung cancer cells. Moreover, overexpression of HuR could reduce the expression of miR-7 in lung cancer cells. Notably, down-regulation of HuR using RNA interference restored miR-7 expression in TLR9 signaling treated lung cancer cells and impaired their enhanced growth and metastatic potential. Finally, TLR9 signaling enhanced HuR expression through Akt pathway. Our findings indicated that HuR could act as regulator in regulating TLR9 signaling associated biological effect in human lung cancer cells through a positive feedback loop, which might be helpful for the understanding of the potential role of HuR in tumor biology.
Key words: Toll like receptor-9, miR-7, Human antigen R, human lung cancer cell

P1.11.107
Choreography and molecular strategy of dendritic cell-mediated NK cell activation in lipopolysaccharide-mediated inflammatory conditions

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Natural killer (NK) cells have anti-tumor, anti-viral and anti-bacterial functions, and efforts are being made to manipulate them in immunotherapeutic approaches. However, their activation mechanisms remain poorly defined, particularly in bacterial infections. Here we show that, upon E. coli or LPS exposure, dendritic cells (DCs) mediate NK cell activation directly in vitro and in vivo. This correlates with the establishment of prolonged DC-NK cell interactions in inflamed lymph nodes. Three DC-derived cytokines - IL-2, IL-18 and IFN-β - are necessary and sufficient to trigger IFN-γ release by NK cells, and IFN-β is also essential to boost NK cell cytotoxicity. IFN-β enhances NK cell activation by inducing IL-15 not only in DCs but, surprisingly, also in NK cells. Both NK cell-derived (cis-presented) and DC-derived IL-15 (trans-presented) contribute to NK cell activation.
Our results shed new light on the mechanisms underlying NK cell activation in vivo in response to E. coli and its major PAMP. A precise understanding of the biological program governing NK cell responses

might provide a framework to build next-generation immunotherapeutic strategies based on the manipulation of NK cell functions.

P1.11.108
Lithium attenuates IFN-β production and antiviral response via inhibition of TBK1/IKK-ε kinase activity

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Lithium salt is widely used GSK3β inhibitor and effective drug for the treatment of psychiatric diseases. However, the effects of lithium in innate immune responses, especially in cellular antiviral responses are unknown. Here we show that lithium chloride (LiCl) attenuates LPS-, poly(I:C)-, and Sendai virus (SeV)-induced IFN-β production and IRF3 activation in macrophages in a GSK3β-independent manner. The ability of the lithium to inhibit IFN-β production was confirmed in vivo as mice treated with LiCl exhibited decreased levels of IFN-β upon SeV infection. In vitro kinase assay demonstrates that lithium suppresses TBK1/IKK-ε kinase activity. Consistently, lithium significantly enhanced the replication of vesicular stomatitis virus (VSV) in vitro and in vivo. Severe infiltration of monocytes and tissue damage were observed in the lungs of control mice, compared to lithium treated mice after virus infection. Our findings suggest lithium as an inhibitor of TBK1/IKK-ε and potential target for the intervention of diseases with uncontrolled IFN-β production. Furthermore, lithium attenuates host defense to virus infection and may cause severely adverse effects in clinical applications.

P1.11.109
Mutated class II transactivator activates the IL-33-upregulated Th2 differentiation via the Nod2-involved NLR signal pathway

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Class II trans-activator (CIITA) is a member of the AD domain-containing NOD-like receptor (NLR) family and also functions as an initiator for the epigenetic control of MHC class II transcription. A negative dominant mutant of CIITA (mCIITA) being depleted its N-terminal shows activity of repressing class II expression by competition with its wild type counterpart. Little is known however about properties of CIITA as a NLR member. Here we show that a mCIITA-overexpressed myeloid cell line Ana-1 (Ana-1-mCIITA) was able not only to down-regulate the class II expression but also to converse immune response patterns from Th1 (IL-2^+IFN-γ^+STAT4^+) to Th2 (IL-4^+IL-5^+IL-10^+IL-13^+STAT6^+) when the Ana-1-mCIITA was collaborated with T cells, resulting in a strong suppression of the syngeneic T cell-mediated MLR. Mechanism analysis indicated that the mCIITA protein stimulated a NLR-related signaling pathway via binding of cytoplasmic Nod2 molecule, which was followed by activating RIP2, IKK-α/β and Caspase 1 for the mobilization of NF-κB and the activation of inflammasome. This ensured an enhanced expression of IL-33 gene, which promoted a skewed secretion of the Th2-related cytokines (IL-4^+IL-5^+IL-10^+IL-13^+IL-2^- IFN-γ^-). The Th2 activation was prevented by the deactivation of Nod2 gene with siRNA or by the blockage of the IL-33-related signaling using a mAb anti-IL-33 receptor. These results indicate that the Th1/Th2 balance could be regulated by the N-terminus-depleted CIITA molecule via the NLR-related signaling, a property valuable for disease controls, especially for inducing transplantation tolerance both via the repression of class II expression and the attenuation of the Th1-dominant response.

P1.11.110
Involvement of toll like receptor 2/3/4 and 9 polymorphisms in the occurrence of cervical cancer in Tunisian women

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Cervical Cancer (CC) occupies the second rank among cancers in women in the developing countries.
It's well established that Human papillomavirus (HPV) is the major causative agent of CC. Most HPV infections are cleared, but a proportion of women are unable to clear this infection, resulting in cervical lesions. Although persistent HPV infection is necessary but it is insufficient for the development of CC. It directly infects cervical keratinocytes and interferes with Toll Like Receptor (TLR) signaling.
Recently, it was reported that stromal up regulation of TLRs may play a role in cervical disease progression. Among several factors affecting the gene expression; single nucleotide polymorphisms (SNPs) or microsatellite polymorphisms particularly those within the regulatory regions of genes often affect expression levels and can serve as disease modifier.
The aim of this study was to investigate whether TLR 2 (−196 to −174 del), TLR 3 (1377 C>T), TLR 4 (Asp299Gly) and TLR 9 (2848 G>A) polymorphisms might be associated with variation of CC risk in 122 Tunisian women with invasive CC and 260 women free from any chronic clinical problem and disease manifestation, as those polymorphisms have an essential role in host immune response. These polymorphisms were genotyped in subjects using PCR amplification and PCR restriction fragment length polymorphism. Results revealed that TLR3 C/C genotype (OR: 1.1453, CI: 0.691-3.054) and TLR 4 Asp/Asp (OR: 4.29, CI:1.781-10.364) are associated with higher risk of CC.

P1.12 Lectins and glycoimmunology

P1.12.01
The interaction of complement-activating lectins with Mycobacterium tuberculosis

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Mannan-binding lectin (MBL) and ficolins (M-, L-, H-ficolin) are pattern-recognition molecules forming complexes with MBL-associated serine proteases (MASP). They bind to the carbohydrate-rich surface structures of numerous microorganisms and may contribute to their clearance thanks to opsonic properties or complement lectin pathway (LP) activation. Numerous reports demonstrated MBL or ficolin deficiency to enhance the susceptibility to infections. MBL and ficolins were shown previously to interact with M. bovis BCG and/or M. avium. However, their role in tuberculosis remains unclear. High MBL serum level or its total deficiency have been suggested to be disadvantageous for the host.
Heat-killed or formalin-fixed Mycobacterium tuberculosis H37RV cells activate lectin pathway in human sera, as demonstrated in a C4-deposition assay. In adult, BCG-vaccinated healthy subjects as well as tuberculosis patients, LP activity accounted for approximately 60% of C4 total serum activation potency (resulted also from antibody-dependent complement classical pathway). In contrast, in cord serum samples, as expected, almost whole activity against MTB came from the lectin pathway. The recombinant MBL, M-ficolin, L-ficolin, and H-ficolin were shown to recognize H37RV cells. MBL, in contrast to ficolins was moreover reactive against BCG strain, M. kansasii and M. gordonae. No interaction of any of tested lectins with M. smegmatis was observed. Further investigation demonstrated that lipoarabinomannan from M. tuberculosis is one of the target structures for MBL but not for ficolins.
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P1.12.02
Pseudo-mannosylated compounds inhibit DC-SIGN mediated HIV infection by competitive inhibition and by interfering with DC-SIGN signal

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Background: DC-SIGN is involved in the initial stages of infection by HIV. DC-SIGN, by binding high mannose glycan on HIV gp120, mediates *trans* infection of CD4 T cells. HIV interaction with DC-SIGN promote immunosuppressive responses that interfere with TLRs signalling, so favouring the persistence of the virus. We synthesized pseudo-mannosylated compounds in the attempt to compete with binding of DC-SIGN to HIV gp120 and interfere with the immunosuppressive DC-SIGN signalling.
Methods: The ability of the compounds to block HIV-1 infection and their toxicity were assessed in cellular and human cervical explant models. Laboratory strain and primary isolates both R5 tropic and X4 tropic were tested. Gene expression profile after treatment of MDDCs with the compounds was evaluated.
Results: Two of the compounds abrogated almost completely the transmission of different HIV strains to CD4 T cells and the infection of cervical explants. This activity was associated with a significant increase in the production of the antiviral cytokine IFNβ and of β chemokines. The production of inflammatory cytokines (IL-6, IL-1 β, TNFα and IFNγ) was increased, whereas IL-10, CCR5 and CXCR4 expression was not modified. The toxicity of the compounds was neglectable.
Conclusion: Pseudo-manosylated compounds competitively inhibit HIV binding to DC-SIGN, blocking infection in *trans*. Furthermore, by interacting with DC-SIGN, the compounds induce immune activation and proinflammatory responses. The production of β chemokines CCL3, CCL4 and RANTES, that compete with the virus binding to CCR5 co-receptor, contributes to antiviral activity. These features make such compounds good candidates to develop new topical microbicides.

P1.12.03
Treatment with interferon beta-1a reduces alpha-2,3-sialylation of CD4+ helper T cells

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The function of immune cells is at least in part determined by carbohydrate residues linked to proteins and lipids on the immune cell surface. We assessed whether treatment of patients with relapsing remitting multiple sclerosis (RRMS) with interferon-beta-1a alters surface glycosylation of immune cells.
Interferon beta-1a treatment lead to reduced alpha-2,3-sialylation of non-activated Helper T cells as determined by flow cytometry using cell-type specific antibodies and glycosylation-specific lectins. Alpha-2,3-sialylated residues were predominantly expressed by effector T cells, particularly by Th2 cells. 60% of Th2 cells, 17% of Th17 cells, 7% of Th1 cells and less than 5% of regulatory T cells displayed alpha-2,3-sialylated residues. Expression of alpha-2,3-sialylated residues was increased after T cell activation and T cells without alpha-2,3-sialylation displayed a reduced capacity to proliferate after polyclonal stimulation. These data collectively indicate a new mechanism of action for Interferon beta-1a in RRMS via reduction of alpha-2,3-sialylation on T cells. Alpha-2.3-sialylation could be a marker for disease activity in RRMS or responsiveness to treatment by interferon beta-1a.

P1.12.04
Jacalin has distinct immunomodulatory effects on early and late stages of experimental colon carcinogenesis

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Inflammation can foster or inhibit cancer growth, depending on the composition of infiltrating cell population and the stage of tumor development. Colorectal cancer represents a paradigm for the connection between inflammation and cancer. Increased expression of the TF antigen is a common feature in malignant epithelia. The lectin jacalin recognizes TF antigens and inhibits the proliferation of human colon cancer cells. In this study, we examined the effects of jacalin on different stages of experimental colon carcinogenesis. C57/BL6 or Balb/c mice were given 4 intrarectal deposits of 0.1 ml solution of Methyl-N'-Nitro-N-Nitroso-Guanidine (5 mg/ml) twice a week for 2 weeks. Jacalin treatment started 2 weeks before, or 24 weeks after carcinogen administration, and the lectin was administered orally (0.5 and 5µg/animal) twice a week for respectively 10 or 4 weeks. In all stages of carcinogenesis, jacalin treatment increased apoptosis and reduced proliferation of colonic/tumor cells. In the early stages, the lectin favored the formation of an anti-inflammatory microenvironment that impaired tumor progression, as shown by the decreased number of preneoplastic lesions. Jacalin administration resulted in increased intestinal production of TGF-β and IL-10 and in reduced COX-2 expression in stromal cells. In the late stages, after tumor establishment, jacalin treatment predominantly induced the production of the pro-inflammatory cytokines TNF and IFN-γ. Although tumor morphology was unaffected, a high number of apoptotic corpuscles was observed in tumors from treated animals. These results demonstrate that jacalin exerts distinct immunomodulatory effects on different stages of colon carcinogenesis, and highlight its potential antitumor activity.

P1.12.05
The change in the glycosylation of human leukocytes in immunosuppressive environment

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Cell and organ transplantation has become a mainstay of therapy for end-stage organ failure.The global effect of immunosuppressive drugs on the immune system predisposes a patient to the development of infection,cancer. An effect of immunosuppressive drugs, crucial for the successful transplantation of organs, on glycosylation of immune cells is not known. Glycans decorate the surfaces of all of the key molecules involved in the innate and adaptive immune response. The proper functioning of immune cells in response to alloantigeny, depends on the process of glycosylation of receptor proteins. Changes in the glycosylation profile of T cell receptor may influence the induction of response against the transplant. The aim of this study was to determine the effect of mTOR inhibitor (rapamycin-RAPA) and calcineurin inhibitors (cyclosporin A-CsA and tacrolimus-Tac), used singly and in two-drug combinations, on the glycosylation of mononuclear human cells (MNCs).MNCs were induced in two-way mixed leucocytes reaction (MLR) in the environment of different regimens of immunosuppression. The surface glycosylation of MNCs was identified by flow cytometry using lectins which selectively recognite specific carbohydrate structures. We observed changes in glycosylation of the analysed cells in the presence of mTOR and/or calcineurin inhibitors. Moreover, the environment of two-drug combinations influenced the glycosylation of MLR immune cells. The immunosuppressive drugs decreased the amount of PHAL- and VVL-positive cells, but the lowest percentage of staining cell it was observed in case of CsA and Tac treated cells. The calcineurin inhibitors reduced of PNA-,UEA- and GNA-positive

glycans, while mTOR inhibitor enhanced glycans recognized by UEA lectin.

P1.12.06
Anti-Polygalacturonic acid antibodies in diagnosis and pathogenesis of rheumatoid arthritis

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Much progress has been made in recent years on the diagnostic value, antigen specificity and pathogenic roles of autoantibodies correlated to the development of rheumatoid arthritis (RA) in humans. However, carbohydrate antigen-specific autoantibodies that may also play important roles in RA have largely been ignored. We herein report that serum levels of Abs against polygalacturonic acid (PGA, major structural component of pectin) strongly correlate with RA in humans. The measurements of PGA-specific Ab (PGA-Abs) in sera are comparable to rheumatoid factors and anti-cyclic citrullinated peptide Abs as serological diagnostic markers for RA in terms of sensitivity and specificity. Immuno-histochemical staining results indicate that the PGA moiety is specifically expressed by synovial membrane cells and chondrocytes in the joints of both humans and rabbits (but not rodents). Induction of PGA-specific Abs by subcutaneous immunization of rabbits with carrier protein-conjugated synthetic PGA leads to severe inflammatory reactions (synovial hyperplasia, small vessels proliferation and inflammatory cell infiltration) in the joints. Injection of affinity purified anti-PGA IgG into the synovial cavity of rabbits resulted in accumulation of pro-inflammatory cytokines such as TNF-α, IL-8 and IL-1β in synovial fluid and also local pathological damage. We conclude that the PGA moiety represents a major autoantigen in joints which can be targeted by autoantibodies capable of triggering arthritogenic responses in vivo.

P1.12.07
Interaction of Siglec-7 and -9 with their ligands inhibit NK cell anti-tumor immune responses

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Malignant transformation is often accompanied with aberrant surface glycosylation patterns. Tumor cells tend to produce increased levels of sialic acid containing glycoconjugates, a phenotype, which has been associated with invasion and malignancy. Glycan expression on the surface of tumor cells can modulate anti-tumor immune responses. Sialic acid binding immunoglobulin-like lectins (Siglecs) -7 and -9 are transmembrane receptors that mediate inhibitory functions, which are expressed on natural killer (NK) cells. Due to their ability to bind sialoglycans they might contribute to tumor escape during anti-tumoral immune responses. In our work we showed that engagement of these receptors inhibits NK cell-mediated cytotoxicity of target cells, as well as, NK cell degranulation and cytokine production. In vivo experiments, in a mouse model with a reconstituted human NK cell compartment, showed an increase in NK cell-mediated cytotoxicity after removal of sialic acid on the surface of the target cells. Even more, in “in vivo” and “in vitro” experiments we are able to significantly increase the lysis of an NK non-susceptible target cell line after enzymatic removal of their ligands. In this sense we suspect that sialylated glycans at the surface of cancer cells play a functional role in shielding them from immune attack. Thus, evidence that Siglecs are implicated in tumor escape, via glycan recognition on the surface of cancer cells by inhibition of NK cells, might become an important step in the future design of novel tumor immunotherapeutic strategies.

P1.12.08
Dectin-1 expressed on human pulmonary epithelial cells is an important pattern recognition receptor for the detection of nontypeable *Haemophilus influenzae*

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The C-type lectin (CTL) receptor Dectin-1 recognizes β-1,3 glucans and is known to be mainly expressed on myeloid cells. The activation of Dectin-1 induces pro-inflammatory immune responses via phosphorylation of the intracellular immunoreceptor tyrosine-based activation motif (ITAM)-like domain. Dectin-1 is known to be important for the host response targeting *Aspergillus fumigatus* and *Mycobacterium tuberculosis*. Moreover it has been suggested that nontypeaple *Haemophilus influenzae* (NTHi), an important bacterial pathogen of the respiratory tract with significant importance in chronic-obstructive lung disease (COPD), interacts with Dectin-1. Given that the majority of the interactions with these pathogens occur at the respiratory epithelium in this study we explored the expression of Dectin-1 on human airway epithelium. We found that immunohistochemical staining of human lung biopsies were positive for Dectin-1 either on bronchial as well as on alveolar epithelium of almost all samples. However, when comparing the intensity of the staining of human lung biopsies with regard to COPD and smoker status of the individuals no correlation was found. Primary normal human bronchial epithelial cells (NHBE) also showed Dectin-1 expression using FACS analysis. Importantly we found that proinflammatory immune responses of bronchial epithelium to infections with NTHi were significantly triggered by Dectin-1. In further experiments using an A549 cell-line overexpressing wildtype Dectin-1 or an ITAM-like phosphorylation mutant we found that ITAM-like phosphorylation was essential for the Dectin-1-triggered response to NTHi.
Taken together our results indicate that recognition of NTHi by Dectin-1 plays a considerable role for the generation of proinflammatory immune responses of airway epithelium.

P1.12.09
Crystal structure of the tetrameric fibrinogen-like recognition domain of FIBCD1

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We have solved the crystal structures of a tetrameric fragment of the C-terminal fibrinogen-like recognition domain of FIBCD1. FIBCD1 is an endocytic receptor that forms tetramers in the membrane of gut and lung epithelial cells. FIBCD1 binds acetylated components including fungal derived chitin and is therefore a pattern recognition receptor. The overall structure shows similarity in structure to the horseshoe crab innate immune protein tachylectin 5A and to the ficolins. Binding of N-acetyl mannosamine in the S1 site shows that the predominant interaction is via the acetyl group with the oxygen interacting with two main chain NH groups, the acetamide nitrogen interacting with the side chain of Tyr431 and the methyl group inserted into a hydrophobic pocket. In the native structure, a crystal contact results in one of the independent protomers binding the first GlcNAc of the Asn340 N-linked glycan on the other independent protomer, shedding light on the mode of binding to chitin itself. In addition, a sulphate ion has been modelled into the electron density at a location similar to the S3 binding site in L-ficolin, while in the native structure an acetate ion has been placed in the S1 N-acetyl binding site and a sulphate ion adjacent to this site. These ion-binding sites are ideally placed to receive the N-acetyl and sulphate groups of sulphated GalNAc residues of glycosaminoglycans. Together, these structures give insight into important determinants of ligand selectivity, demonstrating versatility in recognition and binding in general whilst maintaining conservation in N-acetyl and calcium binding.

P1.12.10

CLEC4F is a Kupffer Cells-Specific C-type Lectin Induced in F4/80-positive Cells upon Entry to Liver

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CLEC4F, a member of C-type lectins, was firstly purified from rat liver extract with high binding affinity to fucose, galactose, N-acetylgalactosamine (GalNAc), and un-sialylated glucosphingolipids with GalNAc or galactose terminus. However, the biological functions of CLEC4F have not been elucidated. To address this question, we examined the expression and distribution of murine CLEC4F firstly, and investigated its function using CLEC4F knockout (*Clec4f-/-*) mice. In this report we show that murine CLEC4F was specifically expressed in liver, worm-like F4/80^{+} Kupffer cells, but was absent in other tissues and cells. During embryogenesis, unlike F4/80, CLEC4F is not detectable in yolk sac, but is expressed in fetal liver of embryonic day 11.5 (E11.5), suggesting the expression of CLEC4F is induced after cells migrating from yolk cells to liver. In addition, CLEC4F is also expressed in both residential Kupffer cells and infiltrating mononuclear cells surrounding liver abscess caused by *Listeria monocytogenes* (*L. monocytogenes*) infection. Moreover, CLEC4F interacts with alpha-galactosylceramide (α-GalCer) in a calcium-dependent manner and participates in the presentation of α-GalCer to natural killer T (NKT) cells. This suggests that CLEC4F is not only a specific marker for Kupffer cells, but also plays an important role in the regulation of hepatic NKT cells.

P1.12.11
Characterization of carbohydrate preference of SIGNR1 as pathogen uptake receptor using carbohydrate-coated liposomes

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The C-type lectin receptors (CLRs) expressed on APCs are particularly important for pathogen recognition and uptake by APCs and therefore, CLRs are potentially useful for antigen targeting. In this context, determination of most preferable carbohydrate ligands for uptake of CLRs is important. However, little is known regarding the functional carbohydrate structures of CLRs as phagocytic receptors. In this study, we examined the uptake of neoglycolipid-coated liposomes mediated by SIGNR1 to understand the carbohydrate preferences of SIGNR1 as a phagocytic receptor.
RAW264.7 cells introduced with SIGNR1 (RAW-SIGNR1) efficiently ingested with mannotriose (Man3), mannopentapse (Man5), and mannobiose (Man2) with Manα1-3Man structure. Furthermore, the cells also efficiently took up liposomes coated with terminal α1,3/4−linked fucose-containing oligosaccharides, such as Lewis A and X antigens. On the other hand, RAW-SIGNR1 barely ingested liposomes coated with Man2 with Manα1-2Man, Manα1-4Man, and Manα1-6Man structures, and those coated with terminal α1,2−linked fucose-containing blood group H oligosaccharide and Lewis B antigen. When carbohydrate preferences of SIGNR1 as a carbohydrate-binding receptor was assessed based on the binding of RAW-SIGNR1 to neoglycolipid-coated solid phases, the cells clearly bound to all of terminal mannose-containing oligosaccharides including Man2 with Manα1-2Man, Manα1-4Man, and Manα1-6Man structures, and all of the terminal fucose-containing oligosaccharides including blood group H and Lewis B oligosaccharides. These results indicate that carbohydrate preferences of SIGNR1 as a phagocytic receptor differ from those as a carbohydrate-binding molecule.

P1.12.12
The role of membrane lectins from subcellular fractions of human prostate in intracellular transformation at different pathologies

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Lectins are biological active glycoproteins, which characterize to bind specifically to the terminal carbohydrates. Lctins with mytogenic activity increase a proliferation of cells that may be precondition of transformation. We studied some biological properties of lectins from human prostate tissues with different diagnoses.
Gal-Lectins was obtained from human post-operational prostate with following diagnoses: benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (BPH+PING$_{(2-3)}$), atypic adenomatous hyperplasia (BPH+AAH). Gal-Lectins were isolated with an aid of affine chromatography. The activity of the Citochrome-C-oxidase complex was determined by change of redox status of active copper and hem.
It has been revealed the sharp increase of lectin activity in all subcellular fractions of BPH+PING$_{(2-3)}$- and BPH+AAH-diagnose prostate as compared with BPH. Gal-1 and Gal-3 have been identified in mitochondria, plasma membrane and nucleus of all tested diagnoses prostate by Western blotting analyze and studied its distribution. Gal-lectins with 60 kD molecular weight have been isolated from mitochondrial and microsomal fractions of prostate tissues. In the modal experiments (the mitochondria and microsome of bovine liver) are studied some of their biological properties. The Gal-lectins from mitochondrial and microsomal fractions of prostate tissues with complicated diagnosis have effect on peroxidation, they inhibit the redox state of Cytochrom-C-oxidase copper and Cytochrom a+a_3 heme.
The obtained data reveal participation of membrane lectins from BPH+PING$_{(2-3)}$- and BPH+AAH-diagnosis prostate in the basic metabolic disorders, which evokes the transformation in the carcinoma and indicate to carry out the prophylactic treatment for avoid the cancer.

P1.12.13
The C-type lectin receptor SIGN-R3 binds to commensal microbiota and influences immune regulation in experimental colitis

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Inflammatory bowel disease is a condition of acute or chronic inflammation of the gut. An important factor contributing to pathogenesis is a dysregulated mucosal immunity against commensal bacteria and fungi. Host pattern recognition receptors (PRRs) sense commensals in the gut and are involved in maintaining the balance between controlled responses to pathogens and overwhelming innate immune activation. C-type lectin receptors (CLRs) are PRRs recognizing glycan structures on pathogens and self-antigens. Recent studies indicate a crucial role for CLRs such as Dectin-1 in the regulation of intestinal inflammatory responses.
In the present study, we examined the role of the CLR SIGN-R3 in the recognition of commensals and its involvement in intestinal immunity. SIGN-R3 is the closest murine homologue of the human DC-SIGN receptor recognizing similar carbohydrate ligands such as terminal fucose or high-mannose glycans. Here we show that SIGN-R3 binds specifically to commensal microbes. To analyze if this interaction impacted the intestinal immunity against microbiota, the dextran sulfate sodium (DSS) model of colitis was employed. Upon colitis induction, SIGN-R3$^{-/-}$ mice exhibited a more severe weight loss compared to wild-type mice. In addition, SIGN-R3$^{-/-}$ mice displayed exacerbated colitis symptoms such as diarrhea, cell infiltration into the colon, and mucosal ulceration. The increased inflammation in SIGN-R3$^{-/-}$ mice was accompanied by a higher TNF-α level in colon of SIGN-R3$^{-/-}$ mice indicating an immune regulatory role for SIGN-R3 during colon inflammation.
Our findings highlight the importance of CLRs in intestinal immunity and demonstrate for the first time an immune regulatory role for SIGN-R3 in colitis.

P1.12.14
Role of galectin-7 in psoriasis by regulating the skin inflammatory response through keratinocytes

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Galectin-7 is predominantly expressed by stratified epithelia, including epidermis. This protein was found to be highly inducible by p53 and modulate epidermal homeostasis after skin injury. However, its role in regulating immune responses in the skin is unknown. In the present study, we found galectin-7 was down-regulated in human psoriatic lesions compared with non-lesional sites. We also demonstrated that knocking down galectin-7 expression in the human keratinocyte cell line HaCaT resulted in increased production of the inflammatory cytokine and chemokine including IL-6 and IL-8, in response to various immune stimuli. In addition, chemotaxis of human neutrophils, but not of lymphocytes, toward galectin-7-knockdown cells was higher than that toward parental cells. The numbers of migratory neutrophils were positively correlated with the levels of IL-6 and IL-8 produced by the cells. Moreover, galectin-7-knockdown cells exhibited dramatically enhanced Erk1/2 phosphorylation upon LPS stimulation, which was associated with LPS-induced IL-6 and IL-8 production. Inhibition of Erk MAP kinase activation using the MEK inhibitor PD98059 suppressed LPS-induced IL-6 and IL-8 secretion from HaCaT cells. Taken together, our results suggest that galectin-7 has a suppressive effect on the inflammatory response in keratinocytes and that galectin-7 gene transfer or other means of specifically inducing galectin-7 expression may be a novel therapeutic approach for psoriasis and other chronic inflammatory skin diseases.

P1.12.15
R-MC 17 antigen - a rat homologue of DEC-205 antigen?

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We previously produced R-MC 17, IgG1 monoclonal antibody (mAb) by fusion of P3X myeloma cells with splenocytes of BALB/c mice immunized with rat thymocytes suspension enriched in non-lymphoid cells. In thymus, immunolabeling with R-MC17 was observed on cortical thymic epithelial cells (TEC), and on scattered cells with dendritic morphology in the medulla. In lymph nodes and spleen, strong R-MC17 expression was observed on dendritic-like cells (DC) throughout the T cell areas. Weak staining was observed in the follicles. In non-lymphoid tissues, staining was observed on single isolated cells in kidney (blood vessels), brain, lung, liver and gut. During ontogenesis (thymus of 16 day old rat embryos) almost all TEC were R-MC17-positive. R-MC17 mAb precipitated a protein of approximate molecular mass of 200 kDa, under non-reducing conditions, from thymus lysate. The staining pattern and biochemical analysis strongly suggest that the mAb recognize rat DEC-205 molecule. Regarding functional characterization of R-MC 17 antigen, we found that R-MC 17 mAb stimulated the engulfment of thymocytes and thymic hybridoma cells by R-TNC.1 cells (a rat epithelilal cell line with nursing characteristics) in vitro. It is interesting that, R-MC 17 mAb also significantly stimulated engulfment of DC by the R-TNC.1 cell line in vitro. The biological significance of this intriguing phenomenon remains to be elucidated. The mAb also suppressed DC-mediated T-cell proliferation. The molecular confirmation of DEC-205 as a binding molecule of R-MC17 mAb is currently in progress.

P1.12.16
Mannose receptor plays a key role in Th1 polarization of Lactobacillus acidophilus NCFM stimulated dendritic cells

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Several strains of Lactobacillus acidophilus have been shown to induce a high amount of antiviral and Th1 polarizing genes in bone marrow derived dendritic cells (BMDCs), which may explain why these bacteria can reduce the incidence and duration of influenza. The induction of antiviral and Th1 polarizing genes is caused by interferon beta (IFN-β), which is secreted in high concentrations upon L. acidophilus stimulation and is also partly responsible for a high IL-12 production. The mechanisms behind this high IFN-β/IL-12 induction are still unknown. In this study, we hypothesized that L. acidophilus NCFM uses receptor-mediated phagocytosis to initiate signaling in DCs and that the mannose receptor (MR) plays a key role. When adding mannan, a ligand to the mannose receptor, before adding L. acidophilus NCFM to BMDCs, the uptake of the bacterium and the induction of IFN- β /IL-12 and CD150, a marker of increased ceramide in the plasma membrane, were increased. In contrast, binding of the dectin-1 ligand zymosan, previously shown to initiate cellular shedding of the MR from the cell surface, reduced MR expression and the L. acidophilus NCFM induced IL-12 production. Our results indicate that the mannose receptor is a likely candidate to participate in the uptake of L. acidophilus either directly, by interacting with the bacterium itself or indirectly, by inducing changes in the plasma membrane that influences the endocytic mechanism of the bacteria, thus affecting cytokine induction.

P1.12.17
An interaction of H-ficolin with lipopolysaccharide contributes to aggregation, phagocytosis and killing of Hafnia alvei 1200 bacterial cells

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H-ficolin (also called ficolin-3 or Hakata antigen) is a complement-activating pattern recognition molecule, possessing the fibrinogen-like domain involved in carbohydrate binding. Amongst ficolins, H-ficolin has the highest concentration in human serum, however its physiological function remains unclear. Its deficiency has been suggested to increase the susceptibility to infections. The specificity of H-ficolin is poorly characterized and currently limited to a few ligands only. We previously presented an interaction of this protein with lipopolysaccharides (LPS) of *Hafnia alvei*, being an opportunistic pathogen. In this report, we present the data concerning the involvement of H-ficolin in aggregation, phagocytosis and killing bacterial cells of *H. alvei* 1200 strain. We observed a strong agglutination of GFP-expressing bacteria, in the presence of recombinant H-ficolin and calcium. H-ficolin augmented phagocytosis of these microorganisms by macrophages. Phagocytosis rate was estimated by flow cytometry and fluorescence microscopy. More than 40% of cells contained phagocytosed bacteria, compared with less than 15% in the case of control (non-opsonized bacteria). These data were confirmed by confocal microscopy. Moreover, we showed much higher bactericidal activity of normal than H-ficolin-depleted human serum. To conclude, H-ficolin, in effect of enhancing of phagocytosis and microbicidal activity may be protective from or influence an outcome of the infection with some bacteria.
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P1.12.18
Increased sialylation of a recombinant IgG mutant produced in human embryonic kidney 293 (HEK293) cells

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The attachment of oligosaccharide, at Asn297, of the IgG-Fc heavy chain is essential for optimal binding and activation of Fcγ receptors and the C1q component of complement[1]. However, it has recently been shown that when fully processed, and bearing terminal sialic acid residues, effector functions are down-regulated, i.e. mediates anti-inflammatory therapeutic effects[2]. The sialylation level of human serum IgG-Fc is 10 - 20% whilst for recombinant IgGs produced from Chinese hamster ovary (CHO) cells sialylation is negligible. It has been shown that sialylation is markedly increased when a mouse-human chimeric IgG3 antibody, bearing a Phe/Ala243 mutation, is expressed in CHO cells[3]. However, CHO-derived IgG is α(2-3)-sialylated whilst human serum IgG is α(2-6)-sialylated. Here, we expressed an IgG1 F/A243 mutant in both human embryonic kidney (HEK) 293 and CHO cells. As expected, sialylation was increased in the F/A243 IgG1 mutant, as for IgG3, and the HEK293-derived IgG1 was α(2-6)-sialylated. Interestingly, increased addition of bisecting GlcNAc residue was also observed for the HEK 293-derived mutant. As sialic acid and bisecting GlcNAc residues are reported to have an opposite influence on antibody-dependent cellular cytotoxicity[4], the effector function profile of a fully sialylated F/A243 mutant antibodies may be of interest for development as therapeutics.

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P1.12.19
C-type lectin receptor Mincle and MCL mediate acquired immunity induced by mycobacterial glycolipid TDM

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We have recently identified the C-type lectin receptors Mincle and MCL are activating receptor for trehalose 6,6'-dimycolate (TDM). TDM is a glycolipid derived from the cell wall of *Mycobacterium tuberculosis*. It is widely known that TDM possesses potent adjuvant activity, however, the molecular mechanisms underlying has been unclear. In this study, we showed that two TDM receptors, Mincle and MCL, mediate TDM-induced acquired immunity using two acquired immunity models, delayed-typed hypersensitivity (DTH) and experimental autoimmune encephalomyelitis (EAE). TDM adjuvant strongly enhanced DTH response against OVA in WT mice, whereas it was impaired in both Mincle- and MCL- deficient mice. MCL-deficient mice failed to develop EAE. In contrast, weak but substantial EAE was observed in Mincle-deficient mice, suggesting that MCL preferentially mediates EAE induced by TDM. Finally, we carried out in vivo infection experiment using *Mycobacterium bovis* bacillus Calmette-Guérin (BCG). IFNγ production from T cells in recall response to the mycobacterial antigen (PPD) was reduced in Mincle- and MCL-deficient mice. These results suggest that Mincle and MCL play an important role in the induction of acquired immunity in response to mycobacterial adjuvant, which may lead to the development of TDM receptor-based vaccines against infectious diseases.

P1.12.20
Anti-inflammatory drugs from bugs: *Mycobacterium tuberculosis* gives us a hand

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Intracellular pathogens, such as *Mycobacterium tuberculosis*, manipulate innate immunity to down regulate inflammatory responses and secure their survival inside infected hosts. Understanding the tactics used by these pathogens and identifying their functional molecules opens new avenues for development of innovative classes of anti-inflammatory molecules. *Mycobacterium tuberculosis* exposes surface lipoglycans at its cell envelope, namely mannose-capped lipoarabinomannans (ManLAM), that inhibit the release of pro-inflammatory cytokines by LPS-stimulated human dendritic cells (DCs), via targeting the C-type lectin receptor DC-SIGN. With the aim of mimicking the bioactive supramolecular structure of ManLAM, we designed and synthesized a new set of poly(phosphorhydrazone) dendrimers grafted with mannose units, called mannodendrimers, that differed by size and the number and length of their (α1→2)-oligommanoside caps. A third-generation dendrimer bearing 48 trimannoside caps (3T) and a fourth-generation dendrimer bearing 96 dimannosides displayed the highest binding avidity for DC-SIGN. Moreover, these dendrimers inhibited TNF-α production by LPS-stimulated DCs in a DC-SIGN dependent fashion. Finally, in a model of acute lung inflammation in which mice were exposed to aerosolized LPS, *per os* administration of 3T mannodendrimer was found to significantly reduce neutrophil influx via targeting the DC-SIGN murine homolog SIGNR1. The 3T mannodendrimer therefore represents a novel type of fully synthetic powerful anti-inflammatory molecule, with an original mechanism of action, that may have therapeutic applications.

P1.12.21
Galectin-3 regulates dendritic cell responses and hematopoietic cell mobilization during acute peritonitis induced by pristane

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Galectin-3 adjusts cell-cell and cell-extracellular matrix interactions. We demonstrated that galectin-3 incites myeloid differentiation and inhibits B lymphocyte maturation in distinct lymphoid tissues. Here, we have investigated the role of galectin-3 in dendritic cell responses and hematopoietic cell mobilization during acute peritonitis. BALB/c (WT) and galectin-3$^{-/-}$ mice (gal-3$^{-/-}$) intraperitoneally injected with pristane (immunologic adjuvant and plasmacytoma inducer) were studied 18h after the injection. Pristane induced a significant increase in peritoneal cellularity of WT and gal-3$^{-/-}$ mice, although it was observed 4-fold more cells in both mice. Cell cycle analysis of peritoneal cells of stimulated mice reinforced the recruitment hypothesis because 98.5% of WT cells and 98.8% of gal-3$^{-/-}$ cells were in G1 phase. Phenotypic and morphological analysis revealed that CD11b^{+}Gr-1^{+} granulocytes and CD11b$^{+/low}$Gr-1^{-} monocytes were intensively mobilized to peritoneal cavity (PC) of WT mice and poorly mobilized in gal-3$^{-/-}$ mice, where the peritoneal exudate was essentially composed by mononuclear cells. Peritoneal CD4^{+}CD8^{-} B220^{-} T lymphocytes and B220^{+}CD19^{+}CD4^{-}CD8^{-} B cells were indifferent to pristane, although there were 3-fold fewer B cells and reduced CD8^{+} T cells in control- and induced-gal-3$^{-/-}$ mice, respectively. Interestingly, peritoneal dendritic cells (DCs) showed distinct responses to pristane in the absence of galectin-3. Paradoxically, peritoneal CD11c^{+}CD11b^{+} myeloid DCs were more evident in WT mice and B220^{+}CD11c^{+}CD11b^{-} plasmacytoid DCs were widely detected in gal-3$^{-/-}$ mice after pristane-injection. The results suggested that galectin-3 regulates dendritic cell responses and hematopoietic cell-fate decisions during acute peritonitis induced by pristane. We considered galectin-3 as an important target to understand immune and inflammatory responses.

P1.12.22

Galectin-8 knockout mice have sex-dependent altered homeostasis of t and b lymphocytes and autoimmune features

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Galectin-8 (Gal-8) regulates a variety of cellular activities interacting with beta-galactosides of cell surface glycoproteins. Its effects in the immune system include stimulation of apoptosis of thymocytes and activated T cells, proliferation of "naïve" T cells and differentiation of B lymphocytes into plasma cells. We have also described function-blocking autoantibodies in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis. However, the requirement of Gal-8 in immunological functions has not been studied "*in vivo*". Here we analyze the expression of Gal-8 in different organs and lymphocyte subpopulations in a Gal-8-null/LacZ knock-in mice (Gal-$8^{-/-}$). LacZ histochemistry show Gal-8 is expressed in different organs at different levels. Strikingly, only female Gal-$8^{-/-}$ mice, examined at 8-12 weeks, have increased spleen populations of CD4 and CD8 T lymphocytes and decreased population of B-lymphocytes, without differences in dendritic cells. By the age of 14 months, these female Gal-$8^{-/-}$ mice develop alopecia and splenomegaly accompanied by a significant decrease in the population of CD4 and CD8 T lymphocytes and B-lymphocytes. These mice also generate anti-ANA and anti-dsDNA autoantibodies. These features suggest an autoimmune disease such as SLE. These results reveal an important contribution of Gal-8 to the homeostasis of T and B cells, with sexual differences, such that alterations in its function might lead to autoimmunity. (Financed by Fondecyt postdoctoral project Nº 3120061, Fondecyt Nº 1100921 and CONICYT project Basal #PFB12/2007).

P1.12.23
The binding specificity of *H. pylori* LPS to DC-SIGN receptor

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The main pathogenic feature of Gram-negative bacilli-*Helicobacter pylori* is their ability to induce chronić infections. This feature allows them to avoid and to modulate the host immune responses. *H.pylori* lipopolysaccharide (LPS) demonstrates strong immunomodulatory properties, with rather suppressive effect on immune cells. The presence of Lewis epitopes in the O-specific region of LPS, possibly enables these bacteria to omit the immune responses, resulting in the phenomenon of antigenic mimicry. In addition, specific bacterial adhesins may recognize the dendritic cell receptor DC-SIGN. The binding of *H.pylori* to the DC-SIGN receptor, may result in the host immune response inhibition, which would encourage the enhancement of the infection. The aim of this study was to assess the binding of *H.pylori* LPS (with or without Lewis epitopes) with the DC-SIGN recombinant receptor in the optimized in-house ELISA assay. The plates were coated with various concentrations of recombinant human DC-SIGN-Fc chimeric peptide (RnD Systems), and the whole cell preparations or LPS with or without Lewis determinants were added. The specificity of this interactions was evaluated by the binding-inhibition-assay, with monoclonal antibodies against Lewis determinants, fucose, or galactose. Our results indicate that both *H.pylori* LPS with and without $Le^{x/y}$ antigenic determinants mediates binding of bacteria to DC-SIGN recombinant receptor. The residues of fucose present in the structure of Lewis determinants are responsible for binding specificity of *H.pylori* LPS to DC-SIGN receptor, whereas the residues of galactose determine binding specificity of the *H.pylori* LPS without Le antigens (LPS GalE) to this receptor.

P1.12.24
Galectin-3 plays a role in murine NK cell-mediated INF-γ production

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Natural killer (NK) cells serve as a crucial first line of defense against tumors and a diverse range of pathogens in the innate immunity. Highly related to receptors recognizing tumor cells, the activation receptors trigger cytotoxicity and cytokine production. IFN-γ, secreted by NK cells, plays a critical role in suppressing pathogen challenge, both to contain the initial infection, and to promote an appropriate adaptive response. Galectin-3, a member of β-galactoside-binding animal lectin, acts as immune modulators implicated in a variety of biological functions such as inflammation, tumor metastasis, and cell apoptosis. However, the role of immune regulation of galectin-3 on NK cells is not known. In our study, galectin-3 induced IFN-γ production in murine NK cells in a dose-dependent manner. IFN-γ production of NK cells by galectin-3 was inhibited by either anti-galectin-3 antibody or NF-*k*B inhibitor, but not lactose. These indicated that active site of galectin-3 in IFN-γ generation was in N-terminal but not in lectin domain that has many other physiological function of the molecule. IFN-γ production by galectin-3 was dependent on the activation, translocation and the transcriptional control of NF-kB. Galectin-3 induced IFN- γ production was enhanced through signaling pathway of phosphorylated Erk1/2. Erk1/2 phosphorylation was synergistically increased in galectin-3 treated NK cells and MEK1/2 inhibitor abrogated the phosphorylation of Erk1/2 by galecin-3. Our results provided new insights into mechanism of NK cell activation and, also, a novel function of galectin-3 in immune reaction.

P1.12.25
The MBL paradox: is functional MBL deficiency may be advantageous

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MBL is an ancient molecule, which has probably been subject to a large number of evolutionary pressures. MBL gene polymorphisms persist at high frequencies, suggesting that they offer potential advantages to the host. Changes in IgG glycosylation secondary to the underlying disease results in MBL-associated complement activation and then contributes to chronic inflammation Thus, there exists a balance in which certain individuals benefit from the expression of high levels of the protein, whereas others may benefit from reduced levels of circulating MBL. In the present retrospective cohort study, based on the ambulant visits of paediatrics departments Central Hospital Naestved, 35 patients aged 2-5 years with many RF in prenatal anamnesis, were investigated for serum levels of MBL, HYPA/LYPB genotypes and the MBL pathway function in serum. Conclusions: There was seen correlation between MBL deficiency, HYPA/LYPB gene mutation or reduced MBL pathway function, but MBL status was either advantageous or disadvantageous when considered from the viewpoint of the severity of a particular illness. Thus, those with higher levels of MBL are better able to modulate acute faze of infections, probably through an effect on cytokine responses. In contrast, those deficient in MBL appear to better modulate inflammation via down regulations of cytokine productions and oxidative stress. For these reasons, we believe that analyses of the relevance of MBL should be extended beyond its role in infectious disease and include clinical areas such as autoimmunity and inflammatory disorders.

P1.13 Fc and Fc-like receptors

P1.13.01
CD16 aggregation induced by therapeutic antibody-opsonised targets impairs cytotoxic responses in human NK cells

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The low affinity receptor for IgG, FcγRIIIA, CD16, is the prototype of NK activating receptors and is responsible for the Antibody Dependent Cellular Cytotoxicity (ADCC). It is well recognised that the clinical efficacy of a number of antibody-based therapeutic approaches largely relies on the ability to induce ADCC.
Here we analysed the dynamics of CD16 expression in primary human NK cells during cytolytic interactions with opsonized targets and the impact of receptor trafficking on NK cytotoxic potential. The chimeric Rituximab and the human Ofatumumab anti-CD20 therapeutic monoclonal antibodies were used to opsonize human lymphoblastoid CD20-positive cell lines.
Our findings demonstrate that the interaction with opsonized target cells induces a marked CD16 internalization associated with a reduced ADCC function in the absence receptor recycling.
Interestingly, in opsonised target-activated NK cells we observed a major impairment of the spontaneous cytotoxic response against a panel of sensitive targets. Such CD16-dependent NK cell hyporesponsiveness was largely independent from the exhaustion of cytolytic mediators or from the down-modulation of activation receptor surface expression. Further, exploring the ability of individual receptor/ligand interaction to induce the cytotoxic response, we observed that CD16-induced cross-tolerance regards different activating receptors including NKG2D, DNAM-1 and to a lesser extent NKp46 and 2B4.
Our data support the notion that stimulation via CD16 may lead to NK cell hyporesponsiveness to triggering via other unrelated signalling pathways.

P1.13.02
Streptococcus pneumoniae triggers platelet activation and platelet-leukocyte complex formation in a strain dependent and Toll-like receptor 2 independent manner

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BACKGROUND: S. pneumoniae strains vary considerably in their ability to cause invasive disease, and this is associated with the capsular serotype. The S. pneumoniae capsule inhibits complement- and phagocyte-mediated immunity. Septic patients often develop thrombocytopenia, which is associated with higher mortality. Besides the role of platelets in hemostasis, platelets exert a role in the immune continuum through release of regulatory proteins, platelet-neutrophil complex formation, and secretion of antimicrobial peptides.
AIMS: To determine how S. pneumoniae activates human platelets.
METHODS: S pneumoniae serotypes used: D39, TIGR4 and 6303 and mutant unencapsulated D39. Human platelet aggregation was measured in citrate-anticoagulated platelet rich plasma by light transmission aggregometry. Platelet degranulation and platelet-leukocyte complexes were measured by flow cytometry. Platelet and neutrophil killing capacity was determined in incubation experiments with S. pneumoniae.
RESULTS: Unencapsulated S. pneumoniae induced platelet aggregation in a capsule-dependent manner; aggregation was not induced by encapsulated strains, or by direct toll-like receptor (TLR) ligands. Aggregation was inhibited by IgG receptor blocking antibody anti-FcγRII, but not by anti-TLR2. Whole blood incubation with all S. pneumoniae serotypes resulted platelet degranulation and platelet-granulocyte and platelet-monocyte complex formation. These reactions were inhibited by PGE1 but not by anti-FcγRII, anti-TLR2 and 4. Platelets had no antimicrobial effect on S. pneumoniae, nor did platelet-neutrophil complex formation enhance neutrophil killing capacity.
CONCLUSIONS: S. pneumoniae causes FcγRII mediated platelet aggregation through a mechanism that is inhibited by its capsule. All S. pneumoniae serotypes are potent inducers of platelet degranulation and platelet-leukocyte complex formation in a TLR2 independent manner.

P1.13.03
Association of FCRL3 genotypes with susceptibility of Iranian patients to rheumatoid arthritis

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Rheumatoid arthritis (RA) is a complex disease, the hallmark of which is synovial joint inflammation. The substantial contribution from genetic factors in susceptibility to RA has been well defined. The Fc receptor-like3 (FCRL3) gene is one of the genes that have recently shown a significant association with RA. To determine the possible role of FCRL3-169 C/T and FCRL3-110 A/G gene polymorphisms in the development of RA in Iranian patients, 320 RA patients and 302 healthy subjects were genotyped using the polymerase chain reaction-restriction fragment length polymorphism method. No significant difference was found in genotype and allele frequencies of FCRL3-169 C/T between patients and controls, whereas a positive correlation was observed between this polymorphism and clinical findings, including erosion (P=0.017) and joint deformities (P=0.019). In contrast, at position -110 A/G, the frequency of the AA genotype was significantly decreased in RA patients compared to controls (P=0.005). The frequency of the G allele was also higher in patients than in controls (P=0.007). The results of this study showed a significant association between FCRL3-110 A/G polymorphism and susceptibility to RA. We also noted a significant association between the FCRL3-169 C/T polymorphism and clinical severity in Iranian patients.

P1.13.04
Fca/mR (CD351) regulates inflammatory responses of marginal zone B cells against experimental septic shock

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Fca/mR (CD351), an Fc receptor for IgA and IgM, is preferentially expressed on marginal zone B (MZB) cells and follicular dendritic cells (FDC). MZB cells, located at the border between white pulp and red pulp of the spleen, play an important role in the first line defense against blood borne antigens and quickly differentiate into plasmablast producing first wave of antibodies. However, the functional role of Fca/mR on MZB cells has been unclear.
To address this issue, we injected wild-type (WT) C57BL/6 mice or Fca/mR-deficient mice with LPS. Fca/mR-deficient mice showed significantly lower serum level of pro-inflammatory cytokines such as TNF-a and IL-6 and longer survival than WT mice did. To examine which cell type expressing Fca/mR is responsible for the attenuated inflammatory responses and prolonged survival, we generated mixed bone marrow chimeric mice, in which Fca/mR expression was specifically deleted on MZB cells, by using Fca/mR-deficient mice and CD19-deficient mice. The bone marrow chimeric mice showed the same phenotype as Fca/mR-deficient mice. We also generated bone marrow chimeric mice, in which Fca/mR expression was specifically deleted on FDC, by bone marrow transplantation from WT mice into lethally irradiated Fca/mR-deficient mice. We did not observe any difference in the pro-inflammatory cytokine level and survival between WT and the bone marrow chimeric mice. These results indicated that Fca/mR on MZB cells plays an important role in inflammatory responses against experimental septic shock.

P1.13.05

Association of FCγRIIA(CD32) polymorphism with susceptibility to brucellosis

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Background: Brucellosis is a major bacterial zoonoses of global importance caused by organisms belonging to the *Brucella*, intracellular bacteria. phagocytosis is the main host defense against *Brucell*avia IgG receptors. FCγRIIA receptor plays a centeral role in phagocytosis of IgG2-opsonized bacteria since it is the sole receptor able to interact with IgG2 antibodies. FCγRIIA exhibits allelic polymorphisms (FcγRIIa-R131 or FcγRIIa-H131) with different capacities for binding IgG2 and phagocytosis. cells expressing FcγRIIa-H131, bind more efficiently complexed IgG2 than those expressing the FcγRIIa-R131 variant. the purpose of this study was to evaluate the association of FCγRIIA polymorphisms with susceptibility to or severity of brucellosis. Subject and methods: In this study we determined FCγRIIA polymorphisms (R/R131, R/H131, H/H131) in 67 patients with brucellosis and 67 age ,sex and geographical matched healthy volunteers. FCγRIIA genotyping was performed by using a sequence-specific primer polymerase chain reaction (SSP-PCR). Result: Comparison of the FCγRIIA genotypes distribution in patients with brucellosis and controls showed a higher frequency in FCγRIIA-R/R131 homozygosity in patients than controls (47.8% vs 28.4%). Logistic regression analysis showed that there is a significant correlation between R/R131 genotype and brucellosis (OR=2.3, 95%CI=1.3-4.2, P=0.04). although the frequency of the FCγRIIA-R/R131 was higher in patients with brucellosis compared with controls, we did not find any statistically significant differences(53.8% vs 46.3%, P=0.65). as a result, there was no significant association between FCγRIIA genotype and severity of brucellosis.
Conclusion: The homozygous genotype of FCγRIIA-R/R131 in patient with brucellosis may be associated with susceptibility to brucellosis as a genetic risk factor.

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P1.13.06
The role of Prohibitin (PHB) in the initial reaction of the IgE Fc receptor signal from lipid raft

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To find the novel regulators affect on the initial mechanism of IgE Fcε receptor signaling, we used limited proteome analyses for lipid rafts from antibody stimulated RBL-2H3 cells. Lipid raft associated protein was prepared from stimulated RBL-2H3 cells or non-stimulated cells. Then, we identified an interesting protein, Prohibitin. Prohibitin is already known to be a regulator of cell proliferation and modulator of transcriptional activity. However, currently there is no information about what is the role of prohibitin in FcεRI signaling of mast cells and basophils.
Firstly, we confirmed that the localization of Prohibitin to raft fraction on non-stimulated or stimulated cell by using immunochemical staining. On Confocal laser scanning microscopy, we found that co-localization of raft and PHB both on stimulated cells and non-stimulated cells. By using biochemical technique, we detected small part of PHB in raft fraction and major part was in heavy fraction without stimulation. Interestingly, after stimulation, PHB in the raft fraction went along with stimulation, and quantity decreased. On the other hand, by using ds-RNA, we knocked down (KD) the Prohibitin for protein level expression. As a result, the degranulation reaction was significantly enhanced compared with wild type (WT). Also, on PHB-KD RBL-2H3 cells, it affects the phosphorylation of the MAP kinase and was no tyrosine phosphorylation of 100 kD protein that induced in stimulated WT cells.
Considering with these our findings, PHB seems to act as a negative regulator for Fcε receptor signals.

P1.13.07
Engagement of the IgM Fc receptor (FcμR) with its ligand occurs in *cis* rather than *trans*

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IgM is the first Ig isotype seen during phylogeny, ontogeny and immune responses. An IgM Fc receptor (FcμR) has recently been identified and, unlike FcRs for switched Ig isotypes, is predominantly expressed by adaptive immune cells, both B and T lymphocytes, in humans. FcμR binds pentameric IgM with high affinity (~10 nM) in Scatchard plot assays. To compare the interaction of FcμR with soluble versus membrane-attached IgM ligands, the apoptosis-prone Jurkat human T-cell line was transduced with both FcμR and GFP cDNAs or the GFP cDNA alone and the resultant cells with equivalent GFP expression were subjected to apoptosis assays using an agonistic IgM anti-Fas mAb. Control GFP^+ cells underwent apoptosis, whereas $FcμR^+/GFP^+$ cells did not. This FcμR-mediated anti-apoptotic activity was not affected by addition of 10-fold excess of FcμR-bearing but Fas-negative cells into the assay, suggesting that the predominant interaction of FcμR with the IgM Fas mAb is in *cis* on the same cell surface rather than in *trans* between neighboring cells. At least 50,000 molar excess of soluble IgM immune complexes was required for inhibiting the FcμR-mediated anti-apoptotic activity, whereas <100 molar excess of IgM mAb against CD2 on Jurkat cells did so, suggesting that FcμR has a higher affinity for the Fc portion of IgM attached to plasma membranes via its Fab region than for the Fc portion of soluble IgM immune complexes. These results suggest that FcμR can modulate the function of lymphocyte cell surface receptors/Ags that are recognized by natural or immune IgM Abs.

P1.13.08
Selective immunotherapy of lupus prone mice by biologically active peptides and simultaneous engagement of BCR and inhibitory B-cell receptors

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Autoreactive B lymphocytes play a major role in many autoimmune diseases as precursors of pathological autoantibody-producing plasma cells and as antigen-presenting cells. The appearance of pathological DNA-specific B cells in lupus is regarded as a major event in the development of the disease because of the specificity of the antibodies they produce.
Peptide-based immunotherapies offer an approach for selective target of autoreactive cells and leaving the remainder of the immune system intact. The triggering of the immunoglobulin B cell receptors (BCR) by the specific antigens ultimately induces gene expression patterns that can promote cell activation, anergy or apoptosis. Inhibitory co-receptors such as FcγRIIb and CD22 negatively regulate BCR signaling and thus prevent unphysiological overactivation of the B lymphocytes. These surface receptors on disease-associated B-lymphocytes are potential target molecules for therapeutic intervention.
We constructed several chimeric molecules by coupling different self - mimicking peptides to antibodies against FcγRIIb and CD22. The intravenous administration of the chimeras prevent the development of the symptoms of autoimmune disease in lupus-prone MRL/lpr mice - reduction of the levels of IgM and IgG anti-dsDNA antibodies, anti-Histone 1 IgG antibodies and proteinuria. The treatment protects the animals from skin lesions development, enlargement of the size of lymphoid organs and the deposition of IgG-containing immune complexes in the glomeruli. Kidneys from chimera-treated mice had a preserved histological structure.
The administration of these artificial molecules to lupus MRL/lpr mice suppress selectively the activity of disease-associated B lymphocytes and change the natural course of the spontaneous autoimmune disease.

P1.13.09

FcγRIIa (CD32) polymorphism and anti-malarial IgG subclass pattern among Fulani and sympatric ethnic groups living in eastern Sudan

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Background: A SNP at position 131, in the FcγRIIa gene, affects the binding of the different IgG subclasses and may influence the clinical variation seen in patients with falciparum malaria. This study confirms and extends previous findings, analysing the FcγRIIa (CD32) polymorphism in relation to the IgG subclass distribution seen among two sympatric tribes living in eastern Sudan, characterized by marked differences in susceptibility to *Plasmodium falciparum* malaria.
Methods: Two hundred and fifty Fulani subjects living in an area of meso-endemic *P. falciparum* malaria infection were genotyped for the FcγRIIa-131 polymorphism. For comparison, 101 non-Fulani donors – (Masaleit, Hausa and Four) – living in the same study area, were genotyped. The levels of plasma antibodies (IgG and subclasses) to four malaria antigens (AMA-1, MSP 2 – 3D7 & FC27, Pf332-C231) were measured using indirect enzyme-linked immunosorbent assays.
Results: The FcγRIIa-H/H131 genotype was found to be significantly more prevalent in the Fulani as compared to the non-Fulani ethnic groups (36.0% for Fulani versus 17.8% for non-Fulani, adjusted OR 3.10, 95% CI 1.61–5.97, P value < 0.001). The Fulani showed lower anti-malarial IgG1 and IgG3 antibody levels as compared to the non-Fulani and higher levels of IgG2 antibodies.
Conclusion: The FcγRIIa-H/H131 genotype and H131 allele is at higher frequency in the Fulani ethnic group. The H/H131 genotype was consistently associated with higher levels of anti-malarial IgG2 and IgG3 antibodies, while the R/R131 genotype was associated with higher levels of IgG1 antibodies.

P1.13.10

Greater number of activated T cells and enlarged germinal centers in the spleen of bovine FcRn transgenic mice in the background of the enhanced immune response

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The neonatal Fc receptor (FcRn) plays key roles in IgG and albumin homeostasis, maternal IgG transports and antigen presentation in case of IgG-opsonized antigens. We have previously shown that bovine FcRn (bFcRn) overexpression in transgenic (Tg) mice resulted in mounting robust humoral response against weakly immunogenic antigens and improving hybridoma production efficiency without any sign of autoimmunity. Further studies showed strong expression of bFcRn in peritoneal macrophages and bone marrow-derived dendritic cells (BMDCs) as well as indicated significantly improved T cell proliferation induced by BMDCs after IgG-ovalbumin immune complex phagocytosis *in vitro*.
To gain further insights of this enhanced antibody response, we immunized mice with ovalbumin and found at least twice as many activated, antigen-specific T cells in the spleen of bFcRn Tg mice compared with wild-type (wt) mice. Furthermore, germinal centers of the immunized bFcRn Tg mice were found to be double the size of those observed in wt controls after 3 days of booster injection, indicating significantly improved germinal center reactions in case of Tg mice.
To identify cell populations expressing bFcRn, we developed bFcRn-specific monoclonal antibody that does not cross react with mouse FcRn. We detected strong bFcRn expression in macrophages of splenic marginal zone, but also found bFcRn positive cells in the T cell zone and in the red pulp, possibly dendritic cells and red pulp macrophages, respectively. These new findings provide better explanations for the augmented humoral immune response in bFcRn transgenic mice.
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P1.13.11

Intravenous immunoglobulin treatment suppresses dendritic cell function in humans by stimulation of the IL-33-Th2 pathway

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High-dose intravenous immunoglobulin (IVIg) is used as anti-inflammatory therapy for autoimmune diseases. Studies in mice suggest that IVIg suppresses inflammation by stimulating inhibitory Fcγ-receptor (FcγR)IIb expression on myeloid cells via a cascade of IL-33-Th2 cytokine production. Here, we investigated whether IVIg monotherapy in 29 patients with hypogammaglobulinaemia or autoimmune diseases stimulates the IL-33-Th2 pathway and modulates FcγR expression on circulating leukocytes. Blood was collected before, immediately after, and 7 days after IVIg infusion. Plasma levels of IL-33 and the Th2 cytokines IL-4 and IL-13 increased following IVIg infusion ($p<0.01$ for each cytokine, day 7), while Th1 cytokine levels remained unchanged. Regression analyses suggested an IL-33-dependent induction of IL-4 ($r=0.69$, $p<0.001$) and IL-13 ($r=0.63$, $p<0.001$) production. *In vitro* experiments showed that IVIg induces IL-33 expression in human lymph node cells, and that IL-33 stimulates Th2 cytokine production by human basophils. Interestingly, expression of the activating FcγRIIa on circulating myeloid dendritic cells (mDCs), but not on monocytes, decreased upon IVIg-treatment (day 7: -30%; $p<0.05$), while FcγRIIb expression remained unchanged. Closer inspection revealed reduction of FcγRIIa expression ($p<0.01$) only upon high-dose IVIg infusion (≥0.6g/kg). This finding was corroborated by *in vitro* experiments showing that IL-4 inhibits FcγRIIa expression on blood-derived human mDCs ($p<0.01$), while FcγRIIb expression remained unchanged. Functionally, IL-4-pre-treated mDCs demonstrated reduced production of pro-inflammatory cytokines TNF-α and IL-8 upon immune-complex stimulation ($p<0.05$). Collectively, our data demonstrate that, in contrast to mice, high-dose IVIg inhibit human mDC function via the IL-33-Th2 cytokine axis by reducing FcγRIIa expression, rather than enhancing FcγRIIb expression.

P1.13.12

FcgammaRIIA and FcgammaRIIIB polymorphisms in Brazilian systemic lupus erythematosus patients and associations with clinical manifestations

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Background: Fcg receptors (FcgR), expressed on neutrophils, play essential roles in the immune response to infection and in inflammatory processes. Recent data have provided evidence that genetic polymorphism of Fcg receptors (FcgRIIa H/R-131 and FcgRIIIb HNA-1a/-1b/-1c) is associated with immune abnormalities and risk to development of systemic lupus erythematosus (SLE). Objective: To investigate whether the distribution of FCGR2A and FCGR3B polymorphisms confers risk for disease susceptibility and clinical manifestations of patients with SLE in Brazilian population. Study design and methods: A total of 157 patients (18 male and 139

female) diagnosed at HCFMRP-USP and which fulfilled the ACR classification criteria for SLE and 160 healthy volunteers (82 male and 78 female) were included in this study. Determination of FCGR2A and FCGR3B genotypes were performed using polymerase chain reaction (PCR)-based allotyping methods with allele-specific primers. The clinical features were obtained from the official medical record. Results: In the case of FcgRIIa polymorphism, it was observed an increase in the frequency of homozygous R-131 in patients (p=0.03; OR = 2.09) compared to genotype H-131. With respect to FcgRIIIb polymorphism, no significant skewing was found between patients and controls. The following clinical associations were found: FcgRIIa-H-131 allele is associated to susceptibility to arthritis and anti-DNA antibody production (p=0.05 for both); FcgRIIIb-HNA-2a allele is associated to susceptibility to malar rash (p=0.03) and arthritis (p=0.02). Conclusion: These results demonstrate the association of FcgRIIa polymorphism with susceptibility to SLE in Brazilian. Furthermore, the FcgRIIa and IIIb polymorphisms constitute factors influencing clinical manifestations of SLE.

P1.14 Cytokines and other regulatory mediators of inflammation

P1.14.01
Can Serial Quantification of IL-1 in human peripheral blood predict Outcome of fracture healing?

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BACKGROUND: Early stages of healing recruit variety of cytokines that stimulate the repair process. This project was aimed to elucidate whether these selected cytokines may have potential to predict traumatic diaphyseal delayed or non union early.
METHODOLOGY: Patients (n=102) with simple tibial diaphyseal fractures are enrolled and their blood samples were collected at definite follow ups and plasma level of cytokines was measured using the ELISA. Clinico-radiological examination was done at definite follow-ups and radiological assessment was done by RUST Scoring. Depending on the clinico-radiological parameters, patients were divided into 03 groups; A (n = 72): union achieved; B1 (n= 21): Delayed union; and B2 (n= 09): Non-union.
RESULTS: In group A and B1, mean cytokines levels of IL-1α and IL-1β showed significantly elevated expression within the mean at 4.2 days after fracture, which was increased on mean peak at 18.5 days with maximum mean level were 16.67 & 24.18 pg/mL respectively. But the mean levels of these cytokines was significantly higher in Group-A as compare to B1. The means of RUST scores of group A & B1 were 9.6 and 7.8 respectively. In Group B2, mean levels of these cytokines were poorly expressed throughout the follow-ups. The mean Rust score of 3.4 at 36 weeks of Group- B2 also seems low in comparison to Group- A and B1.
CONCLUSIONS: Plasma levels of these cytokines are closely related to early phase of fracture healing and may be taken as a diagnostic tool for earlier identification of patients at risk.

P1.14.02
IL-1β, IL-6 and TNF-α gene's expressions and receptor's mutations in ovarian cancer tissues

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Ovarian cancer (OC) remains the leading cause of death among gynecological malignancies and is the fourth most common cause of cancer-related death among women. The TLRs/inflammatory signaling mechanism involving epithelial cells are well-known as an important player for the host defense. Inflammatory cytokines were reported to be modulated in different cancer pathologies that involve epithelial cells. This can be through genetic modulation. Genetic variation in the inflammatory cytokine receptor's (IL-1, IL-6 and TNF-alpha), has been shown in many diseases but not in ovarian cancer. This study was designed to compare the expression of the inflammatory cytokine genes and their receptors in the tumor and normal ovarian tissue in Saudi population. To reach such goal we used quantitative RT-PCR and immunohistochemistry procedures on normal (n = 10) and ovarian cancer (n = 10) tissues. These were supported by analyzing the polymorphism of IL-6R, IL-1R and TNFR by exon sequencing.. The results showed an over expression of IL-1, IL-6 and TNF-α in cancer as compared to normal tissues. The level of these cytokines expression is function of the severity of cancer, which supports the evidence of the cytokines role in the development and promoting the ovarian cancer. This was confirmed by exon sequencing showing different mutations in the specific exons of IL-1R, IL-6R and TNF R. Data demonstrated a possible link between gene mutations, inflammatory processes and ovarian cancer initiation/development. Further studies are needed to shed light on possible inflammatory gene silencing and ovarian cancer control.

P1.14.03
Relationship between selenium intake and inflammatory markers in newly-diagnosed patients with B-cell non-Hodgkin lymphoma

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Background: Non-Hodgkin lymphoma (NHL) is the seventh most common cancer in the world. NHL develops from lymphoid cells. Selenium can modulate the expression of multiple cellular signaling pathways leading to decreased inflammatory gene expression.
Objectives: To determine the relationship between selenium intake and inflammatory markers in newly-diagnosed patients with B-cell NHL.
Materials and Methods: Newly-diagnosed patients with B-cell NHL and healthy age- and sex-matched controls were recruited at the Hematology Department of Tlemcen Medical Centre University (Algeria Northwest) for a retrospective case-control study.
Results: Dietary selenium intake was significantly decreased in patients compared controls, and was significantly correlated with high C-reactive protein (CRP) levels and serum protein electrophoresis patterns. Additionally, oxidant/antioxidant balance was altered in patients.
Conclusion: The reduction of selenium intake induces the synthesis of CRP during the acute phase response in B-cell NHL. Additionally, oxidative stress biomarkers and multi-serum proteomic models may be used for diagnosis and prognosis of the disease.

P1.14.04
Emerging roles of eosinophils and eosinophil-derived lipid mediators in the resolution of acute inflammation

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Resolution of inflammation is critical to restoration of normal tissue function following an inflammatory response. We recently demonstrated that eosinophil and eosinophil-derived lipid mediators contribute to this process. In mouse zymosan-induced peritonitis, eosinophils are recruited to the inflamed loci during the resolution phase. In vivo depletion of eosinophils caused a resolution deficit, namely impaired lymphatic drainage with reduced appearance of phagocytes carrying engulfed zymosan in the draining lymph node, and sustained numbers of neutrophils in inflamed tissues. LC-MS/MS-based lipidomics of the exudates revealed that locally activated eosinophils in the resolution phase produced 12/15-lipoxygenase (12/15-LOX)-derived antiinflammatory mediators including protectin D1 (PD1). The resolution deficit caused by eosinophil depletion was rescued by eosinophil restoration or the local administration of PD1. Eosinophils deficient in 12/15-LOX were unable to rescue the resolution phenotype. These results indicate that eosinophils play a role in promoting the resolution through the 12/15-LOX-mediated pathway. Eosinophils are known to be involved in allergic diseases and host protection against parasites through the release of cytokines/chemokines, mediators, and cytotoxic products. Here we provide the first evidence that eosinophils act as specific pro-resolving cells that are recruited and switched on during the

resolution phase of acute peritonitis. Detailed characterization of eosinophil and its functional interaction with other cell types in the resolution phase will provide insights into the molecular mechanisms for resolution of inflammation.

P1.14.05
Fragmented but not integral collagen IV induces IL-10 in human monocytes

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Leucocyte recruitment to site of infection or damage is crucial for inflammatory response. Once in the tissue, differential expression of integrins, selectins and metalloproteinases (MMPs) expression is needed for leucocyte migration. Collagen Type IV, the major protein of epithelial basal membranes, is target of MMPs activity. Both integral and degraded collagen could be recognized by leucocytes for MMPs induction, but it is unknown if it could increase inflammatory cytokines production, as reported for fibrin.
Objective: Determine if collagen IV recognition by human monocytes modifies cytokine production induced by LPS.
Procedures: Human Peripheral Blood Mononuclear Cells (PBMC) were obtained by density-gradient from Buffy Coats. One million PBMC were incubated in wells covered or not with collagen type IV(ColIV), digested or not with collagenase. Cells were stimulated with lipopolysaccharide (LPS,50 ng) from E. Coli(O26:B6). After 8,12,18 and 24 h, supernatants were collected for TNFa,IL-6,IL-1b,IL-8 and IL-10 quantification by cytometric bead array. To identify cytokine producing cells, intracellular staining procedure was done with cells preincubated with brefeldin A and Monensin (10ug) for 6h, and analyzed with flow cytometry.
Results: At 8 h, incubation of MCPBs with degraded collagen and LPS increased supernatant IL-10 concentration compared with LPS activated cells non collagen exposed. For other cytokines quantified, no significant difference were detected, among conditions explored. As evidence for intracellular detection of IL-10 in CD14+ cells, monocytes were the cells responsible for this IL-10 augmentation.
Conclusion: Fragment collagen recognition could induce regulatory pathways that enhance inflammation resolution and promote tissue repair.

P1.14.06
Analysis of c reactive protein levels after an eccentric exercise

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The C-reactive protein (CRP) is a plasma protein produced by the liver, which mainly operates in the acute phase response that is a body's response to inflammation process or an injury. Therefore, it is very important that tests may be done to determine the concentration of CRP in the bloodstream, in order to know some kind of injury or inflammation in the body. The eccentric exercises have been studied to see if they cause changes in inflammatory markers such as CRP. Randomly it was chosen 73 elderly who were sedentary and overweight. The elderly women were subjected to eccentric strength training through the leg extension machine . The maximum load used in the practice had been determined previously and pre-training and post-training examinations were conducted to determine if there were changes in the level of CRP, and the collection post-training were conducted at intervals of 0 and 3 hours. As results of the total elderly chosen : 38 elderly (about 52,02%) showed a reduction in the concentration of CRP, 25 elderly (34,24%) showed an increase and 10 elderly (13,69 %). This work can't set the eccentric training is an alternative for elderly.

P1.14.07
Immunomodulatory properties of bifidobacteria strains

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The use of bacteria in therapy has a long tradition. In the last years the number of clinical studies investigating bacteria in prevention or therapy of diseases has increased. The studies are based on the finding that interaction between microorganisms and the mucosal system of the intestine has important effects on development and function of immune system and on the health of the whole organism**.**
The aim of the study was to analyze the immunomodulatory properties of probiotic bifidobacteria strains from the Collection of Dairy Microorganisms Laktoflora ®.
The question was whether all strains react with blood mononuclear cells in the same way, or if there are differences between strains of the same species in cytokine stimulation and thus the strains may have a specific effect in the prevention and in the treatment of certain diseases. For this research was seleted 15 strains of the genus Bifidobacterium: *B. animalis subsp. lactis, B. longum subsp. infantis, B. longum subsp. longum, B. dentium and Bifidobacterium sp.*
Method: Mononuclear cells isolated from peripheral blood of healthy persons were cultivated with selected Bifidobacterium strain for 3 days at 37^{o} C and 5% CO_2 . Pro-inflammatory and anti-inflammatory cytokines produced during stimulation was determined by Luminex quantitative multiplex method.
Conclusion: It was found that different strains of bifidobacteria acting on the separated blood mononuclear cells stimulate cytokine production in individual manner. These characteristics of bifidobacteria strains could be used in the prevention and treatment of certain diseases. P28/LF1/6 and RO0511

P1.14.08
Higher baseline tumor necrosis factor [TNF]-α and early postoperative interleukin [IL]-10 level are associated with postoperative sepsis in cardiac surgery: A preliminary study

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Background and aim: Sepsis after cardiac surgery under cardiopulmonary bypass (CPB) is a serious complication. Cytokines are the main and first actors in sepsis, therefore the aim of this study was to evaluate pro-inflammatory (IL1, IL-6, IL-8 and TNF-α) and anti-inflammatory (IL-10) cytokines in patients undergoing a CPB as early markers.
Methods: Patients undergoing a CPB were enrolled in a prospective clinical study. They were divided in two groups as septic and non septic patients. The levels of TNFα, IL-6, IL-8, and IL-10 were measured at four different periods (T1: induction, T2: 5 minutes after the end of CBP, T3: 4h after the end of CBP, and T4: 24h post CBP). Quantitative variables are expressed as median (Interquartile range). CRP was measured at day 1, 3, 6 and 9 after surgery.
Results: Nineteen patients were studied. Six patients presented a postoperative sepsis. Only TNF-α at T1 [266.5 UI/ml (9.93; 480) vs. 7.75 UI/ml (6.66; 11.35) in septic vs. non septic patients respectively; p= 0.041] and IL-10 at T3 [6.35 UI/ml (5.37; 6.89) vs. 5 UI/ml (5; 5.01) in septic vs. non septic patients respectively; p= 0.022] were found to be associated with postoperative sepsis. There was no significant difference among CRP measurements.
Conclusion: Monitoring of cytokines, mainly TNFα and IL-10, are more reliable markers then CRP in this procedure. Therefore, these cytokines might be new tools for management of sepsis during CPB. However, the sample size in this preliminary study was too small to allow any definitive conclusion.

P1.14.09
Early diagnostics of the slowed down bone consolidation

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Method of external fixation - complex system allowing considerably to change principles of treatment, in par-ticular, of damages in an obverse skeleton. We study immunological status by 96 patients. On the basis of clinico-renthgenological criteria the patients were divided into two groups - with the slowed down and normal consolidation of bone in conditions of external fixing. Research of blood carried out at receipt of the patient in clinic and in dynamics after its realization (through 3, 10 day, 1-2 months). Reaction is characterized by high activity and short-lived of proc-ess. The data are received that patients with the slowed down consolidation after ostheosynthesis are revealed the spe-cific changes from the party immunological of system. In particular, after operation period the increase of the basic subpopulations leucocytes with change of their functional condition were marked. In process were involved phagocytic part of immunological system normalization of which activity were marked in one month after operation. Changes characterized humoral part (immunoglobulines, activity комплемента, circulating immunological complexes) of immunity. Inflammation reactions for operative intervention were characterized by the greater duration and less expressed changes of concentration acute phase reactants. Dynamics of IL concentration - IL-1, IL-2, IL-8, IL-10, raIL-1, TNF is established.

P1.14.10
Laboratory monitoring at hip arthroplasty

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The laboratory researches carried out in at 110 patients with degenerative and posttraumatic diseases of hip joint. On the basis of the investigated remote outcomes of operative treatment (hip joint replacement) - in terms till 4 years - all patients are divided into groups with the complicated current - sharp pneumonia, resorbtion of bone and instability of endoprosthesis - (57,3 %) and not complicated current implantation (42,7 %).
The estimation diagnostic informers of separate laboratory parameters have allowed allocating criteria of forecasting of complications before occurrence of the developed clinical picture. In particular, the change of functional activity of neutrophils and contents Ig A allows to assume an opportunity of development sharp pneumonia in terms about 11 day after operation. Monocytic and lymphocytic reaction of blood with increase of production osteoresorbtion factors of IL - 1 and TNF raises risk of osteoresorbtion formation around prosthesis in terms till 3 years. The development of sensibilisation to components of prosthesis, in a combination to an oppression phagocytic activity and decrease of serum immunoglobulines assumes formation of instability of prosthesis in terms till 2 years. Is marked, that the monitoring sharp pneumonia in early postoperation period has shown the clinical efficiency not less, than in 73,1 % of cases, bone resorbtion around prosthesis - in 72,3 %, instability of prosthesis - in 70,6 %.

P1.14.11
Effect of human recombinant interleukin-33 on collagen mRNA expression in lung fibroblasts

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The purpose of this study was to investigate the effect of IL-33 on the expression of collagen type I alpha 1 (COL1A1), collagen type I alpha 2 (COL1A2), and collagen type 3 alpha 1 (COL3A1) mRNA in human lung fibroblasts (HLF).
HLF were grown to confluence in RPMI-1640 medium supplemented with 10% fetal bovine serum, then cells were serum starved in media containing 0.5% dialyzed fetal bovine serum, and 24 hours later, treated with different concentrations (2 to 300 ng/ml) of recombinant human IL-33. mRNA expression levels for COL1A1, COL1A2, and COL3A1 were measured by real-time RT-PCR.
Stimulation of the lung fibroblasts with IL-33 led to a dose-dependent increase in the steady state level of COL3A1 mRNA, but not in COL1A1, and COL1A2 mRNA. The maximum effect of IL-33 on COL3A1 mRNA expression (4.3 fold increase, $p < 0.05$) was observed at 10 ng/ml. This finding suggests that IL-33 can enhance type 3 collagen gene expression, forms conditions promoting the development of fibrotic disorders.

P1.14.12
Pro and anti-inflammatory cytokines from periferal blood mononuclear cells in elderly

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Currently, the studies about immunosenescence demonstrate that the functions of innate immunity are more preserved than adaptive immune mechanisms. However, the two systems operate in an integrated way in the body, and an injury in one inevitably affects the other. The aim of this study was to investigate the *in vitro* production of proinflammatory (TNF-α, IL-6, IL-1β, CXCL-8 and MCP-1) and anti-inflammatory (TGF-β and IL-10) cytokines by monocytes, stimulated or not (basal) with lipopolysaccharide, from healthy young and elderly subjects. We also studied if cytokine profile is altered in these different patient groups, in the presence of lymphocytes, under the same experimental conditions. The monocytes from elderly presented higher basal production of TNF-α, MCP-1 and lower of TGF-β than young monocytes, reinforcing the systemic state of i*nflammaging.* In the presence of lymphocytes, the spontaneous production of IL-10 was higher and of TGF-β was lower than monocytes, regardless of age. After LPS-stimulation, the presence of lymphocytes resulted in decreased CXCL-8 and TGF-β and increased IL-6, IL-1β, MCP-1 and IL-10 in comparison to pure culture of monocytes, in young group. The same differences were observed with age, except for CXCL-8 and TGF-β which production was similar between monocytes and PBMC stimulated with LPS. In contrast to PBMC, monocytes of aged subjects showed alterations in cytokines production, suggesting an immunomodulator role of lymphocytes on monocytes.

P1.14.13
Acute inflammation and anemia: hepcidin and innate immunity

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Introduction: Immunity and iron are closely linked. Many studies in vitro and in animal models have recently elucidated the links of anemia with inflammation and how the innate immunity effectively restricts iron availability to microbial invaders. Hepcidin is an IL-6 induced hormone previously identified as an antimicrobial peptide, currently recognized as the master regulator of iron homeostasis and

the key modulator of inflammation-associated anemia. Hospitalization of patients with acute illness are often complicated by the development of anemia.
Aim: This study aims to explore the association between hepcidin and acute anemia of inflammation and the role of monocytes-derived hepcidin expression in patients with acute inflammation.
Patients and Methods: Sixty consecutive patients were prospectical enrolled after admission from the emergency for acute inflammatory illness, indicated by the presence of a proven or suspected infection and elevated C-RP. Blood was drawn at day 1 and 6 after admission for the measurement of hepcidin-25, cytokines profiling, erythropoietin, GDF-15 and iron status. Monocyte-derived hepcidin mRNA was also assessed.
Results: 95% of patients were affected by acute infections (batteremia, pneumonia, urinary tract infections). High IL-6 serum levels were associated with elevated concentrations of hepcidin, low iron levels, high transferrin saturation and very high ferritin serum levels. Between day 1 and 6, 26 patients lost hemoglobin (0.5 to 2.5g/dL; p<0.001). Erythropoiesis was blunted, despite high erythropoietin serum levels. Hepcidin concentration at admission was able to predict anemia occurrence on day 6. Monocytes-derived mRNA hepcidin positively correlated with serum ferritin and negatively correlated with IL-6R (gp130).

P1.14.14
SNARE proteins in the synaptic secretion of IL-12 and in tumor-induced immunosuppression of dendritic cells

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Secretion of IL-12 by pathogen-instructed DCs is crucial for initiation and programming of the T cell response. We have previously demonstrated that during formation of the DC-T immune synapse DCs polarize their secretory apparatus toward the interacting T cells leading to accumulation of IL-12 vesicles at the interaction site. However, the protein responsible for trafficking IL-12 at the immune synapse and the mechanism of its synaptic release are still unknown. The family of SNARE proteins, which regulate the membrane fusion process during the intracellular trafficking of soluble mediators, has emerged as the most informative for the identification of such pathways. Thus we have investigated the role of SNAREs in IL-12 secretion by DCs. These studies led to the identification of the lysosomal-late endosomal VAMP7 as a specific regulator in the polarized secretion of IL-12 during T cell priming. Furthermore VAMP3 was found to downregulate the production of all the cytokines tested, suggesting a role in TLR9 signalling pathway. SNARE proteins were also analysed in a model of tumour-tolerized DCs, in which the pattern of secreted cytokines is skewed. We analysed DCs *in vitro* (co-culture) and *in vivo* (spleen and tumour-bearing lungs) in a model of Lewis lung carcinoma (3LL) and found that tumour-exposed DCs downregulate all the SNAREs and in particular VAMP3. These observations suggest that regulation of trafficking proteins in DCs may represent a novel pathway targeted during immunosuppression.

P1.14.15
EMF Exposure Facilitates Wound Healing Events Modulating Cytokines Expression

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Background: During wound repair inflammatory cytokines are upregulated sequentially leading to the activation of keratinocyte growth and migration. IL-1, secreted by the injured keratinocytes, drives both keratinocyte activation and production of TNFα that maintain keratinocytes in their activated state. Activated keratinocytes are hyperproliferative and migratory. Previous studies showed that extremely low frequency electromagnetic fields (ELF-EMF) are able to regulate cytokine expression.
Objectives: On this basis the present study aimed to investigate the potential therapeutic use of ELF-EMF, evaluating, in HaCaT cells, the expression of cytokines that orchestrates successful wound.
Methods: HaCaT cells were starved for 24 h in serum-free, then wounded with a pipette tip to create a cell-free zone. HaCaT cells were exposed at 1 mT, 50Hz and compared to unexposed control cells. Wound healing was evaluated by measuring surface area, percentage and duration of healing. Cytokines expression was evaluated by RT-PCR in EMF-exposed and non-exposed HaCaT cells.
Results: After 24 hours the scratch area covered by keratinocyte was increased in EMF-exposed compared to non-exposed samples, in accord with the observed cell proliferation induced by EMF. Moreover, in EMF-exposed HaCaT expression of IL-1, TNFa, IL-18 and MMP-2 was increased and IL-10, IL-18BP and MMP-9 was decreased.
Conclusion: The ELF-EMF-enhanced expression of cytokines responsible for HaCaT proliferation and activation suggest an increased capacity of keratinocytes to reepithelialize the margin of wound avoiding the non-healing wounds. The ability of ELF-EMF to modulate cytokines expression highlights the potential therapeutic role of ELF-EMF in wound healing processes.

P1.14.16
Interleukin-1β micro-environment promotes viability and proliferation of malignant glioma cell U87MG

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Glioma is one of the most common and Glioblastoma (GBM) is most malignant tumours of the central nervous system. It is notorious for its highly proliferative, infiltrative and invasive behaviour. Despite substantial progress in early diagnosis and combined modality therapy including surgical therapy, radiotherapy and chemotherapy have failed to drastically change survival. Due to the invasive phenotype and diffuse penetration of glioblastoma cells into normal regions of the brain, patients diagnosed with GBM have mean survival of 10 to 12 months even with combined modality therapy. Their exceptional ability to proliferate and infiltrate normal brain makes complete radical surgical resection virtually impossible and leading in almost all cases to tumor recurrence. In human glioblastomas, pro-inflammatory cytokine IL-1β is expressed and secreted at high concentration. Objective of the current study was to study the role of IL1β on the glioma proliferation and cell viability. The human glioma cell line U87MG was cultured in 5% CO_2 and 95% humidified atmosphere air at 37°C in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% foetal bovine serum (FBS). Cells were trypsinized and 10^5 cells were incubated with 1 ng/mL IL-1β for 24 hours in serum free media. Cell viability was measured by Trypan blue dye exclusion assay and proliferation was analysed by MTT assay. We observed significant increase in viability of cells and proliferation of glioma cells in the microenvironment of IL-1β. We conclude that IL-1β promotes cell viability and proliferation of U87MG glioma cells.

P1.14.17
Clinical relevance of Cytokines Gene Polymorphism (CGP) in recovery of hemorrhagic shock patients

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Introduction: Severe trauma complicated with multiple organ dysfunction syndromes (MODS) are among the leading causes of deaths in intensive care units. Alarmingly, the mortality rate, owing to multiple causes e.g. multiple organ dysfunctions (MOD) with or without sepsis, is now reported to cross the value of 50% and thus need an immediate insight to overwhelm it. The cytokine levels and their associated gene polymorphisms are now contemplated as underlying cause for the severity of trauma.
Objective: This aims to examine if there exists any link between cytokine gene polymorphisms and outburst of pro-inflammatory/anti-inflammatory cytokines in the patients with various complications (sepsis/septic shock, MOF etc.) after traumatic injury.
Methodology: A total seventy trauma patients and fifty healthy controls were recruited in the study. The allèle, génotype, and haplotype fréquences of 13 cytokines gènes have been evaluated

using PCR-SSP. Cytokine concentrations in the individual patient's sera were determined by sandwich ELISA in a serum level dynamics up to 14 days.
Results and Conclusion: In septic patients, changes in the alleles and genotype frequencies in cytokines TGF-β (codon10), IL-10(-1082) and IL-6(-174) have emerged as major cause of sepsis and MOF related mortality. Thus, immune paralysis (IL-10 over expression) and Th17 (enhanced TGF-β/IL-6) concluded as putative cause of death in this study.

P1.14.19
Acute inflammation loci influence tissue repair in mice

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AIRmax (high inflammation) and AIRmin (low inflammation) mouse lines homozygous for *Slc11a1 S* alleles, produced by genotype-assisted mating, differ in ability to completely repair ear holes. AIRmax^{SS} mice showed fast ear tissue regeneration while AIRmin^{SS} mice did not show regeneration after ear punch. In this work, two-millimeter ear holes were done in these mice for the characterization of inflammatory reaction. The local inflammatory response was more intense in AIRmin^{SS} than AIRmax^{SS} mice 24 and 48h after ear punch, which was demonstrated by histomorphometric analysis, multiplex assay for inflammatory cytokines such as IL-1β, IL-6 and MIP-2 and higher levels of MPO. Global gene expression analysis demonstrated distinct over-represented biological themes between AIRmax^{SS} and AIRmin^{SS} control mice. At 24h after punch, both AIRmax^{SS} and AIRmin^{SS} showed significant up-regulated genes related to inflammation. However, angiogenesis, epidermis development and collagen catabolic process were expressed only in AIRmin^{SS}. All down-regulated genes in response to wounding in AIRmax^{SS} were represented to muscle contraction which is known to be involved in healing with scarring. Microarray results were validated by qPCR. These results suggest that the degree of inflammatory response in the early events after injury drives tissue regeneration or wound healing after ear punch.
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P1.14.20
Content of IL-15 in patients with myalgic encephalomyelitis / chronic fatigue syndrome

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Myalgic Encephalomyelitis/Chronic fatigue syndrome (ME/CFS) is considered as a neurological disorder with a dysfunction of the immune system. Patients have cognitive impairment, depression, sleep disorders, headaches and others. Previously we have found that the majority of patients with ME/CFS associated with herpes virus infection had reduced levels of IL-2. It is important to search biomarkers of ME/CFS and new approaches for the treatment. We studied the level of IL-15 - functional agonist IL-2.
IL-15 and proinflammatory cytokines: IL-1β, IL-8, IL-12 were measured by ELISA using BCM Diagnostics Ltd. 19 patients with ME/CFS associated with chronic herpes infection at the age of 22 to 51 years had a mixed infection: HHV 7 and/or HHV 6 and/or EBV in blood samples, saliva and oropharyngeal swabs measured by PCR. The comparison group consisted of 20 patients (21 to 50 years) with genital HPV infection. The control group: 18 healthy volunteers (20 to 45 years).
Results. Levels of IL-1β, IL-8 and IL-12 were increased or had a tendency to increase in patients with ME/CFS, and in the control group. The principal difference was observed in the level of IL-15. Thus, in patients with ME/CFS the level of IL-15 was lower than in the control group - 8,66 ± 0,93 pg/ml and 16,33 ± 2,1 pg/ml, ($p<0,05$), while in the control group the level was increased to 35,72 ± 4,22 pg/ml ($p<0,01$). Data obtained on the reduced level of IL-15, which has antiapoptotic effects, suggest its involvement in the pathogenesis of ME/CFS.

P1.14.21
Phagocytosis of apoptotic or necrotic cells differentially regulates the expression of IL-12 and IL-35 subunits in human dendritic cells

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Uptake of apoptotic or necrotic cells by dendritic cells (DC) is considered to have differential functional consequences, contributing to tolerance or immunity respectively. An important mechanism in this, is the regulation of IL-12 production. IL-12, a heterodimer composed of IL-12p40 and IL-12p35, belongs to a larger family of cytokines which share common chains. IL-35 is a potent immunosuppressive cytokine, consisting of IL-12p35 and Ebi3, which was initially demonstrated in regulatory T cells. Recently we showed that tolerogenic DC (tolDC) maintained expression of IL-12p35 and Ebi3, in the complete absence of IL-12p40.
When comparing tolDC with control immature DC(immDCs), we demonstrated an increased and accelerated uptake of apoptotic cells, which was not observed for necrotic cells. Culture of immDCs with necrotic cells was sufficient to induce IL-12p40 secretion. In contrast, ingestion of apoptotic cells by immDCs led to a strong increased expression of both IL-12p35 and Ebi3, without affecting IL-12p40. Moreover, apoptotic cells actively inhibited the production of IL-12 by DC following maturation. When investigating tolDCs, we found that challenge with apoptotic or necrotic cells did not further increase expression of Ebi3/IL-12p35, and IL-12p40 remained absent.
We conclude that uptake of apoptotic versus necrotic cells by DCs differentially regulates IL-12 family members, and that expression of IL-35 is favoured by apoptotic cells. Cellular therapy is a promising strategy to tailor immune responses in organ transplantation. Our study demonstrates that tolDC have an increased phagocytic capacity and a tolerogenic cytokine expression profile that is not affected by uptake of either apoptotic/necrotic cells.

P1.14.22
Immunoproperties of hypothalamic proline-rich polypeptides for antibacaterial immune response

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Antibiotic resistance and frequent occurrence of nosocomial infections and sepsis remain a major threat for infected people and hospitalized patients. A recent increase of multiresistant methicillin-resistant Staphylococcus aureus (MRSA) strains have developed resistance to started to pose great difficulty in selecting antimicrobial agents. In recent presentation we summarize the impact of hypothalamic neuropeptide PRP-1 also known as galarmin on adaptative immune response putative factors on cytokines and chemokines level both in methicillin-sensitive S. aureus (MSSA) and MRSA infection. Effects were analyzed in terms of animals secretion level for inflammatory markers such as IL-6, IL-8, IL-1b, IL-10, and keratynocyte chemoattractant (KC), and more detailed study was carried out to assay the level of IL-6 and macrophage inflammatory protein (MIP-2, CXCL2) at delayed stages of infection (72 hours post-infection). PRP-1 was compared with its structural analogues Gx-NH2, d-15 PRP-1 and dGx-NH2 to better understand the exact biochemical and biophysical properties required for its protective effect. PRP-1 and analogues stimulates higher production of both pro- and anti-inflammatory cytokines such as IL-6, IL-10, IL-1b, TNFa and KC at early stage of infection, and for more delayed period (72 hours post-infection) had significant impact on the plasma levels of the main markers of inflammation - IL-6 and MIP-2. Received data provide further insight into the intricate cytokine activity involved in the regulation of inflammatory responses and the control of bacterial infections and allow concluding that PRP-1 and its analogues are potential immunostimulatory agents for MRSA-induced infection prophylaxis and treatment in vivo.

P1.14.23
Targeting NO-driven Inflammation in Human Melanoma via IL-1s and CXCL-10

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An inflammatory microenvironment is an essential component of all tumors, including melanoma. We previously reported that the expression of inducible nitric-oxide synthase (iNOS) protein in melanoma tumor cells is strongly correlated with poor patient survival ($p< 0.001$) and is unrelated to the somatic mutations of BRAF, NRAS, and KIT. Therefore, we hypothesized that nitric oxide (NO) produced by iNOS promotes the inflammatory processes associated with poor outcome, and that attenuation of NO and associated effects would be beneficial. To approach testing this, we performed an inflammatory gene PCR array on a series of stage III melanoma of known iNOS-positive and iNOS-negative melanoma patient samples. Results indicated a series of differences including several folds of increase in IL-1β and decrease in CXCL-10, suggesting IL-1β as a marker of poor prognosis, and CXCL-10 as a marker of relatively better survival. Our functional studies revealed that treating iNOS-negative/CXCL-10-positive melanoma cell lines *in vitro* with a an NO donor reduced CXCL10; in contrast the scavenging of NO from iNOS-expressing cell lines increased expression and is hypothesized to potentiate the adaptive immune response. Our study suggests that an NO-driven carcinogenic inflammatory signature of increased IL1 α/β and decreased CXCL10 be targeted for therapeutic advantage in melanoma by reducing the production of NO in melanoma tumors.

P1.14.24
Interleukin 6 and interleukin 8 in maternal serum as markers of bacterial infection and preterm delivery

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Increased rate of preterm deliveries of pregnant women with intrauterine bacterial infections has been identified. IL-6 and IL-8 might stimulate release of prostaglandin, thereby causing preterm contractions and preterm delivery.
Goal is to examine relevance of the infection in occurrence of preterm contractions, as well as to investigate if preterm delivery is linked with increased concentration of IL-6 and IL-8 in maternal serum, as well as if measured values of the interleukins could be used in detection of early intrauterine infection and preterm delivery of pregnant women with the intact membranes.
In the experimental group, 34 of 53 pregnant women, or 64,2% had cervix infection, 26 pregnant women or 49% had positive urinal-culture, while 47 meaning 88,7% of pregnant women had bacterial vaginal infection. 14 pregnant women had all positive results, meaning 26, 4%. IL-6 has been found in blood of 37 pregnant women, meaning 70%. Average value of found IL-6 was 20,6+/-18,2 pg/ml.
IL-8 has been found in blood of 41 pregnant women, meaning 77%. Average value of found IL-8 was 11,2+/-7,3 pg/ml.
In the control group, IL-6 has been found in blood of 15 pregnant women, meaning 50%. Average value of IL-6 was 7,33+/-3,72 pg/ml. IL-8 has been found in blood of 17 pregnant women, meaning 57%, and its average value was 11, 6+/-7,5 pg/ml.
Direct linkage of bacterial infection and preterm delivery has been proven by this research. IL-6, IL-8 could be used as serum biomarker of bacterial infection and preterm delivery only combined with other interleukins.

P1.14.25
Constitutive STAT-3 activation with maintained responsiveness to IL-6 in peripheral lymphocytes of obese women

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Obesity is characterized by chronic low-grade inflammation, which increases risk of type 2 diabetes, cardiovascular disease, cancer and other pathologies. Inflammation in obesity is associated with chronically elevated levels of IL-6 and leptin, both of which signal through the STAT-3 pathway. A pathogenetic role for constitutively active STAT-3 has been demonstrated in chronic inflammatory diseases and cancer, with evidence indicating that activation of STAT-3 in leukocytes significantly influences the immune response. We investigated whether obesity is associated with alterations in STAT-3 activation in peripheral blood leukocytes. Forty women were divided into groups matched by age and ethnicity based on BMI. Plasma levels of IL-6, sIL-6R, sgp130 and leptin were measured by ELISA. Heparinized whole blood was stimulated with increasing concentrations of IL-6 (0-10 ng/ml) for 10 minutes and phosphorylation of STAT-3 (pSTAT-3) in lymphocytes evaluated by flow cytometry. Plasma IL-6 and leptin levels significantly increased with increasing BMI, as expected. However, plasma sIL-6R and sgp130 were not significantly different among groups. The percentage of unstimulated lymphocytes (no IL-6 added) positive for pSTAT-3 progressively increased with increasing BMI (6.1+/-1.5, 11.0+/-1.6, 19.1+/-3.0 and 20.4+/-2.1 with BMI 50, respectively; $p<0.001$). However, obesity did not alter the ability of lymphocytes to dose-dependently respond to IL-6 with activation of STAT-3. No significant differences among BMI groups were observed for basal or stimulated pSTAT-3 in the neutrophil/monocyte population. Our data suggest that constitutively active STAT-3 in obesity with maintained responsiveness to IL-6 may participate in determining the increased risk of chronic pathologies of obese individuals.

P1.14.26
Evaluation of the immunological reactivity of blood cells in early pregnancy for predicting of pregnancy outcomes

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The aim: to evaluate the spontaneous and mitogen-induced production of cytokines in the early pregnancy, related to its outcome. The immunological assessment of 64 women in early pregnancy (4-12 weeks) was made. The first group consisted of 48 women whose pregnancies was complicated by compensated placental insufficiency. The second group - 60 women whose pregnancy was complicated by impairment of the fetoplacental circulation and/or fetal intrauterine growth restriction. The third group included 19 women whose pregnancy was terminated by early spontaneous abortion (before 12 weeks). The comparison group included 112 women with normal pregnancy. Spontaneous and mitogen-induced cytokine production by immune cells was determined ex vivo. It was demonstrated that the pathogenesis of placental insufficiency is associated with induced Th1-dependent immune response, characterized by increased mitogen-induced synthesis of IFN-γ and accompanied by overproduction of proinflammatory mediators. Compensated placental insufficiency was assotiated with a reduction in basal synthesis of IL-2 and increased stimulated production of IL-1β, IL-1Ra, and IFN-γ. The most significant immunological changes occur in early pregnancy terminated by spontaneous miscarriage: reduction of spontaneous production of factors with a pro-inflammatory and angiogenic effect (IL-2, IL-8 and TNF-a) as well as regulatory IL-10, and increase in induced synthesis IFN-γ, accompanied by overproduction of IL-2 and IL-8. Decreased production of tolerogen IL-10 in vivo may indicate the ineffective mechanisms which protected the fetus from maternal aggressive factors by activation of its immune system. Cytokine-producing dysfunction of immune cells may play a key role in endothelial hyperactivity, causing a thrombotic complications and abortion.

P1.14.27

Cytokine's concentrations and monocyte HLA DR expression as markers defining the therapeutic strategy in contemporary behavior in patients with acute pancreatitis

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Acute pancreatitis (AP) has an incidence of approximately 40 cases per year per 100,000 adults. The mortality rate among patients with severe pancreatitis may approach 30% . The development of acute pancreatitis illustrates the requirement for understanding the basic mechanisms of disease progression to drive the exploration of therapeutic options.
Cytokines play a major role in the pathogenesis of AP as underlying systemic inflammatory response, tissue damage and organ dysfunction. Therefore, the determination of their concentrations could provide both early evaluation of severity,systemic complications and dynamic assessment of initiated treatment . However, little is known about circulating concentrations of these and other inflammatory cytokines and their real impact in clinical practice.
Several studies suggest that the prognosis for acute pancreatitis depends upon the degree of intensity of multisystem organ failure generated by the systemic inflammatory response. This suggests an intricate balance between localized tissue damage with proinflammatory cytokine production and anti-inflammatory response that restricts the inappropriate movement of proinflammatory agents into the circulation. Some of these mediators are markers for disease activity, whereas other inflammatory components may actually drive the disease process as important mediators. Implication of such mediators suggests that interruption or blunting of an inappropriate immune response has the potential to improve outcome. Full understanding of these basic mechanisms involves determining not only which mediators are present, but also closely documenting the kinetics of their appearance.

P1.14.28
Macrophage migration inhibitory factor as a regulator of neonatal innate immune responses

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Background: Newborn infants are characterized by a state of immunosuppression which puts them at risk for severe infections. MIF, a pleiotropic cytokine constitutively expressed in blood and immune cells, is an important regulator of innate immune responses. Here, we addressed the role of MIF in neonatal immunity by investigating the developmental profile of MIF expression and the impact of MIF on innate immune responses of newborn monocytes.
Methods: MIF plasma levels were measured by ELISA in 32 premature and 58 term newborns, 100 children (1 month-16 years) and 20 adults. Cord blood monocytes were transfected with MIF siRNA or incubated with the MIF antagonist ISO-1. Cytokines were quantified by ELISA/Luminex in cell-culture supernatants collected 18h after stimulation with endotoxin, bacterial lipopeptide, *Escherichia coli* and Group B *Streptococcus* (GBS).
Results: Circulating MIF levels were 10-fold higher in cord blood compared to adult blood (92.4±51.2 vs 7.1±3.8ng/ml, p<0.0001) and gradually decreased during childhood. Cord blood MIF levels correlated positively with gestational age and birthweight. Newborn monocytes expressed high levels of MIF, and released MIF upon stimulation with *Escherichia coli* and GBS. Inhibition of MIF expression with MIF siRNA or MIF activity with ISO-1 reduced 1.5-5.7-fold the secretion of pro-inflammatory (TNF, IL-1beta, IL-6, IL-8, IL-12p40, IL-12p70 and IL-23) and anti-inflammatory (IL-10, IL-20 and IL-27) cytokines by newborn monocytes exposed to microbial products.
Conclusion: MIF expression is developmentally regulated, with strikingly elevated levels in newborns compared to adults. High MIF levels at birth may act to balance the pro/anti-inflammatory immune responses in newborns.

P1.14.29
TNF-alpha, IL-4 and IL-10 cytokines induces changes in HO-1 and HDC expression in cervical cancer cells

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Background: Chronic infection with human papilloma virus (HPV) has been associated as an important factor in generating cancer. E6 and E7 viral proteins are involved in cell transformation. Likewise tumor cells generate escape mechanisms as the expression of molecules that regulate the immune response. HDC and HO-1 exert anti-apoptotic functions. These enzymes may represent another mechanism of resistance in cervical cancer cells.
Objectives: We analyzed the gene and protein expression of heme oxygenase (HO-1) and histidine decarboxylase (HDC) enzymes in HPV+ HeLa and SiHa cervical cancer cell lines and HPV- C33A cancer cells treated with TNF-α, I-4 and IL-10 cytokines.
Methods: HeLa, SiHa and C33A cervical cancer cells were treated with pro and anti-inflammatory cytokines; mRNA was extracted and analyzed expression of HO1 and HDC by real-time PCR. Protein expression was assessed by flow cytometry.
Results: Cytokines treatment induces a downregulation in HDC gene expression in HPV+ cancer cells in comparison with untreated tumor cells. In HeLa and SiHa cells this gene is downregulated whereas in C33A cancer cells we observed upregulation of HDC gene. In general HDC and HO-1 protein expression was reduced in cancer cells treated with pro and anti-inflammatory cytokines.
Conclusions: HDC and HO-1 protein expression is modified when the cells were treated with cytokines. In HeLa, SiHa and C33 cancer cells the expression of these enzymes is similar, however at the gene level the behavior is different between cancer cell lines, and this could be due to the presence of HPV infection in some cancer cells.

P1.14.30
To study the contribution of activated CD4+ and CD8+T cells arisen from B-T constructs of F1 and V antigens of Yersinia pestis for the antibacterial activity as an improved vaccine

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Yersinia pestis, a Gram negative bacterium, is the etiological agent of pneumonic and bubonic plague. The current efforts are aimed at evaluating whether our vaccine protocol primes memory T cells that rapidly produce IFN-γ and/or perforin in response to peptide or conjugate stimulation.
In the present study, the secretion of IFN-γ and IL-4 by splenocytes, stimulated by different constructs of B and T cell epitopes of F1 and V antigens, was measured by ELISpot assay. We also measured perforin and IFN-γ expression as a function of cell mediated immunity by flow cytometry. We observed that three B-T constructs of F1 and seven B-T constructs of V antigens produced a high number of IFN-γ secreting cells as compared to native antigen and a low number of IL-4 secreting cells. B-T conjugates of F1 and V antigens showed significantly high ($p < 0.001$) percentage of $CD4^+$ IFN-γ+ cells as compared to $CD8^+$ IFN-γ+ cells.
Our study demonstrated that IFN-γ is an effector molecule of activated $CD4^+$ and $CD8^+$T cells arisen from B-T constructs of F1 and V antigens for the antibacterial activity as an improved plague vaccine.

P1.14.31
Resiniferatoxin exhibits anti-inflammatory property by inhibits pro-inflammatory mediators in endotoxin-challenged mice

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Different chemical structural types of natural products exhibit anti-inflammatory activity and are considered to be potential drug candidates against the inflammation-related pathological processes. Vanilloids bind to the transient receptor potential type V1 (TRPV1) channels, nonselective cation ionophores that play an important role in integration of afferent noxious signals generated by inflammatory mediators. Resiniferatoxin (RTX) is an ultrapotent capsaicin analog showed pharmacological effect including anti-inflammatory activity. BALB/c mice were exposed to LPS application (250µg/100g) in order to evaluate the anti-inflammatory activity RTX. Five different groups were used: (i) control group (none stimulus); (ii) LPS; (iii) LPS-dexamethasone; (iv) LPS- RTX (20 µg/kg) and (v) vehicle control. Plasmas samples were collected and stored at -70ºC, 180 min after the last LPS or treatment administration. The ip administration of RTX provided a significant anti-inflammatory effect. 180 min after RTX administration, the pro-inflammatory cytokines (TNF-α and IL-1β) was significantly inhibited ($p<0.005$). Reduction in the nitric oxide levels was significantly ($p<0.001$), this results demonstrate the efficacy and therapeutic potential of RTX in inflammatory process and may be promising drug for targeted inhibition inflammatory diseases.

P1.14.32
Biomarkers of cardiovascular risk in psychiatric disorders

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Cardiovascular disease (CVD) and depressive illness are two of the world's leading health problems. The most common types of CVD are coronary artery disease and cerebrovascular disease. CAD is a leading cause of morbidity and mortality in the industrialized world. Depressive illness is a very common disorder afflicting an estimated 120 million people worldwide. It is the leading cause of disability worldwide. Epidemiological studies have confirmed the high co-morbidity between these two entities and the co-morbidity is bidirectional. Systems accountable for this co-morbidity include the central and autonomic nervous systems, the neuroendocrine system, the immune system, and the vascular and hematologic systems. Pathophysiologic factors include homeostatic imbalance between the sympathetic and the parasympathetic systems with loss of heart rate variability in depression, sympathoadrenal activation, HPA activation resulting in hypercortisolemia, immune system dysregulation with release of pro-inflammatory cytokines and chemokines, platelet activation and hypercoaguability. These abnormalities have been demonstrated in individuals diagnosed with major depressive disorder. Inflammation also occurs in cardiac and cardiovascular pathology independent of the presence or absence of depression. A chronic pro-inflammatory status has been documented in numerous studies of depression and may extend beyond symptom remission. Inflammation is associated with endothelial dysfunction which is a preamble to atherosclerosis and atherothrombosis. Endothelial dysfunction has been detected in depression and may be a trait marker. Thus understanding vascular biology in conjunction with psychiatric co-morbidity is critical. I will present recent data of a pro-inflammatory status in depressed patients and ANS imbalance as reflected in diminished heart rate variability.

P1.14.33
miRNAs in Sera of Tunisian Patients discriminate between inflammatory Breast Cancer and non-inflammatory Breast Cancer

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The microRNA (miRNA) is key regulators of gene expression. Recent studies have shown that aberrant expression of miRNAs has been implicated in many pathological conditions such as cancer and is associated with tumor invasivity, metastatic potential and clinical features of different types of cancers especially breast cancer. Our aim is to study the alteration of circulating microRNA levels in the model of breast cancer in an attempt to characterize the phenotype of inflammatory breast cancer (IBC).
We used quantitative PCR technology to evaluate the expression of 12 miRNAs (hsa-miR-5p, hsa-miR-3p, hsa-miR-320, hsa-miR-30b, hsa-miR-29a, hsa-miR-24, hsa-miR-15a, hsa-miR-548d-5p, hsa-miR-486-3p, hsa-miR-451, hsa-miR-337-5p, hsa-miR-335) in sera from 20 IBC and 20 non-IBC patients.
We identified a set of 4 miRNAs of which expression differed between IBC and non-IBC, making these miRNAs candidate markers for the IBC subtype.
In conclusions, our observations from this pilot study suggest that the altered levels of circulating miRNAs might have great potential for defining a molecular signature of IBC.

P1.14.34
Identification, function and mechanism of novel potential cytokines and membrane molecules of immune cells

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Cytokines and membrane molecules are major mediators of the immune system. They have essential physiological and pathological roles. More importantly, many of them have been developed to be biomedical drugs. Therefore, it will be of great significance to identify novel potential cytokines and membrane molecules. In 1998, we characterized CKLF1 with chemotactic activity on leukocytes using SSH method. Based on *CKLF*, we identified another 8 genes named *CMTM1* to *CMTM8* using bioinformatic analysis and experimental validation. *CKLF* and *CMTM1-8* constitute the novel gene family CMTM. They have important roles in the immune and male reproductive systems, and participate in tumorigenesis. *CMTM3*, *CMTM5* and *CMTM7* are novel potential tumor suppressor genes. In 2008, we carried out genome-wide bioinformatics analysis as well as literature data mining to characterize novel potential cytokines and membrane molecules. Now, for novel potential cytokines, we obtained 212 candidates numbered 1#-212#, constructed the eukaryotic expression vectors of 121 genes and verified 36 secreted proteins, several recombinant proteins including VSTM1-v2, FAM19A4, TMEM98 et al. have modulatory effects on immune cells. For the membrane molecules, we focus on the expression and function of VSTM1-v1,TMIGD2,LRRC25 and TMEM154, which have characteristic expression profile and might involve the proliferation and differentiation of immune cells.

P1.14.35
The effect of type I and III interferons on the adrenal cortex in the context of autoimmune Addison's disease

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Autoimmune Addison's disease (AAD) is caused by immune-mediated destruction of the adrenal cortices. Although the detailed pathogenesis of AAD remains elusive, autoantibodies and T cell responses towards 21-hydroxylase (21OH) of the adrenal cortex are often observed. IFN-α, IFN-β and IFN-λ are a set of cytokines typically induced by viral infections that have been implicated in several autoimmune conditions, like autoimmune thyroiditis, rheumatoid arthritis and type 1 diabetes. Also, treatment of hepatitis C virus infections with IFNα has been reported to trigger production of autoantibodies associated with autoimmune diseases, including antibodies against 21OH. This indicates that interferons may play a role in the induction and/or maintenance of autoimmune responses. To evaluate potential pathogenic effects of interferons in relation to AAD, we performed in vitro cell culture studies on the adrenocortical carcinoma cell line H295R. In accordance with a previous report, we found that the IFN-α/β receptor (IFNAR) was expressed in H295R cells and that stimulation with recombinant IFN-α and IFN-β induced apoptosis in a dose-dependent manner as measured by LDH release. Moreover, H295R cells expressed even higher basal levels of the IFN-λ receptor than IFNAR, and were sensitive to IFN-λ-induced apoptosis. Stimulation with either IFN-α, IFN-β or IFN-λ also upregulated MHC class I expression and sensitized H295R cells to poly (I:C)-induced chemokine secretion. The induction of these interferons in the adrenal cortex, e.g. by a local virus infection, could potentially contribute to AAD in susceptible individuals by the highlighted mechanisms.

P1.14.36
Immune Senescence in an Innate Immunity Drosophila model

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Changes in the immune system are considered a hallmark of aging in many living organisms including mammals. As we age, the ability to fight off invaders decreases, yet components of the inflammatory pathway are upregulated with age, leading to low-grade chronic inflammation. The Fruit fly Drosophila Melanogaster provides a useful model inwhich to study changes in the innate immune system with age. The Drosophila malpighian tubule (MT) provides an isolated ex vivo system in which to dissect the different pathways playing a role in immunosenescence. Our studies suggest that, while the aging malpighian tubule is hyper-responsive to immune challenges, its capacity to respond effectively to these challenges is diminished. This hyperstimulation response is dependent on the NF Kappa B homolog Relish. We also show that physiologycal stresses such as dessication can elicit hyperstimulation.Underlying mechanisms include alterations in protein translation, stability, or secretion, and studies are underway to elucidate between these possibilities.

P1.14.37
Inverse susceptibility to liver and lung carcinogenesis in mouse lines genetically selected for acute inflammatory response

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Hepatocellular carcinoma (HCC) is the most common liver cancer in humans and many inflammatory diseases are risk factors for its development. AIRmax and AIRmin mouse strains were selected for maximum and minimum acute inflammatory responsiveness (AIR), respectively. Urethane induced both lung tumors and HCCs, with an inverse susceptibility to lung versus liver carcinogenesis in AIRmin and AIRmax mice. Herein, we used diethylnitrosamine (DEN), widely used in experimental hepatocarcinogenesis, and found that at 240 days after DEN treatment, most AIRmax male mice had multiple and large HCC and none or few lung tumors, whereas AIRmin male mice carried small lesions in the liver but multiple lung tumors. This inverse susceptibility was also observed in females but to a lesser extent. Early production of TNF-alpha, IL-1beta and IL-6 in liver after DEN treatment was correlated with tumor development in AIRmax mice. Transcriptome analysis of livers of untreated mice showed specific gene expression profiles in each line which may play a role in their differential susceptibility to HCC. These results demonstrate that the genetic control of AIR segregated in AIRmax and AIRmin mice modulates susceptibility to different types of cancer and highlight the role of local tissue cells in the control of immune responses to tumors. Financial support: FAPESP and CNPq (Brazil).

P1.14.38
Platelet-activating factor enhances tumor metastasis via the reactive oxygen species-dependent protein kinase CK2-mediated NF-kB activation

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Platelet-activating factor (PAF), which is a potent lipid mediator involved in a myriad of inflammatory reactions, promotes tumor metastasis via the activation of the transcription factor, NF-κB. In this study, we investigated the role of the protein kinase CK2 in PAF-induced NF-κB activation and tumor metastasis given that PAF has been reported to increase CK2 activity and CK2 plays a key role in NF-κB activation. PAF increased CK2 activity, phosphorylation, and protein expression in vivo as well as in vitro. CK2 inhibitors inhibited the PAF-mediated NF-κB activation and expression of NF-κB-dependent proinflammatory cytokines and antiapoptotic factors. Pretreatment with the antioxidants, NAC, resulted in a significant inhibition in PAF-induced enhancement of CK2 activity, phosphorylation, and protein expression in vivo as well as in vitro. H_2O_2 and the known reactive oxygen species (ROS) inducers such as LPS and TNF-α enhanced CK2 activity, phosphorylation, and protein expression, which was again inhibited by antioxidant. PAF-, LPS- and TNF-α-induced increased CK2 activity, phosphorylation, and protein expression were inhibited by p38 inhibitor. PAF, LPS or TNF-α increased pulmonary metastasis of B16F10, which were inhibited by antioxidant, CK2 inhibitor or p38 inhibitor. Our data suggest that ROS activates CK2 via p38, which, in turn, induces NF-κB activation, and 2) PAF, LPS and TNF-α increase pulmonary tumor metastasis via induction of ROS/p38/CK2/NF-κB pathway.

P1.14.39
Pathophysiological roles of the CX3CL1-CX3CR1 axis in chemical-induced skin carcinogenesis

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We explored the pathophysiological roles of the CX3CL1-CX3CR1 axis in chemical-induced two-step skin carcinogenesis model by using CX3CR1-/- mice. Both WT (C57BL/6) and KO mice were painted with DMBA (100 μg/200 μl acetone) followed by the treatment of TPA (30 μg/200 μl acetone) for 20 weeks. In the skin of WT mice, the gene expression of Cx3cl1 and Cx3cr1 was significantly enhanced at 2 and 10 weeks after treatment. Intradermal macrophages expressed both CX3CL1 and CX3CR1 after treatment. Moreover, CX3CR1 was also expressed by T cells and endothelial cells. In order to explore the pathophysiological roles of the CX3CL1-CX3CR1 axis in skin carcinogenesis, we monitored tumor growth in both strains. About 80% of WT mice developed papillomas by 16 weeks post-DMBA/TPA treatment, whereas less than 40% of CX3CR1-/- mice developed papillomas. The numbers of papillomas were significantly lower in KO mice than in WT ones. However, in CX3CR1-/- mice, epidermal hyperplasia was markedly impaired,

compared with WT mice. Recruitment of F4/80+ macrophage and CD3+ T cells was more evident in WT than in CX3CR1-/- mice. Moreover, intratumor vessel number was significantly less in KO mice than in WT ones. In skin carcinogenesis, several inflammatory mediators such as cytokines, chemokines, and COX-2 played important roles. In the skin samples of CX3CR1-/- mice after DMBA/TPA treatment, the gene expression of Cxcl1, Cxcl2, Il1b, Il6, Tnfa, and Cox2 was significantly attenuated, compared with WT mice. These observations implied that the CX3CL1-CX3CR1 axis was crucially involved in chemical-induced skin carcinogenesis.

P1.14.40
Inflammatory and cytotoxic effect of silver nanoparticles on human endothelial cells

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Due to silver's antimicrobial properties, silver nanoparticles are used in many biomedical applications. The main objective of this study was to elucidate the cellular effects on endothelial cell include cytotoxicity and cytokine production. The size-dependent cellular effects of silver nanoparticles (5, 20, 100 nm) were evaluated using EA.hy926 cells (human endothelial cell).
Silver nanoparticles and their cellular distribution were characterized by transmission electron microscopy (TEM). Cytotoxicity was measured by the cell counting kit-8 (CCK-8) assay. To quantify relative mRNA levels of the genes of interest in target tissue, real-time PCR was performed using primer/probe sets from FastStart DNA Master SYBER green. A total of 28,000 cDNA profiles were
screened using 5 nm, 20 nm and 100 nm silver nanoparticles in a EA.hy926 cell.
5 nm silver nanoparticles (LD_{50} = 0.89 μg/ml) were more cytotoxic on EA.hy926 cells when compared that of 20nm and 100 nm silver nanoparticles after 24h exposure. We selected four genes, intercellular adhesion molecule (ICAM), IL-1ß, IL-6, IL-8, and determined their mRNA levels. mRNA levels of genes increased significantly in the 5 nm exposure group. Also, using cDNA microarray, we found that 5nm silver nanoparticles induced the expression of specific mRNA after exposure.
Therefore, we provide important data to understand and identify the effects of silver nanoparticles on the immune system.
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P1.14.41
Involvement of collagen receptor DDR2 in cytokine production by dendritic cells

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We investigated the mechanism involving collagen receptors in the production of cytokines by dendritic cells. Collagen type I induced the phosphorylation of discoidin domain receptor 2 (DDR2) and enhanced the phosphorylation of mitogen activated protein kinase (MAPK) kinases. NF-κB binding activity was enhanced when the cells expressing NF-κB reporter were exposed to collagen I. In addition, Collagen I enhanced the IL-12 luciferase activity on DDR2 expressing cells compared to empty vector control. Moreover, when IL-12 reporter transfected cells were treated with biochemical inhibitors of c-Jun N-terminal kinase (JNK) and NF-κB, collagen-induced IL-12 promoter activity was significantly downregulated in comparison to non-treated cells. Similarly, confirmatory experiments on murine dendritic cells revealed that IL-12 promoter activity is downregulated upon NF-κB and JNK inhibitor treatment on collagen I stimulation in dose dependent manner. These results suggest that DDR2 is involved in the collagen I-induced gene expression in dendritic cells via NF-κB and JNK signaling pathway.

P1.14.42
The prenylated flavonoids and 2-arylbenzofurans from the root barks of *Morus alba* inhibit airway inflammation

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It is sometimes difficult to successfully control airway inflammation including chronic bronchitis (one form of chronic obstructive pulmonary diseases). In the present study, for finding new alternative agent against airway inflammation from the herbal medicine and to establish the scientific rationale in clinical use, the root barks of *Morus alba* and its major constituents (flavonoids: kuwanone E, kuwanone G, norartocarpanone, 2-arylbenzofurans: moracin M, moracin O, moracin R, mulberrofuran G) were for the first time examined for their pharmacological activity against lung inflammation. On lipopolysaccharide (LPS)-induced airway inflammation in mice, the total ethanol extract of *M. alba* clearly inhibited bronchitis-like symptoms at 200 - 400 mg/kg/day by oral administration when judged by inflammatory cell infiltration, cytokine levels in BALF and histological observation. When the pharmacological activity of the isolated constituents were examined, the flavonoids inhibited IL-6 production in IL-1-treated lung epithelial cells (A549) and NO production in LPS-treated lung macrophages (MH-S) at concentration ranges of 50 - 100 μM. In addition, 2-arylbenzofurans also showed strong inhibitory action on the same parameters. Among the constituents, moracin M showed a highest inhibitory activity (IC_{50} < 10 μM) in IL-6 production of A549 cells and mulberofuran G exhibited strong inhibition (IC_{50} = 28 μM) in NO production of MH-S cells. Taken together, it is concluded that *M. alba* and the major prenylated flavonoid and 2-arylbenzofuran constituents have a potential for new agents to control lung inflammation including bronchitis.

P1.14.43
Dynamic cross-talk model of innate immune response to double-stranded RNA: order emerging from noisy cell responses

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Using experimentation and mathematical modeling, we discovered an intricate and logical pattern of dependencies in the dynamics of the innate immune response (IIR) to double stranded RNA. Dynamics involves crosstalk between the two major effector arms of the IIR: the NF-κB and IRF3 transcription factors that mediate inflammation and antiviral responses respectively. We related the highly variable response of individual cells to the coordinated response of the cell population. We found that, whereas TNFAIP3 and IκBα are purely NF-κB driven genes and ISG54 expression is purely controlled by IRF3, the RANTES and IFNβ expression is dependent on both NF-κB and IRF3. Further, NF-κB /RelA is negatively regulated by the IRF3 signaling pathway, and RelA also negatively regulates IRF3 expression. The computational model can be used in future exploration of the connectivity and dynamics of the IIR. Our work supports the hypothesis that the IIR is an "emergent phenomenon" that has evolved despite highly variable responses at individual cell level.

P1.14.44
Interleukin-33 promotes proliferation of primary human lung fibroblasts

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Interleukin (IL)-33 is a multifunctional cytokine that belongs to the IL-1 cytokine family and expressed by multiple organs and cell types in humans and mice. Recent studies have showed that IL-33 plays an etiological role in several fibrotic disorders and may be involved in the pathogenesis of chronic respiratory diseases, such as asthma, and pulmonary fibrosis. It has been reported that IL-33–induced cutaneous fibrosis is associated with increased fibroblast proliferation and altered expression of extracellular matrix-modifying genes. However, the role of IL-33 in regulating of functions of lung fibroblasts remains unclear. In the present study, we examined the effect of recombinant human IL-33 on proliferation of human lung fibroblasts.
Five primary lines of normal adult human lung fibroblasts were cultured for 3-7 days in the presence of increasing concentrations of IL-33 (1, 2, 10, 50, 100 and 300 ng/ml). The proliferation of human lung fibroblasts was analyzed using non-radioactive cell proliferation assay (MTT).
We have observed that normal human lung fibroblasts responded, in a dose-dependent manner, to treatment with recombinant human IL-33, by increasing proliferation rates 1.5- to 2.3 fold compared with non-stimulated control. The maximum effect of IL-33 on fibroblast proliferation was observed in cytokine concentrations range from 2 ng/ml to 100 ng/ml. These results suggest that IL-33 may play an important role in the regulation of human lung fibroblast proliferation. Human lung fibroblasts activated by IL-33 may act as effector cells not only in the pathogenesis of lung diseases but also in lung remodeling processes.

P1.14.45
Features of the allergic drug reactions in Russian patients: perspectives of a DNA bank development

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INTRODUCTION:
Nowadays, in various scientific world literatures discuss the possibility of association gene's polymorphisms with the development of allergic drug reactions for different groups of drugs.
MATERIALS AND METHODS:
We analyzed anamnesis of 455 patients, 352 - women (mean age 48,39; SD = 15.86) and 102 men (mean age 44,50; SD = 15,72) [p = 0.0299, t = 2.178], who had a history of various allergic drug reactions, in anamnesis, with various diseases.
RESULTS:
The analysis revealed that the most common drugs, which have resulted in the development of allergic drug reactions previously, were penicillin antimicrobials - 93 patients (20.5%), local anesthetics - 36 patients (8%), NSAIDs, and NSAIDs 34 patients (7.5%).
Also, we noted the combinations of drug groups, which have resulted in the development of allergic drug reactions previously. The combination of penicillin and cephalosporin antimicrobials, and the combination of penicillin and aminoglycosides antimicrobials found in 13 patients (3%). The combination of penicillin antimicrobials with sulfonamides found in 17 patients (4%), and combination of penicillin antimicrobials with local anesthetics found in 18 patients (4%).
CONCLUSIONS:
Nowadays researches of polymorphisms associated with drug allergy conducted on a single group of drugs. Based on this data, the research of polymorphisms of genes, which are associated with the development of an allergic reaction to the drugs, should be carried out for combinations of penicillin and aminoglycosides antimicrobials, as the same cephalosporin antimicrobials, sulfonamides, local anesthetics.

P1.14.46
HMGB1 in an experimental model of acute lung injury

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Despite recent therapeutic advances in acute respiratory distress syndrome (ARDS), its physiological and immunological aspects have not been completely understood. Recently, HMGB1, a nonhistone, chromatin-associated protein and a cytokine produced by macrophages, has been associated with sustained inflammatory response after injury or infection of tissue and lung injury. The aim of the study was to measure HMGB1 levels in rabbits with acute lung injury (ALI) induced by tracheal infusion of warm saline.
Methods: Thirty rabbits were instrumented and randomly assigned into two groups: 1) animals with no ALI (Control Group-CG; n = 15); 2) animals with severe ALI (SG Group; n = 15). The groups were ventilated during 4 hours with protective conventional mechanical ventilation (CMV). After ventilation, HMGB1 levels from bronchoalveolar lavage fluid (BAL) and plasma were measured as well as the percentage of white cell from BAL. Left lung was collected for histopathology analysis.
Results:Lung injury decreased pulmonary compliance (SG before: 1.86±0.576 > SG after: 0.67± 0.24) and oxygenation (PaO2 - SG before: 427.92 ± 89.90 > SG after: 68.18 ± 19.08). Percentage of neutrophil (p=0.001) and lung injury score were higher in SG than CG. Higher levels of HMGB1 in BAL were found in SG as compared to CG group [SG: 62 (20.30-79.41) > CG: 15.60 (13.86-25.81); p<0.035]. No statistically significant difference was found in HMGB1 plasmatic levels when groups were compared.
Conclusions:HMGB1 is released earlier in alveolar space in acute lung lesion induced by warm saline and can contribute to the pathogenesis of ALI.
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P1.14.47
Prone positioning attenuates TNF-alpha production in rabbits with lung injury under protective conventional mechanical ventilation (CMV).

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Prone positioning (PP) improves arterial oxygenation, gas exchange, distribution of ventilation toward dorsal lung areas, redirection of compressive forces on lung and facilitates secretion clearance in patients with ADRS. However, the exact mechanics of PP is still under investigation. The aim of this study aims was to measure TNF-alpha levels in rabbits with experimental ARDS under CMV and PP.
Methods: Rabbits were anesthetized, intubated and assigned into three groups: 1) Control Group(CG)- animals without lung injury under CMV in supine position; 2) Prone Group (PG)- animals with lung injury under CMV in prone position; 3) Supine Group (SG)- animals with lung injury under CMV in supine position. Lung injury was induced by tracheal infusion of warm saline. The parameters evaluated were: TNF-alpha in homogenate of lung tissue, bronchoalveolar fluid (BAL) and plasma; histological and cytological analysis of lung tissue and BAL, respectively. Results: Lung injury decreased oxygenation { PaO2 - PG before[median (p25-p75)]: 451 (412- 504) > PG after: 67 (59-76), p SG after: 76 (59-87); p SG: 24,1 (8,1-55,55); p<0,05}. There is no difference of serum and BAL TNF-alpha levels, histopathological lung injury score and BAL neutrophil percentage between PG and SG. Conclusions: Prone positioning with CMV decreased TNF-alpha production in lung tissue in this experimental model.
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P1.14.48
A potential role for the X chromosome in the CD99 expression and proinflammatory cytokine secretion in response to TLR ligands

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In order to evaluate the role of the X chromosome in innate immunity, we studied immune functions depending on X-linked genes in adults of both sexes and patients with sex chromosome aneuploidy.
CD99 expression on monocytes and lymphocytes was higher in women (XX) compared to Turner patients (X0) as well as in Klinefelter patients (XXY) compared to men (XY). CD99 expression was strongly correlated to the number of sex chromosomes, indicating a sex chromosome dose effect on the CD99 expression. In response to LPS 1 ng/mL, monocytes produced less IL-1β, IL-6 and TNF-α in Klinefelter patients compared to men as well as less IL-1β but more IL-8 in response to zymosan, a TLR_2 ligand. We also observed a lower IL-6 production by neutrophils of Klinefelter patients compared to men in response to LPS 1 ng/mL and a higher IL-8 production in response to R848, a $TLR_{7/8}$ ligand. We did not observe any difference in cytokine production between Turner patients and women, probably due to the high rate of mosaics among Turner patients. 17β-estradiol was higher in Klinefelter patients than in men although the range of difference is probably not biologically relevant. In conclusion, our study highlighted important differences in men carrying an extra X chromosome in terms of the innate immune response through expression of a diapedesis marker and secretion of inflammatory cytokines in response to TLR activation. These differences suggest a role for the X chromosome in sex-specific responses to acute inflammatory challenge, independently on hormonal impregnation.

P1.14.49
Study of immunosenescence through the production of cytokines by blood monocytes

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Aging is a complex process that affects a variety of physiological functions, one of the most important biological changes is termed 'immunosenescence' characterized by a functional decline in immunity leading to a progressive immunodeficiency. Currently, there is evidence to show changes in cytokine production, as well as differences in the number, function and activation of monocytes of elderly in relation to young, although some data are contradictory. It is known also that elderly subjects show an imbalance between inflammatory and anti-inflammatory networks, which results in the low grade chronic pro-inflammatory status called inflammaging. Thus, this study evaluated in vitro the cytokine production by monocytes, challenged or not with lipopolysaccharide (LPS) for 18 hours, obtained from peripheral blood of elderly and young subjects. Later, the supernatants were submitted to the enzyme-linked immunosorbent assay for determination of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and anti-inflammatory cytokines IL-10 and transforming growth factor-β (TGF-β). Our results demonstrated that the stimulation with LPS resulted in significantly higher levels of cytokines TNF-α, IL-6 and IL-10, compared with unstimulated monocytes, regardless of age. Comparing young and elderly, the monocytes from elderly showed a lower spontaneous production of TGF-β. Thus, the elderly have not impairment in the production of TNF-α, IL-6 and IL-10 by monocytes, even after challenged with LPS. However, the low production of TGF-β may corroborate state inflammaging, predisposing old persons to numerous diseases, infectious or neoplastic.

P1.14.50
Roles of IL-22 in the innate immune of chronic hepatitis B

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Hepatitis B is a worldwide health problem since it has affected more than 300 million individuals and the number increases gradually year by year. Therefore, it is urgent to investigate the exact pathogenic mechanism of hepatitis B virus (HBV) and find out an effective target to interfere with HBV adhering to hepatocytes and restore the function of the infected hepatocytes. In this study, we analyzed the effects of IL-22 on the immune of liver and the origins of IL-22 in patients with chronic hepatitis B, aimed to find out what roles does IL-22 play in chronic hepatitis B and what is its mechanism. ELISA used to measure the serum concentration of IL-22. Real-time PCR and Immunohistochemical staining were used to detect the expressions of IL-22, IL-22RA1 and IL-22BP in the liver. After that, confocal microscope imaging and flow cytometric analysis were used to confirm the origins of IL-22. IL-22 expression in liver was up-regulated in hepatitis B ($P<0.05$) and it mainly produced by γδT and NKT cells. However there was no significant difference in serum concentration. Conclusively, IL-22 plays important roles in the innate immune in the liver of chronic hepatitis B patients.

P1.14.51
TNF, autoantibodies and soluble receptors to TNF in health and rheumatoid arthritis

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Tumor necrosis factor (TNF) is an important immunoregulatory cytokine with broad action spectrum. In addition, important place occupy molecules that regulate cytokine activity: membrane-bound and soluble receptors to TNF and autoantibodies. Objectives: to determine a possible contribution of autoantibodies to TNF in rheumatoid arthritis (RA) pathogenesis.
The protocol includes a set of affinity chromatography methods and a magnetic separation procedure to obtain a fraction of specific autoantibodies to TNF. The pure fractions of autoantibodies to TNF obtaining from human serum were used as a calibration material for the determination of the absolute content of autoantibodies to TNF by ELISA.
We have shown a significant increase in contents of TNF and soluble receptors in blood serum of RA patients at the acute stage and RA patients responded to therapy in comparison with healthy donors. It has been shown the significant increase in autoantibodies contents of IgG2, IgG3 and IgG4 subclasses in blood serum of RA patients at the acute stage in comparison with healthy donors, as well as the significant increase in contents of IgG2 and IgG4 subclasses in blood serum of RA patients at the acute stage in comparison with RA patients responded to therapy.
It has been shown change of contents not only of soluble receptors and cytokine itself but also autoantibodies to TNF in blood serum of healthy donors and RA patients. In this case taking into consideration significant changes of levels of autoantibodies to TNF sub-classes we might suppose that they make a contribution to RA pathogenesis.

P1.14.52
Markers of cellular damage, inflammation and apoptosis in a model of endotoxic shock

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Immunological process at central nervous system (CNS) have been studied under a large number of stimuli. Preliminary results by us have indicated that cytotoxicity could be generated by certain inflammatory mediators secreted by the same brain cells in a model of endotoxic shock. However, it remains to determine in this model whether exacerbated TNFα production might affect the release of anti-inflammatory cytokines (i.e. IL-4) as well as FasL. In this work, expression of markers of cellular damage, inflammation and apoptosis was investigated. Using indirect immunofluorescence (IFI), it was found that TNFα and FasL mark intensity was significant at 60 and 120 min, but decreasing at 270 min; while caspase3 mark intensity increased during times. By ELISA assay, concentration of FasL was determined. It was 10 times greater than TNFα, reaching its maximum induction at 270 min (22082.8 pg/ml, while TNFα reached its maximum value at 60 min (844.8pg/ml). The concentration of IL-4, showed maximum value at 30 min (331pg/ml); and IL-10 at 270 min (386.47pg/ml). TNFα, FasL and caspase3 expresion detected by IFI suggest that dead of cells is caused by apoptosis, concomitant with the presence of chromatin condensation and apoptotic bodies, as observed by Wright stain. However, we cannot discard cell death by necrosis and pyknosis. Our results suggest that TNFα expression influences FasL, IL-4 and IL-10 induction since the kinetics of the concentrations of these proteins are closely related to TNFα expression.

P1.14.53
Human acidic mammalian chitinase (AMCase); molecular cloning, protein expression, and production of monoclonal antibody

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Human acidic mammalian chitinase (AMCase), is a 50 kDa protein containing a 30 kDa N-terminal catalytic domain that can hydrolyze chitin. This enzyme belongs to family 18 glycosyl hydrolases. High abundant of this enzyme was found in gastrointestinal tract. In lung, interleukin-13 was proposed to play a key role as primary effector that induces secretion of AMCase by airway epithelial cells and macrophages. The secreted AMCase activates elevation of monocyte chemoattractant protein 1 (MCP-1) and eotaxin-1, which induce the recruitment of eosinophils, T cells, and macrophages to site of inflammation in lung epithelial cells. Nonetheless, the mechanisms underlying AMCase mediated cells recruitment and its surface receptor have not been identified. Present study, the gene encoding AMCase was isolated from the human cDNA, cloned into pQE-tri vector, and the recombinant protein was expressed in E. coli M15. The affinity-purified AMCase was further used as immunogen for monoclonal antibody (mAb) production by standard hybridroma technique. Two specific mAbs against AMCase were successfully raised and named as 4G1 and 6E5. Both monoclones belong to IgG1 isotype. Western blot analysis showed that the mAbs strongly reacted with a corresponding protein with molecular weight of about 50 kDa in human acute monocytic leukemia cell line, THP1, and peripheral blood mononuclear cells. In conclusion, the AMCase was cloned and expressed in E. Coli system. Two specific mAbs to AMCase were raised and specifically reacted to human AMCase. The generated mAbs will be used for further functional analysis of the AMCase in regulation of the immune system.

P1.14.54
CTRP4 Plays an Important Role in Endotoxin Shock

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CTRP4, as the fourth member of C1q/ TNF superfamily, was discovered and named by Harvey Lodish in 2004.
In 2011, it was for the first time that our laboratory reported the function of CTRP4 to the world, and we conducted the functional studies mainly in HepG2 cells. We found that CTRP4 was a classical secretory protein. Both overexpression of CTRP4 and addition of rhCTRP4 protein with the concentration of ng/ml could increase the expression of IL-6 and activate NF-κB and STAT3 signaling pathway.
At present, we conduct our studies in immune system. Bioinformatics analysis revealed that the globular structure of CTRP4 was similar to that of C1q. Our studies found that the CTRP4 interacted with LPS directly to inhibit the interaction of LPS and its receptors in vitro, and then inhibited the release of IL-6 and TNF-α, which were induced by the stimulation of LPS. Furthermore, we generated CTRP4 transgenic mice, and we found that the mice were less hyposensitive to endotoxin shock, induced by LPS, and could survive a higher dose of LPS than wild mice. In the endotoxin shock models, CTRP4 transgenic mice had a higher survival rate with significant difference ($p<0.01$). Finally, we found that adding LPS to RAW264.7 cells could inhibit expression of endogenous CTRP4.
In summary, CTRP4 might be a new inhibitor of LPS, and it might play an important role in endotoxin shock. There are some possibilities that CTRP4 has the potential clinical application value.

P1.14.55
Endothelial cells enhances chemokines and reduces inflammatory cytokines release by human monocyte in vitro

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During inflammatory response, recruiting monocytes interact with activated endothelium, both by soluble mediators and direct contact. However, it is unknown if inflammation related functions, as cytokines-chemokines released change after leucocyte migration through endothelium. Our aim was, using an in vitro migration model, to determine the response to inflammatory inducer of human monocyte which have or not have contact with endothelial cells (EC). METHODOLOGY: Isolated human monocytes (ME) that migrated across activated human umbilical vein endothelial cells (activated-IL-1beta ECs) were stimulated with 10 ng of E. coli lipopolysaccharide (LPS), 50 ng of peptidoglycan (PG) or 50 ng of poly I:C during 8 hours. IL-6,TNF,IL-8,IL-10,IL-1β and IL-12, CXCL8/IL-8,CCL5/RANTES,CXCL9/MIG,CCL2/MCP-1 and CXCL10/IP-10 were quantified in supernatant using cytometric beads arrays. Controls include non-migrating monocytes exposed(MS) or not(M) with supernatants of activated ECs. RESULTS: Supernantants from LPS and PG stimulated MS or ME monocytes, show reduced IL-6, IL-1β and TNF concentration compared to LPS and PG stimulated M monocytes (Normalized results from unstimulated monocytes LPS: IL-6 -M: x1.62, MS: x1.14, ME: x0.78, IL-1β-M: x1.86, MS x1.08, ME: x0.70, TNF-M: x2.20, MS x1.18, ME: x0.75, PG: IL-6-M x1.33, MS x1.07, ME: x0.91, IL-1β-M: x1.59, MS x0.87, ME: x0.70, TNF-M: x1.85, MS: x1.21, ME: x0.82). Additionally, IL-8 and MCP-1 levels increased in both LPS stimulated and non-stimulated ME monocytes (IL-8: MvsME 286.67±89.23vs 2912.97±53.00pg/mL, MCP-1: MvsME 93.035±40.23vs594.04±361.75pg/mL). CONCLUSION: Contact with endothelial cells modulates human monocytes toward a recruiting profile over cytokines mediated inflammatory response.

P1.14.56

Pro-inflammatory cytokines analysis in HPV-positive cancer cells

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Recent data have expanded the concept that inflammation is a critical component of tumor progression. Many cancers arise from sites of infection, chronic irritation and inflammation. It is becoming clear that the tumor microenvironment is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. In addition, tumor cells have co-opted some of the signalling molecules of the innate immune system, such as selectins, chemokines and their receptors for invasion, migration and metastasis. Virus-induced tumors, like cervical high risk Papillomavirus-induced Squamous Cell Carcinomas (SCC), represent a paradigmatic example of the tight interplay between inflammatory responses and malignant transformation as inflammation is an integral part of the innate antiviral response.
To establish a tumorigenic role of inflammatory mediators in HPV-positive SCC, we analysed by real time RT-PCR the expression of inflammatory cytokines, chemokines and related molecules (i.e. IL-1α, IL-1β, IL-1RA, IL-1R1, IL-6, IL-8, MIP-1α and MIP-1β) in several HPV-positive carcinoma cell lines as well as in HPV-negative SCC (i.e. C33A). We also analysed human foreskin keratinocytes transduced by E6 and E7 from mucosal (HPV-16) or cutaneous (HPV-38) genotypes.
Our results indicate that in HPV-positive SCC the level of IL-1β mRNA is augmented compared to HPV-negative SCC. Interestingly, despite huge increase in mRNA levels, these cells didn't secrete any IL-1β. On the other hand, IL-6 mRNA appears upregulated in the same cells as well as secreted in the supernatants. Finally, we also tested the effect of the antiviral cytokine IFN-β on the levels of these pro-inflammatory mediators.

P1.14.57
IL-6, IL-6sr, TNF-α, IL-10 and IL-8 levels in patients with chronic periodontitis after periodontal therapy

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Purpose/Objective: The aim of this study was to determine the levels of IL-6, IL-6sr, TNF-α, IL-10 and IL-8 in gingival crevicular fluid (GCG) of subjects with chronic periodontitis before and after non-surgical periodontal therapy. Methods: Clinical measurements of gingival index (GI), plaque index (PI), bleeding on probing (BOP), probing depth (PD) and attachment loss (AL) were carried out in 11 patients with chronic periodontitis before and after scaling and root planing (SRP) and 11 periodontally healthy controls. GCF samples to measure the cytokine levels were collected by sterile endodontic paper points (No 35) from one tooth per quadrant during 30 s, before and after completion of SRP therapy, and analysed by ELISA test. Results: Significant differences were observed in all clinical parameters between patients and control group before and after periodontal treatment ($p \leq 0.05$). The total amount of IL-6, IL-6sr, TNF-α, IL-10 and IL-8 was significantly higher in the periodontitis group before periodontal therapy and compared with control group ($p \leq 0.05$). Conclusion: The levels of the inflammatory mediators obtained in this study were significantly associated with the severity of periodontal disease and the values decreased significantly after treatment, however did not reach the control group values. This research was supported by CDCH PG: 10-00-7070-2007.

P1.14.58
Deficiency of IL-10/IL-12 cytokine exacerbate systemic inflammatory responses in renal injury induced by ischemia and reperfusion

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Renal ischemia/reperfusion injury (IRI) is the major cause of acute kidney injury in native and also in transplanted kidneys, with a complex pathogenesis that involves many components of inflammation. Recent evidences show a critical role of the CD4+ T cell, with the Th1/Th2 paradigm as a possible effectors mechanism. In order to evaluate the role of Th1/Th2 immune profile in this system, IL-12, IL-4, IL-10 deficient mice and IL-12/IL-10 double deficient mice were used. Moreover, for adoptive transfer, IL-12 KO (IL-12 KO → WT or WT → IL-12 KO) and IL-4 KO (IL-4 KO → WT or WT → IL-4 KO) chimeric mice on C57BL/6 background and their controls were also subjected to IRI. Renal function was evaluated by serum urea and renal morphometric analyses. Gene expression of IL-6, MCP-1 and HO-1 were also investigated by Q-PCR . IL-4, IL-10 and IL-12/IL-10 KO deficient animals present the highest impairment in renal function compared to controls. Conversely, IL-12 KO and IL-12 KO (IL-12 KO → WT) chimeric mice animals were absolutely protected from IRI. Gene expression analyses showed higher expression of HO-1, a cytoprotective gene, IL-6, a pro-inflammatory cytokine and MCP-1, a pro-inflammatory macrophage chemokine, in IL-4, IL-10 and IL-12/IL-10 KO deficient animals subjected to IRI after 24 hours. KC chemokine was higher in serum of IL-4, IL-10 and IL-2/IL10 KO deficient mice and IL-4 was absence in serum of IL-10 and IL-12/IL-10 KO deficient mice. Results confirm that Th1/Th2 related cytokines are critically involved in renal IRI.

P1.14.59
HMG-CoA reductase inhibitor simvastatin improves organ dysfunction, inflammatory profile, bacterial clearance and cognitive damage after onset of sepsis

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Sepsis is a clinical syndrome characterized by systemic inflammatory response secondary to infection, which can progress to multiple organ dysfunction and death. Cognitive impairment is a significant consequence of sepsis reported among survivors. Despite research on new therapies, sepsis treatment remains based on life support and antibiotics. Cohort studies have suggested that statin uses are associated with protection from development and mortality of sepsis. Statins have pleiotropic effects and they are emerging as potent inhibitors of inflammatory processes. In this study we aimed to evaluate the effects of simvastatin in a rodent model of sepsis (Cecal Ligation and Puncture-CLP). Swiss Webster mice were submitted to CLP and 6h later they were treated with simvastatin (2mg/kg - iv). Plasma and peritoneal lavage were collected for organ damage evaluation, cytokine (ELISA) and nitric oxide (NO) (Griess method) quantification, cell migration and bacterial clearance (colony forming units-CFU) analysis. Cognitive damage was evaluated through inhibitory avoidance task. Our results showed that simvastatin decreased liver and kidney damage, and significantly reduced neutrophils numbers, CFU counts, TNF-α, MIF, IL-6 and IL1-β levels in the peritoneum. Simvastatin decreased NO levels in the bloodstream, but it enhanced NO production in the peritoneum. Additionally, simvastatin prevented the loss of aversive memory in septic mice. Our study demonstrates that simvastatin improved inflammatory profile and bacterial elimination in sepsis, preventing organ dysfunction and late consequences of sepsis. Our study indicates that statins can be considered potential candidate to future clinical studies as adjuvant therapy for sepsis.

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P1.14.60
Human mesenchymal stem cell adherence and invasivity in vitro after transfection with CD29 specific siRNA

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Background: Mesenchymal stem cells (MSCs) adhere to inflammatory substrates. The aim of this study was to determine in vitro the involvement of integrin beta1 (CD29) in human MSC (hMSCs) adherence and invasivity.
Method: hMSCs were isolated from iliac crest bone marrow, the presence of specific surface markers was evaluated by flow cytometry, and their differentiation potential was determined. After hMSCs expansion, the expression of CD29 was inhibited specifically by transfection with siRNA duplexes, and the transfection efficiency was evaluated by RT-PCR, flow cytometry, flow chamber assays, and immunohistochemistry. The adherence and invasivity of the transfected hMSCs was evaluated by culture in the presence of different inflammation mediators (VCAM-1, ICAM, TNF-alpha, TGF-beta1), using a real-time cell adhesion and invasivity analyzer system.
Results: hMSCs were successfully expanded and kept in culture for up to 8 passages. The isolated cells were positive for specific surface markers (CD29, CD44, CD73, CD90, and CD105), and they differentiated into the adipogenic, osteogenic and chondrogenic lines. Flow cytometry showed an abundance of CD29 on hMSC surface (79.33±3.8%). hMSCs showed a significant decrease of CD29 expression at 72 hours post-transfection. The invasivity capacity of transfected hMSC to VCAM-1-coated surfaces was significantly decreased in comparison with control hMSCs, and in comparison with surfaces coated with ICAM, TNF-alpha, and TGF-beta1. The adherence of transfected hMSCs to VCAM-1 under shear stress conditions was also significantly decreased.
Conclusion: Specific interactions between CD29 and VCAM-1 are involved in hMSCs migration towards inflammation, which makes CD29 a possible therapeutic target in inflammatory disorders.

P1.14.61
In vitro effect of Interleukin-33 on gastric adenocarcinoma cell line (AGS) and intestinal epithelial cancer cell line (Caco-2)

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Interleukin-33, member of the IL-1 Family of cytokines, is a potent inducer of Th-2 immune responses. IL-33 induces a potent proinflammatory response, promotes protection and the return to immune homeostasis, it also has a role during fibrogenesis at the end of dysregulated wound healing. In the gastrointestinal tract, IL-33 appears to play a critical role in maintaining normal gut homeostasis, enhancing mucosal defenses against intestinal infection and trauma. Overexpression of IL-33 has been reported in the inflamed mucosa of IBD patients, where the localization below ulcerative lesions indicates the potential role for IL-33 in wound healing. The hypothesis of this study is that IL-33 has specific effects in regulating epithelial cell functions in gut and stomach, enhancing those features that are prominent in inflammatory bowel disease. AGS and Caco-2 cell lines were used for proliferation and apoptosis assays and scratch test in a wound healing setting. Cells were seeded with growing concentration of rhIL-33 (0ng/ml-10ng/ml) for 0, 6 and 24h. The proliferation assay and wound healing showed an inhibitory effect on AGS at 24h in a dose-dependent way. Apoptosis was augmented with the increase of rhIL-33 concentrations. Conversely, rhIL-33 enhanced Caco-2 proliferation at 24h in a dose dependent fashion, whereas apoptosis was inhibited. Our study investigates the potential role of IL-33 in the regulation of cell proliferation, whose perturbations may lead to uncontrolled cell growth or impaired tissue repair; these preliminary data also suggest a different function of this cytokine in a cell-type dependent setting.

P1.14.62
***In silico* design of cytokine-trap peptides for modulation of the IL-13 signalling pathway in asthma**

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Peptide therapeutics is a promising area in which treatments are being developed to mitigate the symptoms associated with many diseases, including asthma. Novel synthetic peptides designed exclusively from endogenous peptide sequences are advantageous because the potential for drug-associated side effects is minimal. IL-13 is a Th2-associated cytokine, playing a pivotal role in allergic asthma and the development of physiological responses characteristic of this inflammatory disease; mucus hyper-secretion, airway hyper-responsiveness and airway remodelling. IL-13 and IL-4 are closely related cytokines that share a heterodimeric receptor complex comprising IL-13Rα1 and IL-4Rα chains, known as IL-13R or type-II IL-4R.
We used bioinformatics and structural modelling approaches to design peptides specific for the type-II IL-13R signalling cascade to 'trap' IL-13 and attenuate its signalling. IL-13 initially binds to IL-13Rα1, followed by recruitment and binding of IL-4Rα, culminating in the formation of a functional receptor. IL-13 also binds to another "decoy" receptor, IL-13Rα2, with high affinity. Six peptides were designed based on interacting regions of IL-13, IL-13Rα1 and IL-13Rα2, identified by sequence pattern discovery and structural modelling of IL-13 cytokine and receptor complex chains.
We present the *in silico* design of IL-13, IL-13Rα1 and IL-13Rα2-based peptides and their experimental testing, through assessment of the inhibitory effect on IL-13 signalling in cell line A549 by ELISA, qPCR and flow cytometry. Preliminary results for two of the peptides are promising, displaying 10 – 30% inhibition of IL-13 signalling. When validated, this approach may provide a framework for peptide design applicable to other cytokine receptor systems and diseases.

P1.14.63
Chemokines and biomarkers of endothelial dysfunction in chronic urticaria

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Background. Chronic Urticaria (CU) is a common and disabling disease characterized by recurrent itchy wheals with or without angioedema for more than 6 weeks. These symptoms are the consequence of skin mast cells degranulation with release of histamine and other vasoactive mediators. The aim of our study is to investigate the potential involvement of chemotactic mediators (CCL5/RANTES and CXCL8/IL-8) and markers of endothelial dysfunction such as soluble VCAM-1 (sVCAM-1) and ICAM-1 (sICAM-1) in the pathogenesis of CU, and their relation with disease activity. Methods. We measured the levels of CCL5/RANTES, CXCL8/IL-8, sVCAM-1 and sICAM-1 in the sera of 87 patients with CU and 61 sex- and age-matched normal healthy subjects (NHS), using ELISA assays. According to the results of the autologous serum skin tests (ASST), CU patients were classified in the ASST-positive and ASST-negative subgroups. Results. We detected significantly higher concentration of CCL5/RANTES (P<0.0001) but not of CXCL8/IL-8 in the sera of CU patients compared to NHS. The serum levels of sICAM-1 and sVCAM-1 were significantly increased in the CU patients compared to the control subjects (P=0.0121 and P=0.0043, respectively). No differences in either chemokines and soluble adhesion molecules levels were detected in the ASST-positive and ASST-negative subgroups. Positive correlation has been found between sICAM-1 and sVCAM-1 but not between them and CCL5/RANTES. Conclusions. Our study suggests the potential involvement of the C-C chemokine CCL5/RANTES and of the biomarkers of endothelial dysfunction sICAM-1 and sVCAM-1 in the pathogenesis of CU.

P1.14.64
Role of Cytokine Response in Disease Progression and Severe Clinical Outcomes in Leptospirosis

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Background. Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of the genus Leptospira. The role of the immune response in determining clinical outcomes is not yet well understood. We hypothesized that acute-phase serum cytokine response may play a role in disease progression, risk for death, and development of specific clinical outcomes, including severe pulmonary hemorrhage syndrome (SPHS).
Methods. We prospectively enrolled 172 patients with laboratory-confirmed mild (n=23) and severe leptospirosis (n=149) during active outpatient and hospital-based surveillance, respectively. Circulating concentrations of pro- and anti-inflammatory cytokines were measured using Cytometric-Bead-Array. Logistic regression was used to evaluate the association between cytokine concentrations and clinical outcomes.
Results. Concentrations of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17A, and TNF-α were significantly higher (P<0.05) in hospitalized patients with severe disease compared to outpatients with mild disease. Among hospitalized patients, levels of IL-6 (P<0.0001), IL-8 (P=0.0049), IL-10 (P<0.0001), and IFN-γ (P=0.0195) were significantly higher in fatal cases compared to severe nonfatal cases. High levels of IL-6 and IL-10 were independently associated (P<0.05) with fatal outcome after adjustment for age and days of symptoms. Finally, among fatal cases, IL-6 levels were higher among those who had SPHS compared to those who did not (P=0.0519).
Conclusion. Our findings suggest that severe cases of leptospirosis are differentiated from mild disease by the presence of a cytokine storm process, and that IL-6 and IL-10 may play an immunopathogenic role in the development of life-threatening clinical outcomes in human leptospirosis.

P1.14.65
IL-33 signaling modulates the development of adriamycin-nephropathy

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Adriamycin(ADM)-nephropathy (AN) is an experimental model that mimetic the clinical manifestations of human focal and segmental glomerulosclerosis (FSGS), a leading cause of chronic kidney disease. Because different works associated FSGS with Th2 immune responses, we hypothesized that IL-33, a differentiation factor for Th2 lymphocytes, could be implicated in disease pathogenesis. In order to determine the involvement of IL-33 in AN pathogenesis, we took advantage of mice deficient in the IL-33 receptor, T1/ST2. Methods and Results: BALB/c WT or ST2-/- mice received, by a single tail vein injection, 10mg (ADM)/Kg. The renal function was determined by the proteinuria /creatininuria ratio. Proteinuria was evident at day 4 post-ADM and progressed in a time-dependent manner. In the ST2-/- mice, the proteinuria ratio was 2 fold increased in comparison to wild-type group, indicating a more severe disease. In concordance, western blotting analysis of the renal tissue revealed in ST2-/- mice a more significant decrease in the levels of Nephrin, which was associated with an increase in the expression of phospho-Nephrin, in comparison to WT group. This phenomenon was associated with higher levels of phospho-AKT, JNK-1 and PAI-1, connecting a higher proinflammatory response with a more important loss of podocyte structural proteins. Conclusion: taken together our data show that the severity of AN increases in the absence of T1/ST2 receptor, suggesting a regulatory role for IL-33 signaling during FSGS pathogenesis.

P1.14.66
Degradation of IL-1 beta by the Human Papilloma Virus: novel mechanism of innate immune escape

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Infections with human papillomaviruses (HPVs) are involved in the development of anogenital cancer. HPVs evade the innate immune response of their host by dysregulating immunomodulatory factors such as cytokines, thereby creating a microenvironment favoring malignancy. One key player in the immune surveillance is interleukin-1 beta (IL-1β) which mediates inflammation, and links innate and adaptive immunity.
Here, we describe a novel mechanism how high-risk HPV16 abrogates IL-1β processing and secretion in a NALP3 inflammasome-independent manner. We analyzed IL-1β regulation in immortalized keratinocytes that harbor the HPV16 E6 and/or E7 oncogenes as well as HPV-positive cervical tumor cells. While in primary and in E7-immortalized human keratinocytes the secretion of IL-1β was inducible upon inflammasome activation, E6-positive cells did not respond. Western blot analyses revealed a strong reduction of basal intracellular levels of pro-IL-1β that was independent of NALP3 inflammasome, autophagy or lysosomal activity. Instead, we show that pro-IL-1β is degraded in a proteasome-dependent manner in E6-positive cells which is mediated via the ubiquitin ligase E6-AP and p53. Conversely, in E6- and E6/E7-immortalized cells pro-IL-1β levels were restored by siRNA knock-down of E6-AP and recovery of functional p53. In the context of HPV-induced carcinogenesis, these data suggest a novel post-translational mechanism of pro-IL-1β regulation which inhibits IL-1β secretion in virus-infected cells. Clinical relevance of our results was confirmed in HPV-positive tissue samples, where a gradual decrease of IL-1β towards cervical cancer is observed. Hence, IL-1β attenuation by HPV16-E6 oncoprotein is apparently a crucial step in viral immune evasion and initiation of malignancy.

P1.14.67
Nitric Oxide tightly regulates Dendritic Cells life cycle and cytokines release under inflammatory conditions

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Nitric oxide (NO) is an important effector molecule that is involved in immune regulation and host defence. It has been previously reported that NCX 2057, a chemical compound bearing a nitric oxide (NO)-donating moiety linked to the natural antioxidant ferulic acid, shows marked anti-inflammatory properties in lipopolysaccharide (LPS)/interferon-γ-stimulated RAW 264.7 macrophages and in a model of chronic brain inflammation. Dendritic cells (DCs) are the most potent APCs of immune system and are link between innate and adaptive immunity. It is known that NO is able to regulate DCs functions such as apoptosis and antigen presenting activity. Here, we investigated the anti-inflammatory role of NCX 2057, which releases NO with a slow kinetics, on LPS stimulated DCs. NCX 2057 inhibits NO production in LPS-activated DCs by the modulation of the iNOS expression. NCX 2057 exhibits an anti-inflammatory activity on DCs through the down-modulation of genes (IL6, IL12b and IL1b, ISG15) of the inflammatory signatures developed in our laboratory. The inflammatory genes modulated were further confirmed by ELISA assay. We observed a significant reduction of IL-6 production in presence of NCX 2057, but a slight reduction of IL-1beta and TNFα compared to controls. As previously reported for other NO-donors, NCX 2057 inhibits LPS-induced cells death in a dose dependent manner, with a significant reduction at 50uM. The anti-apoptotic effect of NCX 2057 seems to have a role in DCs-induction of T cells proliferation**.** The data highlight a role of NCX 2057 as anti-inflammatory compound, modulating DCs during LPS-induced activation.

P1.14.68
Correlation of magnesium levels with inflammatory markers in thiazide induced hypomagnesemic rats

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Among cardiovascular diseases, cause of one third deaths worldwide, hypertension is playing major role in cerebrovascular disease, ischemic heart disease and cardiac and renal failure. The most prevalent type of hypertension affecting 90-95% of hypertensive patients is Primary hypertension. A thiazide-type diuretic is usually prescribed as first-line pharmacotherapy. Use of hydrochlorthiazide, although vital in the management of hypertension can have several unfavorable effects such as electrolyte disorders (hyponatremia, hypokalemia and hypomagnesemia), hyperlipidemia, hyperuricemia and impairment of glucose metabolism in addition to depletion of volume. Hypomagnesaemia is associated with considerable morbidity also influencing inflammatory response by affecting the endothelial proliferation due to an up regulation of interleukin-1 (IL1) and sVCAM-1, pro inflammatory cytokines, CRP. The objectives of the study was to evaluate the effects of Magnesium Supplementation on inflammatory markers (CRP s-VCAM 1) in thiazide administered rats. Ninety male Sprague Dawley rats of age 90 to 120 days were selected, divided into three groups of control, Hydrochlorthiazide administered control and Hydrochlorthiazide +Magnesium administered experimental group. Blood samples were taken and levels of inflammatory markers were determined by ELISA and those of serum Magnesium. The results showed levels of inflammatory markers (sVCAM-1 and CRP) are increased in HCTZ administered group thus having a negative correlation with serum magnesium levels whereas levels of these parameters decreased in HTCZ + Mg2+ administered group thus showing that magnesium supplementation helped in normalizing the derangements in inflammatory markers.

P1.14.69
Anti-inflammatory, immunostimulatory and antiviral effects of Coffee fruit (Coffea arabica)

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Chlorogenic and quinic acid are found in coffee fruit. Previous studies have demonstrated that polyphenols possess anti-inflammatory, immunostimulatory and antiviral properties. Therefore, due to their increased amount in coffee fruit these immunological activities were evaluated.
LD50 and IC50 assays were performed to assess the toxicity of the coffee fruit extract (CFE). Anti-inflammatory activity was evaluated by pro-inflammatory models, CBA and histological techniques. Immunostimulatory activity was assayed by T cell proliferation with MTT and antiviral activity was supported by T cell subpopulations analysis by flow cytometry.
LD50 value was 13.8g/kg while the IC50 value obtained was 2.9mg/ml. Anti-inflammatory activity assessed with carrageenan-induced paw edema model exhibited a dose-dependent inflammatory inhibition for CFE (1136.4mg/kg, 39.5% vs. Indomethacin, 44.8%). LPS-stimulated PBMC supernatants showed a decreased secretion of TNFα for CFE (1136.4mg/kg). Additionally, air pouch model demonstrated decreased cellular infiltration for CFE (1136.4mg/kg, $0.78x10^6$cells vs. positive control, $6.75x10^6$cells) and inflammatory exudates showed decreased production of IL6, TNFα and MCP-1. T cell proliferation assays demonstrated an immunostimulatory activity for CFE (500ug/ml). After viral simulation with Poly I:C stimulation, CFE (1000ug/ml) increased the percentage and activated cytotoxic T cells (CD8/CD44+/+). These results were statistically significant ($p<0.05$).
CFE showed anti-inflammatory properties on cellular and humoral levels supported by histological techniques. CFE decreased the secretion of pro-inflammatory cytokines such us IL6, TNFα and also MCP-1, thus diminishing cellular infiltration. Conversely, under viral simulation, CFE stimulated T cell proliferation and increased the percentage and activated cytotoxic T cells. Therefore, these results could attribute coffee fruit immunomodulatory and antiviral properties.

P1.14.70
Proinflammatory Effect of the P2X7 Receptor during Sepsis

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Background: Sepsis is characterized by an imbalanced inflammatory response, leading to organ damage, shock and death. P2X7 receptor (P2X7R) activation induces proinflammatory events and may be associated to sepsis.
Aims: To analyze the role of P2X7R in modulating the inflammatory response observed in cecal ligation and puncture (CLP) induced sepsis.
Methods: CLP was induced (2 punctures of 21G) in C57BL/6 and P2X7KO mice. After 48 h, CFUs were counted at 37°C in agar plate. Seven-day survival curve was determined. Inflammatory neutrophil and macrophage populations were counted by flow cytometry (anti-CD11b, Ly6G antibodies). Cytokines (IL-1β, IL-6, IL-17, TNF-α, and TGF β) and NO were dosed in the peritoneal wash and blood (IL-1β) by ELISA and Griess method, respectively.
Results: P2X7KO animals were partially protected from sepsis showing 30% higher survival and reduced bacteremia after CLP. In the peritoneal lavage Ly6G+ cells increased only in WT animals and CD11b+ cells increased in both septic groups. Cytokines such as IL-1β, IL-17 and TGF- β beta were reduced in P2X7KO animals after sepsis induction.
Conclusion: The absence of the P2X7R attenuates the inflammatory response and protects against. Thus more studies may contribute to the development of new therapeutic strategies.
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P1.14.71
Role and correlation of inflammatory cytokines, angiogenic markers and insulin growth factors in post treatment (neoadjuvant chemoradiation) disease mapping in advanced stage carcinoma of uterine cervix

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Background: Carcinoma of uterine cervix accounts for most common malignancy in Indian women. Inflammation is a critical component of tumour progression and many cancers arise from sites of infection & inflammation. Angiogenesis and Insulin-like growth factors promotes tumour cells survival, proliferation and metastasis. Currently chemo-radiation is mainstay of treatment. Post treatment (Neoadjuvant Chemotherapy) disease mapping using these markers in carcinoma cervix patients needs to be explored.
Material and Methods: 40 patients of cancer cervix stage IIIb were recruited. 20 healthy women were taken as controls. Circulatory levels of TNF-α, IL-8, VEGF, Ang-2 and IGF-I & II, were measured before and after chemoradiation, using ELISA and correlated with therapeutic response. mRNA levels were quantitated using Q-PCR and data was statistically analyzed.
Results: Levels of all theses molecules were significantly ($p<0.001$) higher in patients than in controls. After treatment their levels were significantly ($p<0.001$) declined. Out of 40 patients, 33 were complete responders and 7 were non-responders when they were clinically assessed. On comparison of before and after treatment levels of these molecules complete responders showed significant decline whereas non responders showed insignificant decrease in their levels.
Conclusions: Higher levels of angiogenic factors indicate role played by them in disease progression aiding angiogenesis. It may be possible that these angiogenic factors function via up-regulation of inflammatory cytokines and IGFs. These markers may serve as useful tools in post treatment disease mapping which otherwise may not provide true picture with available imaging methods.

P1.14.72

S100A8 and S100A9 induce the expression of cytokines and regulate the NLRP-3 inflammasome via ROS-dependent activation of NF-κB

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S100A8 et S100A9 are members of the Myeloid-Related-Proteins (MRP) sub-family, representing up the 30% of neutrophil cytosolic proteins. These proteins are released upon cell damage or cell activation, acting as danger signal for the organism by signaling, at least partially, through toll-like receptors. High extracellular concentrations of S100A8 and S100A9 are found in sera from patients suffering from various chronic inflammatory disorders. In this study, we have investigated the role of S00A8 and S100A9 on cytokine production in peripheral blood mononuclear cells. First, using an antibody array assay approach, we show that S100A8 and S100A9 induce the expression of inflammatory analytes, including TNF-α, IL-6, IL-8, Gro-α and MIP1α/β. Results were confirmed using specific ELISA for IL-6 and IL-8. Next, using an antisens strategy and a pharmacological inhibition approach for NF-κB, we show that S100A8 and S100A9 induce secretion of IL-6 and IL-8 via translocation of NF-κB. We next investigated the potential activation of the NLRP-3 inflammasome and observed an increase in the expression of the pro-IL-1β and the NLRP-3 proteins in S100A8- and S100A9- induced cells. Furthermore, pretreatment with S100A8 and S100A9 increased the IL-1β production and caspase-1 processing after stimulation with ATP, a known inflammasome activator. Inhibition experiments revealed the importance of NF-κB and ROS in the expression of pro-IL-1β and NLRP-3 as well as in IL-1β secretion. Taken together, our results show the importance of S100A8 and S100A9 in the secretion of inflammatory cytokines, especially in the production of IL-1β controlled by the NRLP-3 inflammasome.

P1.14.73
Mice selected for maximal inflammatory response display a higher lung inflammation induced by Tityus serrulatus scorpion venom

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Tityus serrulatus venom (TsV) may induce cardiac failure and pulmonary edema, leading to death by scorpionism. TsV induces activation of a systemic inflammatory cascade with the release of different mediators including cytokines. The aim of this study was to evaluate TsV activity in the lung of mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response and observe if genetic factors are involved in this response. A subletal dose of TsV was inoculated in mice and after different periods, lungs were collected, for histopathological analysis, myeloperoxidase (MPO) quantification, cellular phenotyping and cytokine and chemokine quantification. Perivascular cellular infiltrate, haemorrhage and edema were observed in the lung of TsV-treated mice after venom injection, with higher intensity in AIRmax compared to AIRmin mice. No cardiac alterations were observed. MPO activity showed that TsV induces a significant higher migration of neutrophils in AIRmax compared to AIRmin mice. Phenotypic analysis of lung cellular populations of TsV-treated mice showed that AIRmax presented significantly more neutrophils ($Ly6G^+CD11b^+$ cells) and macrophages ($F4/80^+CD11b^+$ cells) when compared to their controls and to AIRmin. Moreover, AIRmin mice displayed an increase in $Ly6G^+CD11b^+$ and $F4/80^+CD11b^+$ cells later after venom administration compared to AIRmax. Lungs of AIRmax TsV-treated mice presented higher levels of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and the chemokines MCP-1, MIP-1β and RANTES, compared to AIRmin. Our results demonstrated that TsV induced higher acute pulmonary inflammatory response in AIRmax mice, suggesting that the response to scorpion venoms is modulated by genetic factors.
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P1.14.74
Melaleuca alternifolia and its main component, terpinen-4-ol, inhibit the production of IL-1β, IL-6, IL-4 and IL-10 by activated monocytes

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The antimicrobial activity of oil of Melaleuca alternifolia (TTO) is well established, however the mechanisms of modulation of inflammatory and anti-inflammatory cytokines properties are still unclear. The present study investigated in vitro, the capacity of TTO and its main component, terpinen-4-ol, in the modulation of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-4 (IL-4) and interleukin-10 (IL-10). Initially, we determined the cytotoxicity of oil in U937 monocytes differentiated into macrophages with 40ng/mL Phorbol 12-myristate 13-acetate (PMA) for scanning the mitochondrial activity reduction assay methyl tetrazolium (MTT). The ability of TTO and terpinen-4-ol in modulating the production of cytokines such as IL-1β, IL-6, IL-4 and IL-10 after stimulation with LPS of *Porphyromonas gingivalis* (TLR2 agonist) and *Escherichia coli* (TLR4 agonist) was determined by ELISA assays. Data normality by the Shapiro-Wilk test ($p < 0.05$). In the assessment of cytotoxicity was used ANOVA with Tukey post test. In assessing cytokine production was used in the t-test. The TTO and terpinen-4-ol, were effective in decreasing the amount of these cytokines: IL-1β, IL-6 and IL-10. The TTO had no effect on IL-4 since the terpinen-4-ol significantly reduced levels of IL-4. Thus, it was concluded that the TTO and terpinen-4-ol can suppress the production of inflammatory mediators and anti-inflammatory in human monocytes activated.

P1.14.75
Inflammatory responses to low volume high load and high volume medium load protocols during dynamic leg press exercise

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One bout of resistance exercise triggers an aseptic inflammatory response which involves leukocytosis and stimulation of pro-inflammatory cytokines (Freidenreich & Volek 2012). The purpose of the present study was to identify differences in the acute immune response between low volume high load (gains in maximal strength, MAX) and high volume medium load (gains in muscle mass, HYP) resistance exercise protocols. In a cross-over design twelve healthy men participated in bilateral press exercise consisting of 5 sets of 10 RM and 15 sets of 1 RM. The inter-set rest period was three minutes for MAX and 2 minutes for HYP. Venous blood samples were taken at baseline, immediately after and 15 (P15) and 30 (P30) minutes after the exercise. Basic blood count was analyzed using Sysmex KX-21N (TOA Medical Electronics Co., Ltd., Kobe, Japan). Concentrations of IL-6 and monocyte chemotactic protein-1 (MCP-1) in serum samples were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents. Both exercises induced acute significant leukocytosis ($p<0.001$). Leukocytosis was significantly higher after HYP ($p<0.01$). Significant lymphopenia was observed only after HYP ($p<0.01$). IL-6 and MCP-1 concentrations increased significantly immediately after both exercises and remained elevated in HYP at P30. The acute MCP-1 response was significantly higher in HYP. Hypertrophic resistance exercise triggered significantly stronger inflammation response. As expected, manipulation of the rest period and load in resistance exercise alters the inflammatory response. This should be taken into account when planning resistance training e.g. periodization for peak performance as well as for special populations e.g. the elderly.

P1.14.76
Cytokines profile in a social defeat animal model of depression and effects of a novel anti-asthenic drug ladasten

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Based on evidence, including the findings of clinical depression studies and animal models, the cytokine hypothesis of depression, was formulated. Ladasten® (N-(2-adamantyl)-N-(para-bromophenyl)-amine), an anti-asthenic drug with immunomodulatory, psychostimulatory and anxiolytic activities, was registered in Russia in 2008. The aim of the present study was to investigate the cytokine profile in an animal model of depression, and examine the effects of ladasten treatment .
Mice subjected to social defeat stress for 3 (T3), 15 (T15) and 30 (T30) days developed a depressive-like phenotype. Ladasten (30 mg/kg,i.p.) was administered for 5 days after the stress-days. Serum cytokines were measured using a multiplex immunoassay by flow cytometry. The forced swim test (FST) was used to evaluate the depression state of the mice.
T15 and T30 group mice from FST exhibited significantly shorter latency to the first float period by 29 and 44%, and longer time of immobility by 15 and 20%. T15, T30 ladasten-treated groups behaved as control mice. T3 was accompanied by a significant increase in IL-6 (7.9-fold), IL-17 (16.3-fold), IL-4 (6.0-fold). T15 was the peak level of IL-6 (46.5-fold), but the levels of cytokines at T30 were not different from controls. Thus, administration of ladasten prevented the expression of inflammatory cytokines in response to social defeat stress. In ladasten-treated mice, the cytokine levels were similar to unstressed mice. These findings support the notion that pro-inflammatory cytokines play an important role as mediators of the stress response. Targeting this pathway may represent a novel therapeutic approach for the treatment of depression, and ladasten could be considered as a potential drug candidate.

P1.14.77
sICAM-1 and sUPAR as Immune Markers for Treatment Response in Ethiopian TB-Patients with and without HIV Co-Infection

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Background: Soluble Intercellular Adhesion Molecule 1 (sICAM-1) and soluble Urokinase Plasminogen Activator Receptor (sUPAR) were among potential host immune markers for TB patients. This study aimed to assess the prospective of sICAM-1 and sUPAR as treatment response markers in TB patients with or without HIV in Ethiopia.
Methods: For this particular study, 34 TB+HIV-, 23 TB+HIV+ cases and 52 healthy controls participants were selected from GC6-74 cohort project conduct from 2005 to 2010 aimed to assess biomarkers of TB in relation to HIV/AIDS in Africa. Plasma levels of sICAM-1 and sUPAR were measured using Quantikine sICAM, R &D systems (USA) and suPARnostic, ViroGetes (Denmark) ELISAs, respectively at baseline and 6 months after TB treatment.
Results: Mean sICAM-1 levels at baseline were 393.8±251.1ng/ml in TB+HIV- and 698±588.4ng/ml in TB+HIV+ cases, while 195.9±125.6ng/ml in controls groups. There was decline to 250.6±147.9ng/ml and 135.6 ± 176.6ng/ml in TB+HIV- and TB+HIV+ cases, respectively after TB treatment. The mean sUPAR levels at baseline were 10.1±5.9ng/ml in TB+HIV- and 18.0±14.2ng/ml in TB+HIV+ cases and 4±2.8ng/ml in healthy controls. sUPAR levels declined to 5.3±2.7ng/ml and 9.5±7.0ng/ml in TB+HIV- and TB+HIV+ cases, respectively after TB treatment. The declines in the level of both markers after treatment were statistically significant (P-value= 0.05).
Conclusion: Significantly higher levels of sICAM-1 and sUPAR were observed in patients than controls group before TB treatment; while the levels declined significantly after 6 months treatment. This shows the potential of sICAM-1 and sUPAR as treatment response markers in TB patients irrespective of their HIV status.

P1.14.78
State of adaptive mucosal immunity among immunocompromised patients with allergic rhinosinusitis.

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Survey of immunocompromised patients with allergic rhinosinusitis reveals significant breaches in the system of adaptive mucosal immunity among these patients. Changes in the local cytokine response with violation of IL-4, IL-8, γ-interferon, α-TNF, and violation of serum fractions cytokine state are accompanied by the activation of an opportunistic infection on "allergic" mucosal. A key role in the formation of pathology among these patients plays herpetic infection.
Major disturbances in the mucosal immune system, that are clearly seen in a case of immunocompromised patient with rhinosinusitis are disturbances in the system of anti-viral defense. Violations of interferon status are easily spotted during the evaluation of local interferone response on mucosal among patients with allergic rhinosinusitis.
A common case for this group of patients is the case of IgA fractions content change.
Lack of change or insignificant changes in the phagocytic immune system, cell system, a unit responsible for the immune defense system, are fully compensated by opposite changes in local immunity among patients with allergic rhinosinusitis.
Continuous-recurrent nature of the underlying disease, complicated by infectious syndrome clinic, the presence of treatment-resistant opportunistic infection among this group of patients is the result of ineffective approaches to the treatment of mucosal immune system among immunocompromised patients with allergic rhinosinusitis.

P1.14.79
High sensitivity C-reactive protein and insulin levels in obese and non-obese patients with polycystic ovarian syndrome

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The purpose of this study was to compare the levels of hs-CRP and insulin levels in obese and non-obese patients of PCOS. The study design was Cross-sectional and comparative study. A total of 72 patients having Polycystic Ovarian Syndrome (PCOS) were selected, who had been diagnosed as per Rotterdam diagnostic criteria. Blood sampling was done for glucose, insulin and high sensitivity C-reactive protein (hs-CRP) in fasting state. Insulin Resistance (IR) was calculated by homeostatic model for assessment of insulin resistance (HOMA-IR) method. The obese PCOS women had significantly higher levels of insulin, HOMA-IR and hs-CRP levels as compared to the overweight and normal weight PCOS women. The overweight PCOS women had significantly higher insulin, HOMA-IR and hs-CRP levels as compared to the normal weight PCOS women. Our results revealed that obese PCOS patients had increased rate of IR and low grade inflammation as compared to the non-obese PCOS women. Therefore it was concluded that BMI is an important factor, that plays crucial role in the etiology and the complications associated with PCOS.

P1.14.80
HMGB1 is a major mediator of pathology in mice surviving sepsis

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Severe sepsis is a life-threatening complication of infection and tissue injury. Survivors have reduced quality of life, cognitive impairment, and five-year mortality of 80%. The pathophysiology is largely unknown, with no specific treatment available. High-mobility group box 1 (HMGB1) is a cytokine known to mediate sepsis pathogenesis by causing endothelial dysfunction, inflammation and organ damage. We hypothesized that persistently elevated levels of HMGB1 mediate immune dysfunction in sepsis survivors.
In BALB/c mice, sepsis was induced by cecal ligation and puncture (CLP) and survivors were followed for 12 weeks. Circulating HMGB1 and inflammatory cytokines were determined weekly. Cell phenotype was determined by flow cytometry. Mass spectrometric analysis was used to characterize the redox state of HMGB1.
Splenomegaly and leukocytosis were observed in mice surviving sepsis for four weeks. Levels of HMGB1 were elevated for eight weeks after CLP. IL-6 and CXCL-1 were only transiently increased in the first two weeks. No increase was observed in TNF, IL-1β, or IFN-γ. Splenocytes from sepsis survivors had an augmented response to LPS ex vivo. Treatment of CLP-survivors with an anti-HMGB1 antibody reversed splenomegaly, leukocytosis and reverted splenocyte sensitization. Administration of exogenous HMGB1 to naïve mice replicated the immunophenotype observed in sepsis survivors. Analysis of circulating HMGB1 from sepsis survivors demonstrated a stepwise increase of reduced all-thiol isoform (chemoattractant) during the first 3 weeks, followed by the disulphide isoform (cytokine-inducing) 4-8 weeks after CLP. Our findings suggest that sustained increase in circulating HMGB1 is crucial in inducing immune changes observed in sepsis survivors.

P1.14.81
Association of polymorphism of TNF-alpha receptors genes with expression levels of membrane-bound receptors and serum levels of soluble receptors

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Tumor necrosis factor alpha (TNF) is a pleiotropic cytokine, the biological effects of which are mediated by interaction with specific membrane-bound receptors (TNFRs). Biological activity of TNF depends on TNFRs expression levels on cells, which in turn can be regulated by allele polymorphism. Levels of TNFRs expression were determined by flow cytometry using QuantiBRITE PE calibration beads. Serum levels of soluble TNFRs were measured using enzyme-linked immunosorbent assay. Single nucleotide polymorphisms (SNPs) of TNFRI and TNFRII were genotyped by polymerase chain reaction–restriction fragment length polymorphism. In the study sample (n=150 healthy individuals), carriers of the T allele in rs4149570 of the *TNFRI* gene showed significantly lower level of soluble TNFRI (TT vs GG, p=0.006). Number of membrane-bound TNFRI on $CD14^+$ cells of CC genotype carriers in rs4149569 of *TNFRI* gene was significantly lower than that of GC genotype carriers (p=0.012). Healthy individuals with a CC genotype in rs590368 of the *TNFRII* gene had the lower percentage of $CD14^+$ cells bearing membrane-bound TNFRII (CC vs CT, p=0.015). The results of this study provide evidence of association between SNPs in *TNFRI* and *TNFRII* genes and expression levels of membrane-bound TNF receptors types I and II on mononuclear cells and soluble TNFRI levels in serum of 150 healthy individuals. Thus, allele polymorphism of TNFα receptors genes is a one of the mechanisms influencing TNFα system, functioning through the formation of individual variability of the expression of membrane-bound receptors.

P1.14.82
Comparison of cytokine production in human lymphocytes and human tissue culture THP 1 stimulated by metallic ions

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Introduction: Physiological interaction of foreign materials (including metallic ions) with oral mucosal tissue can lead to oral tolerance induction. However, a minority of humans induce an intense reaction to metal dental reconstruction. Cytokines produced during the interaction of lymphocytes and macrophages play an important role in activation and regulation of immune response. Aim and methods: Our study aimed at exploring cytokine production in lymphocytes of mercury and nickel hypersensitive patients (verified with a test of lymphocyte proliferation) and in a monocyte-macrophage cell line of human origin THP 1. In all cases, the cells were cultivated for five days with an addition of mercury and nickel salts. Cytokines in culture media were determined by Ray Bio® Human Inflammation Antibody Array III and compared with results of control group cells, cultivated without addition of metallic salts. Results: Lymphocytes of hypersensitive patients predominantly produced pro-inflammatory cytokines, for example IL-1 alpha, IL-11, IL-17, IFN-gamma, TNF-beta, sTNFRI, sTNFRII and TIMP-2. These cytokines were observed in both cases of metallic salts stimulation. On the other hand, cell culture THP 1 predominantly produced anti-inflammatory cytokines after stimulation with both metallic salts. The results suggested that in the case of hypersensitivity diagnosis, to follow the reaction of both lymphocytes and monocyte-macrophage cells should be recommended.
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P1.14.83
TNF and IL-10 in a bitter-sweet battle in taste buds

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Taste buds are chemosensory structures widely distributed on the surface of the oral cavity and larynx. Taste cells, exposed to the oral environment, face great challenges in defense against potential pathogens. While immune cells are rarely found in taste buds, our recent studies suggest that some immune-response-associated molecules are expressed at higher levels in taste cells than in nontaste tongue epithelial cells. It remains unclear how protective responses are regulated in taste buds. In this study, we investigated the expression of the pro-inflammatory cytokine TNF and the anti-inflammatory cytokine IL-10 in the posterior taste tissues. Both TNF and IL-10 were found exclusively in subsets of type II taste cells, the receptor cells for sweet, bitter and umami tastes. Further analysis revealed that TNF was co-expressed with the sweet and umami receptor T1R3, but not with the G-protein Gustducin (mostly bitter receptor cells). In contrast, IL-10 was co-expressed with Gustducin, but not with T1R3. Meanwhile, there were few taste cells co-expressed both cytokines. These data suggest that these two cytokines are produced by different populations of taste cells in the posterior taste tissues: TNF by sweet and umami cells and IL-10 by bitter cells. Furthermore, TNF receptors were expressed in all taste cells at differential levels, while IL-10 receptors were preferentially expressed in T1R3+TNF+ cells, suggesting that TNF could act on all taste cells under inflammatory conditions, while IL-10 may preferentially target TNF-producing-taste cells to limit TNF production. This study revealed a novel regulatory mechanism of inflammation in taste buds.

P1.14.84
The role of Notch signaling in IL-12 production in murine macrophages

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Macrophages are innate immune cells which play crucial roles in both innate and adaptive immune responses. Signaling through interferon (IFN)γ receptor together with Toll-like receptor (TLRs) such as LPS in macrophages leads to secretion of various pro-inflammatory cytokines including IL-12. Previous studies showed the involvement of Notch signaling in regulation of biological activities of TLR activated macrophages such as expression of cytokines, IL-6, TNF-a and IL-12. However, the involvement of Notch signaling pathway in IL-12 production has not been fully elucidated. In this study, we investigated the role of Notch signaling in regulating expression of the IL-12 especially focusing on the p40 subunit. We found that using a pharmacological inhibitor (GSI) to suppress Notch signaling in IFNγ/LPS-activated macrophages derived from wild type mice decreased il12p40 mRNA levels and IL-12p40/70 secretion. We found that nuclear translocation c-Rel, one of key factors to control il12p40 transcription, was inhibited and Erk1/2 activation was compromised by GSI treatment. However, activated macrophages derived from Cre+/-Notch1flox/flox mice did not show a reduction in the level of il12p40 mRNA nor the protein level whereas activated macrophages derived from Cre+/-CSLflox/flox mice phenocopied that of the GSI treatment. Treatment with GSI in activated Cre+/-Notch1flox/flox macrophages resulted in the reduction of IL12p40/70 secretion similar to GSI treatment in wild type macrophages or Cre+/-CSLflox/flox macrophages. Currently, silencing expression of CSL by siRNA is under investigation for its effect on IL-12 production. Taken together, Notch1, but not CSL or gamma secretase activity is dispensable for IL12p40/70 production.

P1.14.85

Cortex Magnolia Officinalis extract prevents gastric ulcer by stimulating CB2 gene expression and reducing IL-6 and TNF-alpha productions in stomach tissue

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A variety of factors including stresses, acid, Helicobacter pylori, NSAIDs and ethanol have been known to be involved in the pathogenesis of gastric mucosal injury and gastroduodenal ulcer disease. This study investigated the effects of water extracts of Curcumae Radix and Cortex Magnoliae Officinalis, on cell activities of gastric mucosa cell in vitro and protective effects on gastric mucosal injury in vivo. Results showed that Curcumae Radix and Cortex Magnoliae Officinalis extracts in vitro specifically promoted the cell viability and decreased the production of reactive oxygen species (ROS) of the gastric epithelial cells in the acidic medium as compared with those of control cells. In the animal model of Shey's ulcer ligation, the Cortex Magnoliae Officinalis extracts stimulated the gene expression of the cannabinoid receptor type 2 (CB2) and decreased the productions of tumor necrosis factor-alpha (TNF-alpha) and interleukin 6 (IL-6) of gastric tissue and thus, significantly decreased the gastric ulcer index in the pylorus-ligated animals. Thus, Cortex Magnoliae Officinalis extracts may protect the gastric mucosal tissue from the acidified injury by enhancing gastric epithelial cell survival rates and decreasing ROS, TNF-alpha and IL-6 productions in the injured gastric tissue.

P1.14.86

An essential role for eosinophils in neutrophil accumulation in response to eotaxin

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The roles of eosinophils in defense against infection, unlike their contribution to allergic inflammation, remain controversial. The 5-lipoxygenase (5-LO) pathway is required for the recruitment of eosinophils to the peritoneal cavity of allergic mice by both antigen and eotaxin. The relevance of this requirement to host defense is unknown. We evaluated whether eosinophil recruitment by eotaxin in nonallergic mice: a) required 5-LO; b) contributed to innate immunity. Wild-type (BALB/c; PAS) and mutant (5-LO-deficient ALOX; eosinophil-deficient GATA-1-mutants) mice were injected i.p. with eotaxin, eosinophils or both, and leukocyte accumulation was quantified up to 24h. Recruitment of eosinophils by eotaxin in BALB/c (50 ng/cavity) was significant at 4h, 12h and 24h, and accompanied by recruitment of neutrophils and macrophages in numbers respectively 8.2 and 9.9-fold larger. These effects were abolished by eotaxin neutralization and 5-LO-pathway blocker MK886. In ALOX (but not PAS) mice, eotaxin recruitment was abolished for eosinophils and reduced by≈55% for neutrophils. In GATA -1 mutants, eotaxin recruited neither neutrophils nor macrophages. Transfer of purified eosinophils from BALB/c donors, or from ALOX donors, into GATA-1 mutant recipients, i.p., restored eotaxin recruitment of neutrophils, and showed that the critical step dependent on 5-LO is the initial recruitment of eosinophils by eotaxin, not the secondary attraction of neutrophils. Eosinophil-dependent recruitment of neutrophils in naive BALB/c mice was associated with increased binding of bacteria. This points to an indirect mechanism through which eosinophils contribute to innate immunity in nonsensitized mice.

P1.14.87

Interleukin-1 signaling prevents bacteremia in enteric infection with *Citrobacter rodentium*

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Attaching and effacing pathogens, including enterohemorrhagic *Escherichia coli* (EHEC) in humans and *Citrobacter rodentium* (*C. rodentium*) in mice, raise serious public health concerns. Here we demonstrate that interleukin-1 receptor (IL-1R) signaling is indispensable for protection against *C. rodentium* infection in mice. Oral challenge with *C. rodentium* ($5x10^9$ CFU) showed severe loss of body weight and high mortality in IL-$1R^{-/-}$ mice at 7-14 days post infection (dpi) while IL-1R intact wild-type (WT) mice restored from mild symptoms within three weeks. The hemorrhage and thickened cecum and colon were observed in IL-$1R^{-/-}$ mice, and higher levels of inflammatory cytokines such as IL-6 and MCP-1, but not IL-22 and IL-23, were determined in IL-$1R^{-/-}$ mice than in WT mice at 10 dpi. Moreover, an enlarged spleen with neutrophil infiltration and bacteremia in systemic tissues (i.e., spleen and peripheral blood) were found in IL-$1R^{-/-}$ mice but not in WT mice. Interestingly, extracellular *C. rodentium* resided in $CD18^{high}Ly6C^{+}Ly6G^{+}$ neutrophils and $CD18^{high}Ly6C^{+}Ly6G^{-}$ monocytes from cecum and colon of IL-$1R^{-/-}$ mice, but no bacteria were found in those cells from WT mice. These results suggest that the IL-1 signaling plays a pivotal role in bactericidal activity of phagocytes and inhibits bacterial dissemination from mucosal tissues to bloodstream.

P1.14.88
IL-27 affects helper T cell responses via regulation of PGE2 production by macrophages

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IL-27 is a heterodimeric cytokine that regulates both innate and adaptive immunity. IL-27 acts as an immune suppressor by the induction of Tr1 type T cells, which produce IL-10 and inhibit effector T cells. IL-27 also suppresses function of macrophages and dendritic cells by regulation of cytokine secretion. On the other hand, effects of IL-27 on regulation of inflammation via mediators other than cytokines remain poorly understood. To address this issue, we examined immunoregulatory effects of conditional medium of bone marrow-derived macrophages (BMDMs) from *WSX-1* (*IL-27Ra*) KO mice to find enhanced IFN-g and IL-17A secretion by $CD4^+$T cells over that of control BMDMs. EP2 and EP4 antagonist inhibited this enhancement, suggesting possible involvement of prostaglandins. PGE_2 secretion and *COX-2* expression by BMDMs from *WSX-1* KO mice was increased compared to control macrophages in response to LPS. To explore how IL-27/WSX-1 signal regulate COX-2/PGE_2 production, murine *WSX-1*-expressing Raw264.7 cells (mWSX-1-Raw264.7) were established. mWSX-1-Raw264.7 showed phosphorylation of both STAT1 and STAT3 in response to IL-27 and produced less amounts of PGE_2 and COX-2 compared to parental Raw264.7. Finally, siRNA knockdown study was performed toreveal siSTAT1, but not siSTAT3, resulted in increased production of PGE_2 and COX-2. In addition, BMDMs from *STAT1* KO mice showed higher *COX-2* expression than those from control mice. Collectively, our result indicated that IL-27/WSX-1 regulated PGE_2 secretion via STAT1-COX-2 pathway in macrophages and affected helper T cells responses in a PGE_2-mediated indirect fashion.

P1.14.89
High Concentration Of Interleukin-23 And Soluble Vascular Endothelial Cadherin In Sera Of Patients With Behcet disease

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Introduction and Aims: Behcet's disease (BD) is a chronic multisystem vasculitis disease that can affect any organ and usually is combined with hyperactivation of neutrophils. Involvement of inflammatory cytokines such as Interleukin (IL)-12 in BD has been shown. However, IL-12 shares a p40 subunit with IL-23 which has additional inflammatory effects apart from IL-12. IL-23 increases neutrophils' transmigration therefore could contribute in BD induction or progression. Moreover, endothelial cells express vascular endothelial cadherin adhesion molecule (VE-cadherin), which plays critical roles in angiogenesis and endothelial integrity. VE-cadherin may shed into the circulation as soluble (sVE-cadherin) which inflammatory cytokines can increase this process. We therefore assessed the correlation between IL-23 concentration and amount of sVE-cadherin in sera of Behcet patients.
Methods: We enrolled 44 healthy persons and 53 cases of BD with different disease activities and examined their serum concentrations of IL-23 and sVE-cadherin using commercial ELISA kits (Bendermed Systems, Austria).
Results: A significant correlation was found between the concentrations of these two factors only among patients. Comparing sVE-cadherin mean concentration in patients and controls showed a significant difference, this difference for IL-23 was negligible. Results showed higher IL-23 levels in sera of patients with uveitis. Moreover, there was a meaningful correlation between IL-23 content and disease activity.
Conclusion: These results extend the biological effects of IL-23 in BD and introduce sVE-cadherin as a potential new biomarker in BD pathogenesis.

P1.15 Inflammasome

P1.15.01
Malaria-induced NLRP12/NLRP3-dependent caspase-1 activation mediates inflammation and hypersensitivity to bacterial superinfection

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Cyclic paroxysm is a hallmark of malaria, being associated with high levels of the pyrogenic cytokine, IL-1β. Here we report that mice infected with P. chabaudi show a signature for expression of inflammasome-related genes, and that caspase-1 activation was dependent on IFNγ-priming, MyD88 activation, ASC, P2X7R, NLRP3 and NLRP12, whereas expression of pro-IL-1β required MyD88, IFNγ and TNF receptor. Importantly, therapeutic intervention with an IL-1 receptor antagonist prevented bacterial-induced septic shock in the hypersensitive rodent malaria model. We also report a significant increase in the frequency of circulating CD14+CD16-Caspase-1+ and CD14dimCD16+Caspase-1+ monocytes, which was associated with pronounced production of IL-1β by peripheral blood mononuclear cells from febrile malaria patients. Furthermore, we demonstrated that the inflammasomes from these patients contained either NLRP12 or NLRP3 oligomerized with ASC. Thus, NLRP12/NLRP3-dependent activation of caspase-1 is a key event in mediating systemic inflammation and hypersensitivity to secondary bacterial infection during malaria.

P1.15.02
Molecular differences of NLRP3 inflammasome activation in LPS-activated human monocyte-derived macrophage subtypes

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IL-1beta is a "master" cytokine that has indispensable roles in orchestrating effective innate and adaptive immune responses. It is produced in an inactive precursor form that is cleaved to active cytokine by protein complexes called Nlrp3 inflammasomes. While our knowledge on the general mechanisms involved in Nlrp3 inflammasome function and on its regulation is rapidly increasing, it is also getting clear that the actual outcome of the activation (like IL-1beta production) strongly depends on the cell type and on the presence or absence of various intracellular or extracellular modulators. Depending on their localization macrophages can develop into a wide range of phenotypes. Macrophages differentiated in the presence of GM-CSF (GM-MFs) develop inflammatory phenotype, while cells differentiated in the presence of M-CSF (M-MFs) possess anti-inflammatory characteristics and function in wound healing and tissue repair. Our results show that following LPS treatment GM-MFs produce high IL-1beta, while M-MFs produce low IL-1beta with a different time- and concentration kinetics. We found significant differences in basal and LPS-induced expression of Nlrp3, procaspase-1 and ASC between the two MF types. We found that LPS-treated GM-MFs are able to release ATP and produce IL-1beta, while M-MFs require ATP supplementation for IL-1beta secretion. We have also found expression differences in the proteins responsible for ATP release, recognition and degradation as well as in the activation of key signal transduction pathways. Furthermore, we will show that different nucleosides have strong and differential regulatory effects of NLRP3 inflammasome function. This work was supported by TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" grant.

P1.15.03
Acidosis drives DAMP-induced IL-1β secretion via a caspase-1-independent pathway

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Inflammation in the absence of infection is sterile and contributes to the worsening of disease. The pro-inflammatory cytokine interleukin-1β (IL-1β) is a mediator of sterile inflammatory responses, and is known to drive the worsening of brain injury in numerous experimental models. In the brain IL-1β is synthesised by microglia as an inactive precursor (pro-IL-1β). Cleavage of pro-IL-1β to a mature secreted form follows stimulation of the activated cell by Damage Associated Molecular Patterns (DAMPs), endogenous disease or injury associated molecules. DAMPs are sensed by the pattern recognition receptor NLRP3, whose activation results in the formation of an inflammasome, the activation of caspase-1 and the cleavage of pro-IL-1β. To-date, studies on the inflammasome in vitro have all been conducted under standard culture conditions. However, during disease there are profound changes in the intercellular milieu, and how DAMPs signal under these conditions is not known. Acidosis is a common consequence of disease and so we investigated whether acidosis influenced DAMP-induced IL-1β release. When LPS-primed glial cells were stimulated with DAMPs under acidic conditions (pH 6.2) the predominant IL-1β form secreted was 20kDa in size rather than the 17kDa caspase-1-dependent species. Lactic acidosis, induced by the addition of 25mM lactate to the culture also induced the release of 20kDa IL-1β. This 20kDa product was produced independently of the NLRP3 inflammasome and caspase-1 but could be inhibited by the cathepsin D inhibitor pepstatin A. These data suggest that under disease relevant culture conditions, DAMPs induce the secretion of IL-1β independently of the inflammasome.

P1.15.04
The impact of Butyrate on the NOD-like receptor pyrin domain-containing protein 6 (NLRP6)-inflammasome in humans

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A recent study using a knock-out (KO) mouse model has suggested a specific interaction between the NLRP6-inflammasome and the intestinal microbiome. KO mice presented a higher susceptibility to chemically induced colitis compared to their wild-type controls. Interestingly this phenotype was transferable upon cohousing before treatment. The investigators suggest that a shift in the commensal microbial community (dysbiosis) and a decreased expression of IL-18 at baseline could be responsible for this phenomenon. The role of NLRP6-mediated immune regulation in human health and disease is still unknown (Elinav et al., 2011).
Aim of this study was to examine if bacterial structural components (microbial associated molecular pattern, MAMPs) and bacterial by-products (butyrate) affect NLRP6 and IL-18 gene expression in the intestinal epithelium.
Time and dose-dependent effects of MAMPs, infection (Campylobacter jejuni) and sodium butyrate on intestinal NLRP6 and IL-18 gene and protein expression were analysed by quantitative real-time PCR, Elisa and Western blotting.
Butyrate led to a major increase in epithelial IL-18 and NLRP6 gene expression whereas the presence of other inflammasome components such as NLRP3 and Caspase-1 were not affected.
Butyrate-producing commensal bacteria have been associated with health benefits for the host including increased epithelial barrier function and protection against colorectal cancer (Fung et al., 2012). Our study indicates a putative novel role for butyrate-mediated modulation of the inflammasome that may have an impact on the onset of inflammatory driven diseases. Further studies are needed to investigate the association between the NLRP6 inflammasome and the microbiome.

P1.15.05
Activation of the NLRP3 inflammasome promotes the pathogenic Th1/Th17 responses in type 1 diabetes murine model

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Introduction: Autoimmune diseases including type 1 diabetes (T1D) are thought to have a pathogenic dual Th1/Th17 response, but the underlying mechanisms of innate immunity driving the activation and polarization of these subtypes is not fully understood.
Objective: Thus, we addressed the role of the NLRP3 inflammasome in the T-cell mediated response during the development of experimental T1D.
Methods: Nlrp3 deficient mice and their wild-type (C57BL/6) were inoculated intraperitoneally with 40mg/Kg of streptozotocin for 5 consecutive days. Mice inoculated with vehicle (sodium citrate) were used as control mice. The pancreatic lymph nodes (PLNs) were removed to assess the myeloid and lymphoid cell frequency and number by flow cytometry. The pro and anti-inflammatory cytokine levels were determined in pancreatic tissue homogenates by ELISA assay.
Results: Initially, our results demonstrate that Nlrp3 deficient mice developed lower hyperglycemia and reduction of 50% in the disease incidence compared to wild-type mice. In agreement, mice lacking Nlrp3 had less inflammatory infiltrate (insulitis) and reduced IL-1β production in the pancreatic islets. In parallel, they exhibited augmented number of mast cells in the PLNs and significant increase of the IL-4 production in the pancreatic tissue. In addition, the Nlrp3 deficiency caused a decrease in IL-17 (Th17) and IFN-γ (Th1) producing CD4 T cell population in PLNs. Conclusion: These results suggest that NLRP3 inflammasome activation promotes the pathogenic Th17/Th1 response skewing and possibly contributes to pancreatic islet damage and T1D development in mice.
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P1.15.06
Caspase-1 and MyD88 are involved in adaptive immune response induced by cytosolic flagellin

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TLR activation in dendritic cells leads to its maturation and production of proinflammatory cytokines, providing favorable conditions for the activation and differentiation of naïve T cells. Flagellin is a highly effective adjuvant for humoral and CD4 T cell responses. It is recognized by TLR5 and by cytosolic NLRs members NAIP5/NLRC4. TLR5 activates inflammatory genes through MyD88 pathway whereas NLRC4 and NAIP5 assemble multiprotein complexes called inflammasomes, leading to caspase-1 activation. Besides the well-described adjuvant properties of flagellin, little is known about the mechanisms through which TLR5 and inflammasomes act during the shaping adaptive of immune responses. Thus, we analyzed the role of inflammasome activation in the maturation of dendritic cells and activation of T cells. Purified flagellin in its free form was able to stimulate BMDCs via TLR5 and MyD88. Cytosolic flagellin induced upregulation of costimulatory molecules independent on TLR5, NLRC4 and Caspase-1, but also dependent on MyD88. In addition, cytosolic flagellin- stimulated OVA-pulsed BMDCs induced the production of IFN-γ by OT-II splenocytes, which was independent on TLR5 and dependent on caspase-1 and MyD88. From these data we can infer that in addition to TLR signaling, inflammasomes might be shaping the flagellin-induced immune response through Caspase-1 and MyD88.

P1.15.07
A novel, Cyclosporine A (CyA)-sensitive P2RX7 SNP haplotype linked to survival of immature antigen presenting cells (APC) in autoinflammation

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Adenosine-3-phosphate (ATP) is a major danger associated molecular pattern (DAMP) released by trauma. Concentrations of >1mM stimulate P2XR7 and trigger the inflammasome. We enriched in vivo activated antigen presenting cells (APC) from patients at the onset of septic shock and autoinflammatory diseases. Genotyping of functional single nucleotide polymorphisms (SNPs) of P2RX7 revealed a higher frequency of haplotypes bearing SNP489 C>T and SNP 1513 A>C in patients. ATP stimulated IL-1β secretion was lower in the mutated genotype than in wild-type (wt) and so was the ATP-induced ion flux signal (p=0.0004). When ATP was co-administered with Cyclosporine A (CyA), the ion flux of SNP489/1513 mutated cells was upregulated (p=0.03), but never reached the activity of wt cells. ATP did not stimulate but rather decrease both spontaneous and staurosporine (STS) induced caspase-3/7 release in cultured APC. This effect was more pronounced when CyA was added to ATP. Generally, STS-induced caspase-3/7 release was lower in P2RX7 mutant- than in wt cells (p<0.002). In summary, we describe the properties of a novel P2RX7 SNP haplotype in in-vivo activated APC of patients with autoinflammatory diseases. This haplotype has both a reduced ion channel activity, IL-1β-secretion and caspase activation. The phenotype encoded by this haplotype may explain persistence of chronic inflammation by impaired activation of counterregulatory pathways in vivo.

P1.15.08
Deregulation of NLRP3 inflammasome is involved in progression of hepatocellular carcinoma

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The nucleotide-binding domain, leucine rich family (NLR), pyrin containing 3 (NLRP3) inflammasome, has been shown to orchestrate multiple innate and adaptive immune responses, however little is known about its role in cancer. This study is to investigate the role of NLRP3 inflammasome in the development and manipulation of hepatocellular carcinoma (HCC),a typical inflammation-induced cancer. Three cohorts of patients were investigated in this study for the expression of NLRP3 inflammasome components in liver cells. Our data showed expression of all of the NLRP3 inflammasome components was either completely lost or significantly down-regulated in human HCC, and loss of NLRP3 inflammasome components was significantly correlated with advanced stages and poor pathological differentiation. Reconstitution of NLRP3 inflammasome in HCC cells had antitumor effects and significantly reversed malignant behaviors of these cancer cells. Our data offered the basis for potential immunological cancer therapy by reconstitution of NLRP3 inflammasome in HCC patients.
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P1.15.09
Mistletoe lectin-mediated mmunostimulatory effect implicates the activation of NLRP3 inflammasome

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Inflammosomes are the molecular complexes that are activated upon cellular stimulation that trigger the maturation and secretion of pro-inflammatory cytokines like IL-1β, thereby driving innate immune defence mechanisms. NLRP3 inflammasome plays an important role in the regulation and stimulation of anti-cancer immune response. Several cancer chemotherapeutics efficiently promote the anti-tumor immunity by activating the NLRP3 inflammasome. Mistletoe lectins are one of the important bioactive molecules present in the therapeutic preparations of Viscum album (VA) which are used as complementary therapy in cancer. They exert anti-tumor activities which involve the cytotoxic properties, induction of apoptosis, inhibition of angiogenesis and several other immunomodulatory mechanisms. They also exert immunostimulatory effect by inducing maturation and activation of human dendritic cells thereby enabling them to stimulate tumor-specific CD8 T cell response; however the underlying cellular and molecular mechanisms are not clear. In this study, we hypothesized that mistletoe lectins present in Viscum album preparations induce the activation of NLRP3 inflammasome in human dendritic cells thereby activating them for the secretion of pro-inflammatory cytokine IL-1β. We demonstrate that, treatment with mistletoe lectin-containing VA preparations alone can induce a moderate secretion of mature IL-1β, however along with the TLR4 stimulation they significantly up-regulate the secretion of mature IL-1β. We are currently dissecting the mistletoe lectin-mediated activation of inflammasome at different regulatory levels of caspase-1 and purinergic signaling pathways. Our results thus demonstrate a novel molecular regulatory mechanism by which mistletoe lectin-containing Viscum album preparations exert immunostimulatory effect.

P1.15.10
Fine mapping of the mouse *Irm1* locus controlling interleukin-1beta production

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Irm1 (Inflammatory response modulator 1) in chromosome 7 is a major locus regulating the acute inflammatory response through modulation of IL-1beta production by circulating leukocytes, after lipopolysaccharide and ATP stimulation of the Nlrp3 inflammasome. In order to fine map this locus, we carried out genome-wide linkage analysis of 879 informative single nucleotide polymorphisms (SNPs) in a new and larger F2 intercross population (n=693 compared with the previous analysis with 280 mice) of outbred mouse lines selected for the intensity of acute inflammatory response (AIRmax x AIRmin). We confirmed the mapping of the Irm1 locus (the LOD score increased from 9 to 72) and the 1-LOD confidence interval decreased from 14 to 3.5 Mb. Deep-sequencing of this chromosomal region was carried out in 8 F2 mice with extreme high IL-1beta production and 8 extreme low responder F2 mice. Interval of the locus region was restricted to about 420 kb where 10 protein-encoding candidate genes map. Functional assays will be carried out in order to characterize the role of genetic variants evidenced in this region to the differential modulation of IL-1beta levels in AIRmax and AIRmin mice.

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P1.15.11
ASC inflammassome mediates IL-1β production and host resistance to Paracoccidioides brasiliensis

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An efficient recognition of the fungus *Paracoccidioides brasiliensis* by the host's innate immune system is essential for protection against paracoccidioidomycosis (PCM), a systemic human mycosis highly prevalent in Latin America. The NOD like receptors (NLRs), unlike TLRs, are intracellular pattern recognition receptors that form a molecular platform, called inflammasone, which activates caspase-1 and induces the production of active IL-1β and IL-18. Here, we evaluated the importance of inflammasome in the experimental PCM. We showed that *P. brasiliensis* triggers the secretion of IL-1β in murine bone marrow-derived macrophages (BMMs) and that its release in the active form requires the activation of caspase-1 and the involvement of the ASC inflammasome, since we found high caspase-1 activation and IL-1β production in BMMs from WT mice, but not from $ASC^{-/-}$ mice. To understand the role of inflammasome regarding protection to *P. brasiliensis* infection, WT, $ASC^{-/-}$ and caspase-$1^{-/-}$ mice were intravenously infected with Pb18 strain. We verified that the knockouts mice displayed higher mortality index and fungal loads in the lung after 30 days of infection compared with WT mice. The increased susceptibility is related with reduced amounts of IFN-γ in the same period of infection. Moreover, the histological analysis of the lung revealed that in the absence of caspase-1 there was significant increased in the amount of fungus after the infection on the 15th day after infection. However, this is not followed by impaired compact and well defined granulomas formation, revealing a new signaling pathway implicated in the regulation of pulmonary antifungal defenses.

P1.15.12
ATP-mediated inflammasome activation is efficiently inhibited by acetylcholine

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The release of IL-1beta by mononuclear phagocytes is tightly controlled. Lipopolysaccharide induces synthesis of pro-IL-1beta. A second danger signal such as ATP triggers inflammasome activation resulting in cleavage and secretion of mature IL-1beta. Acetylcholine is known for its anti-inflammatory effects on the innate immune system. In this study, we test the hypothesis, that acetylcholine dampens ATP-mediated activation of the inflammasome.
Human monocytic U937 cells were primed with lipopolysaccharide for 3 hours. In addition, mononuclear blood leukocytes from healthy human donors and from blood vessels of renal rat allografts undergoing acute rejection were investigated. Cells were stimulated with BzATP or nigericin in the presence or absence of cholinergics and inhibitors of NO-synthases. The release of IL-1beta was measured by ELISA.
Acetylcholine and nicotine efficiently inhibited BzATP-induced release of IL-1beta from U937 cells and primary blood leukocytes. Mecamylamine, strychnine, alpha-bungarotoxin and inhibitors of NO-synthases reversed this effect. Nicotinic agonists did not inhibit IL-1beta release induced by nigericin. Activated leukocytes from allografts, which are known to synthesize acetylcholine, did not release IL-1beta unless acetylcholine esterase was added together with BzATP. As revealed by RT-PCR, alpha-9 subunits of acetylcholine receptors and NO-synthases 1-3 were expressed.
We describe a novel anti-inflammatory mechanism of acetylcholine. Acetylcholine inhibits ATP-mediated inflammasome activation. Our pharmacological data suggest that P2X7 receptors, alpha-9 nicotinic receptor subunits and NO-synthases are involved in the signaling cascade. We suggest that this novel anti-inflammatory pathway is active in renal allografts during acute rejection, where endogenous leukocytic acetylcholine prevents IL-1beta secretion into the blood stream.

P1.15.13
Activation of NLRP3 inflammasome is associated with the progression of Alzheimer's disease

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Inflammatory mediators are responsible for the neuroinflammation observed in Alzheimer's disease (AD),a phenomenon that might be the culprit of disease or, possibly, a reaction to pathology. In particular IL-1β and its key regulator, the inflammasome protein complex, are strongly suggested to play a role in this disease. To better investigate the possible involvement of the inflammasome in AD we stimulated with lipopolysaccaride (LPS) for 4 hours and β-Amyloide peptides (Aβ) for 21hours PBMC of 20 individuals with a diagnosis of mild cognitive impairment (MCI)(MMSE score>24), 20 Alzheimer's disease (AD) patients with either moderate(MMSE score 19-23), and 10 severe (MMSE score<19) Alzheimer's disease (AD) patients, and evaluated by RT-PCR the expression of 84 genes involved in the inflammasome pathway.
Proinflammatory cytokines-expressing immune cells were measured by FACS analyses in the same individuals upon stimulating cells with antigenic β-amyloid peptides.
Results showed that a number of genes including inflammasome components (AIM 2, NLRP3 and NPLR-9), downstream signaling (IRF-2 and RAGE) and effector molecules (IFNγ, IL12, IL1β, IL6, TNFα and PTGS2) are different regulated in AD compared to MCI individuals, with the highest degree of upregulation seen in patients with the lower MMSE scores. Proinflammatory cytokines, (IL-1β, TNFα, IL-6 and IFNγ), expressing β-amyloid peptides-stimulated immune cells were upregulated in severe AD compared to AD and MCI individuals; These results suggest an involvement of the inflammasome pathway in AD-associated neuroinflammation. Therapeutic strategies targeting NPLR3 modulation could be useful in the therapy of AD.

P1.15.14
Cytosolic flagellin induces caspase-1/11-independent lysosomal cathepsins-mediated cell death that correlates to the control of S. typhimurium

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Cytosolic flagellin is sensed by NAIP5/NLRC4 inflammasomes leading to caspase-1 activation. Caspase-1 is responsible for IL-1β/IL-18 processing and secretion and inflammatory cell death, named pyroptosis. Regardless of the well-established role of pyroptosis as an effector mechanism to clear intracellular infections, its molecular regulation remains to be solved. We demonstrate that purified flagellin from *Bacillus subtilis* delivered directly into macrophages cytosol by transfection lipid vesicles leads to NLRC4, ASC and caspase-1/11-dependent IL-1β secretion but cell death still occurs in the absence of either of these molecules as assessed by fluorescence microscopy according to ethidium bromide (EtBr) incorporation and loss of vital acridine orange (AO) staining. Also, flagellin-induced cell death strongly correlates to the control of *S. typhimurium* by wild-type and caspase-$1/11^{-/-}$ macrophages. Cytosolic flagellin-induced caspase-1/11-independent cell death displayed some apoptotic features, such as cell shrinkage and formation of membrane blebs. Nevertheless, unlike actinomycin D-induced apoptotic cell death, cytosolic flagellin-induced caspase-1/11-independent cell death does not require caspase activation and results in the loss of membrane integrity and release of the known damage signal IL-1α. Finally, cell death induced by cytosolic flagellin seems to rely of lysosomal pathways in which cathepsins B and D

have a redundant role. These results demonstrate that cytosolic flagellin induces a novel pro-inflammatory caspase-independent lysosomal-dependent form of cell death that could be considered an additional effector mechanism to restrict flagellated bacterial infections.

P1.15.15
Selective inhibition of the NLRP3 inflammasome by targeting to promyelocytic leukemia protein

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The functional activities of the tumor suppressor promyelocytic leukemia protein (PML) are mostly associated with its nuclear location. In the present study, we discovered an unexpected role of PML in NLRP3 inflammasome activation. In PML-deficient macrophages, the production of IL-1beta was strongly impaired. The expression of pro-IL-1beta, NLRP3, ASC, and procaspase-1 was not affected in PML-knockout macrophages. PML deficiency selectively reduced the processing of procaspase-1. We further showed that PML is required for the assembly of the NLRP inflammasome in reconstitution experiment. All PML isoforms were capable of stimulating NLRP3 inflammasome activation. In PML-knockout macrophages, the generation of reactive oxygen species and release of mitochondrial DNA were decreased. The involvement of PML in inflammasome activation constitutes an important activity of PML and reveals a new mechanism underlying the inflammasome activation. In addition, downregulation of PML by arsenic trioxide suppressed monosodium urate (MSU)-induced IL-1beta production, suggesting that targeting to PML could be used to treat NLRP3 inflammasome-associated diseases.

P1.15.16
Caspase-1-dependent iNOS activation require NF-κB and acts as a non-redundant effector mechanism agaisnt *S. typhimurium*

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Introduction: Flagellin recognition is shared by transmembranic TLR5 and cytosolic Nlrc4 and Naip5 innate receptors. Although both TLR5 and Naip5/Nlrc4 pathways are known to be involved in the clearance of infections, little is known about the anti-pathogen effector mechanisms operated through each of them. In this sense, we have recently described a novel pathway for iNOS activation by Naip5/Nlrc4 inflammasomes in response to cytosolic flagellin in peritoneal macrophages (PM).
Methods and Results: Extending our findings, here we found that NF-κB
was required for caspase-1-mediated iNOS activation in response to cytosolic flagellin, since iNOS activation was abrogated in the presence of a selective IκB-α degradation inhibitor, PDTC. Caspase-1-induced pyroptosis has been described as the main effector mechanism against *S. typhimurium.* However, at late time points of infection, when the frequency of cell death was similar in both, wild type (WT) and caspase-$1^{-/-}$ PM, we found a higher CFU number inside of remaining caspase-$1^{-/-}$ PM when compared to WT PM ones. Using a selective inhibitor of iNOS, aminoguanidine (AG), we found that iNOS expression and NO production contribute to the control of *S. typhimurium.* Moreover, flagellin was required for caspase-1-mediated iNOS activation in MP, showing that it could represent a non-redundant additional effector mechanism along with cell death to the control of *S. typhimurium.* **Conclusion:** Our results indicate that NF-κB is required for caspase-1-mediated iNOS activation in response to cytosolic flagellin and it activation contributes to the control of *S. typhimurium* infection. **Financial support:** CAPES; CNPq, FAPESP.

P1.15.17
NLRP3 inflammasome controls the cellular response to DNA damage during oxidative and genotoxic stress

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The NLRP3 inflammasome is a cytoplasmic protein complex, which mediates inflammatory responses to a broad array of signals by driving caspase-1 activation and promoting the secretion of pro-inflammatory cytokines IL-1β and IL-18. It is unclear whether NLRP3 participates in other cellular processes. This study was undertaken to identify new NLRP3-regulated pathways induced by monosodium urate (MSU) crystals in DCs. Using a transcriptomic approach, we initially found that NLRP3-/- DCs responded to MSU with differential expression of genes involved in DNA damage response and apoptosis. Upon exposure to MSU or other ROS-mobilizing stimuli (rotenone, gamma-radiation), DNA fragmentation was markedly ameliorated in NLRP3-/- and caspase-1-/- DCs compared with WT DCs. Moreover, NLRP3-/- DCs showed a significant reduction in oxidative damage of DNA mediated by ROS. A significant decrease of the expression of several genes involved in double-strand and base-excision DNA repair was observed in WT DC. Suboptimal cellular DNA repair capacity in WT DC resulted in activation and stabilization of p53 in vitro and in vivo, which resulted in increased cell death compared to NLRP3-/- cells. These data provide evidence for the novel involvement of the NLRP3 inflammasome in DNA damage responses induced by cellular stress.

P1.15.18
Intracellular Shigella remodels its LPS to dampen the innate immune recognition and evade inflammasome activation

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Lipopolysaccharide (LPS) is a potent bacterial effector triggering the activation of the innate immune system following binding with the complex CD14, MD-2 and TLR4. The LPS of the enteropathogen *Shigella flexneri* is a hexa-acylated isoform possessing an optimal inflammatory activity. Symptoms of shigellosis are produced by severe inflammation due to the invasion process of *Shigella* in colonic and rectal mucosa. Here we addressed the question of the role played by the *Shigella* LPS in eliciting a dysregulated inflammatory response of the host. We unveil that (i) *Shigella* is able to modify the LPS composition, e.g. the lipid A and core domains, during proliferation within epithelial cells; (ii) the LPS of intracellular bacteria (iLPS) and that of bacteria grown in laboratory medium (eLPS) differ in the number of acyl chains in lipid A, iLPS being the hypo-acylated; (iii) the immunopotential of iLPS is dramatically lower than that of eLPS; (iv) both LPS forms mainly signal through the TLR4/MyD88 pathway; (v) iLPS down-regulates the inflammasome-mediated release of IL-1β in *Shigella*-infected macrophages; and (vi) iLPS exhibits a reduced capacity to prime PMNs for an oxidative burst.
We hypothesized that the two forms of LPS might govern different steps of the invasive process of *Shigella*. In the first phases the bacteria, decorated with hypo-acylated LPS, are able to lower the immune system surveillance, while in the late phases *shigella* harboring immunopotent LPS are fully recognized by the immune system which can then successfully resolve the infection.

P1.15.19
Molecular mechanisms of caspase-11-mediated NLRP3 inflammasome activation

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Caspase-11 is the central inflammatory caspase responsible for NLRP3 inflammasome-dependent maturation of caspase-1 and the subsequent production of inflammatory cytokines IL-1 and IL-18 in enteropathogenic bacterial infections. Caspase-11 also functions to facilitate pyroptosis (inflammatory cell death). While the effector functions of caspase-11 have been well known, the proximal signaling events that trigger caspase-11 activation remained unknown. Here, we have identified a novel innate immune pathway that licenses caspase-11 activation. A systematic investigation of inflammasome activation by enterohemorrhagic *Escherichia coli* (EHEC) and *Citrobacter rodentium*, two model Gram-negative enteropathogens, revealed a role for TLR4 and TRIF in caspase-11-mediated responses as macrophages lacking TLR4 or TRIF failed to activate caspase-11 and as a result process caspase-1 and secrete IL-1β and IL-18. By engaging TLR4-TRIF, EHEC and *C. rodentium* activate caspase-11 via the type I interferon (IFN-β) signaling. IFN-β was found to regulate caspase-11 activation by up-regulating caspase-11 expression in an autocrine/paracrine manner. Remarkably the transcriptional induction of caspase-11 by IFN-β was both necessary and sufficient to promote caspase-11 auto-activation suggesting a model wherein transcriptional induction of pro-caspase-11 is coupled to its auto-activation. Once activated via the TLR4-TRIF-IFN-β pathway, caspase-11 ultimately synergizes with the NLRP3 inflammasome to coordinate caspase-1 dependent production of active IL-1β and IL-18. The IFN-β-caspase-11 axis is specifically required for NLRP3-caspase-1 inflammasome activation by Gram-negative but not Gram-positive bacteria. Overall, the identification of TRIF-type I interferon as a key regulator of caspase-11-dependent NLRP3 activation provides new insights into the integration of TLR and NLR pathways during Gram-negative infections.

P1.15.20
NLRP3-driven IL-1β production by *Cryptococcus neoformans* infection

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Cryptococcus neoformans is an encapsulated human pathogenic fungus that affects primarly immunocompromised individuals. It has a prominent capsule that is an important virulence factor, which is involved in immune response evasion and fungal dissemination. In the present study we investigated whether *C. neoformans* was able of triggering inflammasome activation and the role of its capsule in this event. The acapsular mutant of *C. neoformans*, but not the wild type, induced high levels of IL-1β secretion. In parallel, caspase-1 was activated upon infection with acapsular but not with WT *C. neoformans*. IL-1β secretion induced by *C. neoformans* was assessed in WT, *Asc*$^{-/-}$, *Casp1*$^{-/-}$, *Nlrp3*$^{-/-}$ and *Nlrc4*$^{-/-}$ bone marrow-derived macrophages, showing IL-1β secretion to be dependent on NLRP3 inflammasome. In addition, inflammasome components were dispensable for *C. neoformans* uptake or killing. The mechanisms underlying IL-1β processing and release were dependent on reactive oxygen species (ROS), lysosomal destabilization, potassium efflux and Syk tirosine kinase signaling. We also demonstrate that IL-1β signaling is not required to restrict intracellular yeast, but limited the rate of infection by an unknown mechanism. Together, our data show that acapsular *C. neoformans* activates the NLRP3 inflammasome and IL-1β secretion in a caspase-1 dependent manner and that the mechanisms involved in this event rely on ROS, lysosomal damage, potassium efflux and Syk signaling, leading to restriction of infection rate. Furthermore, our results show that the encapsulated *C. neoformans* is able to dimish inflammasome activation.

P1.15.21
Inflammation triggered by oncogene KRas in pancreatic tumor cells

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Pancreatic ductal adenocarcinoma (PDAC) has one of the worst prognoses among all cancers and current therapeutic approaches are limited and largely ineffective. PDAC is almost invariably associated with mutations in the KRas gene, which is essential for its maintenance. However, how KRas mutations promote pancreatic carcinogenesis is not fully understood. KRas drives cancer-associated inflammation and its contribution to tumor development and possibly to Epithelial to Mesenchymal transition (EMT) needs to be elucidated. In order to address this, we transduced with oncogenic KRasG12V the normal immortalized human pancreatic cell line HPDE and obtained various cloned sub-lines with distinct epithelial and mesenchymal features. Here, we report that differential expression of KRas activity results in different cellular phenotypes: “epithelial” with higher expression of E-cadherin and “mesenchymal” with increased expression of Vimentin, loss of E-cadherin and enhanced expression of transcription factor responsible Twist and Zeb2 for EMT. Significant amounts of pro-inflammatory cytokines (IL-6 and IL-8) are secreted from all clones, whereas IL-beta and PTX-3 are produced more in mesenchymal clones. By using IL-1 receptor antagonist, we observed higher suppression of IL-6, IL-8 and PTX-3 in epithelial clones, whereas significantly lower degree of suppression was observed in mesenchymal clones. This suggests that cells with EMT phenotype are less dependent on IL-1 for production of inflammatory mediators, indicating a link between oncogenic KRas induced inflammation and EMT. Future studies involving silencing of IL-1 or IL-6 receptors and in vivo growth in immune deficient mice may reveal important aspects of inflammatory mediators in tumor progression and EMT.

P1.15.22
Uric acid-induced NLRP3 inflammasome contribute to diabetic kidney injury

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Background: Although hyperuricemia is frequently found in a diabetic nephropathy, the definite cause and effect between hyperuricemia and kidney injury have not well investigated yet. Recently, reports showed IL-1β secreting NLRP3 inflammasome in cytoplasm plays a role as a sensor of the innate immune injury in metabolic disease. Therefore, we investigated the cause and effects of hyperuricemia and kidney injury in diabetic nephropathy to demonstrate the role of NLRP3 inflammasome in uric acid-induced kidney injury in diabetes.
Methods: We designed four animal groups as following; 1) LETO (Long Evans Tokushima Otsuka); 2) OLETF (Otsuka Long Evans Tokushima Fatty); 3) OLETF + HFD (high fructose diet) for 16 weeks; 4) OLETF + HFD + allopurinol (10mg/dL). HK-2 cells and THP1 cells were cultured and stimulated with uric acid.
Result: OLETF + HFD group showed a higher serum uric acid, urinary albumin creatinine ratio and the expression of NLRP3 and IL1β than OLETF group. Immunohistochemical staining of CD68^{+} cells showed significant increase in HFD group compared to OLETF group. Allopurinol attenuated HFD induced hyperuricemia and NLRP3 activation-related renal inflammation. Uric acid-induced NLRP3 activation and IL-1β secretion in THP1 cells were also observed. THP1 and HK-2 co-culture confirmed that IL-1β, secreted by THP1, plays a pivotal role in activating IL-1R1, MyD88 and IRAK4 signaling in HK-2 cells. This up-regulated IL-1R1 resulted in NF-κB activation in HK-2 cells.
Conclusion: According to these results we can conclude that hyperuricemia activates NLRP3 inflammasome of macrophage and contribute in renal injury by secretion of IL-1β.

P1.15.23

Transcriptional regulation of the anti-inflammatory NLRP12/Monarch-1 by NF-kB

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NLRP12 (NLR family, pyrin domain containing 12) is a NLR (Nucleotide-binding domain and leucine-rich repeat containing) protein which is expressed predominantly in cells of myeloid origin. It has been described as a negative regulator of both canonical and non-canonical NF-kB activation. Accordingly with its inhibitory function, NLRP12 expression is down-regulated following activation by TLR agonists or exposure of cells to inflammatory cytokines, whereas the expression of most NLR proteins is typically induced. It is hypothesized that the downregulation of NLRP12 expression is necessary for an appropriate immune response. After the removal of inflammatory stimuli, the expression level of NLRP12 should be restored to prevent excessive inflammation. To test this hypothesis, we focused on the control of NLRP12 expression at the transcriptional level. We asked if a NF-kB-driven negative feedback mechanism causes the down-regulation of NLRP12 transcription. We will show our recent findings about the modulatory effects of NF-kB on the NLRP12 promoter. Moreover, work is in progress to analyze the modulation of NLRP12 protein expression during inflammation and differentiation of myeloid cells.

P1.15.24
The role of autophagy in macrophage-derived foam cells after Salmonella enterica serovar Typhimurium infection

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Mycotic aneurysm are bacteria infected cardiovascular diseases. The mortality rate is extremely high without surgical treatment. Salmonella Typhimurium, which belongs to the type of non-typhoid, can cause gastroenteritis, bacteremia, and at times mycotic aneurysm. Foam cells are major immune cells in mycotic aneurysm. In our hospital, we found the most common pathogens isolated was nontyphoid Salmonella. We want to characterize the immune response in foam cells during Salmonella infecetion. We used Salmonella Typhimurium strain, SL1344 to infect macrophages cell line THP-1 cells and foam cells, using ELISA to analysis IL-1βsecretion and Western blot to detect activated caspase-1 and LC3-I and II which can trace autophagy activation. Foam cells derived from THP-1 cells by treating oxidized LDL. ELISA data shows more secretion of IL-1 β inTHP-1 cells after SL1344 infection, but after SL1344 infection and NH4Cl treatment, there were not significant difference between foam cells and THP-1 cells. In Western blot data, Salmonella infection or NH4Cl treatment, foam cells can activate more autophagy than THP-1 cells. And active caspase-1 in foam cells were more than in THP-1 cells after NH4Cl treatment and SL1344 infection. We suggest in foam cells, the hyper-activation of autophagy in foam cells may cause inflammasome degradation after Salmonella infection.

P1.16 Complement system

P1.16.01
Transcript levels of complement regulatory proteins (CD35 and CD59) in rheumatoid arthritis patients and their relationship with disease activity and prognosis

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Objectives: Complement regulatory proteins (CRPs) protect the host from tissue injury mediated by activated complement system. In rheumatoid arthritis (RA) exaggerated complement activation is the key event, due to which CRPs CD35 and CD59 may influence the disease pathology and prognosis. Therefore, we aimed at elucidating the leukocyte-CD35 (L-CD35) and L-CD59 transcript levels and the relationship of these two CRPs with the disease activity of RA patients.
Methods: 66 controls and 45 RA patients were recruited for this study. Out of 45 RA patients, 11 patients volunteered for the longitudinal study. L-CD35 and L-CD59 transcript levels were determined by real-time PCR and correlated with Circulating immune complexes (CIC) levels and Disease Activity Score-28 (DAS28).
Results: Transcript levels of L-CR1 ($p < 0.05$) and L-CD59 ($p < 0.01$) were lowered in patients as compared to controls. In longitudinal study, L-CR1 transcript levels ($p < 0.05$) increased in patients which responded to treatment which correlated with lowered CIC levels ($p < 0.05$) and DAS28 scores ($p < 0.05$) at W24 as compared to W0. No significant difference was observed for L-CR1 transcript levels in non-responder group and for L-CD59 transcript levels in responder and non-responder group at W24 as compared to W0.
Conclusions: This is the first study to assess the L-CR1 and L-CD59 transcript levels in RA patients. The findings indicate an intimate relation of L-CD35 transcript levels with the disease activity and prognosis in RA patients, and thus suggest L-CD35 transcript as a potential disease marker in RA.

P1.16.02
Anti-mitochondria antibodies type 2 and neurological manifestations: Report of 5 cases

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Introduction: Anti-mitochondria antibodies type2 (AMA2) are often described as a specific marker of primary biliary cirrhosis(CBP).AMA2 associated with neurological symptoms were rarely described in the literature.
Objective: Clinical and biological features of patients with AMA2 and neurological manifestations.
Patients and methods: From January 2010 to March 2011 we recorded all cases with neurological manifestations and AMA2.
In all cases, AMA2 were detected fortuitously when screening sera for ANA on Hep-2 cells (granular cytoplasmic fluorescence).
AMA2 were confirmed by indirect immunefluorescence on tissue rat sections and/or immunodot using the subunit E2 of pyruvate dehydrogenase as antigen (Euroimmun).
Results: There were 5 patients, all of them were women. The average age was 50 years. Neurologic signs were weakness and impairment of speech in 1 case, functional neurological deficit in 2 cases, a pyramidal syndrome with myelitis in 1 cases and cerebellar syndrome associated a mood disorder in the last case. Anti-gp 210 antibodies were associated with AMA2 in 1 case. Hepatic assessment, available in 2 cases, showed a cholestasis suggestive of PBC.
Conclusion: Neurological manifestations associated with cholestasis are rare. However this association should evoke PBC. Physiopathologic mechanisms of this association are not yet elucidated.

P1.16.03
Immunotoxicity of work environment exposure in some welders: implications for occupational health

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Background: The welding process generates fumes of toxic metals oxides and ground level ozone. Exposure to these complex mixtures may have deleterious effect on metabolic functions including the immune system.
Methods: To investigate this live of thought, fifty welders and Forty controls were recruited for the study. Interviewer styled questionnaire was administered for information on work history and biodata. Urine was estimated for welding related toxic metals, namely chromium, Iron, Lead, manganese, zinc, nickel and lead using AA.
Serum obtained from whole blood was used for the estimation of IgG, IgA, IgM, IgE and C3 by turbidmetic method and WBC of whole blood by haematology analyzer.
Results were input into the computer and statistical analysis was done using Student T-test, Mann-Whitney U and Pearson correlation.

Results: There was significantly increased concentration of all the toxic metals analysed in welders compared to the control with a pronounced increased value in iron concentration P = 0.0002, There was also significant reduction in levels of WBC (P = 0.004), IgA P = 0.001, IgM P =0.001, C3, P = 0.012 but not in IgG P = 0.128 and IgE P = 0.115. There was positive correlation between level of iron and reduced concentration of the immune markers.
Conclusion: The welders studied are at increased risk of respiratory tract infections due to the depression in the immune system particularly IgA and in case of infections, like Mycobacterium tuberculosis, severity could be supported by reduced C3 and increased body iron levels.

P1.16.04
Hereditary C1 esterase inhibitor deficiency in Tunisia: a seven years laboratory experience

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Introduction: Hereditary C1 esterase inhibitor (C1-INH) deficiency is characterized by occurrence of subcutaneous and submucosal swellings in any parts of the skin, the respiratory and gastrointestinal tracts, called hereditary angioedema (HAE). The epidemiological, clinical and biological characteristics of C1 INH deficiency were reported in many series in Europe and USA. In Tunisia data are not available. Here we report our laboratory experience to define the frequency, the clinical and biological aspects of C1 INH deficiency.
Materials and methods: From May 2005 to August 2012, we enrolled all cases sent for suspicion of C1-INH deficiency. Complement investigations included (i) C3 and C4 measurement (ii) functional activity of the classical pathway (CH50), (iii) C1 INH antigenic and functional assays; Type I HAE is defined by low plasma levels of a C1-INH protein. Type II is characterized by dysfunctional C1-INH.
Results: Twelve patients among 150 analysis were diagnosed as C1-INH deficiency. Ten were type I HAE and two were type II. Four patients were diagnosed in a context of a familial investigation. C1-INH deficient patients were 6 men and 6 women with a median age 21years and 41years respectively. The main clinical features were skin and mucosal swellings (5 patients), and chronic urticaria in 2 patients. Ten out of 12 cases had low levels of C4 and CH50.
Conclusion: C1-INH deficiency seems to be rare in Tunisia compared to European and other Maghrebin series. Improvement of knowledge to the various forms of HAE is needed to assess our real prevalence of C1-INH deficiency.

P1.16.05
Humoral Immunity in the healthy population of the central region of Cuba

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In order to study the humoral immune response in healthy population of the central region of Cuba we establish reference values of immunoglobulins IgG, IgM and IgA and C3 and C4 complement proteins in three different and interesting moments in life from the immunological point of view (birth, childhood and adulthood) in apparently healthy population of the province of Villa Clara, Cuba. METHODS: With serum samples from 200 individuals were formed 3 study groups comprised 80 new born, 60 children and 60 adults. In each case were 50% female and 50% male. Using nonparametric tests found no significant differences between gender at each group was taken as a single sample. The values of reference were established for the immunological parameters according to age, taking the central interval (95%) delimited by the 2.5th and 97.5. RESULTS: It was observed that new born had lower serum complement proteins, while children showed slightly higher serum concentrations than adults. The immunoglobulins were significantly different in each study group. CONCLUSIONS: This work is unique in the region as it also characterizes the humoral immunity, and provides reference values to facilitate further studies to make decisions about possible prognosis and treatment of patients exhibiting immunological symptoms.

P1.16.06
Whole exome sequencing in a Finnish family with hereditary angioedema with normal C1 inhibitor

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Hereditary angioedema (HAE) is an autosomal dominant disease characterized by episodic, local swelling of the submucosal, dermal or subcutaneous tissue. HAE is divided into type I and II HAE with low or functionally defective serum C1 inhibitor, respectively. Both forms are caused by mutations in the C1 inhibitor gene. In addition, there is a group of patients with symptoms typical of HAE, but normal C1 inhibitor. Mutations in coagulation factor XII have been identified in 20% of these patients. The aim of our study is to find a mutation predisposing to the described symptoms in families of Finnish descent. For this purpose we collected five patients from four different families. They presented with symptoms typical of HAE, normal C1 inhibitor levels, functional activity and C4. These patients did not respond to treatment with high dose antihistamines, corticosteroids or adrenalin. Two of the patients are mother and daughter from the same family in which the late grandmother and cousins from the mother's side also had similar symptoms. We sequenced the coding and regulatory regions of the factor XII gene in these patients. We found only previously identified gene polymorphisms that were also present in control subjects. Thus, genetic causes other than those in C1 inhibitor or FXII may cause angioedema. Since the predisposing cause for the same phenotype is probably heterogeneous, we chose the patients from the family affected in three generations for whole exome sequencing. The analyses are underway and may reveal a new genetic cause for hereditary angioedema.

P1.16.07
Effect of immune complex and cytokines on expression and modulation of neutrophil complement regulatory proteins in SLE

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Modulation of neutrophil DAF, MCP, CD59 & CR1 transcripts in SLE and their close correlations with SLE disease activity had been documented. To gain further insight, we studied effects of serum opsonized heat aggregated immunoglobulins (sHIgG), IFN-γ, TNF-α, IL4 and, IL10 on the expression and, modulation of these proteins in SLE.
Neutrophils isolated from the venous blood of patients and controls were treated in vitro with different effecters. The transcript levels of different complement regulatory proteins in treated and untreated cells were determined by real time PCR, data were compared between patients and controls.
sHIgG down-regulated the levels of CR1 transcript but up-regulated the levels of MCP and DAF with no significant effects on CD59 expression both in controls and patients. CR1 correlated negatively with MCP, DAF, & CD59. Levels of all these proteins were upregulated by IFN-γ and were down regulated by TNF α. IL4 and IL10 significantly up regulated MCP and DAF, respectively. The upregulation by all the effecters ranged from 1.5 -4 fold and down regulation ranged from .25-.8 fold as compared to the untreated cells. Mostly, the modulation was more marked in patients compared to controls. Observations were statistically significant ((P<.05-0.001, Mann-Whitney test).
Our study documents modulation of neutrophil complement regulatory proteins by cytokines and hence, suggests close interplay of

cytokines with functional attributes of complement in health and diseases like SLE where complement proteins have disease modulating roles. Further studies are needed to confirm our findings and their translational implications.

P1.16.08
Contribution of C5a measurement to explain some clinical biological discrepancies in monitoring of lupus

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Introduction: Besides the search for anti-dsDNA, complement investigations and measurement of CH50, C3 and C4 specially, have long been considered as good parameters for monitoring lupus (SLE). Very few works explores the degradation products of complement in this disease. Aim: The objective of our study is to determine the role of C5a assay in explaining some discrepancies clinical biological of lupus.
Patients and methods: Our study included 142 patients with SLE based on the American College of Rheumatology (ACR) criteria. Patients were stratified according to state's activity of disease. Classical exploration of complement was done. C5a was detected by an enzyme-linked immunoassay kit (Quiedel, USA) in sera of patients in clinical relapse with normocomplementemia only.
Results: Positive correlation between complement variation and SLE disease activity (r=0.69; P=0.0001) was detected in 81.5% (97/120) of SLE sera. Clinical biological discordance was found in 23 patients, among them 10 were in remission and showed deceased of complement C4 with or without C3. However 13 patients were in relapse and normocomplementemia, 5 among these 13 patients have very increased level of C5a.
Conclusion: Normal level of C3 with increased level of C5 would support the involvement of a new pathway for generation of C5a independently of classical, alternative and lectin ones. The presence of thrombin seems to be the major parameter to generate C5a as reported recently.

P1.16.09
Complement activation in patients with systemic lupus erythematosus without nephritis

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INTRODUCTION: The complement system has long been known to be activated in exacerbations of systemic lupus erythematosus (SLE), particularly reflecting nephritic activity. The aim of this study was to evaluate the association between disease activity and complement activation in SLE patients with mild disease and with no renal involvement.
METHODS: We studied 42 SLE patients (36 females, 6 male) without nephritis. Levels of serum complement C3 and C4 were measured by nephelometry. Total complement haemolytic activity was measured with assays specific for the classical pathway (CH50). Terminal SC5b-9 complement complex (TCC), as an activation product from the final common pathway, was quantified by ELISA.
RESULTS: Lupus flares were observed in 11 patients (26%). Decreased CH50 was found in 13 (31%) patients but was not associated with increased complement activation. Slightly to moderately decreased C4 (0.05-0.10 g/l) was found in five patients and severely decreased C4 (<0.05 g/l) in three patients. Three patients had decreased C3 in conjunction with positive anti-C1q antibodies and low C4 concentrations. Complement activation products (TTC) were either normal or slightly elevated. None of the variables tested (C3, C4, CH50 and SC5b-C9) correlated with disease activity.
CONCLUSION: Our data suggest that complement tests are of limited value in routine examination of SLE without nephritis.

P1.16.10
MASP2 and MBL: Dynamics and Intrathecal synthesis

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Background: There is an increasing interest to look for the possible participation in lectin pathway components in the immune response in CNS. Methods: MASP2 and MBL were assayed in 20 control samples of CSF and serum with an ELISA, coated with anti MASP2 and MBL antibodies. Albumin-, immunoglobulin- CSF/serum quotients, oligoclonal IgG and cell count were used to characterize the patient groups. Groups comprised firstly, control patients without organic brain disease with normal CSF and normal barrier function and secondly, patients without inflammatory diseases but with increased QAlb, with a blood CSF barrier dysfunction. Results: MASP2 concentration in CSF was at least two-fold higher than expected for a molecular-size-dependent passage from blood and CSF MBL was at least five-fold 2) in a QMASP2 vs QAlb regression line 9/20 cases showed an intrathecal fraction and QIgM/QAlb quotient diagram (Reibergram) 9/13 cases showed an intrathecal fraction in some cases over 80% of total CSF MBL concentration 3) The higher inter-individual variation of MASP2 concentrations in CSF of the control group (CV = 55%) compared to the MASP2 concentrations in serum (CV = 40%) indicate that MASP2 is mostly a blood protein and the smaller inter-individual variation of CSF MBL of the control group compared to serum MBL concentration indicate an independent source of CSF MBL 4) The absolute MASP2 and MBL concentration in CSF increases with increasing QAlb. Conclusions: MASP2 in CSF is predominantly blood-derived. MBL dynamics indicate that only the leptomeningeal proteins shower a linear increase with decreasing CSF flow rate.

P1.16.11
Human skin-derived mast cells express complement C3 and C5

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This study examines whether complement factor C3 or C5 is synthesized by the cultured human skin-derived mast cells and whether their synthesis is regulated by cytokines. C3 and C5 mRNAs were expressed and baseline protein levels/10^6 cultured mast cells measured by ELISA were 0.9 and 0.8 ng, respectively, and detected by flow cytometry and Western blotting. Both proteins were located in the cytoplasm outside of secretory granules assessed by confocal microscopy. Similar mRNA and protein levels were also found in freshly purified skin-derived mast cells as the cultured mast cells. C3 accumulated in mast cell culture medium over time and by 3 days reached a concentration of 9.4 ± 8.0 ng/ml, whereas C5 levels were not detectable. Three-day incubations of mast cells with IL-1α, IL-1β, IL-17, IFNγ or IL-6 did not affect C3 protein levels in culture medium, whereas incubations with TNFα, IL-13 or IL-4 enhanced levels of C3 1.7- to 3.3-fold. Importantly, treatment with TNFα together with either IL-4 or IL-13 synergistically enhanced C3 (but not C5) production in culture medium by 9.8 or 7.1 fold, respectively. This synergy was blocked with neutralizing anti-TNFα Ab, soluble TNFR, or an inhibitor of NF-κB, or by attenuating the IL-4/13 pathway with Jak family or Erk inhibitors. This study shows for the first time that human mast cells can produce and secrete C3, while beta-tryptase can act on C3 to generate C3a and C3b, raising the likelihood that mast cells engage complement to modulate immunity and inflammation in vivo.

P1.16.12
Probiotic spores: a missing link between innate immunity and nutritional health

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Spores of the human-friendly bacterium Bacillus subtilis represent a new generation of probiotics due to their high stability and additional utilization as non-refrigerated antigen-delivery vaccines. An interesting premise for probiotics is their capacity to modulate the immune system. The Complement System (C) is a major effector of innate response and is under the control of three different pathways of activation (Classic, CP; Alternative, AP; and Lectin pathway, LP). However, the understanding of the probiotic effect on C is waiting. Using a battery of bacterial genetic techniques, in combination with specific methods to evaluate C activation, we demonstrate that B. subtilis is capable of activating the three C pathways without MAC formation. This activation is under the control of the master transcriptional regulator of biofilm development SinR. Using different genetically-constructed B. subtilis mutants, we uncovered each component of the biofilm involved in C regulation. B. subtilis mutants deficient in the synthesis of exopolysaccharide (EPS) and the antimicrobial and antibiotic-repellent proteins TasA and BslA, respectively, were severely affected in C activation. Furthermore, we demonstrate a specific role for each biofilm component on each C activation-pathway. TasA was essential and specific for CP-activation whereas BslA and EPS were essential and specific for the activation of the AP and LP, respectively. Overall, our results show for the first time how different components of the bacterial biofilm (which colonize the human mucosa) modulates the activity of C and contribute to a better understanding of the beneficial effects of probiotics on innate immunity at molecular level.

P1.16.13
Hereditary complement C8 deficiency in a Turkish family found in a girl with recurrent meningitis and her asymptomatic sister

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The complement system plays important roles in both the innate and the adaptive immune defence and also acts as a bridge between these arms of immunity. C8 is one of the five terminal complement components required for the formation of the membrane attack complex and composed of three polypeptide chains. Complete absence of C8 Complete absence of C8 results in increased susceptibility to gram-negative bacteria especially Neisseria species. Two functionally distinct C8 deficiency states (C8 alpha-gamma and C8 beta deficiency) in all together thirteen patients have been described until now. The patient, a seven years old girl was admitted to hospital with high fever and diffuse, purple-coloured skin lesions. Her symptoms gave the diagnose meningococcal meningitis. She had also earlier been diagnosed with the same disease when she was 5 years old. A sister to the patient had died from meningitis at 3 years of age. She has also one older and one younger sister. There is no consanguinity between her parents. The laboratory analyses of the classical pathway measured as complement hemolytic activity (CH50) and C8 concentration revealed no activity and absence of C8, respectively. Analysis of serum from her younger sister showed the same results, while her older sister's CH50 and C8 levels were found normal. Thus, our patient and her younger sister were diagnosed with hereditary C8 deficiency. The genetic analyses have not been completed yet. We here report the first cases of C8 deficiency in Turkish patients.

P1.16.14
Complement component C3 variant (R102G) and the risk of neovascular age realated macular degeneration in a Tunisian population

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Purpose: To explore the association between the polymorphism(S/F) R102G in the complement component 3 (C3) gene and age-related macular degeneration (AMD) in a Tunisian population.
Methods: The molecular study was performed by PCR-SSP in 135 control subjects free of any eye disease (Fundus normal) and 91 patients with exudative AMD. The CH50 activity and quantification of C3 and C4 have been made by technical home method and nephelometry respectively.
Results: The prevalence of C3 F allele polymorphism was significantly higher in AMD patients compared to controls OR: 2.051, IC95% [1.29-3.26], p=0.002. However, no correlation between this allelic variant and the type of neovascularization. Similarly, there is no association between this polymorphism and the presence of functional and / or quantitative hypocomplementemia.
Conclusions: The C3 F allele of the gene could be a susceptibility factor for AMD in the Tunisian population. However, it does not seem to influence the clinical profil of the disease.

P1.16.15
The Yersinia pestis Outer Membrane Protein Ail Recruits C4b-Binding Protein Leading to Factor I-Mediated Inactivation of Covalently and Non-covalently Bound C4b

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The outer membrane protein Ail of Yersinia pestis mediates several virulence functions, including serum resistance. It is highly expressed at 37oC and indispensable in a rat model of infection. The Ail protein from the closely related enteropathogen Y. pseudotuberculosis functionally recruits C4b-binding protein (C4BP), the primary fluid-phase inhibitor of the classical and lectin pathways of complement. Here, we demonstrate that Y. pestis Ail also binds C4BP as well as C4 and C4b in a non-covalent manner. C4BP bound to Ail can act as a cofactor to the serine protease factor I (fI) in the cleavage of fluid-phase C4b. Employing a panel of C4BP alpha-chain mutants, each deficient in a single complement control protein domain (CCP), we observed that the absence of CCPs 6 and 8 reduced binding to Ail. Immunoblot analysis of normal human serum (NHS)-treated bacteria revealed minimal C4b alpha'-chain complexes with the bacterial outer membrane targets. The addition of the anti-C4BP monoclonal antibody MK104 to NHS restored the emergence of C4b-alpha' chain target complexes, suggesting that C4b binds covalently to targets on the Y. pestis surface. C4b bound to Ail non-covalently is also cleaved in a C4BP and fI-dependent manner, leaving the C4c fragment bound to Ail. The addition of MK104 to NHS also prevented the cleavage of non-covalently bound C4b. Collectively, these data suggest that when C4BP is bound to Ail, fI can cleave and inactivate C4b that has bound covalently to bacterial surface structures as well as C4b that has bound non-covalently to neighboring Ail molecules.

P1.16.16
Neutralizing complement resistance improves cell-mediated killing (CDCC) of HER2 positive breast cancer cells by Trastuzumab and Pertuzumab

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The therapeutic potential of anti-cancer mAbs is hampered by the ability of malignant cells to acquire resistance to complement, primarily by the over-expression of membrane complement regulatory

proteins (mCRPs) such as CD46, CD55 and CD59. Trastuzumab and Pertuzumab are anti-HER2 monoclonal antibodies approved for the treatment of HER2-positive breast cancers, which exert only minor complement-mediated cytotoxicity (CDC). Upon opsonisation of tumor cells with the C3 split product iC3b, complement receptor 3 (CD11b/CD18) on NK cells and macrophages mediates complement-dependent cellular cytotoxicity (CDCC). Here, we investigated whether treatment of HER2 positive tumor cells with trastuzumab and pertuzumab not only leads to direct destruction by CDC but also induces CDCC and if silencing of mCRPs with siRNA enhances cell-mediated cytotoxicity.
Small interfering RNAs (siRNAs), which are chemically stabilized by 2´-O-methyl sugar modifications, were encapsulated in liposomes (AtuPLEX) for delivery to Her2/neu high expressing BT474 breast tumor cells to knockdown synthesis of CD46, CD55 and CD59.
Upon mCRPs knock down, CDC was significantly increased when BT474 tumor cells were treated with both trastuzumab and pertuzumab. Complement-induced apoptosis and caspase activation was augmented if mCRPs were neutralized in tumor cells. Incubation of tumor cells with both antibodies also led to increased C3 deposition (opsonization), and further augmented tumor cell killing by M1 and M2 macrophages. Furthermore, incubation of mCRP neutralized BT474 cells with PBL or NK92 cells further augmented cell-mediated cytotoxicity.
Thus, the combination of both trastuzumab and pertuzumab induces CDCC and neutralization of complement regulators further enhances their anti-tumor effects

P1.16.17
Dissecting the modular nature of properdin using recombinant thrombospondin repeats TSR4 and TSR5

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Properdin upregulates the alternative complement pathway by binding and stabilising the C3 convertase complex (C3bBb). Properdin is a 53kDa soluble glycoprotein and its flexible rod-like monomers form cyclic polymers (dimers, trimers and tetramers). The properdin monomer consists of six thrombospondin type I repeats (TSR) each having about 60 amino acids, which are similar or homologous to circumsporozoite and thrombospondin-related anonymous proteins (TRAP) of *Plasmodium* species, ETP100 of *Eimeria tenella*, various complement components C6-C9 and thrombopondins I and II. Using deletion constructs, TSR4 and TSR5 have been shown to be involved in C3b binding and stabilising C3 convertase, suggesting that these modules contribute significantly to properdin functions. However, individual TSR4 or TSR5, expressed in *E. coli*, failed to bind properdin ligands. Here, we have expressed in *E. coli* biologically active TSR4 and TSR5, together in tandem (TSR4+5), in *E. coli* and examined its interaction with C3b, sulfatides, Bacillus Calmette-Guerin (BCG) and apoptotic cells. The results of binding studies and modulation of the alternative pathway by recombinant TSR4+5 will be presented.

P1.16.18
Comparison of C3 and C4 with CH50 in North West of Iran

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Background and Aim: The complement system comprises a group of serum proteins and cell membrane receptors that function primarily to fight infection. These components interact in three activation pathways. The central results of activation of these pathways are to deposit the opsonic C3b on bacteria to promote phagocytosis, to lyse bacteria by the assembly of the terminal membrane attack complex and to promote inflammation. The aim of this study was to compare the rate of increase or decrease in C3 and C4 in patients is CH50.
Methods: This cross-sectional study on 281 patients referred to the Zanjan's Clinical Laboratory, measured C3 and C4 by nephelometry method, CH50 levels were measured using SRID. Then the data were analyzed by the SPSS13 Software.
Results: The results of tests on patients revealed that the female:male ratio was 1/16. The mean age of the patients was 35.31±16.21. (5.5 percent) 15 cases with high C3, (8.7 percent) 24 cases with high C4, (3.4 percent) 9 cases with high CH50. The CH50 data not showed a significant increase in patients.
Conclusions: This study showed that CH50 measurements alone cannot be the expression of the complement system function. Other measures such as the complement component like C1q, C3, C4, C5, and C9 with CH50 are recommended.

P1.16.19
Myeloperoxidase directly interacts with properdin and induces alternative pathway complement activation

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Properdin is well known as a stabilizing factor for the alternative pathway (AP) C3 convertase, thereby promoting complement activation. More recently it has been demonstrated that properdin can also act as a pattern recognition molecule, recognizing for instance microbial surfaces or apoptotic cells, providing a platform for AP activation. Neutrophils are a major source of properdin and the AP has been implicated in many neutrophil-mediated diseases. Here we investigated the ability of properdin to interact with various neutrophil components which are released upon neutrophil priming and activation. Using purified components, properdin showed a clear dose-dependent binding with several neutrophil enzymes, including myeloperoxidase (MPO), elastase, lysozyme and cathepsin G, but not with azurocidin. However, only MPO showed the ability to induce C3 activation when exposed to serum as a complement source. Complement activation could be completely prevented by EDTA, but was not affected by MgEGTA, confirming that MPO specifically activated the AP. Activation of the complement cascade could be demonstrated both by the deposition of C3, but also resulted in deposition of C5b-9. MPO initiated AP activation was absent in properdin deficient serum. Furthermore, we could show that MPO can bind properdin directly which then induces AP C3 deposition in-vitro. In summary we show a novel mechanism by which properdin can interact directly with neutrophil components, and for MPO results in AP mediated complement activation. This unique interaction may be an important contribution to the central role for the AP in neutrophil-mediated diseases such as rheumatoid arthritis and ANCA vasculitis.

P1.16.20
Marine algae Bostrychia tenella fractions and extracts from associated endophytic microorganisms modulate the complement system

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Exotic sources of natural products such as marine environment, including marine endophytic microorganisms, have gained interest in the search for novel bioactive substances. Very few studies have explored the pharmacological potential of the marine algae Bostrychia tenella. Therefore, this study aimed to evaluate whether different fractions of this macroalgae and extracts of endophytic microorganisms isolated from it possess immunomodulatory activity on the human complement system (CS), which is recognized as one of the major effectors of the inflammatory process. CS plays an important role in various inflammatory reactions and in various pathogenic mechanisms of tissue damage. Hemolytic assays of complement activity were used for screening 20 samples. Ten samples were selected because they were able to modulate this activity in a pool of normal human serum. The 10 samples (fractions, subfractions and extracts of B. tenella and its endophytic microorganisms) were able to induce the generation of fragments of C3, which were detected by immunoelectrophoresis and Western blot. Also, we observed the formation of the membrane attack complex in enzyme immunoassay, indicating that there was total

activation of the CS. From the results obtained in Western blot for fragments of C4 and Factor B was possible to identify which pathways are activated in CS, when correlated with the results of hemolytic assays for the classical/lectin (CP/LP) and alternative pathway (AP). Thus, we conclude that the 10 selected samples have modulatory effects on human CS because they were able to activate it either by CP/LP or AP. Financial support: CAPES, FAPESP.

P1.16.21
Developmental programming of low levels of C3 and C4 complement factors by gestational chronodisruption

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The hypothesis of developmental origins of health and disease (DOHaD) states that exposure to adverse intrauterine environment leads to fetal maladaptation, increasing the risk for diseases in adulthood. In this context, epidemiological and animal model studies have been mainly focused in the effects of gestational undernutrition on the offspring development. Chronodisruption (a significant disturbance of the circadian rhythm of physiological parameters) along pregnancy has been associated with an increased risk of preterm delivery and low birth weight, both strong predictors of chronic diseases in adulthood. The long term goal of our research is to determine the effects of developmental chronodisruption on the immune system of the offspring. Our results of gene expression analysis by means of DNA microarray and real time RT-PCR in the fetal liver, demonstrated that exposure of pregnant rats to constant light along the second half of gestation downregulated the expression of several genes of classic and alternative complement activation pathway as well as the membrane attack complex, relative to gestation under control photoperiod. Interestingly, in a parallel cohort analyzed at postnatal day 90, we found that plasma circulating levels of C3 and C4 complement factors were significantly lower in the offspring gestated under constant light, relative to normal photoperiod ($P<0.05$). Shift work affects about one fifth of the workforce, including pregnant woman, which means that detrimental impact on health by intrauterine exposure to chronodisruption may have important public health implications.
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P1.16.22
Dietary restriction and fasting downregulate complement activity via MBL-pathway

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Introduction: Preoperative dietary restriction (DR) offers robust protection against renal ischemia-reperfusion injury (IRI) in mice. However, the mechanism is unknown. We hypothesize that immunomodulation via the complement system plays a pivotal role, and investigated the impact of DR on complement activation pathways.
Materials and Methods: Male C57Bl/6 mice were fed *ad libitum* (AL), underwent 72hrs fasting (F) or 2 weeks 30% DR (n=8/group). Functional activity of the complement activation pathways (classical (CP), lectin (LP) and alternative pathway (AP)) was assessed, and mRNA studies were performed for all relevant upstream and downstream complement proteins.
Results: Significant downregulation in CP, LP activity by DR and in CP, LP and AP activity by F was observed. Activation of C3, C9, and MBL-A and -C concentrations in DR and F were significantly decreased ($p\leq0.002$) in CP, LP and AP. C1q concentration was only significantly lower in the F group ($p\leq0.0001$). The mRNA studies showed a significant downregulation in liver MBL expression in both DR and F ($p\leq0.004$) groups. However, C3 levels (both in kidney and liver) were found to be elevated in both DR ($p\leq0.03$) and F ($p\leq0.05$) groups.
Discussion: Dietary interventions downregulate complement activation pathways. The most prominent effect of DR and F was observed on the MBL pathway. To our knowledge, our data for the first time show that DR and F cause downregulation of complement activation pathways. This may be one of the mechanisms by which dietary interventions protect against renal IRI.

P1.17 Allergic mediators

P1.17.01
Parvalbumin: as a fish-allergen

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Parvalbumin is a small, acidic, Ca^{2+}-binding protein found abundantly in the muscle sarcoplasm of fish, frog and other vertebrates. IgE-binding studies have shown that purified parvalbumins from several fish species were recognized by a large number of individuals allergic to fish. Therefore, fish allergy has been considered as one of the most common food allergies in the countries where, fish is a staple in the diet. However, in the present study boiled parvalbumin samples from several commonly consumed Indian fish species (*Channa* sps. and *Clarias* sps.) were run on native 10% polyacrylamide gels. The electrophoretic studies revealed that the parvalbumins remained pretty stable at boiling temperatures for about one hour. Thus, the fact that parvalbumins have remarkable thermo-stability and remain allergic to the susceptible people suggests that the immunologic-domains of the protein also remain active even after cooking. Therefore, molecule-based research would be of immense help in better understanding of the parvalbumin-allergy and diagnosis.

P1.17.02
Clock genes and eosinophil-specific mediators are expressed in a circadian manner in human eosinophils

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Allergic diseases are frequently exacerbated between midnight and early morning suggesting an involvement of the biological clock. Eosinophils are main effector cells of allergic diseases and some eosinophil-specific markers such as eosinophil cationic protein (ECP) exhibit circadian variation. Thus, the aim of our study was to analyze whether the circadian clock is functional in eosinophils. Human eosinophils were isolated from peripheral blood of both non-allergic and allergic subjects and purified by magnetic cell separation. Subsequently, cells were harvested and analyzed every 3 hours around the circadian cycle. Clock gene (hPer1, hPer2, hCry1, hBmal1 and hClock) expression and expression of eosinophil-specific mediators including hEcp as well as eosinophil-derived neurotoxin (hEdn) was analyzed by real-time RT-PCR, and protein levels of ECP were measured by Western blotting. All clock genes, hPer1, hPer2, hCry1, hBmal1, and hClock are expressed and exhibit a circadian rhythm in eosinophils of both healthy and allergic persons. In addition, the expression of eosinophil-specific mediators ECP and EDN also revealed a circadian oscillation. In summary, circadian clock genes as well as eosinophil-specific mediators oscillate in human eosinophils. Thus, the biological clock controls eosinophils leading to circadian expression of their mediators and therefore may play a pivotal role in the pathophysiology of allergy.

P1.17.03
Heat shock protein 70 is potent to suppress chemotactic activity of ATP and migration of dendritic cells

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Heat shock protein 70 (Hsp70) responds for ATP-dependent folding and degradation of proteins. The pool of extracellular Hsp70 possesses immunomodulatory activity. Extracellular ATP has gain attention as a key mediator of asthma. ATP has been shown to modify the recruitment and function of inflammatory cells including dendritic cells (DCs). Due to high affinity of Hsp70 to ATP that results in ATP dephosphorylation, Hsp70 might decrease ATP-mediated lung inflammation. To prove this hypothesis in the present study we tested the influence of Hsp70 on ATP-dependent chemotaxis of dendritic cells in vitro.
Bone marrow-derived DCs (BMDC) were cultivated in presence of GM-CSF for 9 days. Migration assay was carried out using 24-well Transwell chambers. To induce the chemotaxis we used 1uM of ATP or ADP. To estimate the suppressive effect 1uM of Hsp70 was added to ATP. PBS served as a control. BMDCs were added in concentration 0,5 mln cell/well and incubated at 37C. After 90 minutes migrated DCs were collected and counted. Chemotactic index was calculated as the ratio of the cells migrated in response to chemoattractants and to PBS.
ATP revealed the highest chemotactic activity compare to other stimuli. The minimal chemotactic index was observed in response to ADP. Although Hsp70 along induced slight cell migration, addition of Hsp70 to ATP in 1:1 ratio two-fold decreased the chemotactic index.
Thus, Hsp70 reduced ATP chemotactic activity and subsequently ATP-induced migration of BMDCs.
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P1.17.04
Regulation of allergen-specific IgA and IL-17 responses by intestinal epithelial cell IKKβ reshapes allergic inflammation at distant sites

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Regulation of allergic responses by intestinal epithelial cells remains poorly understood. Using a model of oral allergen sensitization in the presence of cholera toxin as adjuvant and mice with cell-specific deletion of IKKβ in intestinal epithelial cells IECs (IKKβΔIEC), we addressed the contribution of IECs to allergic sensitization to ingested antigens and allergic manifestations at distant mucosal sites and the skin. Cholera toxin induced higher proinflammatory responses and altered the profile of the gut microbiota in IKKβΔIEC mice. Antigen-specific IgE responses were unaltered in IKKβΔIEC mice, but Th1 responses and associated IgG2a Abs were enhanced. Interestingly, allergen-specific Th17 and IgA Ab responses were enhanced in IKKβΔIEC mice. Upon nasal antigen challenge, these mice developed lower levels of allergic lung inflammation, which correlated with higher levels of IgA Abs in the airways. However, IKKβΔIEC mice recruited a higher number of gut-sensitized T cells in the airways after nasal antigen challenge and developed airway hyper-responsiveness, which were suppressed by treatment with anti-IL-17A. Orally sensitized IKKβΔIEC mice were also protected against allergic inflammation in response to epicutaeous challenge. Ongoing studies are investigating the relative contribution of IgA and T helper cell subsets to the protection achieved in the skin.

P1.17.05
Regulation of human skin mast cell histamine release by PDE inhibitors

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Mast cell and basophil are thought to be central to inflammation that has an allergic basis as allergens activate these cells in an ige-dependent manner to generate mediators such as histamine, eicosanoids and cytokines. Phosphodiestres is known to exist as multiple molecular forms of enzyme that metabolize the second messengers. studies of our own have shown that, of a variety of isoform-selective drugs, the phosphodiestres4-selective inhibitors, such as rolipram, attenuate the ige-mediated release of histamine from human basophils but not from human lung mast cells . The main aim of the present study was to characterize the type and role of phosphodiestres regulating human skin mast cells by using selective and non-selective phosphodiestres inhibitors
Cells were pre-treated for 15 min with these agents and then challenged with an optimal releasing concentration of anti ige (1:300) for a further 25 min for the release of histamine. the data show that all the selective phosphodiestres inhibitor compounds (10^{-5} m) were ineffective whereas the non-selective phosphodiestres inhibitor, theophylline (10^{-3} m), inhibited histamine release from skin mast cell (74±4% inhibition; $p<0.05$). None of the selective phosphodiestrases inhibitors had any effect on histamine release from lung mast cells whereas, in basophils, compounds with activity at phosphodiestrases 4 (rolipram, denbufylline, ro-2017, org 30029) were effective inhibitors of histamine release.
Data suggest that unlike most inflammatory cells, phosphodiestres-selective inhibitors are ineffective stabilizers of skin mast cells activity which is similar to lung mast cells.

P1.17.06
Interferon-α antagonizes IL-3-mediated immunoregulatory functions of human basophils

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Human basophils are major inflammatory cells in maintaining chronic allergic asthma. It has been published that interferon-α (IFN-α) improves clinical symptoms of asthma patients. In contrast, IL-3 exacerbates airway inflammation by inducing IL-4, IL-8 and IL-13 secretion from human basophils thus regulating their immunoregulatory functions. Furthermore, IL-3 exceptionally promotes survival of basophils. Here, we assessed cellular response of human basophils treated with IFN-α alone or in combination with IL-3.
Our data show that IFN-α enhances apoptosis in purified human blood basophils compared to spontaneous apoptosis of controls or IFN-γ treated cells. Furthermore, we demonstrate that both IFN-α and FasL enhance apoptosis in human basophils with similar efficiency in a rather additive than synergistic way. IFN-α inhibits IL-3-induced survival to a minor degree. Particularly however, it suppresses IL-3-induced de-novo production of IL-8 and IL-13 up to 80%. In contrast, the production of IL-4 is not affected. Analyses of signaling pathways reveal that IFN-α promotes prolonged phosphorylation of STAT1/STAT2. By using a pan-JAK inhibitor the phosphorylation of STAT1/STAT2 is inhibited and most importantly the pro-apoptotic effect of IFN-α is abolished. Although the phosphorylation of p38-MAPK in IFN-α-treated cells is comparable to non-treated cells, inhibition of p-p38 activity abrogates IFN-α-enhanced apoptosis as well.
We conclude that IFN-α-enhanced apoptosis is tightly regulated by the cooperation of JAK/STAT and p38-MAPK pathways. Our study identifies IFN-α as a novel inhibitor of IL-3-induced IL-8 and IL-13 production of human basophils. Taken together our study may explain the improved clinical symptoms of asthma patients treated with IFN-α.

P1.17.07
Serum IgE and Eosinophil level in patients Infected with giardiasis

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Background: Several studies have described the existence of allergic symptoms such as eosinophilia and elevated levels of IgE in patients with giardia infection. Due to discrepancy of the results of these studies, lack of recent studies showing the relationship between the Giaridsis and allergy among higher age groups, it seems essential to design and perform the currents study.
Materials and methods: Amongst patients who were infected with giardiasis and their infection was definitely diagnosed using stool exam 30 individuals were randomly selected as the case and 30 healthy individuals were also selected as the control group. Total IgE was measured using ELISA while CBC was count using Sysmax cell counter. Data were analysed using Logestic regression and Mann-whitney U test.
Results: The mean CBC among the case and control groups was 6.69 × 103 and 7.45 × 10 3 respectively. The mean Hb was 13,37 and 13.74 among the case and the control groups respectively. The difference of mean absolute neutrophil count (ANC) between the case and the control groups was statistically significant (P<0. 01). The mean IgE of all patients in all ages was 134.±12 for case and 50.57± 66 for the control group. The odd ratio for IgE of case and control group was 2.7.
Conclusion: The strong relationship of IgE and giardiasis showed a prediction value for this antibody amongst the giardiasis patients so that with increasing the probability of giardiasis the level of IgE can be increased few times.

P1.17.08
Anti-TIM-4 mAb ameliorates allergic lung inflammation by inhibiting TIM-4-mediated mast cell stimulation

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T-cell immunoglobulin and mucin domain (TIM)-4 plays an essential role as the phosphatidylserine receptor in maintaining the homoeostatic function of macrophages, but the function of TIM-4 in allergic responses is not fully understood. In this study, we investigated whether TIM-4 regulates mast cell activation and allergic response. TIM-4 extracellular domain/Ig Fc region fusion protein (TIM-4-Ig) could bind to bone marrow-derived mast cells (BMMCs) and induced pro-inflammatory cytokine production, but not degranulation, by naïve and IgE/Ag-stimulated BMMCs. It was confirmed that these effects of TIM-4-Ig was induced by the interaction with leukocyte mono-Ig-like receptor 5 (LIMR5) by the findings using LMIR5 siRNA-transfected BMMCs or LMIR5$^{-/-}$ BMMCs. These results indicated that LMIR5 works as functional receptors on mast cells for TIM-4. Thus, we further investigated the effects of anti-TIM-4 mAb on a murine model of mast cell-dependent airway inflammation. Accumulation of eosinophils and production of Th2 cytokines in the lung were significantly reduced in the anti-TIM-4-treated mice. Importantly, TIM-4-trasfected cells and macrophages released a soluble form of TIM-4 (sTIM-4) through proteolytic cleavage of surface TIM-4. Moreover, high amount of sTIM-4 was detected in bronchoalveolar lavage fluids and sera in the allergic lung inflammatory mice. Given that sTIM-4 could induce pro-inflammatory cytokine production by BMMCs, it is possible that TIM-4 can be an appropriate target for the therapeutic treatment and/or the diagnostic marker of allergic diseases.

P1.17.09
Role of IL-6 in the development of dendritic cell-induced allergic inflammation

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IL-6, a pleiotropic inflammatory cytokine, secreted by dendritic cells (DCs) is recognized as an important factor for the development of T_H2 and T_H17 cells. However, the role of IL-6 in allergic asthma is still undefined. In this study, to understand the effect of IL-6 on *Dermatophagoides pteronyssinus* (Der p)-induced airway inflammation, mice were sensitized through the peritoneum and intratracheally challenged or instilled through the airway using Der p. Significant decreases of airway hyperreactivity (AHR), recruited eosinophils and neutrophils in the inflamed airways, and cytokine (IL-4, IL-5, IL-13, and IL-17) production of IL-6$^{-/-}$ mice were found in both models of allergic asthma. Moreover, the suppressive function of $CD4^+CD25^+$ regulatory T cells from lungs and Foxp3 expression from lung draining lymph nodes and spleens were all down-regulated in Der p-sensitized and -challenged wild-type mice, but not affected in IL-6$^{-/-}$ mice. Further exploration on bone marrow-derived DCs (BMDCs) from IL-6$^{-/-}$ mice revealed reduced expression of DC maturation markers, MHC class II and CD86, after Der p stimulation. Studies on the adoptive transfer of Der p-primed BMDCs showed a functional defect of BMDCs from IL-6$^{-/-}$ mice due to their inability to induce T_H2 and T_H17 responses and allergic airway inflammation in recipient mice. Hence, IL-6 plays important roles in regulating DC functions and subsequent T_H2/T_H17-mediated airway inflammation and AHR, and is thus a potential target for the treatment of allergic asthma.

P1.17.10
Role of the CC-chemokine receptor CCR9 as a modulator of the inflammatory process during allergic airway inflammation

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BACKGROUND. Recruitment of inflammatory cells to airway epithelium is a pathological feature of allergic airway disease. It remains unclear if the chemokine-receptor CCR9 has a function as a modulator of the inflammatory process. AIM. To analyze wheather exists a potencial regulatory role of CCR9 in the inflammatory process in a murine model of allergic airway disease. METHODS. Mice were sensitized by i.p. injections of 10 µg of OVA and 10 µL of AL(OH)3 (Alum) diluted in 100 µL of PBS for each one, on days 1 and 8. Then, aerosolized OVA was administrated as local challenge (1% in PBS) for 30 min on days 15 to 20 and 34 (5% in PBS). RESULTS. There is a reduction in the eosinophils recruitment as it was observed in a differential cell count. Furthermore, we observed a significant reduction in the eosinophilic population-recruitment of CCR9 -/- mice compared to WT mice as we determined by flow cytometry. Histological and morphometric analysis demostrated a reduction in celluar recruitment in the absence of CCR9. The recruitment of different cell types was analyzed by FACS using anti-CD4 and anti-CD8 as expressing T- lymphocytes, CD19 expression was not modified in CCR9 -/- mice.These results suggest that CCR9 could be involved in the modulation of asthmatic response in airways. Interestingly, mucus production and IgE levels in CCR9-deficient mice were not affected. CONCLUSIONS. These results suggest a potential role of CCR9 as a regulator of eosinophils recruitment into inflammed airways. Further investigation is in process to corroborate these findings.

P1.17.11
Multiplex immunoassay for allergens using oligonucleotide tethering

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Allergy to cow's milk affects about 2.5% of all children under age 3. While most infants overcome this allergy, milk intolerance is prevalent in most countries. Milk allergy is often associated with other food allergy, as well as, respiratory allergy.
Microplate-based multiplexed immunoassays are useful for the simultaneous measure of multiple analytes from a single sample in a single well. This allows for the creation of disease-specific panels potentially leading to a more accurate prognosis and/or diagnosis of the disease state.
The A-squared (A^2®) MicroArray System (QuantiScientifics, Irvine, CA, USA) is a microplate-based platform for quantitative multiplexed immunoassays. Arrays of oligonucleotides are covalently attached to the bottom of the plate well. Complementary strands are in turn linked to antibodies or antigens. The prepared oligo-antibody (-antigen) conjugates are pooled and applied to the wells. Through the process of hybridization self-assembly of the hybrids leads to the creation of a diagnostic multiplex panel in each well of the plate.
In this study, several milk proteins recognized as allergens were assembled into a multiplex panel using oligonucleotide tethering. Here, purified milk proteins whose epitopes are known to be associated with the allergy, as well as, crude milk allergen extracts are conjugated to oligonucleotides to create the panel. The panel included F2 crude extract, beta-lactoglobulin, alpha-lactalbumin, IgG, bovine serum albumin, lactoferrin, and 3 caseins. 13 patient serums were screened for the presence of specific IgE providing a profile and subsequent identification of principal component allergen.

P1.17.12
Microfibrillar-associated protein 4 deficiency attenuates experimental allergic airway inflammation

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Microfibrillar-associated protein 4 (MFAP4) is a matricellular protein abundant in lungs and other elastin-rich tissues. It contains a fibrinogen-related domain, characteristic of many proteins engaged in innate immune responses, and an integrin-binding domain. MFAP4 is known to interact with surfactant proteins SP-A and SP-D, collectins critical for pulmonary host defence. No studies so far have addressed the possible immunomodulatory effects of MFAP4.
The aim of the current study is to investigate a potential pro-inflammatory role of MFAP4 in the murine acute model of allergic asthma.
BALB/c MFAP4 KO and WT mice were sensitized and subsequently challenged intranasally with ovalbumin (OVA) during three consecutive days. Immune cell infiltration and cytokine production were measured in bronchoalveolar lavage (BAL). Lungs were processed for histology and evaluated for signs of inflammation and airway remodeling.
OVA-treated MFAP4 KO mice exhibited significantly lowered cell counts in the BAL, corresponding to diminished numbers of eosinophils and neutrophils. Alveolar macrophages, although similar in number, were less activated in KO animals than in WT littermates. There was also a tendency towards reduced production of Th2-related cytokines IL-4, IL-5 and IL-13 in the BAL of MFAP4-deficient mice. Moreover, histological stainings revealed significant differences in lung morphology: more prominent parenchymal inflammation and more pronounced early airway remodeling events in WT mice, including increased epithelial thickness and goblet cell hyperplasia.
Obtained results show that absence of MFAP4 attenuates OVA-induced allergic airway disease. It indicates that MFAP4 may serve as a potential therapeutic target in the treatment for acute allergic asthma.

P1.17.13
S1P/SphK signalling in mast cells: relevance to airway inflammation, asthma and anaphylaxis

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Airway inflammation is primarily characterized by increased numbers of eosinophils, macrophages, and T lymphocytes in the airway mucosa and lumen recruited through the release of chemokines from resident structural and inflammatory cells like mast cells. The airway inflammation is driven by increased mast cell activation and Th2 cytokines/chemokines. Developing new therapies for chronic airway inflammation has been shown to be challenging. The sphingosine kinases (SphK1 & SphK2) may be the potential targets for allergic anaphylaxis, refractory asthma and chronic airway inflammation. Two isoenzymes SphK1 and SphK2 generate bioactive sphingosine-1-phosphate (S1P). Using Ingenuity Pathway Analysis (IPA) software, we have constructed the biological networks initiated through FcεRI receptor in mast cells and the intracellular molecular activation networks regulated by SphK during the activation process in allergic responses. In our preliminary in vivo studies, we have specifically silenced SPHK1 or SPHK2 in vivo, using specific siRNAs, and demonstrate that SphK1, but not SphK2, is indeed required for the effector responses triggered by Ag/FcεRI in mice models. We have used both Passive Cutaneous Anaphylaxis (PCA) and Passive Systemic Anaphylaxis (PSA) models to study the isoform specific in vivo role of SphKs which has not been well-characterized. Hence, dissecting the S1P/SphK signalling in mast cells could provide novel cues for the effective treatment and management of an extensive range of allergic disorders and asthma, which remain unmet clinical need in the world.

P1.17.14
Identification of a novel quantitative trait locus and candidate genes for airway hyperresponsiveness

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The objectives of our study are to identify novel candidate genomic regions and genes associated with airway hyperresponsiveness (AHR), a heritable polygenic phenotype seen in allergic asthmatics where their airways are susceptible to bronchospasms.
To identify candidate genes for AHR, we used a unique genetic panel of 33 recombinant congenic strains (RCS) of mice originating from airway hyperreresponsive and hyporesponsive strains, A/J and C57BL/6J respectively. An informative RCS that has 85% of its genetic material originating from C57BL/6J, but has an AHR phenotype similar to A/J was used to generate F2 mice. Quantitative trait loci (QTL) analysis was done using the F2 mice and genes within the QTL containing non-synonymous or splice site coding mutations were selected as candidates. Candidate regions and genes were validated at the chromosome and gene expression levels.
One QTL containing 15 candidate genes was identified. Chromosome substitution strain mice, for the chromosome containing the candidate QTL, had an AHR phenotype similar to A/J strain and significantly different from C57BL/6J strain. Interestingly, this chromosome was not associated with other phenotypes of allergic asthma, such as atopy. Gene expression analysis revealed one candidate gene as having a two-fold increase in expression in the lungs of C57BL/6J mice compared to A/J, the informative RCS and the chromosome substitution strain.
Our approach provides a manageable list of candidate genes for AHR and can be applied to identify candidate genes for other phenotypes of asthma. Furthermore, it allows us to genetically segregate the phenotypes of asthma and study them individually.

P1.17.15

Circulating human basophils lack the features of professional antigen presenting cells

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Recent reports in mice demonstrate that basophils function as antigen presenting cells (APC). They express MHC class II and co-stimulatory molecules CD80 and CD86, capture and present soluble antigens or IgE-antigen complexes and polarize Th2 responses. Therefore, we explored whether circulating human basophils possess the features of professional APC. We found that unlike dendritic cells (DC) and monocytes, steady-state circulating human basophils did not express HLA-DR and co-stimulatory molecules CD80 and CD86. Interestingly, basophils remained negative for these molecules following stimulation with soluble Asp f 1, one of the allergens of Aspergillus fumigatus, Bet v 1, the major birch allergen, stimulation via TLR2-ligand or even upon IgE cross-linking. Further, DC pulsed with Asp f 1 promoted the Th2 response as analyzed by the secretion of IL-4 in the DC-CD4+ T cell co-culture. Whereas, Asp f 1-pulsed basophils did not promote Th2 responses from CD4+ T cells. Together, these results demonstrate that although basophils from mice show the expression of antigen presenting and co-stimulatory molecules, human basophils from circulation show the inability to function as professional APC.

P1.17.16
Intratracheally administration Asian sand dust activates NF-kappaB in immune cells

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Asian sand dust (ASD) is derived from air pollutants in East China that migrate to Japan. ASD contains particles of SiO_2 with microbial materials, sulfate, and nitrate. As increasing flying ASD, there are concerns regarding its possible adverse health effects. Although our group has reported that ASD induces inflammatory responses in lung tissue and causes respiratory diseases in mice. It is still unclear whether ASD influence lymphoid organ. In this study, we investigated the effect on splenocytes in an ASD-administered mice model. ICR mice were intratracheally administered ASD and were sacrificed at days 1 and day 3. After administration cell number of bronchoalveoar fluids (BALF) was significantly increased after ASD administration at days 1 and 3. Spleen cells were prepared and cell lysates of splenocytes were resolved by western blotting. Immunoblotting demonstrated that phosphorylation of p65 was induced at days 3 after ASD administration but not at day 1. Furthermore, electrophoretic mobility shift assay demonstrated that ASD induced active form of NF-κB in splenocytes after ASD administration at days 3 but not day 1. In addition, ASD could increase the expression of TNF-α production after 3 days administration. Finally, ATP level in splenocytes was measured, and high level of ATP was observed after ASD administration for 3 days. These results suggest that at different time point of lung response, ASD affects peripheral lymphoid tissue, and may modulate cell survival or activity.

P1.17.17
Receptor for advanced glycation end products (RAGE) - a central mediator of allergic sensitization

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Allergic sensitization to house dust mite (HDM) is a significant risk factor associated with asthma onset and progression. Activation of innate pattern-recognition receptors (PRRs) can induce the release of endogenous tissue alarmins that modulate the magnitude of the inflammatory response. RAGE is a PRR with the unique capacity to recognise a diverse repertoire of endogenous alarmins, and can collaborate with toll-like receptor (TLR)-4 (known to 'sense' HDM), yet its role in the development of allergic sensitization to HDM and other allergens remains obscure. Here, we report that HDM-induced airway inflammation was diminished in RAGE-deficient mice. Strikingly, the early induction of HDM-induced IL-33 and IL-25 was TLR4-dependent; whereas a second 'wave' (persistent expression) of these T_H2-instructive cytokines was regulated downstream of RAGE activation. HDM sensitization also induced nuclear to cytoplasmic translocation of endogenous alarmin, high mobility group box-1 (HMGB1), and elevated levels in bronchoalveolar lavage fluid. Like innate cytokines, the early HMGB1 activation was TLR4-dependent and later on sustained via RAGE signalling. Moreover the magnitude of the T_H2 immune response was abolished when HMGB1 was neutralized, implicating the functional role of HMGB1. With evidence that RAGE is engaged secondary to TLR4, we hypothesised that it might potentially function as a common pathway down-stream of various PRRs involved in initial allergen recognition/sensing. Of note, we demonstrated that RAGE-deficient mice were also protected against cockroach-extract induced allergic inflammation and had attenuated levels of HMGB1. Together, these studies address HMGB1-RAGE signalling as a critical innate mechanism of allergic sensitization and development of airway inflammation.

P2.01 Leukocyte signalling

P2.01.01
Hydrophobic amino acid cluster in the cytoplasmic tail of interleukin-2 receptor β chain acts as a sorting signal for lysosome

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Lysosomal degradation of cell surface receptors contributes to regulate the expression levels of the receptors, and attenuation of signal transduction from the receptors. It is well known that ubiquitin modification of the cell surface receptors serves as a sorting signal for endocytic trafficking to lysosome. Hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) is a key molecule in this process that captures ubiquitinated receptors and sorts them to the lysosomal pathway. On the other hand, we previously demonstrated that lysosomal trafficking of interleukin-2 receptor β chain (IL-2Rβ) does not require the ubiquitin modification, although undergoing Hrs-interaction process. We also reported that hydrophobic amino acid cluster (HC) in the cytoplasmic tail of IL-2Rβ (365-FFFHL-369) is essential for its binding to Hrs and trafficking to lysosome. HC is necessary for lysosomal sorting of IL-2Rβ, but it has been unknown whether the motif is sufficient to act as a sorting signal for lysosome. To elucidate this, we constructed chimeras comprised of IL-2R α chain (IL-2Rα) fused with various fragments including HC. Because IL-2Rα has no sorting signal, after internalized, it is rapidly recycle back to plasma membrane. The IL-2Rα chimeras were sorted to lysosome, while chimeras including only HC or fragments lacking HC were not sorted. These results indicated that fragments (more than 30 residues) including HC are functional sorting signal for lysosome via interaction with Hrs.

P2.01.02

Apurinic/apyrimidinic endonuclease1/redox factor-1 (Ape1/Ref-1) is essential for IL-21-induced signal transduction through ERK1/2 pathway

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IL-21 is a pleiotropic cytokine that regulates T-cell and B-cell differentiation, NK-cell activation, and dendritic cell functions. IL-21 activates the JAK-STAT, ERK, and PI3K pathways. We report here that Ape1/Ref-1, which has many functions through the regulation of redox, through endonuclease activity and also through unknown mechanisms, has an essential role in IL-21-induced cell growth signal transduction. Overexpression of Ape1/Ref-1 enhances IL-21-induced cell proliferation, but it is suppressed by overexpressing an N-terminal deletion mutant of Ape1/Ref-1 that lacks the redox domain. Furthermore, knockdown of the Ape1/Ref-1 mRNA dramatically compromises IL-21-induced ERK1/2 activation and cell proliferation with increasing cell death. Activity of JAK1 and JAK3, which are the key kinases for IL-21-induced signaling, seems not to be reduced in the Ape1/Ref-1 knockdown cells. These impaired activities are recovered by the re-expression of Ape1/Ref-1 in the knockdown cells. Our findings are the first demonstration that Ape1/Ref-1 is an indispensable molecule for the IL-21-mediated signal transduction through ERK1/2 activation. We are now studying how Ape1/Ref-1 controls the ERK activation.

P2.01.03

Notch receptors and ligands expression in human T cell development

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The thymus is a primary lymphoid organ responsible of maturing T lymphocytes. Several signaling pathways act during T lymphocytes maturation including Notch signaling pathway, comprising in receptors (Notch1-4) and ligands (DLL1-3-4, Jagged1-2). We sought to verify the expression of Notch components in human developing lymphocytes. Thymic tissue fragments were stained by immunohistochemistry and Notch receptors/ligands were quantified. Total thymocytes were sorted into CD4-8-, CD4+8+, CD4+8- and CD4-8+. RNA expression was evaluated on each population. In thymic tissue we detected all Notch components (mean of positive cells). Notch receptors and ligands proteic expression showed no significant difference.The CD4+8+ subset presented higher percentage among all sorted populations (51,82%). RNA expression showed that all Notch receptors and ligands genes were detected in lymphocytes subsets, except DLL3 gene. NOTCH1 gene was more expressed at earlier stages of development (CD4-8-), and JAG2 gene was more expressed on CD4+8+ subset. The other genes showed no difference among lymphocytes subsets. NOTCH1 gene may have a key role in the first stages of T lymphocytes development due to its higher expression in early stages, different of NOTCH2 gene which showed significant expression at later stages of thymic subsets. JAGGED2 gene may represent a consequence of TCD4+ maturation owing to its prior expression at CD4+8+ subset. Different from genes analysis, the homogeneous distribution of Notch components in thymic tissue suggests that a new approach should be considered for Notch pathway during human T lymphocytes development and maturation.

P2.01.05

Site-wide quantitative phosphoproteomics reveal non-redundant pathways regulated by IL-7 and IL-15 in memory CD8+ T cells

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Self-renewing memory CD8+ T-cells require IL-7 and IL-15, which preferentially promote survival versus cell division/growth. Although both signal through a common gamma-chain and JAK/STAT5, their non-redundant networks are unknown. Here, we compared highly purified primary memory-phenotype (CD44high) lymphocytes after IL-7 or IL-15 equipotent stimulation *ex vivo*. IL-15 was shown to greatly increase, besides proliferation and size, blast formation and migration/contact. To identify specific signaling components, the differential downstream phosphorylation events were measured. Since conventional mass spectrometry (MS) demands samples in the range of mg, we faced the challenge of robust and large-scale quantification of post-translational modifications in primary cells. This was overcome with our automated online liquid chromatography-MS/MS platform combining nanoflow electrospray ionization with a novel 3D fractionation. By enabling examination of minute protein amounts (~50 µg/condition), we efficiently (>8000 unique phosphopeptides) completed a quantitative high-throughput site-wide (Ser-/Thr-/Tyr) phosphoproteome profiling. Notably, despite the extreme biological variability that hinders these analyses, we succeeded in detecting selected peptides (mapped to 24 proteins) that in cells from independent animal pools were consistently differentially phosphorylated (by >2-fold). This led to reveal IL-15-induced signatures: 1) AKT over-activation (as confirmed by phosphorylated AKT and substrate levels after IL-15-stimulation and selective AKT-inhibition); and 2) phosphorylation of potential effectors with unexplored roles (i.e. membrane sculpting, calcium signaling and mRNA splicing) in self-renewal. The relationship between IL-15-induced phenotypes, AKT activation and candidate effectors is being determined. In conclusion, we provide a powerful approach for global phosphoproteomics of immune cell subsets and contribute to define the non-redundant effects of IL-7/IL-15.

P2.01.06

Quantifying differential T cell responses using tuneable material surfaces

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Upon the interaction between the T-cell receptor (TCR) and its cognate ligand, activated T-cells elicit powerful anti-tumour responses and can destroy pathogen-infected host cells. It is well established that the binding affinity and the density of the stimulating TCR ligand has directly proportional consequences on the overall T-cell response. Understanding the combination of these parameters that result in optimal T-cell activation has significant applications in the field of immune monitoring of vaccines. Therefore, the purpose of this study is to develop a functionalized platform that allows controlled deposition of TCR ligand parameters for the characterization of the resulting T-cell response.
Functionalized platforms were created by covalently attaching streptavidin on solid material surfaces for immobilizing biotinylated ligands. The concentration of streptavidin on the surfaces was altered to control deposition of ligands, which included anti-CD3 f(Ab) fragments, and OVA-specific peptide-MHC monomers. When primary T-cells were then activated on the material surfaces of altered ligand density, differential expression of activation markers and early calcium flux was observed. To interrogate the early T-cell response, calcium oscillations within single cells were assessed and found to differ based on ligand density, suggesting that intracellular calcium flux may be used to differentiate T-cell outcomes. To explore this, we are developing an assay to measure and correlate early calcium flux

with late stage activation markers within individual cells. Therefore, the ability of functionalized material surfaces to 'tune' TCR ligand density can be employed in combination with altered ligand affinity for fully quantifying differential T-cell responses for clinical application.

P2.01.07
Levodopa-induced neutropenia in Parkinson's disease

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We report the case of a male PD patient, who came to our attention for progressive resting tremor, hypokinesia and rigidity. The patient was thus started on Levodopa-Carbidopa. At the beginning of therapy, the patient showed reduction of hypokinesia and rigidity but also a significant reduction in leukocytes count (2,470 x mm3), particularly neutrophils (0,710 x mm3), confirmed by a second control. After these findings, Levodopa-Carbidopa therapy was stopped with normalization of blood studies. Then the patient began treatment with Levodopa-Benserazide to control motor symptoms. For the second time the blood tests underscored an important neutropenia (2,40 x mm3), that forced the suspension of therapy followed by normalization of leukocyte count. To date, the patient is taking dopamine agonists with benefit and no evidence of adverse effects. By means of rt-PCR we showed that, in comparison to circulating neutrophils from healthy subjects, cells from our patient underexprressed mRNA for D2-like dopamine receptors (DR) and overexpressed mRNA for D1-like (D5) DR as well as for tyrosine hydroxylase, the first and rate-limiting enzyme in the synthesis of dopamine.
Our case shows an adverse reaction to LD that to our knowledge has not been previously reported. Normalization after LD dechallenge as well as relapse after rechallenge with LD in association with a different inhibitor of L-Dopa decarboxylase supports a strong causal relationship between LD and leukopenia. Additional studies are warranted to assess whether DR and/or TH mRNA expression in circulating neutrophils may be a marker of vulnerability to LD-induced leukopenia.

P2.01.08
Correlation between low tyrosine phosphatase activity of inhibitory SHP-1 and high proliferation in chronic lymphocytic leukemia (CLL) B-cells

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The clinical course of B-cell chronic lymphocytic leukemia (CLL) is very heterogenous, with some patients presenting an indolent disease and others having a worse outcome. Biological active inhibitory molecules, such as Src homology domain-containing protein tyrosine phosphatase-1 (SHP-1), can decrease cell viability or proliferation in CLL cells. In this study, expression levels of IgM, SHP-1, ZAP70 were investigated together with SHP-1 phosphatase activity of different CLL cell lines. The intracellular phosphatase activity was assessed to evaluate a potential role of SHP-1 on cell proliferation. We show that CLL cells with high expression levels of SHP-1 also displayed high SHP-1 phosphatase activity, lower levels of ZAP70, and lower cell proliferation. On the other hand, in cells with low SHP-1 expression and phosphatase activity, increased proliferative activity was determined. To our knowledge, this is the first time that the SHP-1 phosphatase activity has been shown to correlate with proliferative activity in CLL.

P2.01.09
Functional regions in the Ankyrin Repeat Domain 54/ Liar protein which regulate nucleocytoplasmic shuttling of Bruton's Tyrosine Kinase (Btk) through a novel SH3-dependent interaction

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Bruton's tyrosine kinase (Btk) is a cytoplasmic protein kinase that belongs to the Tec family of tyrosine kinases (TFKs). It is essential for B-lymphocyte development and is involved in multiple signal transduction pathways. Abrogation of Btk signaling causes human X-linked agammaglobulinemia (XLA) and murine X-linked immunodeficiency (Xid).
We have recently employed affinity-purification of Flag-tagged Btk, combined with tandem mass-spectrometry analysis to capture and identify novel interacting proteins. The results were validated by using endogenous as well as ectopic expression of both proteins in a co-immunoprecipitation assay. We further characterized the interaction with Ankryin repeat domain protein 54, also known as Lyn-interacting ankyrin repeat protein, Liar (Gustafsson et al. Mol Cell Biol. 32:13; 2440-2453).
Liar mediated Btk shuttling was enriched for activation loop, nonphosphorylated Btk and entirely dependent on Btk's SH3 domain. Liar also showed reduced binding to an aspartic acid phosphomimetic SH3 mutant. We have now generated site-directed mutants where different regions in Liar have been removed or altered. This allows us to assign functional entities within the protein, which govern intracellular transport of Btk.
We identified a novel interaction between Liar and the Btk SH3 domain and it was not dependent on classical polyproline-rich (PPR) PXXP motifs, since the Liar ankyrin repeats does not contain such motifs. Moreover, mutagenetic analysis revealed that three of four ankyrin repeat domains of Liar are required for the interaction and nuclear shuttling of Btk.

P2.01.10
Role of the long-chain fatty acids receptor GPR40 in bovine neutrophils activation

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Long chain fatty acids (LCFA) bind to G-protein coupled receptors such as GPR40, a receptor with known functions in pancreas. Recently, we described the presence of GPR40 in bovine neutrophils and demonstrated that oleic and linoleic acids, two LCFA, stimulate responses such as superoxide production, granules release and MAPK phosphorylation. In this study, we investigated the function of GPR40 on bovine neutrophils activation. Neutrophils isolated from blood samples of healthy heifers were treated with GW9508, a synthetic GPR40 agonist, or linoleic and oleic acid, natural ligands of GPR40, in presence or absence of the selective GPR40 antagonist GW1100. Protein phosphorylation was analyzed by immunoblot, NF-κB pathway was assessed as IκBα levels by flow cytometry, IL8 mRNA by RT-qPCR, matrix metalloproteinase-9 (MMP9) activity by zymography and superoxide production by flow cytometry. We observed that GW1100 (10 μM) reduced the ERK1/2, p38 MAPK and Akt phopshorylation induced by GW9508 (10 μM), linoleic (100 μM) or oleic acid (300 μM). GW9508 and fatty acids significantly decreased IκBα levels, at 30 min of treatment, suggesting activation of the NFκB pathway. The reduction of IκBα levels induced by ligands of GPR40 was inhibited by GW1100. GW9508 and natural ligands increased the IL8 mRNA levels and GW1100 reduced this effect. Finally, GW1100 reduced the MMP9 activity and superoxide production induced by GPR40 ligands. We conclude that GPR40 activation modulates the function oxidative and non-oxidative of bovine neutrophils, exerting a potential role in innate immune response.
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P2.01.11

Role of SLP-76, ADAP and Pyk2 on chemokine-stimulated T cell adhesion mediated by the integrin *Alpha4-Beta1*

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Lymphocyte extravasation at sites of tissue injury or infection depends on chemokine-activated adhesion mediated by the α4β1 and αLβ2 integrins. This activation must be rapid to deliver tight cell attachment to resist the blood shear stress. The chemokine-dependent integrin activation step in lymphocytes requires an inside-out signalling that impinges on the integrin β subunit cytoplasmic domains, a key event for transmitting conformational changes to the extracellular regions that leads to increase in integrin affinity. Talin is a recipient of this inside-out signalling that promote their binding to the β cytoplasmic domains, finally stimulating the transition to high-affinity α4β1 and αLβ2 conformations. A key inside-out molecule that regulates the activation of α4β1is the guanine-nucleotide exchange factor Vav1, which associates with talin forming a key complex that controls chemokine-dependent, α4β1-mediated T cell adhesion. SLP-76 and Pyk2 are Vav1-binding proteins with the potential of influencing α4β1-mediated T cell adhesion in response to chemokine stimulation. We are investigating the role of SLP-76, its binding partner ADAP, and Pyk2, as possible regulators of this adhesion by inside-out mechanisms arising from chemokine stimulation.

P2.01.12
Regulation of apoptosis by CD45 in T cells

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CD45 is a transmembrane tyrosine phosphatase expressed in all leukocytes. We have previously showed that CD45- cells (HPB45.0 and JK6.6) were resistant to nuclear apoptosis, as observed by a lack of chromatin condensation and DNA fragmentation. The latter is majorly under the responsibility of the DNA fragmentation factor (DFF), an heteromeric complex of the endonuclease DFF40 and its inhibitor DFF45. Nuclear apoptosis is an important process that reduces the risk of mutated genes transfers to neighbouring cells. The aim of this study was to identify if the CD45-dependant signalisation pathways can be responsible for the lack of DNA fragmentation. HPB-ALL (CD45+) T cells and HPB45.0 (CD45-) T cells were exposed to the mitochondrial perturbing agent, tributyltin (TBT), to induce apoptosis and selected biochemical pathways were analysed by immunoblot and antibody array. The absence of CD45 expression increased the activity of downstream protein kinases such as p38, JNK, Akt and PKC-delta after apoptosis induction. However, specific protein kinases inhibitors did not restore nuclear apoptosis in CD45- cells, suggesting an intramolecular defect in the regulation of apoptosis with the lack of CD45 expression. Interestingly, the phosphorylation, 0-glycosylation and acetylation level of DFF is altered in CD45- cells. Understanding the control of DNA fragmentation is important considering that the expression of CD45 is lost in nearly 10% of patients with acute lymphoblastic lymphomas and was associated with poor prognostic.

P2.01.13
The SNARE protein VAMP7 controls T cell activation by regulating recruitment and phosphorylation of Lat subsynaptic vesicles to the TCR activation sites

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The mechanisms by which the linker for activation of T cells (Lat), a key adaptor in the T cell receptor (TCR) signaling pathway, and the TCR come together after TCR triggering are not well understood. Nevertheless, it is now known that Lat is contained in intracellular compartments that are recruited close to the TCR activation sites. We reasoned that fusion/docking of these Lat containing compartments might be important for T cell activation.
We show here, by using silencing approaches and genetically modified mice, that the vesicular soluble N-ethylmaleimide-sensitive factor attachment protein receptor (v-SNARE) VAMP7 is required for the recruitment to the TCR activation sites of Lat-containing sub-synaptic vesicles, which do not fuse with the plasma membrane. VAMP7, which colocalized with Lat on the vesicles, controls Lat phosphorylation, TCR-Lat-signaling complex formation and ultimately T cell activation.
Our findings suggest that transport, docking and perhaps fusion of Lat-containing vesicles with target membranes containing the TCR are required to regulate TCR-induced signaling.

P2.01.14
Dual phosphorylation of Btk induced by Akt/PKB kinase regulating shuttling and attenuating signaling in B cells

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Bruton's tyrosine kinase (Btk) is a key regulator of B-cell activation and development. Mutations in the Btk-gene cause X-linked agammaglobulinemia (XLA) in humans and X-linked immunodeficiency (Xid) in mice. In the present study, we employed mass spectrometry to identify a new partner protein as a novel regulator of Btk in B-cell lines and primary B-lymphocytes. In addition, we herein demonstrate that phosphorylation of two bona fide Akt (PKB) target sites is responsible for mediating functional interaction of BTK with a new partner.
The interaction-sites on Btk were mapped to phospho-serine/threonine in the kinase-domain. Mutation of both residues to alanine abolished binding of Btk to the new identified partner, while phosphomimetic mutant conversely demonstrated enhanced interaction. The PI3-kinase inhibitor LY294002 abrogated Btk serine/threonine phosphorylation and disrupted Btk's interaction with that particular partner.
Moreover, a newly characterized Btk inhibitor, PCI-32765, interrupted Btk binding. Down-regulation of the novel partner of Btk elevated nuclear translocation of Btk, while over-expression of that particular partner resulted in the perinuclear accumulation of Btk. Finally, phosphorylation of these sites is a prerequisite for the tyrosine phosphorylation, ubiquitination and degradation of Btk by the 26S proteasome and has implications in the regulation of the B cell receptor signaling pathway.

P2.01.15
Role of SRC family kinases in the activation of ITK-SYK

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The chromosomal translocation t(5;9)(q33;q22) generating the interleukin-2 (IL-2)-inducible T cell kinase (ITK)-spleen tyrosine kinase (SYK) fusion tyrosine kinase was identified as a recurrent event in PTCL. SRC family kinases (SFKs) initiate intracellular signaling in response to ligation of many antigen receptors. This leads to recruitment of SYK, by binding of the SYK SH2 protein domain to the phospho-tyrosines (pY) on ITAM residues, and activation of SYK allowing it to phosphorylate downstream substrate. To investigate the role of the SRC family kinases in ITK-SYK activation and signaling, we used a SFK-deficient cell line, SYF, and transfected it with ITK-SYK mutants. Other cell lines that contain SRC family kinases, like COS-7 cells, were used as a control. The results show that coexpression of ITK-SYK and ITK-SYK mutants with SLP-76 in SYF cells leads to phosphorylation of SLP-76, while there is no detectable phosphorylation of ITK-SYK. We then used COS-7 cells as control, and when we cotransfected ITK-SYK and SLP-76, there was conversely detectable phosphorylation of both SLP-76 and ITK-SYK. From these results we can conclude that the activation and phosphorylation of ITK-SYK is independent of SFKs.

P2.01.16

CD137 Costimulation may compromise cytotoxic mediators of Natural Killer Cells

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Background: In spite of T cells, costimulatory effects of CD137 in NK cells have not been fully understood. In this study, we investigated the consequence of CD137-CD137L interaction on activation and functionality of human NK cells.
Methods:
MCF-7 breast cancer cells were infected in advance by non-replicative adenovirus vectors to over-express 4-1BBL transgene or GFP (as control). Human NK cells were negatively purified from peripheral blood and activated with suboptimal concentrations of IL-2 and IL-15 and cocultured with the infected tumor cells. The cytotoxic function of the NK cells was examined by CD107a mobilization and granzyme-B staining. Moreover, killing of target cells was visualized on CFSE labeled K562 cells by flow cytometry.
Result: CD137 signal in IL-2/IL-15 activated NK cells resulted in decrease in the frequency of $CD107a^{+}GrB^{+}$ cells. More NK cells turned to $CD56^{-}$ $CD16^{-}$ after exposure to CD137L. There was an increase in the frequency of $CD107a^{+}GrB^{+}$ cells in the $CD56^{+}CD16^{+}$ subpopulation compared to other subsets. However, results of CFSE cytotoxicity assays of the whole population showed no significant difference between the experimental conditions.
Conclusion: CD137-CD137L interaction may affect the activity of various sub-populations of NK cells differently as it augmented the cytotoxcity of only CD56+CD16+ subpopulation but not others.

P2.01.17
NFkB alternative pathway is activated in IgE class switching

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We previously established B cell specific alpha4 KO mouse and showed that alpha4 was essential for B cell activation through BCR, IL4 receptor, LPS and CD40. Mature B cells undergo class switching to IgE by IL4 and CD40 signaling. It was later found that alpha4 associated with Protein phosphatase 2Ac and regulated its enzymatic activity. Alpha4 and PP2A regulate CaMKII activity in the central nervous system to control learning and memory. Alpha4 deficient mice also showed impaired class switching with T cell dependent Ag. We therefore analyzed whether alpha4 was involved in class switching to IgE. Real Time PCR of epsilon germ line transcript revealed that IL4 and CD40 induced IgE class switching in M12, a murine mature B cell line. IL4 stimulation of M12 induced serine and threonine phosphorylation of alpha4. Although canonical and alternative NFkB activation by CD40 signal was well characterized, IL4 receptor engagement was shown to activate Stat signaling and only canonical NFkB pathway. IL4-induced class switching to IgE and alpha4 phosphorylation was inhibited by KN93, a potent CaMKII inhibitor. These results suggest that alpha4/CaMKII is involved in IgE class switching via NFkB alternative pathway.

P2.01.18
Level of CD44 expression and its function distinguishes TH17 from TH1 cells

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CD44 is a prominent activation marker, which distinguishes memory and effector from naïve T cells. CD44 also has a role in early T cell signaling events: It is bound to lymphocyte-specific protein kinase (Lck) and can enhance TCR signaling (Taher et al. 1996). We found that the level of CD44 distinguishes TH17 from TH1 cells. We hypothesize, that CD44 serves as a co-stimulus for TH17 cells and facilitates its development.
MACS-purified CD4+ cells from C57Bl/6 mice were either stimulated with allogeneic bone-marrow-derived dendritic cells (BMDCs) from Balb/c mice or polyclonally with anti-CD3 and splenocytes. Four days later cells were analyzed by fluorescence cytometry.
Stimulation of CD4+ T cells with allogeneic BMDCs results in the formation of three distinguishable populations: CD44low, CD44intermediate and CD44high. In vitro and in vivo generated allo-reactive TH17 cells are mainly CD44high compared to TH1, which are CD44int. This effect is strengthened under polarizing conditions: TH17 polarization leads to a shift towards a CD44high-population whereas TH1 polarization diminishes the CD44high and increases the CD44int-population. Furthermore, blocking CD44 with neutralizing antibodies decreases IL17 secretion, while IFNγ is barely affected. Titration experiments revealed that low TCR and low CD28 stimulation supports TH17 rather than TH1 development. Under exactly these conditions CD44 can replace CD28 signaling and enhance TCR signaling (Föger et al. 2000). Indeed, rested CD44high cells contain already more phosphorylated Zap70 compared to their CD44int counterparts.
Our results support the notion that CD44 enhances TCR signaling strength by delivering LCK, which is required to support TH17 development.

P2.01.19
CD28 and NKG2D-mediated costimulation regulates CD8+ T cell chemotaxis through different signaling pathways

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Introduction: CD28 is the prototypical costimulatory receptor in T cells, which propagates its signals through the YMNM signaling motif. NKG2D is a transmembrane receptor mainly expressed on NK cells and CD8+ T cells. It associates with DAP10, which contains a YINM motif, similar to CD28. Here we study the potential role of NKG2D as a costimulatory molecule in CD8+ T cell chemotactic response.
Material and methods: Human CD8+ T cells were assayed for their ability to migrate towards a CXCL12 gradient upon stimulation with anti-CD3, anti-CD28 and anti-NKG2D alone or in combinations. The activity of the Rho GTPases Rac1 and Cdc42 was analyzed by G-LISA. N-WASp was knocked-down using siRNA. Pharmacological inhibition of N-WASp by wiskostatin was used to analyze proteins involved in TCR signaling and cytoskeleton dynamics by western blot.
Results: Costimulation via CD28 and NKG2D inhibited cell migration. Rac1 is activated upon T cell receptor activation and costimulation through CD28 and NKG2D, whereas Cdc42 is activated only upon CD3/NKG2D activation. Knock-down of N-WASp partially abolished CD3/NKG2D-mediated impairment of T-cell chemotaxis. Vav, ERK1/2 and ERM proteins are regulated by CD3-mediated signaling, and pretreatment with wiskostatin did not affect their phosphorylation status. Costimulation of the TCR together with CD28 or NKG2D induces cofilin dephosphorylation. Administration of wiskostatin further decreased upon CD3/NKG2D activation, but not after CD3/CD28 engagement.
Conclusions: Costimulation via CD28 and NKG2D inhibits CD8+ T cell migration through different signaling pathways. CD3/NKG2D stimulation activates the Cdc42/N-WASp axis, and N-WASp is required for CD3/NKG2D-mediated inhibition of CD8+ T cell chemotaxis.

P2.01.20
Inhibition of T cell receptor signaling by CD147

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In the defence strategy of the body against harming pathogens, the activation of T cells is a central but tightly regulated event. To make the grade, T cells not only upregulate costimulatory but also inhibitory receptors upon antigen recognition to modulate the response. A potential new inhibitory receptor is CD147, a member of the immunoglobulin supergene-family, which is upregulated upon T cell activation. Previous studies reported that lymphocytes from CD147 knockout mice possess enhanced mixed lymphocyte reactions and a CD147 monoclonal antibody can interfere with T cell activation. These observations already pointed to a negative crosstalk of CD147

signals with the T cell antigen receptor or co-stimulatory signals. Consistent with these studies, we found that RNA interference (RNAi) with CD147 in Jurkat T cells augments the secretion of the T cell growth-factor interleukin-2 (IL-2) upon T cell activation. With the help of reporter gene assays, we show that this upregulation is at least partially due to an increased activity of the nuclear factor of activated T cells (NFAT), which resulted in an enhanced IL-2 promoter activity. However, biochemical and cellular assays revealed that the increased transcriptional activity of NFAT in CD147 silenced cells was independent from calcium mobilization or the activity of MAP kinases. By reconstituting the RNAi-mediated knockdown with various truncated RNAi-resistant forms of CD147, we identified the immunomodulatory sub-domain of CD147.
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P2.01.21
IL4-induced IgE class switching is regulated by NFkB alternative pathway

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Alpha4 was identified as a signal transduction molecule which associated with Ig-alpha, a component of B cell antigen receptor (BCR) complex. Alpha4 was ubiquitously expressed and gene targeting experiment revealed that alpha4 was essential for cell survival. B cell specific gene targeting confirmed that alpha4 was not only essential for BCR signal transduction but also for CD40, IL4 and LPS signals. At the same time, we found that alpha4 associated with a protein phosphatase PP2A catalytic subunit and regulated its enzymatic activity. Neuron specific gene targeting of alpha4 revealed that alpha4/PP2Ac associated with CaMKII to regulate learning and memory. In this study, we show that IL4 receptor signaling activates alternative as well as canonical NFkB signal transduction pathway. IL4 induced the serine and threonine phosphorylation of alpha4. Both alternative NFkB pathway and threonine phosphorylation of alpha4 were inhibited by a potent CaMKII inhibitor KN93. These results suggest that alpha4/CaMKII molecules are involved in IL4 receptor-induced NFkB alternative pathway. These molecules also regulate IgE class switching induced by IL4 and CD40. Establishment of M12 B cell transfectant which inducibly express TRAF3 will be also discussed.

P2.01.22
During human T cell activation ERK binds to a panel of promoters and regulates the transcription of target genes

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Extracellular signal regulated kinase 1/2 (ERK1/2) is a serine threonine kinase, which is conserved across all mammals. It has been shown to regulate proliferation, division and differentiation in many different cell types. We recently reported that upon primary human T cell activation ERK is recruited to IL-4 promoter and activates its transcription leading to Th2 differentiation (Tripathi et al., Immunol Cell Biol. 90, 676-87, 2012). It was therefore of interest to investigate the global profile of its binding throughout all the promoters of known human open reading frames during the activation of primary human $CD4^+$ T cells. We found ERK to bind to a panel of gene promoters including those of the immune system. This binding appears to be phosphorylation dependent as it was sensitive to treatment with a MEK inhibitor U0126. We also identified the consensus binding motif for ERK. Further analysis of target genes identified olfactory receptor family as the most abundant gene family arguing for a dominant role of this protein in sensory development. Expression analysis of the target genes indicate a complex relationship between ERK binding and the expression of target genes, suggesting for a context dependent role of ERK.

P2.01.23
Paracrine ATP signalling between T lymphocytes

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Extracellular nucleotides such as adenosine-5'-triphosphate (ATP) serve as important signals in many biological processes and is released by many immune cells during an inflammatory response. The binding of T Cell Receptor to the ligand triggers a cascade of signals resulting the release of calcium from intracellular stores and the opening of Calcium Release Activated Channels to promote the subsequent activation processes. The increased concentration of cytosolic free calcium and mitochondria uptake lead to the synthesis and release of ATP by T lymphocytes. Many studies now show that autocrine ATP signalling via purinergic receptors is vital to sustain and promote T cell activation. We report that ATP can also signal in a paracrine manner between CD4+ lymphocytes and trigger calcium waves in murine ex vivo lymph node slices. Using a series of of pharmacological agents, we identified P2X4 and P2X7 as the key purinergic receptors for paracrine ATP signalling. ATP acts as a paracrine messenger to reduce the motility of lymphocytes and this may represent an additional mechanism contributing to the orchestration of cell trafficking in lymph nodes.

P2.01.24
Pleural B cells protect against pneumonia via a GM-CSF-IgM axis

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Pneumonia is a major cause of mortality worldwide and a serious problem in critical care medicine. The host mechanisms that promote microbial recognition and clearance are essential to effective protection and operate in a broad immuno-physiological system that encompasses lung anatomy and the immune system. Although many of these recognition and clearance mechanisms have been identified, their in vivo context is still poorly understood. Here we show that the pleural cavity, a space between the lung and chest wall that aids in respiration, is an immune site harboring a crucial anti-microbial function. Using different genetic, fate mapping and rescue approaches we show that in response to airway infection, pleural space B1a B cells relocate to the lungs and secrete protective immunoglobulin-M (IgM). The process requires innate response activator (IRA) B cells which license IgM production via direct granulocyte-macrophage colony stimulating factor (GM-CSF) signaling. B1a and IRA B cells, the study indicates, are strategically located in the pleural space to provide effective early frontline defense against microbes invading the lungs. The study identifies the pleural space as the primary source of IgM in the airways; describes an important, previously unrecognized, GM-CSF-IgM axis; and positions IRA B cells as orchestrators of protective IgM immunity.

P2.01.25
The Rho Family Guanine Nucleotide Exchange Factor, FARP1 negatively regulates T cell response

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Rho small GTPases are known to function in the transduction of T cell receptor (TCR) signaling which is essential for survival and maturation, although the mechanisms underlying this event remains unclear. Here, we present evidence that the FERM domain-containing guanine nucleotide exchange factor, FARP1 is negatively regulates the T cell response upon the engagement of TCR. To explore the function of FARP1 in the immune system, we analyzed immune response in FARP1-deficient($FARP1^{-/-}$) mice. Interestingly, we

detected the higher level of FARP1 transcripts in the activated CD4+ lymphocytes, but not in naïve T cells. Moreover, the expression of FARP1 was induced by anti-CD3 stimulation in a dose dependent manner. FARP1$^{-/-}$ mice had normal numbers and cell surface markers for each lymphocyte subset, suggesting that FARP1 is dispensable for lymphocyte development. To investigate a role of FARP1 during T cell activation, we immunized wild-type and FARP1$^{-/-}$ mice with keyhole limpet hemocyanin in complete freund adjuvant. After 5 days, CD4^{+} lymphocytes from FARP1$^{-/-}$ mice exhibited enhanced antigen-specific T cells responses as higher amount of IFN-γ production than that of wild type. On the other hands, the proliferation of CD4^{+} lymphocytes from FARP1$^{-/-}$ mice was comparable to wild-type mice. In addition, FARP1$^{-/-}$ CD4^{+} lymphocytes showed hypereactive to produce IFN-γand IL-17 upon anti-CD3 and CD28 stimulation *in vitro*. Collectively, these results suggest that FARP1 negatively regulates the T cell-mediated immune response.

P2.01.26
Interaction between Siglec-9 and prohibitins down-regulates T cell receptor signaling

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In this study, we provided that new markers of activated T lymphocytes, and these markers may become a new therapeutic target. First, we demonstrated that prohibitin-1 and -2 (prohibitins) were induced on the cell surface of T lymphocytes in response to activation. Furthermore, we found that prohibitins play a role as counter receptors for Siglec-9 expressed on macrophages and dendritic cells. Siglec-9 bound to prohibitins in a sialic acid-independent manner. Mutated Siglec-9 with Arg120 changed to Ala lost the binding activity, suggesting a specific ionic peptide-peptide interaction. Prohibitins were co-localized with CD3 on the surface of Jurkat cells. ERK1/2 was phosphorylated in Jurkat cells and preactivated T lymphocytes on treatment with anti-CD3 antibody immobilized beads, whereas phosphorylation of ERK1/2 was markedly diminished on treatment with anti-CD3 antibody and Siglec-9 co-immobilized beads, indicating that engagement of prohibitins with Siglec-9 inhibits ERK1/2 phosphorylation. Phosphorylation of c-Raf was also reduced, maybe due to inhibition of the c-Raf-prohibitin interaction by Siglec-9 ligation. In parallel with inhibition of the ERK cascade, IL-2 and IFN-γ production were markedly decreased in Jurkat cells and preactivated T lymphocytes, respectively. Anti-PHBs antibodies also reduced T cell receptor mediated cell signaling. Therefore, cell surface PHBs could be a target of immunoregulator to cure autoimmune diseases. In addition, PHBs were also induced on the surface of activated B lymphocytes. Thus, this interaction may be a useful immunotherapeutic target on T and B lymphocytes.

P2.01.27
PD-1 - PD-L1 interaction does not affect the ability of dendritic-like cells to activate human lymphocytes in vitro

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Dendritic cells (DC) are professional antigen presenting cells and are responsible for antigen capture and T-cells activation. Recently a PD-1 (Programmed death 1) receptor was discovered which is involved in inhibition of lymphocyte activation. Two ligands for the PD-1 receptor, namely PD-L1 and PD-L2, were identified. Both are present on DC.
In this study a myeloid KG-1 cell line was used as a known model of dendritic cells. After appropriate stimulation they differentiate into so-called dendritic-like cells (DC-like cells) which exhibit many properties of DC. In previous studies we have shown that the KG-1 cells express PD-L1, but not PD-L2, in activation dependant fashion.
The aim of this study was to determine if the expression of PD-L1, ligand for PD-1 receptor, on activated KG-1 cells affects their ability to activate T lymphocytes in MLR (mixed leukocyte reaction).
KG-1 cells were stimulated with PMA and ionomycin to receive DC-like cells. Then the PD-L1 ligand on KG-1 cells was blocked by using blocking antibodies. Prepared in such way KG-1 cells were then used in MLR with human lymphocytes. The results show that the blockade of PD-1 - PD-L1 signaling didn't affect the ability of the DC-like cells to activate T lymphocytes.
Research on DC is difficult because of high costs and problematic procedure. Using a cell line, such as KG-1 line of DC phenotype, overcomes these difficulties. The presented study demonstrates that the PD-1 - PD-L1 interaction does not take part during activation of T lymphocytes by KG-1 cells in MLR.

P2.02 Nuclear receptors

P2.02.01
A comparison of the biological features of prostate cancer with (PSA+,PSMA+) profile according to RKIP

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To investigate differences in the biological features of the most immunoexpressed prostate cancer (PC) profile (PSA+,PSMA+) according to the RKIP. Formalin-fixed and paraffin embedded tissue blocks from 19 PC with dominant Gleason grade ≥ 8 were studied. Expression of PSA, PSMA, RKIP, Raf-1, MEK-1, ERK-1, ERK-2, p-Akt (T308),p-Akt (S473),NF-κB p50 and NF-κBp65 were detected immunohistochemically. The most relevant results showed that loss of RKIP in the most immunoexpressed PC (PSA+,PSMA+) profile was associated with increased of PSA and PSMA expression. Intensities of immunoreactions to PSA and PSMA were higher in cancer epithelial cells negative for RKIP (12.51±1.6 and 34.95±1.92) compared to those positive for RKIP (4.68±1.11 and 28.56±0.91). In parallel, missing of RKIP expression in PC patients with (PSA+,PSMA+) profile was connected with increased of components of both Raf-1/MEK/ERK and NF-κB (p65/p50); whereas Akt is activated independently to RKIP. Although characterized by the same (PSA+,PSMA+) profile, PC phenotype missing the RKIP related to invasive potential and greater biological aggressiveness reflected in overexpression of components of Raf-1/MEK/ERK and NF-κB (p65/p50) in which Akt is activated independently to RKIP. Taking into account the PC phenotypes according to RKIP among PSA-PSMA profiles may improve distinguish from cancers that will become more aggressive; and therefore adapt the therapeutic strategies in those patients.

P2.02.02
Matrix metalloproteinase 3 activates NFκB in cell nucleus during Dengue virus infection

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Dengue virus (DENV) is a mosquito-borne virus that endangers an estimated 2.5 billion people globally. Study of the host anti-viral mechanism is important for us to understand the process of Dengue fever disease. Matrix metalloproteinase 3(MMP3), a downstream effector of JAK-STAT pathway, was found to be induced during DENV infection. Silencing MMP3 in macrophage, the DENV infection level significantly increased, while the anti-viral cytokine (eg: IFNβ,IL6) production impaired. Further experiments shown that MMP3 was translocated into cell nucleus upon DENV infection and positively influenced the activity of transcriptional factor NFκB. Immunofluorescence assay showed that MMP3 were co-localized with NFκB in the cell nucleus after DENV infection, and co-immunoprecipitation experiment suggested that MMP3 may directly interact with NFκB p65 subunit in cell nucleus. These data suggested that, during DENV infection, MMP3 may move to cell nucleus, interact with NFκB, and further enhance the transcriptional activity of NFκB to promote the anti-viral cytokine production. This will be the first time to show the novel anti-viral function of MMP3 in cell nucleus, and provide new evidence for the role of MMPs in immune modulation.This work will also contribute to the development of new anti-viral drugs based on MMPs.

P2.02.03
Tumor inflammatory environment modulates expression of PPAR-γ, inflammasome activation and triggers prostate cancer cell death in vitro

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Inflammation is one of the most well-known risk factors for the development of prostate cancer. Prostatic inflamed areas are characterized by focal epithelial atrophy and immune cells infiltration. To address the relation between the tumor cells and inflammation process, tumor environment was simulated using a conditioned medium of peripheral mononuclear blood cells that were previously stimulated by standard inflammatory inducers. All assays were performed using prostate cancer cell lines DU145 and LNCaP clone FGC. Our results showed that prostate cancer cell lines secreted IL-1β in response to inflammatory environment, indicating that these cells could trigger inflammasome activation. IL-6 and TNF-α were also quantified and no significant secretion level was observed. It was also observed constitutively expression of PPAR-γ in the cell line LNCaP that is androgen sensitive. In the other hand, DU145 an androgen insensitive cell line showed no constitutively PPAR-γ expression. When subjected to proinflammatory medium both cell lines showed increased expression of the nuclear receptor PPAR-γ. Furthermore, after 24h of stimulation both cell lines presented an increased rate of cell death although only DU145 had a decreased level of cell proliferation, as assessed by DNA fragmentation assay and CFSE staining, respectively. These results suggest that a proinflammatory microenvironment modulates secretion of IL-1β and PPAR-γ expression, which seems to trigger cell death and decrease proliferation rate in prostate cancer cell lines in vitro. Therefore, our data suggest that inflammasome and PPAR-γ could be an interesting chemotherapeutic target in prostate cancer treatment. CNPq, FapDF

P2.02.04
Glucocorticoid receptor-dependent immunomodulatory effect of ursodeoxycholic acid on liver lymphocytes in mice

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Although ursodeoxycholic acid (UDCA) has long been used for patients with chronic cholestatic liver diseases, particularly primary biliary cirrhosis, it may modulate the host immune response. This study investigated the effect of UDCA feeding on experimental hepatitis, endotoxin shock, and bacterial infection in mice. C57BL/6 mice were fed a diet supplemented with or without 0.3% (w/v) UDCA for four weeks. UDCA improved hepatocyte injury and survival in concanavalin-A (Con-A)-induced hepatitis by suppressing IFN- production by liver mononuclear cells (MNC), especially NK and NKT cells. UDCA also increased survival after lipopolysaccharide (LPS)-challenge; however, it decreased survival of mice following *Escherichia coli* (*E. coli*) infection due to the worsening of infection. UDCA-fed mice showed suppressed serum IL-18 levels and production of IL-18 from liver Kupffer cells, which together with IL-12 induces potent IFN- production from liver NK/NKT cells. However, unlike normal mice and immune-compromised burn-injured mice, exogenous IL-18 pretreatment did not increase the serum IFN- levels after *E. coli* challenge in the UDCA-fed mice. Interestingly, however, glucocorticoid receptor (GR) expression was significantly upregulated in the liver MNC, especially NK and NKT cells, of the UDCA-fed mice but not in their whole liver tissue homogenates. Silencing GR in the liver MNC abrogated the suppressive effect of UDCA on LPS-induced IFN- production. Furthermore, RU486, a GR antagonist, restored the serum IFN- level in UDCA-fed mice after *E. coli* infection. Taken together, these results suggest that IFN--reducing immunomodulatory property of UDCA is mediated by elevated GR in the liver lymphocytes in an IL-12/18 independent manner.

P2.03 Epigenetic control of immune responses

P2.03.01
Polycomb repressive complex-1 is critical for peripheral B cell differentiation and germinal center responses

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B cell development is a step-wise, tightly regulated process whereby multipotent stem cells progressively acquire immunological competence, ultimately becoming antibody- secreting cells. Transition from one developmental stage to the next entails changes in gene expression mediated by the activity of stage-specific transcription factors and chromatin remodeling enzymes.
Polycomb group proteins (PcG) are a family of chromatin remodelers that repress targeted loci within complexes named Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2). The former monubiquitinates lysine 119 of histone H2A whereas the latter trimethylates lysine 27 of histone H3. In pre- and post-natal life, PcG proteins regulate lineage specification and restrict cell identity.
Using conditional gene targeting, we studied the role of the PRC1 catalytic subunits Ring1a and Ring1b in peripheral B cell development and germinal center (GC) B cell responses.
We found that PRC1 ablation in transitional (T) B cells leads to a differentiation block at the T2 stage, resulting in decreased numbers of mature B cells. Moreover, PRC1 inactivation in GC B cells revealed an essential requirement for the complex in the generation and/or maintenance of memory and antibody-secreting cells, as shown by a reduction in antibody titers produced during both primary and recall T-cell dependent immune responses. Transcriptome analyses of PRC1-deficient splenic B cells suggest that PRC1 activity is needed to prevent diversion to other lineages and orchestrate B cell differentiation.
Identification of direct PRC1 targets in peripheral B cells will help to unravel the molecular mechanisms through which the complex controls late B cell differentiation.

P2.03.02
The rheumatoid factor in idiotypic regulation of autoimmunity

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Rheumatoid factors (RFs) were defined originally by the ability to react with Fc region of IgG and useful marker for the diagnosis of rheumatoid arthritis. Further studies showed that RFs are able to bind the Fab region of IgG, and to enter into idiotypic interactions with antibodies of different specificity. Facts about RF multiplicity and heterogeneous behavior require a new look at the role of RF. Taking into account important role of idiotypic interactions in autoimmunity regulation we assumed that RFs might regulate autoimmune response through idiotypic interactions. We have investigated RF levels in rats resistant and susceptible to arthritis, encephalomyelitis, atherosclerosis and idiotypic interactions between RFs and antibodies to antigens-inductors of autoimmunity. We revealed that in the autoimmune disease-resistant rats the increase of RF level preceded antibodies appearance to given antigens, on the contrary it was not observed in autoimmune rats. Thus resistance to induction of autoimmune diseases in rats is associated with high RF production within the initiation of immune response, and low RF levels in this period may be used as a preclinical marker of autoimmune manifestations. RF containing sera compete with antigen, if sera were obtained by this antigen immunization, and nonspecifically inhibit binding of different antigen-antibodies pairs. These facts suggest that RFs are anti-idiotypic antibodies carrying individual and shared

paratopes. Individual paratope recognizes antigen-binding sites of antibodies. Shared paratope is bound to recurrent idiotype in antibodies of different specificity. Thus RFs are involved **in** negative idiotypic regulation of lymphocytes specific to antigens-inductors of autoimmune diseases.

P2.03.03
Interleukin-10 suppresses CXCL8 gene transcription in LPS-stimulated monocytes via modulation of histone deacetylases (HDACs) recruitment

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Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine, very effective at suppressing pro-inflammatory cytokine produced by myeloid cells. The mechanistic bases of the immuno-suppressive/immuno-modulating activities of IL-10 largely involve transcriptional regulation. In the last years, we demonstrated that the ability of IL-10 to modulate LPS-induced IL-1ra transcription relies on IL-10 capacity to modify the acetylated state of local promoter-associated histones. Herein, we extended this study to the mechanisms utilized by IL-10 to inhibit the pro-inflammatory CXCL8 gene transcription, particularly focusing on the epigenetic modifications at the CXCL8 promoter level.
Chromatin immunoprecipitation (ChIP) was employed to characterize the molecular events triggered by IL-10 at the level of CXCL8 gene promoter in LPS-stimulated human monocytes.
ChIP analysis revealed that IL-10 reduces the recruitment of CDK9 induced by LPS and, as a consequence, inhibits LPS-induced activation (i.e. ser-2-phosphorylation) of promoter-bound Pol II. Furthermore, we demonstrate that IL-10 acts upstream of CDK9, by triggering histone H4 deacetylation and preventing, in this manner, the subsequent recruitment of bromodomain-containig protein Brd4. Indeed, histone H4 deacetylation blockade with HDAC inhibitors, abrogates the ability of IL-10 to inhibit LPS-induced CXCL8, further suggesting the involvement of an HDAC in the CXCL8 transcriptional inhibition by IL-10. Most importantly, PBMCs from patients with alterations in the HDAC activity show a reduced responsiveness to IL-10-mediated inhibition of CXCL8, but not TNF-α, transcription triggered by LPS.
Collectively, our data unravel a novel mechanism utilized by IL-10 to inhibit LPS-induced CXCL8 transcription, likely proceeding via histone deacetylase(s) (HDAC) activation.

P2.03.04
Bacteria-induced epigenetic modifications in gingival innate immunity

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We previously reported exposure to different oral bacteria resulted in differential epigenetic modifications and in altered expression of inflammation-associated genes. The goal of this study was to investigate which bacteria-induced epigenetic modifications regulate gingival innate immunity *in vitro* and if amount of periopathogens is correlated to epigenetic status *in vivo*.
Chromatin Immunoprecipitation examined if trimethylation of histone H3 at lysine4 (H3K4Me3) and at lysine9 (H3K9Me3) interacted with promoters of innate immune genes hBD-2, CCL20 and IL-8 following exposure of gingival epithelial cells (GECs) to periopathogen *Porphyromonas gingivalis* (Pg) or to non-pathogen *Fusobacterium nucleatum* (Fn). Western analyses of total and acetylated H3 and H4 investigated differential modulation of chromatin structure by Fn vs. Pg. Unstimulated GECs were used as controls. Correlation between CXCL5 methylation level of periodontal patients and amount of periopathogens recovered was determined by Spearman correlation.
Interaction between H3K4Me3 and hBD-2, CCL20 and IL-8 promoters was suppressed with Pg, while increased with Fn. Fn decreased acetylation of H3 and H4, while Pg decreased acetylation of H4 only. Amount of Fn recovered from plaque of periodontal patients was correlated with decreased CXCL5 methylation.
Our data show bacteria-specific histone modification and suggest oral bacteria affect the accessibility of chromatin to transcription factors, subsequently affecting gene transcription. Hypomethylation of CXCL5 with increased *Fn* may cause an inappropriate over-transcription of CXCL5 in periodontal patients. This study highlights potential interplay between epigenetic modifications and oral bacteria in periodontitis, and may ultimately lead to the development of innovative treatment and diagnostic modalities.

P2.03.05
Methylation patterns near and inside of the TSLP promoter in an african descendent population of Colombia

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Rationale: Thymic stromal lymphopoietin - TSLP is a gene that affects many cell lineages and can directly or indirectly leads to various outcomes, the expression of it is detected in the airways of asthmatic patients, and correlates with disease severity. For this reason we decided to analyze the methylation patterns of the TSLP in Afro-Colombian population.
Methods: We analyze the methylation patterns close and inside of the TSLP promoter in 94 African-descent individuals of Colombian Caribbean coast. From TSLP isoform 1 precursor sequence reported by NCBI, we selected the sequence from chromosomal location chr5: 110405778 to 110413722, that containing 5'UTR and promoter regions from TSLP. Using Methprimer the parameters for prediction of CpG Islands were CpG islands >100bp, GC>50% and Obs/Exp >0.60.
Results: Methprimer analysis predicted two CpG Islands, where primers for methylation specific PCR (MSP) where designed, one in the 5'UTR region (Island 1 with 190bp) and the other one in his promoter (Island 2 with 231bp). We found, that Island 1 showed a variable methylation pattern with unmethylated, methylated and hipermethylated individuals (24.5%, 44.7% and 30.9%, respectively. While, the Island 2 was almost always hipermethylated (92.6%, n=87).
Conclusions: Although this study was performed with peripheral blood samples, the Island 1 in the 5'UTR is more informative than Island 2, and could be regulating gene expression of TSLP and may be associated with the levels of TSLP protein in serum.

P2.03.06
Tumor Necrosis Factor Gene Polymorphisms in Tunisian Patients with Non -Small Cell Lung Cancer

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Lung cancer (LC) is one of the most lethal malignant disorders; it's divided into two groups: small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC). In our present study we have been interested to NSCLC. Several approaches were adopted to study the etiology or pathophysiology of this disease, as recent reports which focused on the genetic susceptibility to this disease, among the many candidate genes studied, we chose the TNF in view of the major role it plays in the immune pro inflammatory and its association with increased risk of a variety of human cancers. We have investigated three polymorphisms in the promoter region of the TNFα gene (-308 G/A and -238 G/A) and TNF β +252A>G for their susceptibility to non-small cell lung cancer (NSCLC) in Tunisian population. We compared the distribution of these polymorphisms between 133NSCL patients and 174 healthy controls using a polymerase chain reaction restriction fragment length-polymorphism (PCR-RFLP) analysis. The frequencies of the two TNFα (-238 and-308) "A" alleles were significantly higher in NSCLC patients than in healthy controls respectively (p =0.01; OR= 1.92; 95% CI 1.14_3.23 and p =0.000008; OR=3.65; 95% CI 2.12_6.30), whereas the frequency of the TNF β +252 G allele was approximately similar in the two compared groups. This study support a relation between TNFα-238G/A and TNFα -308G/A polymorphisms and the susceptibility to lung cancer. Contrary to other studies, the -308 A and -238A alleles have an inductive

action on Lung cancer development and progression in our Tunisian population.

P2.03.07
Locus-specific biochemical epigenetics / chromatin biochemistry by insertional chromatin immnoprecipitation (iChIP)

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Comprehensive understanding of mechanisms of epigenetic regulation requires identification of molecules bound to genomic regions of interest *in vivo*. To perform biochemical and molecular biological analysis of specific genomic regions, we developed the insertional chromatin immunoprecipitation (iChIP) [1-3].
The scheme of iChIP is as follows:
i) The recognition sequence of LexA, an exogenous DNA-binding protein, is inserted into the genomic region of interest in the cell to be analyzed.
ii) The DNA-binding domain of LexA is fused with a tag(s) and a nuclear localization signal(s) and expressed in the cell.
iii) The resultant cell is crosslinked and lysed, and the crosslinked DNA is fragmented.
iv) The complexes including the LexA protein are immunoprecipitated with an antibody against the tag. The isolated complexes retain molecules interacting with the genomic region of interest.
v) Reverse crosslinking and subsequent purification of DNA, RNA, or proteins allows their identification and characterization.
We applied iChIP to identification of proteins interacting with the single-copy *Pax5* promoter in the chicken DT40 B cell line. Pax5 is known as a key factor for B cell lineage commitment. iChIP combined with mass spectrometry identified proteins interacting with the *Pax5* promoter. Knock-down of an identified protein reduced *Pax5* transcription. Therefore, the protein should be a novel regulator of *Pax5* expression. iChIP is a useful tool for identification of proteins bound to endogenous genomic regions *in vivo*.
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P2.03.08
The Research on IFN-γ,IL-4 Gene Methylation of Uighurs and Han Patients with Allergic Rhinitis in Xinjiang of China

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Objective To investigate the methylation level of IFN-γ,IL-4 gene in allergic rhinitis (AR) patients of Uighur and Han in Xinjiang. Methods we randomly selected sample via epidemiological survey in Yili area, which includ healthy peoples and AR patients. In this study, the genomic DNA was extracted from peripheral blood and was modified using bisulfate modification kit. Then, the methylation of DNA was analyzed by methylation specificity polymerase chain reaction (MS-PCR). Results We found that the positive rate of IFN-γ, IL-4 gene methylation in Han and Uighur AR patients was no significant differences as compared with healthy groups ($P > 0.05$). Also, there are no significant differences between Uighurs and Han AR patients ($P > 0.05$). However, the positive rate of IFN-γ, IL-4 gene methylation in Uighur healthy group was significant higher than that of Han healthy control ($P< 0.05$). Conclusion This research suggest that the low methylation of IFN-γ gene and high methylation of IL-4 in Han healthy groups may play an important role in the pathogenesis of AR in Han patients.

P2.03.09
Pronounced co-localization of immunoglobulin genes and their enhancers in transcription factories at the nuclear periphery in plasma cells

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A single plasma cell is estimated to secrete its own mass in antibody molecules each day. To uncover some of the molecular secrets behind such remarkably high levels of gene expression, we have investigated the nuclear organization of transcribing immunoglobulin (Ig) genes at various stages of mouse B cell development, utilizing 3D RNA immuno-FISH and ChIP-3C-Seq technologies. After the pro-B cell stage, ~80% of Igκ and IgH genes are bi-allelically transcribed. As B cells differentiate to become better suited for high-level expression of antibody molecules, their Ig genes become progressively co-localized in transcription factories, which peaks at the plasma cell stage, with ~48% co-localization between Igκ-IgH, Igκ-IgJ, and IgH-IgJ gene pairs. Remarkably, ~26% of the plasma cell nuclei exhibit triplet Igκ-IgH-IgJ gene co-localizations in transcription factories, with ~45% of the transcribing Ig genes located within <0.5μm from the nuclear periphery. In plasmablasts we identified the trans-chromosomal interacting sequences between Ig genes in transcription factories as their enhancer elements. Moreover, plasma cells from Igκ gene E3′ knockout mice exhibit significant reductions in Ig gene co-localizations at transcription factories, nuclear periphery locales, and Ig gene transcript levels. Furthermore, Ig gene transcripts arising from genes co-localized in the nuclear interior are channeled together between dense chromatin regions from their sites of synthesis toward a subset of nuclear pores. We conclude that plasma cells have optimized coordinated transcriptional and post-transcriptional control of Ig genes by co-localizing them in transcription factories often near the nuclear periphery through their transcriptional enhancers.

P2.03.10
Testing the resource-reallocation hypothesis: influence of maternal testosterone on immune system of Japanese quail (Coturnix japonica) chicks

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Immunomodulatory effects of testosterone are frequently discussed but the effects of maternal testosterone (mT) are less studied. Deposition of mT into the egg yolk is generally considered as epigenetic mechanism, enabling transfer of information from the mother to her offspring and better coping with environmental challenges after hatching. In our study we challenge the hypothesis about reallocation of resources between mT-enhanced growth and immune system in Japanese quail selected for high (HET) and low (LET) egg testosterone content since HET quail exhibit faster growth than LET quail. From day 4 after hatching, half of animals (n= 40) was subjected to protein restriction (control group 21%, restricted group 15% of protein content). Subsequently, cell-mediated immune response to phytohemmaglutinine (PHA) and acute phase response to lipopolysaccharide were examined. Acute phase response was evaluated as plasma corticosterone concentrations (Cort), heterophil/lymphocyte (He/Ly) ratio and total immunoglobulin (IgY) levels. Protein restriction negatively influenced body weight and performance in PHA-test. The increase of basal and stimulated Cort and He/Ly ratio and no changes in total IgY levels were observed. HET quail showed higher body mass and total IgY levels and lower He/Ly ratio than LET quail, while PHA index and Cort concentration did not differ between lines. No significant interaction was found between food restriction and genetic line. Therefore, it is unlikely that reallocation of resources induced by mT acts as the main mechanism affecting the immune system of offspring and a compromised immune response probably does not limit selection for high maternal androgen deposition.

P2.03.11
Heterogeneity of Polycomb repressive complexes and long-noncoding RNA dictate the regulation network of thymic T cell development

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The thymic microenvironment principally formed by thymic epithelial cells (TECs) provides a specialized niche for T-cell development and controls thymocyte cellularity.
The epigenetic regulator Polycomb group (PcG) gene products form multimeric protein complexes (PRC1, PRC2), which maintain the repressed state of target genes during multiple rounds of cell divisions through histone modifications. We reported that different PRC1 subunit plays a crucial role in thymocyte development on different point of action, which might be due to the heterogeneity of PRCs. Bmi1, one of PRC1 subunit, acts on the repression of p19Arf but not p16Ink4a, and p19Arf deficiency completely restores the Bmi1-/- thymocyte defects in vitro. However, unlike cases with thymocytes, the deletion of p16Ink4a and p19Arf could not restore the defects in Bmi1-/- TEC, indicating a distinct role for Bmi1 in regeneration process of TECs.
Recently we and others noticed that long-noncoding RNA can bind the PRC complex, therefore, may leading to the target gene specificity. One of these is ANRIL that is anti-sense RNA encoded in INK4 locus.
In human, sequence polymorphisms in a 58-kilobase (kb) interval on chromosome 9p21 (including INK4 locus) confer a markedly increased risk of coronary artery disease (CAD), the leading cause of death worldwide. And it was shown that deletion of the orthologous 70-kb non-coding interval on mouse chromosome 4 affects cardiac expression of neighboring genes, as well as proliferation properties of vascular cells. Therefore, we will discuss the Heterogeneity of PRCs on thymopoiesis and Polycomb connection between CAD and regeneration process of thymus.

P2.03.12
Epigenetic regulation of T cell Ig mucin 3 expression in Th1 cells reveals a novel control of adaptive immunity

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The T cell Ig mucin 3 (Tim-3) is expressed selectively on the terminal differentiated Th1 cells and acts as a negative regulator of the Th1 immunity. The dysregulated expression of Tim-3 on Th1 cells is linked to several autoimmune phenotypes and chronic viral infections, indicating that Tim-3 pathway regulates homeostasis of the T cell response under pathogenic condition. In this study, we investigated the epigenetic regulation of *Tim-3* and dissect the possible mechanisms of dysregulated Tim-3 expression under pathogenic conditions. By analyzing the sequences of putative *Tim-3* promoter region of human and mouse, we found that there are three conserved regions and one CpG island within the Tim-3 promoter. We identified the promoter regions of the *Tim-3* gene and explored the effects of DNA methylation on the promoter activity by using a CpG free vector for *in vitro* methylation experiment and reporter assays. Finally, treatment of 5-Azacytidine (5-Aza) enhanced Tim-3 expression on $CD4^+$ T cells under the Th1 polarization condition, which is correlated with the demethylation of the CpG island within the promoter region. Surprisingly, 5-Aza induced Tim-3 expression under a Th2 condition but not under a Th17 condition. These data suggested that the epigenetic process selectively cooperates with lineage-specific factors in the controlling of Th1 cell development, whereas under certain conditions, epigenetic control displayed different hierarchy in the control of Tim-3 expression. In conclusion, these regulatory mechanisms of Tim-3 provide novel insight into the understanding of the Th1 cell biology.

P2.03.13
DNA methylation changes in allergic patients correlate with symptom severity and reflect altered CD4+ T-cell population structure

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Altered DNA methylation patterns in CD4+ T-cells indicate the importance of epigenetic mechanisms in inflammatory diseases. However, the identification of these alterations is complicated by the heterogeneity of most inflammatory diseases. Seasonal allergic rhinitis (SAR) is an optimal disease model for the study of DNA methylation because of its well-defined phenotype and etiology. We generated genome-wide DNA methylation and gene expression profiles of CD4+ T-cells from SAR patients and healthy controls using Illumina's HumanMethylation450 and HT-12 microarrays, respectively. DNA methylation profiles clearly and robustly distinguished SAR patients from controls, during and outside the pollen season. Moreover, we found that this methylation signature correlated with symptom severity. In agreement with previously published studies, gene expression profiles of the same samples failed to separate patients and controls. Separation by methylation, but not by gene expression was also observed in an in vitro model system in which purified PBMCs from patients and healthy controls were challenged with allergen. We associated these differences, not with epigenetic dysregulation in a given CD4+ T-cell subset, but with changes in the proportions of central memory T-cell populations between patients and controls. Thus, quantitative DNA methylation data can act as a sensitive proxy measure of changes in cell population structure in disease. Our data highlight the potential of epigenomics in the stratification of immune disease and represents the first successful molecular classification of SAR using CD4+ T cells.

P2.03.14
Targeted disruption of Calcium/NFAT signaling reveals a left-right determination disorder in the pharyngeal arch artery

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An immunosuppressant was injected into the pregnant mice in order to investigate whether the immune response is involved in differentiation during embryonic development. In saline-injected fetuses, the cardiac outflow tract (aorta) arose from the left ventricle, ascended for a short distance and then curved to the left and descended through the left side of the chest. Injection of FK506, an inhibitor of carcineurin, into the mother mouse on days 6.5, 7.5, and 8.5 of pregnancy induced an left-right (L/R) determination disorder, in which the cardiac outflow tract ascended toward the right side, formed an arch and then descended through the right side of the chest. Injection of 3.0mg/kg of FK506 increased the penetrance of the right aortic arch by 32% (ten of 31) relative to saline injection. FK506 is known to work by restricting NFAT (nuclear factor activated T-cell) dephosphorylation. An L/R determination disorder in cardiac outflows appeared when an NFATc4 siRNA was directly injected into amniotic fluid. As for the mechanism, Pitx2, which is normally expressed on the left-hand side, was found to be expressed also on the right-hand side. Furthermore, it turned out that administration of FK506 also prevented the dephosphorylation of NSFL1 cofactor p47. When an siRNA targeting p47 was introduced into amniotic fluid of FK506-treated fetuses, both of the dorsal arteries - which should normally become one - remained. The finding is that mother's immune system contributes not only to self-defense but also to remodeling processes in her fetus morphogenesis.

P2.03.15
Activation of Aryl hydrocarbon receptor (AhR) triggers epigenetic and microRNA modulation and consequent differential regulation of Tregs and Th17 cells and amelioration of Experimental Autoimmune Encephalomyelitis (EAE)

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AhR is a member of the family of basic-helix-loop-helix transcription factors. Recent studies have suggested that AhR may regulate T cell differentiation. We examined the effect of AhR activation on micro RNA (miRNA) profile in T cells, and epigenetic modulation of Foxp3 and IL-17 genes, using a wide range of AhR agonists such as TCDD and dietary indole derivatives, indole-3-carbinol (I3C), and diindolylmethane (DIM). Upon activation of AhR, there was a

significant difference in the profiles of various miRNAs in T cells. Analysis of some of these miRNAs showed their role in regulation of Foxp3 and IL-17. AhR activation also led to significant epigenetic modulation including demethylation of Foxp3 promoter but methylation of IL-17 promoter. Addition of AhR ligands in vitro and administration in vivo triggered increased induction of Tregs but a decrease in Th17 cells. Using an EAE murine model of multiple sclerosis, we examined the effects of AhR activation on EAE symptoms. There was significant amelioration of EAE disease symptoms in mice treated with AhR ligands. I3C and DIM acted in vivo as AhR ligands inasmuch as AhR inhibitors reversed the protection afforded against EAE. Also, the effect of these compounds on Tregs and Th17 cells was not demonstrable in AhR knockout mice. Together, these data demonstrated that AhR activation regulates epigenetic modulation and can have differential effects on T cell differentiation into Tregs/Th17 leading to immune suppression. Our studies also suggest that natural dietary AhR ligands may serve as novel treatment modalities against inflammatory diseases.

P2.03.16
Epigenetic regulation of cytokine production in sepsis and endotoxin tolerance

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Endotoxin tolerance (ET), defined by a transient unresponsiveness to endotoxin (LPS) following a first exposure to LPS, is a protective mechanism against excessive inflammation. However, it plays a major role in the mortality of sepsis patients suffering from secondary infections.
A previous publication demonstrated chromatin remodeling through histone modifications with a transient silencing of a class of tolerizable genes including pro-inflammatory cytokines in endotoxin-tolerant murine macrophages (Foster et al. 2007).
The aim of our study is to understand ET on the chromatin level and the impact of anti-inflammatory cytokines in human monocytes during ET induction. Therefore, human $CD14^+$ monocytes were desensitized with a first LPS treatment overnight followed by a second LPS challenge on Day 2. Histone modifications were analyzed by chromatin-immunoprecipitation (ChIP) combined with sequencing and quantitative PCR analysis.
In contrast to the findings from Foster et al., our results did not reveal any differences between endotoxin-responsive and endotoxin-tolerant genes in human monocytes for two markers of active transcription - H3K4me3 and H4ac. These modifications were still present in the promoter regions of pro-inflammatory cytokines in desensitized cells suggesting that other epigenetic changes might play a role. Therefore, we are currently analyzing histone modifications determining transcriptional repression and combining different ChIP assays of positive and negative histone markers as well as transcriptional co-regulatory proteins.
This study could help to clarify the complex picture of induction/inhibition of cytokine production in ET.

P2.03.17
Impact of Wnt pathway activation in liver infiltrating T lymphocytes

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Hepatocellular carcinoma (HCC), a malignancy caused mainly by chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), is a highly fatal disease.
Wnt/β-catenin pathway is frequently activated in HCC, therefore we hypothesize that Wnt ligands or activation of Wnt/β-catenin pathway in inflamed/tumor liver tissues, can establish micro-environmental conditions that influence T cells functions and phenotypes.
Recent studies indicate that Wnt/β-catenin signaling is critical for the differentiation, polarization and survival of mature T lymphocytes. In order to asses the Wnt pathway activation on T cells that infiltrate peritumoral (LIL) and tumoral liver tissues (TIL), we performed flow cytometry analysis in peripheral or intrahepatic T cells to evaluate citoplasmatic accumulation of β-catenin in patients with HCC related to HCV and HBV infection. Preliminary data show significant citoplasmatic accumulation of β-catenin in CD4+CD127hi Foxp3low T cells (Tconv) from LIL and TIL as compared to PBMC from patients or healthy donors ex vivo. The β-catenin accumulation was also evident in CD4+CD127lowFoxp3hi Treg cells derived from the same tissue districts. The effect of β-catenin accumulation on CD4 T cell proliferation and differentiation was evaluated by inhibiting GSK-3β with the pharmacological compound TWS119 in vitro. Both CD4+ Tconv and Treg cells treated with TWS119 proliferated less than untreated cells, and Tconv cells displayed cytokine production impairment. Correlation between Wnt/β-catenin pathway activation in liver cancer and T cells in inflamed or tumor tissue, is crucial to better understand the behavior of immunological effector/memory phenotypes in this pathological condition.

P2.03.18
Evidence for in vivo DNA demethylation at Interferon-γ promoter site CpG island during human endotoxemia

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Introduction: Activation of inflammatory gene transcription is vital for host immune defense against pathogens. This response is often deleterious to the host due to exaggerated or undermined inflammatory protein production, representing common manifestations in sepsis. Modifications to histones and DNA sequence nucleotides by nuclear proteins, including methylation of cytosine residues (5-mC) at CpG islands (CGIs), support mRNA transcription initiation, repression and/or elongation. We here sought to unravel the role of DNA methylation at an interferon-γ (IFNG) promoter CGI in connection with inducible IFNG transcription in vivo. To this aim we adopted the well-established human model of lipopolysaccharide (LPS)-induced systemic inflammation.
Methods: Eight healthy subjects were enrolled for an intravenous LPS challenge (4 ng/kg). Whole-blood was collected prior to and 3, 6, 20 hours post-LPS challenge. Ex vivo LPS-stimulated whole-blood at each time point was analyzed for pro-inflammatory (TNFα, IL1ß, IL6, IL8 and IFNG) and anti-inflammatory (IL1RN and IL10) gene transcription and protein release. DNA methylation was assessed by methylation-specific PCR (MSP) of bisulfite converted DNA.
Results: In line with previous studies, we observed a significantly impaired ex vivo induction of pro-inflammatory cytokines in the 3 and 6 hour samples given the "second hit" of LPS. Anti-inflammatory cytokine release was not impaired. By MSP analysis we detected a statistically significant increase in demethylation, in parallel to a decrease in methylation, at the IFNG CGI that coincided with a repressive state of IFNG mRNA transcription ex vivo.
Conclusion: Our findings suggest LPS-induced active DNA demethylation at an IFNG promoter CGI in vivo.

P2.03.19
Differential effects of histone-deacetylase inhibition during lipopolysaccharide-induced lung inflammation in mice

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Introduction: Histone-deacetylases (HDACs) can influence gene transcription by increasing the interaction between histones and DNA. HDAC inhibitors have been implicated as possible new therapeutic agents in inflammatory diseases. The aim of this study was to determine the effect of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) on lipopolysaccharide (LPS)-induced lung inflammation.

Methods: C57BL/6 mice received either SAHA (200mg/kg) or vehicle intraperitoneally 30 minutes before intranasal inoculation with Klebsiella LPS (1µg). Mice were euthanized 6 hours after LPS administration for analyses. MH-S cells (alveolar macrophages) were used to study the effect of SAHA at cellular level.
Results: SAHA pretreatment resulted in a 10-fold reduction in TNFα and a 4-fold reduction of IL-6 levels in bronchoalveolar lavage fluid (BALF) upon LPS instillation (both P< 0.001). In contrast, SAHA increased LPS-induced release of the CXC chemokines KC and MIP-2 in BALF (both P< 0.001). In accordance, SAHA significantly inhibited LPS-induced TNFα release, while significantly enhancing LPS-induced KC and MIP-2 secretion by MH-S cells in vitro. These effects were associated with reduced NFkB activation in the presence of SAHA, suggesting a NFkB independent mechanism of chemokine upregulation.
Conclusion: HDAC inhibition results in differential effects in the bronchoalveolar space during LPS-induced lung inflammation characterized by inhibition of proinflammatory cytokine release with concurrent enhancement of chemokine secretion.

P2.04 MicroRNAs in immune regulation

P2.04.01
miR-146, miR-21, and miR-31 expression in patients with active tuberculosis and subjects with latent tuberculosis infection

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There is a growing amount of evidence that microRNAs (miRs) play critical roles in complex host-pathogen interaction networks. Different studies have identified a role for mir-146 in regulation of inflammatory cytokines, and for mir-21 and mir-31 in FOXP3 expression, a master regulatory gene for both development and function of regulatory T cells. Tuberculosis (TB) as a chronic infectious disease remains a world-wide public health problem around the world. 5%-10% of individuals infected with *Mycobacterium tuberculosis* will eventually develop active disease and more than 90% of the people who become infected develop a latent TB infection (LTBI). Immune effector mechanisms that lead to resistance or susceptibility to infection or clinical disease are not fully known.
In the present study the expression patterns of miR-146, miR-21, and miR-31 have been studied in patients with active TB and subjects with LTBI, and healthy individuals. Peripheral blood mononuclear cells were cultured with or without PPD. MiRs were isolated by microRNA isolation kit; and quantitation of miRs was carried out using Taqman microRNA assay kit. The expression level of miRNA was normalized to RNU24.
The results showed that the expression of miR-146, miR-21, and miR-31 were up-regulated in TB-infected individuals compared with those in healthy individuals. Moreover, miR-146, miR-21, and miR-31 expression were higher in patients with active disease compared to subjects with LTBI.
The different expression patterns of the miRs suggest that they may mediate important roles in development of appropriate immune response.

P2.04.02
MicroRNA-mediated regulation of the pattern recognition receptor PTX3

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The long pentraxin 3 (PTX3) is a soluble pattern recognition receptor transcriptionally induced by proinflammatory signals in different cell types, including monocytes and macrophages. Despite its characterized role in innate resistance and inflammation, regulation of PTX3 by microRNAs, fundamental fine-tuners of these processes, has not yet been described. Therefore, we investigated the effect on PTX3 production of microRNAs potentially targeting PTX3 itself or molecules involved in PTX3 expression and correct folding.
Among the microRNAs predicted to target PTX3 3'UTR, miR-29c and miR-181c are constitutively expressed in human primary monocytes and macrophages, whereas miR-9 is expressed in monocytes, as demonstrated by bioinformatic analysis on transcription profiles. Reporter assay experiments demonstrated that miR-9, miR-29 and miR-181c act on PTX3 mRNA. Experiments on *PTX3* promoter showed that miR-29, miR-181c and other microRNAs induced by proinflammatory signals in monocyte/macrophages (miR-146a, miR-132 and miR-155) reduced the transcriptional rate of *PTX3* gene. Finally, miR-181c inhibited the production of ERp18, a thioredoxin superfamily member required for the correct folding of PTX3, by directly targeting its mRNA. When overexpressed in the 8387 fibroblastic cell line which produces PTX3 in basal conditions and upon stimulation with inflammatory mediators (TNFα and IL-1β), these microRNAs downregulated PTX3 production, demonstrating the biological relevance of this network of post-transcriptional regulators.
All together, these data reveal a complex role of microRNAs in regulating PTX3 expression, exerted either by direct targeting PTX3 mRNA or indirectly by regulating key molecules involved in PTX3 induction.

P2.04.03
MiR-217 is an oncogene that enhances the germinal center reaction

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MicroRNAs (miRNAs) regulate virtually all biological processes, but little is known of their role in germinal center (GC) B cells. While the GC response is crucial to ensure a competent immune response, GC B cells are also the origin of most human lymphomas. Here we report that miR-217 is specifically upregulated in GC B cells. Gain- and loss-of-function mouse models reveal that miR-217 functions as a positive modulator of the GC response through the regulation of a DNA repair gene network. Moreover, we show that miR-217 overexpression promotes mature B cell lymphomagenesis. Therefore miR-217 provides a novel molecular link between the normal GC response and B cell transformation.

P2.04.04
The effect of tumor cell derived exosomes on mesenchymal stem cells

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Malignant melanoma (MM) is an especially aggressive skin cancer. The mechanism of its rapid metastasis formation, high genetic variability and effective immune escape mechanisms are not fully explained. Exosomes are among the potential mediators of communication between melanoma cells and their environment. To explore the interaction of melanoma cell derived exosomes (MDCE) and their microenvironment, we investigated their effect of mesenchymal stem cells (MSC). Mouse MSCs from adipose tissue were pretreated with B16 mouse melanoma cell derived exosomes, then the rate of spontaneous apoptosis and expression of multipotent stromal cell markers were analyzed by flow cytofluorometry. We have found marked differences in the expression CD44, integrin alpha 4, CD29 and CD106 after 7 days of induction. The ratio of late apoptotic or necrotic/early apoptototic cells decreased after MCDE treatment (0.43/1 versus 0.98/1). We sequenced the small RNA content of melanoma cell derived exosomes by SOLiD 5500xl technology, and the sequences were annotated in CLC Genomics Workbench version 5.5.1. We have found highest expression values of mir205, mir31, mir21a, mir15b respectively. To explore the potential in vivo tumor-promoting effect of exosomes, B16F1 tumor bearing mice were injected with MDCE-induced-MSCs i.v., the control mice received untreated MSC. The survival rate was 38% after pre-conditioned MSC treatment versus 85% un-induced MSCs treatment at day 42nd. Our data suggest that melanoma cell derived exosomes reeducate mesenchymal stem cells through their miRNA cargo, giving rise to a cell population that support metastasis formation.

P2.04.05
MicroRNA modulations in plasmacytoid dendritic cell hyperactivity in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that affects predominantly young females causing significant morbidity and mortality. The etiopathogenesis of SLE is not completely clear and plasmacytoid dendritic cells (pDCs) are thought to play a major pathogenic role owing to its unique ability to produce large amount of type-I interferon upon nucleic acids stimulation through the toll-like receptors (TLRs)-7/9. We previously demonstrated hyperactivity in pDCs from lupus patients when compared with healthy control subjects. Using the NZB/W F1 mouse model, we aimed to elucidate the regulatory mechanism for pDC hyperactivity in lupus development. Similar to the human setting, bone marrow–derived pDCs from symptomatic F1 mice exhibited heightened up-regulation of co-stimulatory molecules upon TLR-7 and TLR-9 ligands stimulation when compared with pre-symptomatic animals. Functional abnormalities in terms of cytokines production were also compared. The expression profiles of over 700 microRNA (miRNAs) in pDCs upon TLR7 activation were analyzed by low-density arrays and distinct miRNAs were differentially expressed by pDCs from lupus mice. On-going experiments are underway to correlate the perturbed miRNAs induction and the aberrant pDC functions in SLE using miRNA mimics and inhibitors. In summary, our study has revealed miRNA-mediated modulations that may lead to pDC functional abnormalities, thus further demonstrating its pathogenic attributes in SLE development.

P2.04.06
MicroRNA profiling in giant cell arteritis: diagnostic and prognostic potential

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MicroRNAs (miRNAs) are small, non-coding RNAs that suppress gene expression at post-transcriptional level. MiRNAs have a proven role in innate and adaptive immunity and inflammatory networks. They have been shown deregulated in various autoimmune diseases emerging not only as biomarkers but also as novel therapeutic targets. No data exist on miRNA expression in giant cell arteritis (GCA), an autoimmune inflammatory vasculitis affecting large and medium-sized arteries.
The present study aimed to evaluate the diagnostic and prognostic potential of miRNAs in GCA.
Expression of 1209 miRNAs was profiled with a microRNA array (Ocean Ridge Biosciences, Palm Beach Gardens, FL) in temporal artery biopsies (TABs) from 7 GCA patients (harboring a transmural infiltrate) *versus* normal TABs obtained from 8 patients thought to have GCA instead having a different disease. MiRNAs showing a >2 fold, statistically significant differential expression with a FDR <10%, were selected for further analyses. Subsequent real-time PCRs confirmed that miR-146b-5p, -146a, -155, -150 and -21 were significantly more expressed in TABs from patients with GCA. Expression of miR-146b-5p was particularly promising in a diagnostic perspective because it was possible to set a threshold level which correctly classified TABs as diseased or normal. MiR-146a and miR-21 are negative regulators of the physiologic immune responses inhibiting respectively IRAK1 and PDCD4. No correlations were found between miR-146 and IRAK1 and between miR-21 and PDCD4 levels in TABs indicating defective mechanisms. Ongoing patients' follow up will let to determine if miRNA levels might predict response to therapy and correlate with GCA aggressiveness.

P2.04.07
MicroRNAs dysregulation in human Chagas cardiomyopathy

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MicroRNAs (miR) are 22 nucleotide-long small RNA molecules that regulate tissue-specific mRNA and protein expression by interaction with complementary 3'UTR sequences and are involved in virtually all cellular processes. Several studies have described the fundamental role of different miR in heart development and cardiovascular disease (CD), but until now there are no data on their role in Chronic Chagas disease Cardiomyopathy (CCC), an inflammatory dilated cardiomyopathy occurring in 30% of Trypanosoma cruzi-infected individuals. We therefore analyzed the expression of eight miRs (miR-1, -133a-2, -133b, -208a, -208b, -214-3p, -146a-5p, -155-5p and -150-5p) by real-time quantitative PCR (qPCR) in myocardial tissue samples from end-stage CCC and idiopathic dilated cardiomyopathy (DCM) patients, using samples from heart donors as controls (CONT). Five miRNAs (miR-1, 133a-2, 133b, 208a, 208b) were significantly downregulated in CCC samples as compared to CONT and the miRNAs: of those, miR-1, -133a-2 and -208b were significantly less expressed among CCC than DCM samples. These three downregulated MiRs targeted multiple genes concordantly upregulated in CCC myocardium, which belong to pathways associated with progression of cardiomyopathy. We also confirmed by qPCR a negative correlation between expression of miR-1 and its target gene, cyclin D1 (cyD1), important in cardiomyocyte hypertrophy. Our results indicate that miRs may play a fundamental

role in CCC pathogenesis by controlling expression of key genes and proteins. Further in-depth studies will assess the full extent of miRs contribution to CCC.

P2.04.08
Does HCMV employ miRNA mediated regulation to counter the antiviral mechanisms to maintain long latency periods in Humans? An in silico study

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MicroRNAs are gaining significance, for their regulatory role in RNA directed gene expression regulation in various spectrums of biological systems such as cell development, differentiation, carcinogenesis and disease pathogenesis to prognosis. Reports pertaining to the immunoevasion role of HCMV miRNAs are beginning, this study was initiated to understand the role of HCMV miRNAs on the human antiviral mechanism such as apoptosis and autophagy. The 3'UTR regions of the genes were probed for the potential binding sites of HCMV miRNAs by RNAhybrid and RNA22 algorithms. The results revealed that genes involved in both the intrinsic and extrinsic pathway of apoptosis and autophagy contain potential binding sites for HCMV miRNAs. Further, HCMV appears to regulate these mechanisms by targeting multiple genes in any pathway with more than one miRNA, suggesting that HCMV uses miRNA mediated regulation in controlled manner. The obtained results strengthens the hypothesis that HCMV employes miRNA mediated regulation for its tissue tropism and long latency periods in humans.

P2.04.09
Anti-microRNA-378 Enhances Wound Healing Process by Up-regulating Vimentin and Integrin beta-3

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Background: Impaired wound healing evolves to be a major public health issue worldwide. Until recently, microRNAs has emerged as key regulators of wound healing. Methods: We developed an anti-miR-378 sponge by inserting multiple-tandem microRNA binding sites. It sufficiently blocks the process of precursor microRNA. Anti-miR-378 transgenic mice were generated and subjected to skin biopsy, causing full-thickness, excisional wounds. Wound size was measured and tissue samples were collected for immunohistochemistry study. Mouse fibroblasts NIH/3T3 was transfected with anti-miR-378 fragments and subjected to function tests. Results: Anti-miR-378 sponge could block mature miR-378 generation in vitro and in vivo. Compared to control mice, enhanced wound healing process was shown in anti-miR-378 transgenic mice. In addition, we found vimentin and integrin beta-3, modulators which are important in wound healing process, increased remarkably in transgenic mice samples. Wound scratch and transwell migration tests showed a greater mobility in anti-miR-378 transfected NIH/3T3 cells, which was due to the up-regulation of vimentin and integrin beta-3. They are confirmed as targets of miR-378, thus their expression could be rescued by anti-miR-378. Overexpression of vimentin could also contribute to fibroblasts differentiation and up-regulation of integrin beta-3 by anti-miR-378 is responsible for angiogenesis. More interestingly, further analysis of inflammatory cytokines in situ revealed a pro-inflammation pattern, which favors wound healing as well. Conclusion: Taken together, we demonstrated that knockdown of miR-378 by endogenous-integrated antisense-fragments can increase the expression of its targeted proteins, vimentin and integrin beta-3, which enhanced wound healing.

P2.04.10
Global effect of IL-10-induced modulation of miRNAs expression on cytokine production by LPS-stimulated human monocytes

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IL-10 negatively regulates inflammation via transcriptional and post-transcriptional inhibition of proinflammatory cytokines production. With respect to the mechanisms acting at the post-transcriptional level, we have recently identified miR-187 and miR-146b as a direct targets of IL-10, and uncovered a novel miR-187-mediated pathway triggered by IL-10 to inhibit LPS-induced TNFα, IL-6 and IL-12p40 production. Moreover, we demonstrated that IL-10 is able not only to increase the expression of LPS-induced miR-187 and miR-146b, but also to down-modulate the expression of LPS-induced miR-9, miR-155, miR-146a and miR-132. In this study, we analyzed the global contribution of IL-10-down-modulated miR-9, miR-155, miR-146a and miR-132 and of IL-10-upregulated miR-187 and miR-146b in the cytokine response of monocytes to LPS. AntagomiRs targeting each of the LPS-induced miRNAs (i.e. miR-9, miR-155, miR-146a and miR-132) together with miR-187 and miR-146b mimics were simultaneously transfected into monocytes, in order to reproduce the effect of IL-10. Cytokine production were qualitatively and quantitatively evaluated in the supernatants of transfected monocytes stimulated with LPS or left untreated. Our data show that while the overexpression and/or silencing of each individual miRNA partially modifies the levels of cytokine produced, only the combination of all miRNA inhibitors and mimics correctly replicates the effect of IL10 on LPS-induced cytokine production. Collectively, our data provide evidence for the differential role played by miRNA during the development of the inflammatory response and candidate the miRNA system as a novel mechanism through which IL-10 can tailor the overall monocyte responses to LPS.

P2.04.11
TNF influences microRNA expression during T cell activation

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MicroRNAs (miRNAs) are recently described posttranscriptional regulators. Altered expression of miR-155 is associated with several immunological diseases, including rheumatoid arthritis (RA). MiR-181a deficiency leads to the increase in the proportion of autoreactive lymphocytes. Dysregulated expression of miR-146a was described in more cell types that are relevant in RA.
In this study, we focused on miRNAs, which may influence the activation of T lymphocytes, and assumed to be involved in the pathomechanism of RA. The aim was to find connection between the dysregulated miRNAs and the presence of proinflammatory cytokines like TNF. The expression of miRNAs was determined in anti-CD3/CD28 activated human CD4+ T lymphocytes by RT-PCR method.
The expression of miR-155 was significantly upregulated during T cell activation, and according to our data this can be attenuated by TNF pretreatment. In case of miR-181a, a downregulation was observed, and this effect was inhibited when cells had been primed with TNF before activation. The activation of T cells leads to increase the expression of miR-146a with the maximal expression at 2 hours after stimulation, and surprisingly the TNF treatment shifted this peak to 6 hours.
Among the characterized targets of these miRNAs there are some proteins of TNF signal transduction (miR-146a: TRAF6, IRAK1) and they can regulate several other key components of T cell signal transduction (miR-155: various targets in fate decisions and Treg homeostasis; miR-181a: PTPN22, DUSP6) as well. Bioinformatic analysis using predicted targets was performed to delineate the extent of involvement of these miRNAs in these pathways.

P2.04.12
The miR-17-92 cluster is regulating naive T cell homeostasis as well as effector and memory differentiation

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The miR-17-92 cluster, encoding 6 different microRNAs, is upregulated upon T cell activation and has been shown to have important effects on T cell-mediated immune responses. To study its role in CD8+ T cells, we used mice expressing CRE recombinase under the control of the CD4 promoter in combination with a floxed miR-17-92 locus. In naïve mice, CD8+ T cell numbers in lymphoid organs and blood were reduced by about 50%. This was also observed in chimera mouse bearing a mix of wild type and 17-92 fl/fl bone marrow. Upon activation in vitro, early T cell proliferation was strongly impaired. In order to follow a virus-specific immune response in vivo, we infected mice with LCMV and analysed the effector and memory response from day 8 to several months after infection. We found that again virus specific and IFN-γ producing cells were strongly reduced. In order to identify intrinsic roles for miR-17-92, we adoptively transferred TCR transgenic OT-1 specific or gp-33 specific knockout CD8 T cells into wild type recipients before infection with either OVA-expressing L. monocytogenes or LCMV. At the early memory phase upon infection, knockout CD8+ T cells were skewed towards central memory cell differentiation (CD127high, CD62Lhigh, KLRG1low). Together, these results demonstrate an important role for the miR-17-92 cluster in the homeostasis and differentiation of CD8+ T cells. Future experiments will explore the potential of modulating these miRNAs for the improvement of immunotherapy with cytolytic T lymphocytes.

P2.04.13
Double stimuli by TLR7 and CD11c+ may change microRNA expression in murine plasmacytoids dendritic cells

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Dendritic Cells (DC) are central key in starting an immune response activating or inhibiting T lymphocytes in specific way. PAMPs as messenger RNA (mRNA) or plasmideal DNA (pDNA) are molecules able to activate DC through toll-like receptor 7 and 9, respectively. However these cells need a regulation, avoiding nonspecific response. The microRNAs (miRNA) are involved in a fine-tuned cell-response at control posttranscriptional level. Then we verified whether different miRNAs and targets are induced by mRNA or pDNA stimuli.
We used DC (anti-$CD11c^+$ or anti-mPDCA-1 magnetic beads separation) from spleen of Balb/c mice stimulated with 10µg of mRNAhsp65 or 10µg of pDNA-HSP65 and incubated per 30 minutes. The miRNA were obtained in triplicate in microarray analysis, using the Agilent platform. Our results showed just one differentially express miRNAs, miR-690 was down-regulated ($p<0,05$) with mRNA-stimuli. Moreover, after the use of anti-mPDCA-1(plasmacytoid dendritic cell especific) beads, DC was not able to show miRNA differential expression, when we use anti-$CD11c^+$ before anti-PDCA-1 we recovery the miRNA differential expression. In addition *in silico* and *in vitro* analysis suggests that prostaglandin E synthasis would be a possible target for the miRNA. Taken together, mRNAhsp65 and $CD11c^+$ stimuli may induce change in miR-690 expression and in target, which may play important role gene therapy.
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P2.04.14
Anti-inflammatory function of miR-146a in human primary keratinocytes and atopic dermatitis

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Previously, miR-146a has been shown to regulate immune responses in different cell types. We carried out miRNA expression profiling and determined elevated expression of miR-146a in keratinocytes and skin from atopic dermatitis (AD) patients. Our results show that miRNA-146a is up-regulated by proinflammatory cytokines, such as TNF-alpha and IL1-beta, but not by IFN-gamma in primary keratinocytes. To study miR-146a functions in human primary keratinocytes, we transfected miR-146a precursors or inhibitors into keratinocytes treated with IFN-gamma, TNF-alpha or IL1-beta. These experiments reveal that miR-146a hinders the capacity of primary keratinocytes to produce several inflammation-related cytokines and chemokines, such as CCL5, IL-8 and IL-6 both in mRNA and protein level. Over-expression of miR-146a suppresses proliferation and cytokine induced apoptosis of primary keratinocytes. mRNA array and pathway analysis of miR-146a-influenced genes demonstrates that miR-146a down-regulates mRNAs encoding proteins from NF-kappaB pathway as well as proteins involved in regulation of apoptosis and proliferation. siRNA inhibition of two miR-146a targets from NF-kappaB pathway, CARD10 and IRAK1 show that both these proteins are needed for production of IL-8, however, only CARD10 influences the expression of CCL5. In addition, our preliminary results indicate that miR-146a-deficient mouse acquires similar Th2 type skin inflammation as wild type mouse in mouse AD model and that the anti-inflammatory function of miR-146a becomes more important in the chronic phase of the skin inflammation. Together, our data show that miR-146a has a strong anti-inflammatory effect in human keratinocytes and its expression level may influence the course and intensity of skin inflammation in AD.

P2.04.15
Negative regulation of Toll-like receptor 4 signaling by the IL-10-dependent miR-146b

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Toll-like receptors (TLRs) play key roles in detecting pathogens and in initiating inflammatory responses but their activity must be tightly regulated to avoid excessive inflammation which could lead to the development of immunopathology. Our work has identified that in human monocytes, stimulation with the anti-inflammatory IL-10 induced the expression of miR-146b and was able to potentiate the effect of LPS which induces miR-146b expression only at later time points. Blocking endogenous IL-10 autocrine signaling resulted in a 40% reduction of miR-146b induction by LPS and when the JAK/STAT signaling pathway activated by IL-10 was blocked, the LPS-mediated induction of miR-146b and the production of IL-10 were significantly reduced. To gain additional insight on the role of IL-10 in the transcriptional regulation of miR-146b in monocytes, we demonstrated the recruitment of Pol II and the enrichment of STAT3 to the miR-146b promoter region after stimulation with IL-10, consistent with IL-10-mediated up-regulation of miR-146b expression. To demonstrate the role of miR-146b in the context of inflammation, we over-expressed miR-146b in the THP-1 cell line using a lentiviral-based system approach. We identified as targets the major components of the TLR pathway including TLR4, MyD88, IRAK1 and TRAF6 and observed a significant reduction in the production of several pro-inflammatory cytokines. Our results show miR-146b as an IL-10-responsive miRNA with an anti-inflammatory activity in monocytes based on multiple targeting of the TLR4 pathway and candidate miR-146b as a new feedback modulator of LPS response possibly involved in the resolution of inflammation.

P2.04.16
The role of microRNA 155 in innate immunity and arthritis

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Background: MicroRNA 155 (miR155) has been demonstrated to be essential for the development of collagen induced arthritis by controlling the generation of autoreactive T and B cells. However, the contribution of miR155 in innate immune cells is not known.
Materials and Methods: We analyzed activation and cytokine production of macrophages and dendritic cells (DCs) in vitro and in vivo. T-cell stimulatory capacity of DCs was measured. MiR155 deficient mice were crossed into hTNFtg mice and arthritis development was analyzed clinically as well as histologically.
Results: MiR155 deficiency did not alter the expression of costimulatory molecules or MHCII expression after stimulation of macrophages and DCs in vitro and in vivo. We also Facs-sorted DCs after stimulation with LPS in vivo and determined the production of proinflammatory cytokines such as IL-23, IL-6 as well as TNF. We did not detect differences between wt and miR155-/- mice. In addition, the T cell stimulatory capacity of wt and miR155-/- was identical. When we analyzed hTNFtg/ miR155-/- mice compared to wt mice, we did not detect differences in the clinical signs and symptoms of arthritis. Histologically, we even found slightly increased synovial inflammation in hTNFtg/ miR155-/- mice compared to wt mice.
Conclusion: In contrast to the pivotal role of miR155 in autoimmunity requiring the adaptive immune system, the role of miR155 in innate immunity seems to be limited. This is emphasized by the fact that miR155 hardly influences the course of TNF-driven arthritis, which is mainly dependent on components of the innate immune system.

P2.04.17
Association of microRNA-146a target gene IRAK-1 polymorphism with enthesitis related arthritis category of juvenile idiopathic arthritis

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Purpose of the study: MicroRNAs are non-coding RNA molecules that play pivotal role in modulating the expression of multiple target genes at the post-transcriptional level. SNP in pre-miRNAs can alter miRNA expression, and polymorphism in target molecules can affect binding to target mRNA. Studies have shown there is association of *miR-146a* target *IRAK*-1 polymorphisms in autoimmune disease. Thus we studied the association between SNPs of *miRNA-146* and it's target *IRAK*-1 with susceptibility to ERA-JIA.
Methods: 115 patients with JIA-ERA were included in the study. 216 blood donors (201 male) with a mean age of 30.5 years served as controls. *miR-146a* (rs2910164) and it's target *IRAK*-1 (rs1059703) at Exon 12 region and *IRAK*-1 (rs3027898) at 3'UTR polymorphisms were done using PCR-RFLP method.
Results: Among 115 patients (103 males), the mean age at onset of disease was 11(4-17) years with mean disease duration 4.6(0.5-12) years. 21 had uveitis, 16 had positive family history, 51 had enthesitis, 57 had inflammatory back pain and all had arthritis. 88 were HLA B27 positive. The genotype frequency for *miR-146a* was similar in controls and patients. *IRAK*-1(rs1059703) genotype frequencies in controls and patients were also similar. But *IRAK*-1(rs3027898) genotype frequencies were different among control and patients [CC (59.72% vs 65.22%), CA (34.26% vs 20.87%) with 95% CI= 0.56 (0.32 - 0.96) and AA (6.02% vs 13.91%) with 95% CI= 2.12 (1.02 - 4.64)].
Conclusion: Polymorphisms at 3'UTR of *IRAK*-1 (rs3027898) with AA genotype was associated whereas polymorphism in *miRNA-146a* (rs2910164) was not associated with susceptibility to ERA-JIA.

P2.04.18
Regulated expression of microRNA-21 is involved in memory T-cell survival

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Objectives: MicroRNAs (miRNAs) are important post-transcriptional gene regulators. Immune cell-type specific miRNA expression patterns have been described. However, the specific role of single miRNAs in T cell activation is still not well known. In this study we investigated the function of miR-21 in the process of T cell activation. We report that miR-21 is a pro-survival factor in the context of memory T cell function.
Methods: Using qRT-PCR we analyzed miR-21 expression in course of anti-CD3/CD28 stimulation of naive (CD4+CD45RO-) and memory (CD4+CD45RO+)-FACS sorted human primary T cells. Viral vector harboring miR-21 inhibitor sequence served to stably inhibit endogenous miR-21. Apoptosis was assessed by measuring mitochondrial potential. Experimental analysis of T-cell specific miR-21 target genes was performed by RISC immunoprecipitation followed by gene expression analysis in Jurkat cell line.
Results and conclusions: Resting memory CD4+ T cells expressed five times more miR-21 than naive cells. Moreover, miR-21 was strongly upregulated upon TcR engagement implicating it's contribution to the activation process. MiR-21 inhibition resulted in an increased rate of spontaneous apoptosis in in vitro activated T cell cultures, especially in the memory T cell population, and induced massive spontaneous apoptosis in Jurkat cells. RISC immunoprecipitation in Jurkat cells overexpressing miR-21, followed by gene expression analysis identified several potential miR-21 target genes relevant to T cell biology. In conclusion, our data provide evidence that miR-21 is a key component in the process of T-cell activation by regulating T-cell survival signaling, especially in the memory T-cell population.

P2.04.19
Aged T cell subsets are characterized by a distinct microRNA signature

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Objective: Decline of immunological responsiveness in elderly correlates with changes in the composition of the T cell compartment. Recently, microRNAs (miRNA) have emerged as important players in the regulation of T cell development, differentiation and function. However, comprehensive insight into the contribution of miRNAs to T cell age-related changes remains elusive. The aim of this study was to investigate the age associated changes in miRNA expression within defined T cell subsets in young and old healthy individuals.
Materials and Methods: T cell subsets (naïve, memory, CD4 and CD8 cells) derived from young and elderly healthy subjects were sorted based on CD3, CD4, CD45RO and CCR7 expression. MiRNA expression patterns were determined using the agilent human miRNA microarray platform (V2) based on Sanger miRbase (release 10.1). Results were validated by miRNA-specific qRT-PCR.
Results: Hierarchical clustering showed differential expression of miRNAs mainly between naive and memory T-cell subsets. Age related differential expression was observed predominantly within the naive CD45RO- T-cell population. 17 miRNAs showed at least 2 fold up- or downregulation in aged naïve T cells. Analysis of individual samples revealed a statistically significant age related upregulation for miR-21, miR-223 and miR-451 by qRT-PCR. Moreover, age-related upregulation of miR-21 was associated with age-dependent accumulation of end-stage differentiated CD8 T cells.
Conclusion: Age-related changes in miRNA expression are found predominantly within the CD45RO- T cell compartment that converges with the accumulation of end-stage differentiated CD45RO- T cells.

P2.04.20

MicroRNA-mediated regulation of TLR4 by immunosuppressive agent increases susceptibility to bacterial infections

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Urinary tract infections (UTIs) are responsible for 7 to 10 million cases each year in the United States. They constitute a major cause of morbidity and mortality in humans, particularly in renal transplanted patients. Acute pyelonephritis (APNs) are frequent complications after renal graft and represent an independent risk factor associated with graft survival. Uropathogenic *Escherichia coli* (UPECs) cause more than 80% of the community-acquired APNs and about half of the APNs in hospitalized patients. Bacterial attachment to mucosal epithelial cells represents the initial step in the pathogenicity and TLR4-mediated recognition of UPECs by epithelial cells elicits potent inflammatory response. The consequences of long-term immunosuppressive drugs on TLR function in transplanted patients have not been yet investigated. We showed that mice treated with cyclosporine A (CsA), widely used for the prevention of graft rejection, are more susceptible to UPEC infection. Moreover, the expression of TLR4 and phosphorylated form of p65 subunit were decreased in LPS-activated epithelial cells incubated with CsA. Increasing concentrations of CsA induced a dose-dependent inhibition of the level of TLR4 mRNA concomitantly with the increase of the expression of the microRNA let-7. The inflammatory response, as well as TLR4 expression, was restored by using an anti-let-7. Taken together, these results show that CsA induces an increase of let-7 downregulating the expression of TLR4, which could explain the greater sensitivity of patients to APN after transplantation. Consequently, let-7 may represent a therapeutic target to prevent interstitial injury and progressive alteration of the kidney function of transplanted patients.

P2.04.21
Genome-wide identification of differentially expressed microRNAs in human plasma cell differentiation revealed crucial microRNAs involved

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MicroRNAs (miRNAs) are the classes of short endogenous small noncoding RNA molecules. They regulate the expression of genes by inhibition of translation or degradation of target mRNAs via binding to the partially complementary sites in the 3'-untranslated region (3'-UTR) of target mRNAs. Recently, accumulating studies indicate that the expression of some miRNAs can be highly tissue-specific in many important cellular pathways. In this study, we focus on identification of the expression profiles of miRNAs and their target genes by genome-wide approaches, deep-sequencing of miRNAs and microarray analysis of cDNA expression, in primary human plasma cell differentiation. A total of 134 miRNAs were detected to be either up- or down-regulated after differentiation. Combined computational analysis with gene expression profile data revealed that gene ontology of miRNAs target genes is indistinguishable from that of overall gene expression. Given that plasma cell differentiation is controlled by some key transcription factors, such as BCL6, BACH2, and PRDM1, we examined the relationship between the differentially expressed miRNAs and key transcription factors. Interestingly, our analysis revealed that the expression of these key transcription factors could be co-regulated by clusters of differentially expressed miRNAs. We are examining the functional role of these miRNAs in plasma cell differentiation.

P2.04.22
Evaluation of expression pattern of miRNA146a in Pakistani rheumatoid Arthritis Patients

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Objectives: The objectives of the present study were to identify the role of miRNA146a in rheumatoid arthritis (RA) pathogenesis and to evaluate expression of miRNA146a and its target genes in RA patients.
Methods: In the present study, expression of microRNA146a (miRNA146a) and its target genes were analyzed using Real time PCR and Semi-quantitative Reverse transcription PCR in blood and synovial fluid of rheumatoid arthritis patients.
Results: An average of 0.7 fold increase was observed in expression of miRNA146a in blood and 14 fold in synovial fluid in rheumatoid arthritis patients as compared to healthy controls. Tumor necrosis factor receptor-associated factor 6 (TRAF6) was down-regulated and its expression correlated with miRNA146a in blood but not in synovial fluid. Interleukin-1 receptor-associated kinase 1 (IRAK-1) and Tumor Necrosis factor alpha (TNF-α) was up-regulated in both blood and synovial fluid. IRAK-1 expression did not correlate with miRNA16a in both blood and synovial fluid while TNF-α correlated with miRNA146a expression in synovial fluid only (R square = 1.4). **Conclusion:** Increased expression of miRNA146a and its statistically significant correlation with expression of TNF-α suggests that miRNA146a can be used as a potential diagnostic biomarker in rheumatoid arthritis.

P2.04.23
Functional analysis for microRNA involving in arthritis

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microRNAs (miRNAs), which are class of post-transcriptional regulators such as short 19 to 23-nucleotide non-coding RNAs, complementarily bind seed sequences in the 3'-untranslational region of multiple target mRNAs, resulting in their suppression of translation or degradation [1]. In the former case, since the mRNA expression of the targets does not any change, transcriptomics approach, such as expression array, cannot identify the targets.
Recent studies shed light on the fine-tuning mechanism of miRNAs in myriad biological processes including development [2], tumorigenesis [3] and inflammation [4]. We have identified enhancement of mir-146a expression in synoviocyte and macrophages of rheumatoid arthritis [5], whilst suppression of them in osteoarthritis [6]. However, the targets of mir-146a and its molecular mechanisms are not still fully identified.
In this study, in order to identify the targets of them, we established cell-based screening for miRNA using luciferase tandemed full length mRNA. We also performed liquid chromatography-tandem mass spectrometry-based shotgun proteomics in gain of function models using adenovirus- and CMV promoter-mediated overexpression.

P2.04.24
Mir-34a promotes the development of Plasmacytoid dendritic cell

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MicroRNAs are a class of small non-coding RNAs that negatively regulate gene expression, causing translational repression and/or messenger RNA degradation. They play pivotal roles in cell development, activation and some diseases occurrences such as tumor, infections and autoimmune diseases. First we established mir-34a chimerical and transgenic mice model, FACS analysis showed that over-expression of mir-34a could induce the increasing of the number of plasmacytoid dendritic cells, as well as conventional dendritic cells, either in chimerical mice or in transgenic mice. Further analysis suggests that mir-34a affected the check-point at PreDC to pDC. Brdu and Annexin V staining indicated that mir-34a did not affect the proliferation and apoptosis of PreDC and pDC, also did not

induce the activation of pDC by detection of CD80/CD86/MHCII. Luciferase assay showed that WNT1 may the target of mir-34a in pDC, indeed, western-blot showed that WNT1 was down-regulated in the BM and spleen cells from mir-34a-TG mice, compared to that in wild type cells. Moreover, over-expression of WNT1 in pDCs could rescue the phenotype of the number increasing of pDCs in mir-34a-TG mice; suggest that WNT1 is the functional target of mir-34a in pDCs. Thus, our studies suggest that mir-34a promotes the development of preDC to pDCs by targeting the expression of WNT1.

P2.05 Immune responses in aging

P2.05.01
How M. leprae Hsp65 influences the immune response in genetically selected aged mice?

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Heat shock proteins may trigger innate immune responses and are involved in immunosenescence and autoimmunity. We characterized some cellular and humoral alterations after intraperitoneal administration of 2.5µg *M. leprae* Hsp65 in genetically selected mice for High (H_{III}) or Low (L_{III}) antibody production (9-months-old) and in its F_1 hybrids. Aged H_{III} female injected with Hsp65 presented a survival decrease of 42% when compared to untreated group (control); no changes in IgG1 or IgG2a anti-Hsp production were observed in H_{III} and L_{III} mice. Regarding the cellular changes, aged H_{III} female Hsp65-group presented amplified frequency in $CD4^+CD154^+CD28^+$ cells (p<0.01) and reduced percentage of B and activated $CD11c^+$ cells (p<0.01) in the spleen, and increased percentage of $CD11c^+$ and $NKG1A/C/E^+$ cells (p<0.01) in the blood compared to control. Hsp65 acts like an imbalance trigger: post-injection, the aged F_1H female Hsp65-group died 2 months after the first death, as observed in aged H_{III} females; however there was no statistically significance compared with F_1H control group. Furthermore, aged F_1H and F_1L female showed amplified frequency of naïve T cells and CD11c cells in spleen (p<0.001). In conclusion, our results confirm the sex dichotomy (sex effect) of the Hsp65 interference in the immunity of aged mice, becoming evident in females. Next, we will characterize innate immune cells in peritoneal cavity after Hsp65 inoculation; in addition, the role of myeloid-derived suppressor cells will be investigated as these cells are increased during ageing process and have been associated with attenuation of experimental autoimmune diseases.

P2.05.02
Interferon signaling pathway: Intrahepatic expression of STAT 1 and interferon resistance in HCV patients

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Hepatitis C virus (HCV) has victimized more than 210 million of the world population and about 7 million of Pakistani population. The only widely used approved therapy for the disease is pegylated interferon alpha but in Pakistan the standard combination therapy interferon plus ribavirin is preferably recommended for HCV genotype 3 (most prevalent genotype i.e., between 75-90% in Pakistan) because of its higher response rate i.e., up to 80%. There are number of viral and cellular factors causing failure to respond to standard interferon treatment. In order to evaluate the cellular factors, interferon signaling pathway was studied. The current study aimed to investigate the role of intrahepatic expression analysis of STAT1 in treatment failure taking age of the patient into consideration. The study was first approved by Institute's Ethical Committee. Liver biopsies and blood from 26 HCV patients resistant to interferon therapy were collected under sterile conditions. Serum markers i.e., liver function tests (LFTs), HCV viral load, genotyping and pre-biopsy tests were taken into consideration. The liver biopsy was then subjected to both liver histopathology status evaluation and PCR based detection of STAT1. STAT1 mRNA was detected in about 96% of the HCV patients resistant to interferon therapy. The investigation showed that STAT 1 expression is not a major factor behind interferon resistance in HCV patients and mean age of the normal and responders (to interferon treatment) group is lower than that of the non-responders.

P2.05.03
Impact of Cytomegalovirus seropositivity on the lymphocytes counts in middle age individuals: increase of CD4+CD62L-CD28- lymphocytes in a sub-group of CMV+ individuals

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The peripheral blood T, B and NK lymphocytes and their subpopulations were numerated by flow cytometry by using four antibody panels of 8 antibodies each, in 200 healthy blood donors of both genders, 18 to 68 years old, of known cytomegalovirus serology (120 CMV- and 80 CMV+).
The T lymphocytes counts are significantly increased in the CMV+ group but are not influenced by gender or age. CD4+ T lymphocytes counts are increased in women and CMV+ subjects. B-lymphocytes are increased in CMV+ individuals but not influenced by gender or age. Absolute counts of NK cells are not influenced by gender age or CMV serological status. CMV+ individuals have lower proportions of NK cells expressing NKp30, NKp46 or KIR3DL1/NKB1, and a higher proportion of HLA-DR+ NK lymphocytes.
The CMV+ subjects have lower frequencies of naive CD8+ T lymphocytes and a higher percentage of CD8+ "effector memory" and terminally differentiated "EMRA" lymphocytes which have lost CD28 and/or CD27 markers. Moreover, an increase of the CD4+ T EMRA / total CD4+ T cells ratio (above 3%, up to 27%) is observed in 25% of CMV+ individuals but in none of CMV- controls. These CMV+ individuals with a high proportion of CD4+ T EMRA might progress toward an Immune Risk Phenotype at a later age.

P2.05.04
Reduced naïve CD8+ T-cell priming capacity in elderly and HIV infected individuals

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Naïve CD8+ T-cells are necessary for the induction of adaptive immune responses upon priming with specific antigens. However, advanced age as well as HIV infection are known to affect T-cell production and the pool of naïve CD8+ T-cells. This deterioration may alter CD8+ T-cell priming capacity, thus making old individuals or HIV infected patients vulnerable to new or reemerging pathogens and less responsive to vaccination. Our objective is to study the effect of age and HIV infection on the priming capacities of antigen-specific CD8+ T-cell precursors, and thus the mounting of effective T-cell responses in humans.
We have developed a simple in vitro assay to assess CD8+ T-cell priming in humans. We used Melan-A as a model antigen, due to the high frequency of Melan-A specific naïve CD8+ T-cell precursors in HLA-A2 healthy donors. Using an accelerated co-cultured of dendritic cells, we analyze antigen specific CD8+ T-cells upon in vitro priming. We compared the naïve CD8+ T-cell priming capacities between healthy donors, elderly individuals and HIV infected individuals.
We found that elderly individuals and HIV infected patients have reduced priming capacities compared with healthy donors. Priming capacities were directly correlated with naïve CD8+ T-cell frequencies. Moreover, we observed that elderly individuals with

better in vitro priming capacities responded better to vaccination in vivo.
Alterations in priming capacity could explain the lack of response to new or recall antigens in elderly and HIV infected individuals. This assay could be used to predict vaccination response in humans.

P2.05.05
Is angiotensin-I converting enzyme (ACE) associated with immunosenescence?

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Aging has been associated with changes in the immune system which interfere with responses to infections and efficacy of vaccination. Angiotensin-I converting enzyme (ACE) plays a role in inflammation and in the generation of progenitor cells from bone marrow. Our aim was to evaluate ACE and immunological parameters in individuals from 61 to 65 years old and in adults (20-30 years old). Blood was collected for ACE activity evaluation (fluorogenic assay), ACE expression in non-lymphocytes cells (CD143+) and T lymphocytes phenotype by flow cytometry. In individuals older than 60 years (G1) we observed a higher median of plasmatic ACE activity (125 nM/min/mg) in comparison with adults (G2, 103 nM/min/mg) in opposition to ACE expression in the membrane of non-lymphocytes (G1=57.4% x G2=71.6%). The median of CD4+ T cells was higher in older individuals (G1=54.1% x G2=49.7%) whereas CD4+ naïve T cells were decreased in this population (CD4+CD45RA+CD27+, G1=40.5% x G2=48.4%). CD8+ T cells percentage was decreased in elderly individuals (G1=16.6% x G2=25.1%) and the same was observed for CD8+ naïve T cells (CD8+CD45RA+CD27+, G1=41.2% x G2=47.3%). Memory CD4+ T cells were higher in elderly individuals (G1=47.1% x G2=33.2%) and the same was observed for CD8+ T cells (G1=22.9% x G2=14.3%). Individuals older than 60 years presented decrease in naïve T cells and also in non-lymphocytes expressing ACE in opposite to the increase in memory T cells and higher activity of ACE. In conclusion, ACE modulation during aging could play a role in immunosenescence.

P2.05.06
SRC homology 2 domain-containing phosphatase-1 (SHP-1) is a negative T cell regulator of T lymphocyte responses associated with aging

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With aging the immune response is decreasing. Our aim was to study the role of phosphatases in T cells in elderly under TCR/CD28 stimulation. We showed that a dysregulation of the Csk/PAG loop in activated T cells from elderly individuals favored the inactive form of phosphorylated Lck (Tyr505). Dynamic movements of these regulatory proteins in lipid raft microdomains were also altered in T cells of aged individuals. We showed that SHP-1 activity was upregulated in T cells of aged donors compared to young subjects. Pharmacological inhibition of SHP-1 resulted in recovery of TCR/CD28-dependent lymphocyte proliferation and IL-2 production of aged individuals to levels not significantly different than those of young donors. Furthermore, we report differences in the active (Y394) and inactive (Y505) phosphorylated forms of Lck in response to T cell activation in elderly donors. Our data suggest that the regulatory role of SHP-1 in T cell activation extends to its involvement in Lck-associated negative feedback in aging. Modulation of SHP-1 activity could restore altered T cell functions in aging, suggesting a powerful tool for improvement of immunosenescence. Our data suggest that the tyrosine phosphatase SHP-1, a key regulator of T cell signal transduction machinery is, at least in part, responsible for the impaired T cell activation in aging.

P2.05.07
MF59-induced ATP-signaling pathway is not impaired by immunosenescence thereby contributing to an efficient adjuvant activity in the elderly

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MF59-adjuvanted influenza vaccine has been developed specifically for the elderly, since this age group typically responds inadequately to traditional influenza vaccines, due to immunosenescence. Several studies have been performed to identify MF59 mechanism of action in pre-clinical models. However, most of these studies have been conducted in young mice (6-8 weeks), which do not reflect the immune response of the elderly. Taking into consideration that several signalling cascades are altered during aging, we wanted to assess which MF59-activated immune events were still active in old mice (> 18 months), and performed a comparison to young mice in most of the relevant assays for MF59 activity.
We found differences in MF59-induced cytokines, with a lower pro-inflammatory response in the elderly. Yet, our results also show that MF59 still acts as adjuvant in elderly mice, enhancing immune cell recruitment, antigen-translocation to draining LNs, CD4 T cell responses and restores HI titers similar to those from young mice immunized with unadjuvanted vaccine. Further, since we recently showed that MF59 induces rapid and transient ATP-release in young mice, we tested functionality of this signalling pathway also in the elderly. Indeed, abrogation of ATP-signaling with apyrase – an ATP-hydrolyzing enzyme - completely blocks MF59-activity also in elderly mice suggesting an important role for extracellular ATP also in this age group. Understanding in elderly mice how the immune system is altered and how MF59 helps to overcome age-related limitations, will give us useful insight for future tailor-made vaccine solutions for this growing patient age group.

P2.05.08
The significance of thymic involution and the function of re-entryed T cells in thymus

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During the aging, the phenomena named thymic involution show that the size of thymus is shrinking and the cellularity is decreased and the structure is disordered. Thymic involution is associated with reduced number of thymic output, meanwhile the number of T cell pool is maintained by the homeostatic expansion of T cells in the periphery. Thymic involution is the key point of immune senescence. Since the aged has worse resistance to tumor, infection and self-immune diseases, thymic involution is not thought as a benefit process to the individual. However, the reasons of thymic involution are not clear until now. Some believe that deficiency of hemopoietic stem cells is the major concern of thymic involution, but most agree with the point that the change of microenvironment is the essential reason of thymic involution. Some results suggest that peripheral T cells could go back to the thymus through some unknown mechanism, most of which are CD44hi, an activated phenotype. The function of these re-entryed T cells is not known. We find that thymic involution might be a beneficial process for individuals as increased numbers of memory T cells which is helpful to the immune response to the secondary infection with the same pathogen. These re-entryed activated T cells may play a role in thymic involution, especially of Th2 subsets.

P2.05.09
Targeting macrophages rescues age-related immune deficiencies in geriatric mice

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Changes to innate cells, such as macrophages and myeloid-derived suppressor cells (MDSCs), during aging in healthy or tumor-bearing hosts are not well understood. We compared macrophage subpopulations and MDSCs from healthy young (6 - 8 weeks) C57BL/6J mice to those from healthy geriatric (24 - 28 months) mice. Spleens, lymph nodes and bone marrow of geriatric hosts contained significantly more M2-macrophages and MDSCs than their younger counterparts. Peritoneal macrophages from geriatric, but not young, mice co-expressed CD40 and CX3CR1 that are usually mutually exclusively expressed by M1 or M2 macrophages. Nonetheless macrophages from geriatric mice responded to M1 or M2 stimuli similarly to macrophages from young mice, although they secreted higher levels of TGF-β in response to IL-4. We mimicked conditions that may occur within tumors by exposing macrophages from young versus geriatric mice to mesothelioma or lung carcinoma tumor cell-derived supernatants. Whilst both supernatants skewed macrophages towards the M2-phenotype regardless of age, only geriatric-derived macrophages produced IL-4, suggesting a more immunosuppressive tumor microenvironment will be established in the elderly. Both geriatric- and young-derived macrophages induced allogeneic T cell proliferation, regardless of the stimuli used, including tumor supernatant. However, only macrophages from young mice induced T cell IFN-γ production. We examined the potential of an IL-2/agonist anti-CD40 antibody immunotherapy that eradicates large tumors in young hosts to activate macrophages from geriatric mice. IL-2/CD40 activated macrophages rescued T cell production of IFN-γ in geriatric mice. Therefore, targeting macrophages with IL-2/anti-CD40 antibody may improve innate and T cell immunity in aging hosts.

P2.05.11
Crucial role of Menin in the regulation of CD4 T cell immunosenescence

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Although altered CD4 T cell functions play an important role in immunosenescence, the molecular mechanisms remain unclear. Menin is a tumor suppressor encoded by the *MEN1* gene that is mutated in patients with multiple endocrine neoplasia type 1. We assessed the role of Menin in T cell functions and found that Menin controls senescence of CD4 T cells. *Menin*-deficient (Men KO) effector CD4 T (Th) cells normally proliferated after antigenic stimulation during the initial activation phase. However, Men KO Th cells showed a markedly reduced proliferation rate in comparison to the control wild-type (WT) Th cells from day 5 after the initial antigenic stimulation, even in the presence of exogenous IL-2. Menin KO Th cells also showed a senescence-like phenotype more rapidly than WT Th cells, based on the expression of senescence-associated β-galactosidase activity and cell cycle regulators. A prolonged activation of RelA/p65, a characteristic feature of senescent cells, was also detected in Menin KO Th cells. In addition, Menin KO Th cells expressed large amounts of pro-inflammatory cytokines, chemokines, enzymes, and angiogenic factors including *Il6*, *Opn, Ccl3, Ccl4, Ccl5, Cxcl2*, *Gzms, S100a4, Pdgfα,* and *Vegfc*. These findings suggest that Menin KO Th cells exhibited a senescent-associated secretory phenotype (SASP). Therefore, Menin KO CD4 T cells underwent premature senescence after antigenic stimulation.

P2.05.12
Gender specific immune status differences in children and adults

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150 healthy children 7 months-18 years and 45 adults 25-45 years of age balanced by sex were studied to identify gender specific immune status differences dependent from sex hormone concentrations. As result of study it was found that up to 14 years of age the major gender-dependent difference was found among innate immunity phenomena: higher efficacy of phagocytic leucocytes was identified in males. The level of phagocytic activity was correlated with serum progesterone concentration. In older age groups: adolescents 15-18 years and adults the adaptive immunity parameters demonstrated gender-specific differences.Th1 lymphocytes absolute count was increased in male adolescents 15-18 years of age and was correlated with dehydroepiandrosterone sulfate level. Th2 lymphocytes level was significantly higher in females than in males and was correlated with estradiol concentration. IgG and IgM concentrations were higher in adolescent girls and females than in male subjects. Innate and adaptive immunity quantitative factors' gender-specific differences correlate with changes of sex hormones concentrations.

P2.05.13
Lipid laden multilocular cells in the aging thymus are phenotypically heterogeneous

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Age-related thymic involution is characterized by decreased thymopoiesis and increased white adipose tissue mainly in the septa region of the thymus. Previously, we showed lipid-laden multilocular cells (LLMC) expressing pro-inflammatory factors inside the aging thymus. Herein, we characterized the distribution, morphology and phenotype of intrathymic LLMC in middle-aged Balb/c mice. LLMC were found in subcapsular region (SC-LLMC) and deeper in parenchyma (P-LLMC), including in perivascular area (PV-LLMC). The number of SC- and P-LLMC increase in the thymus with age. Furthermore, LLMC exhibit fibroblastoid, globular or stellate morphologies in distinct regions of the thymus. Ultrastructural features noticed in LLMC includes numerous lipid droplets of different electrondensities, lipofuscin granules and autolipophagossomes. Autophagic activity was confirmed by immunofluorescence staining for beclin and perilipin (Plin) in these cells. In addition, we noticed membrane contacts between P-LLMC and thymic epithelial cells, lymphocytes or mast cells, which number increase in the thymus with age. Some P-LLMC in a juxtacrine interaction with lymphocytes presented long membrane protrusions resembling telocytes. PV-LLMC contacting thymocytes and endothelial cells were observed as well. Phenotypically, intrathymic LLMC were positively stained for PPAR-γ2 or UCP1, markers for preadipocytes or brown adipocytes, respectively. Moreover, Iba1+Plin+ macrophages, α-SMA+Plin+ or NG2+Plin+ pericytes were identified in the aging thymus. LLMC were negative for cytokeratin expression. In conclusion, intrathymic LLMC are able to interact with various microenvironmental cells and correspond to a population of distinct cell types. LLMC of different phenotypes might play distinct roles in the physiology of the aging thymus and in its involution process.

P2.05.14
Ageing impairs macrophage phagocytosis: impact of altered cellular microenvironment?

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Ageing results in deterioration of the immune system and is associated with an increased susceptibility to infection and impaired wound healing in the elderly. Phagocytosis is an essential process in both wound healing and immune defence. We hypothesised that macrophage phagocytosis is impaired with age which may be due to age-related cellular changes in the peritoneum of aged mice. We investigated the efficiency of peritoneal and bone-marrow derived macrophages from young and aged mice to phagocytose fluorescent particles. Phagocytosis was analysed by flow cytometry and immunofluorescence microscopy. In addition, *in vivo* phagocytosis was measured by intraperitoneal injection of fluorescent particles in young and aged mice. Proportions of immune cell types in the peritoneum of young and aged mice were also characterised. Phagocytosis was significantly reduced in peritoneal macrophages from aged mice compared to young mice, both *in vitro* and *in vivo*. Interestingly, ageing did not significantly alter phagocytosis in bone marrow-derived macrophages generated from young or aged mice in our experimental system. We found a consistent decrease in the percentage of macrophages in the peritoneum of aged mice and this was accompanied by an increase in the percentage of $B220^+$ cells and $CD3^+$ cells. In conclusion, phagocytosis is impaired in macrophages from aged mice. This finding has important implications for defence against infection and tissue repair in the elderly. Future work will investigate impact of increased $B220^+$ and $CD3^+$ cells on macrophage function.

P2.05.15
The effect of anti-CMV response differs in nonagenarians and young controls: a transcriptomic analysis

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Cytomegalovirus (CMV) is a common herpes virus; seroprevalence in the population increases with age, being 85-95% in elderly individuals. CMV seropositivity is associated with dramatic changes in the immune system, especially in the T-cell pool. The exact mechanisms of how CMV affects the immune system are yet to be identified. However, several epidemiological studies show that the strength of the anti-CMV response, quantified as the anti-CMV IgG antibody titer, is of significance in the pathogenesis. The high anti-CMV titer has for example been associated with shortened lifespan. To get information about the immunological processes associated with high anti-CMV response we now performed a genome-wide transcriptomic analysis of peripheral blood mononuclear cells (PBMCs) of 138 nonagenarians and 16 young controls (aged 19-30 years). We identified 1305 and 441 transcripts whose expression correlated ($p<0.05$) with anti-CMV titer in nonagenarians and young controls, respectively. Further, an IPA canonical pathway analysis (Ingenuity® Systems) identified 95 pathways in nonagenarians and 26 pathways in young controls that were associated with the CMV titer. Of these pathways, only 10 were common to nonagenarians and young controls. The most enriched signaling pathway categories in nonagenarians were “Cellular Growth” and “Proliferation and Development” and in the young controls the most enriched categories were “Cellular Immune Response” and “Cytokine Signaling”. From our results it appears that in the nonagenarians the CMV titer has a wide-spread effect on basic cellular functions, whereas in the young controls the main effect is the viral defense.

P2.05.16
Fetal microchimerism induces positive effects on the immune system of elderly individuals

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After a pregnancy small numbers of cells originating from the fetus persist in the mothers, a phenomenon called fetal microchimerism (FMc). It has been suggested to be associated either with positive or negative (e.g. autoimmunity) health effects. We decided to find out whether FMc can still be detected in elderly individuals (nonagenarians) and whether it has any effect on the aging of the immune system. The study cohort consisted of 106 nonagenarians who had given birth to 0-5 sons. DNA was extracted from the whole blood and the Y chromosome specific DYS-14 multicopy gene was used for the quantification of fetal cells with quantitative PCR. 34 samples out of 106 gave positive DYS-14 signal with the mean of 18 fetal cells in milliliter of blood. The quantity of fetal cells correlated positively with the proportion of CD4(+) helper T cells ($p=0.015$, $r=0.238$) and negatively with the proportion of CD8(+) cytotoxic T cells ($p=0.046$, $r=-0.196$). In addition, whole genome transcriptome analysis indicated 635 significantly up-regulated and 1083 down-regulated genes. Further gene pathway analysis revealed 125 significantly ($p<0.05$) overrepresented pathways of which several were immunologically related such as positive regulation of T cell activation ($p=0.0001$) and regulation of lymphocyte activation ($p=0.0003$). In addition 19 pathways were significantly ($p<0.05$) underrepresented. In conclusion, the presence of male fetus derived cells in nonagenarian females seems to induce positive effects on their immune system. This phenomenon appears to be long-lasting as their last pregnancy took place several decades ago.

P2.05.17
Bone marrow T cells from the femur are similar to iliac crest derived cells in old age and represent a useful tool for studying the aged immune system

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$CD4^+$ and $CD8^+$ T cells reside in the human bone marrow (BM) and show a heightened activation state. However, only small sample sizes are available from the iliac crest. Larger samples can be obtained from the femur shaft. Therefore it was the goal to compare the phenotype and function of BM T cells from different sources from elderly persons and to investigate how femur derived BM T cells can serve as a tool to gain a better understanding of the role of adaptive immune cells in the BM in old age. As expected the yield of mononuclear cells was higher from femur than from iliac crest samples. There were no phenotypic differences between BMMC from the two sources. Compared to PBMC, both BM sample types contained fewer naïve and more antigen-experienced $CD4^+$ and $CD8^+$ T cells, which, in contrast to peripheral cells, expressed CD69. Cytokine production was also similar in T cells from both BM types. Femur derived BM samples allowed the generation of T cell lines using non-specific and specific stimulation. On the one hand such lines can be used for studies on the interaction of different types of BM cells. On the other hand the generation of pp65 CMV_{NLV} specific T cell lines can be used in studies on the clonal composition of antigen specific BM T cells. In conclusion, our results demonstrate that BMMC from the femur shaft are a useful tool for studies on the role of T cells in the BM in old age.

P2.05.18
Association analysis between CTLA-4 polymorphisms and bipolar disorder

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Background: The immunogenetics of bipolar affective disorder (BPD) comprises a complex area of study with growing importance. In BPD, massive immune activation might be due to immune dysregulation. The current study investigated whether is a contribution of CTLA-4 SNPs in a Caucasian population with bipolar affective disorder (BPD).
Methods: This case-control association study assessed the association of four common CTLA-4 SNPs (-318 C/T, -1661 A/G and -1722 C/T in the promoter region and +49A/G in exon 1) with BPD subjects. Genotyping was performed in 312 BPD patients and 308 healthy controls by polymerase chain reaction-fragment length polymorphism or amplification refractory mutation system analysis.
Results: The allelic and genotype frequencies of only -1661 A/G SNP were more prominence in BPD patients than that controls (0.0003 and 0.04, respectively). Multivariate logistic regression analysis was demonstrated that G allele carriage genotypes had significantly increased risk of BPD up to 1.9 fold (95% CI; 1.3- 2.7, p= 0.0008). Gender stratification was revealed CTLA-4 -1661 A/G and +49 A/G SNPs are associated with increased susceptibility to BPD only in female patients. In addition, female patients who carried -1661G allele, experienced more depressive episodes than male patients.
Conclusion: Our results suggest that CTLA-4 gene variants might be associated to increased risk of BPD, particularly, in a gender-specific manner.

P2.05.19
Survival, proapoptotic proteins and TNFR induced in senil cataract

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Purpose: Various studies in murine models have suggested alterations in apoptosis of epithelial cells of the lens to cause cataract. Apoptotic proteins have not been described in human, so we consider apoptotic proteome study of senile cataract for a greater understanding of its pathogenesis.
Methods: We performed a continuous curvilinear capsulorhexis to obtain lens epithelium, both patients with senile cataract patients as well as healthy crystalline underwent phacorefractive surgery, samples were placed in a lysis buffer. Once all samples were collected, we proceeded to perform the apoptotic protein microarray, as suggested by the vendor (R&Dsystems) adjusting to a 300mg of protein. After processing the microarray membrane were visualized with iBOX and analyzed density of each point with Vision Works LS software. Data were normalized relative to the positive control and the negative control. We considered as a biological significance more than 2-fold change.
Results: Apoptotic proteome analysis in senile cataract showed that apoptotic and anti-apoptotic proteins were differentially expressed. By analyzing the proteins with which were greater increases, we found that the profile type is found antiapoptotic and survival: clusterin, XIAP, (>7fold change, fc), (6-7fc) (5-6fc) (4-5fc), (3-4fc) (2-3fc)
Conclusions: We observed a survival proteome in general. Importantly we observed a increased expression of TNFR1SFA1, we need more studies to know if this increased is due to a inflammatory microenvironment or is due a response of the signaling of survival in the lens epithelial cells

P2.05.20
CMV infection associates to the expansion of polyfunctional CD8+CD57+ T cells in young individuals

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CMV infection plays is a major factor involved in the development of T cell age-associated changes (immunosenescence). CMV infection is associated in elderly individuals to T cell repertoire shrinkage and clonal expansion of CMV-specific CD8+ T cells with limited functional capacities. It also has a deleterious effect on the efficacy of influenza vaccination in the elderly, suggesting that CMV chronic infection in old age restricts immunological diversity and impairs the immune system functionality. The efficiency of the T cell response is associated with the capacity of responding cells to produce several cytokines (“polyfunctionality” as a marker of quality) rather than with the percentage (quantity) of specific T cells.
The aim of this work is to study in young healthy donors the effect of CMV infection on CD8+ T cells polyfunctionality, degranulation (CD107a) and INFg and TNFa production in response to CMV proteins (pp65/IE-1) and to a nonspecific antigen (SEB).
CD8+ T cells from CMV-seropositive individuals are more polyfunctional than CD8+ T cells from CMV-seronegative, not only in response to CMV peptides, but also in response to SEB stimulation, indicating that CMV infection improves the quality of CD8+ T cells in young individuals. In addition, CD8+CD57+ T cells are expanded in CMV-seropositive young individuals. These CD8+CD57+ T cells are more polyfunctional than CD8+CD57- T cells. Therefore, CMV infection in young individuals enhances the functionality and consequently the quality of CD8+ T cells and this effect is most probably due to the expansion of polyfunctional CD8+CD57+ T cells.

P2.05.21
Association between early development of cellular immunity to cytomegalovirus and Graft-Versus Leukemia effect in pediatric transplantation

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Background: A favorable impact, on graft-versus leukemia effect (GVL), from early cytomegalovirus-(CMV) reactivations after allogeneic hematopoietic-stem cell transplantation (HSCT) has been suggested. The role, in this process, for the immune response triggered by CMV remains to be addressed.
Methods: 108 children (median age 8 years) were included at HSCT following myeloablative conditioning for primary acute leukemia. HSCT were from HLA-matched related (42%) or unrelated (44%) donors. 15% of patients received cord-blood units. CMV-DNAemia was programmed weekly for at least 3 months post-HSCT. Immunity to CMV was evaluated sequentially since the first month post-HSCT using 3H-Thymidine incorporation assay (T-cell proliferation) and intracytoplasmic cytokine accumulation assay (IFNγ secretion).
Results: Median follow-up from transplant was 40 months; the cumulative incidence of recipients with CMV-DNAemia at day 120 was 31% (median time to onset: 26 days) and of recipients with anti-CMV immunity (T-cell proliferation and/or IFNγ secretion) at that time was 38% (median time to onset: 2 months). Among the 89 patients without disease at day 120 multivariate analysis revealed different risk factors for relapse according to anti-CMV immunity and CMV-reactivation (p=0.039), with the lowest risk (7%) in recipients with immunity but no reactivation and the highest risk (40%) in recipients with reactivation but no immunity (HR: 0.06, 95%CI 0.005-0.69, p=0.024).
Conclusions: An association between early development post-HSCT of immunity to CMV and GVL was observed. The mechanisms that may be involved in this process will be discussed.

P2.05.22

Age influences the systemic inflammatory response in patients with community-acquired pneumonia

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Introduction: Community-acquired pneumonia (CAP) is a major cause of morbidity and mortality worldwide. Increasing age has been associated with elevated circulating levels of proinflammatory mediators.
Aim: To determine the impact of aging on the systemic inflammatory response to CAP.
Methods: 201 CAP patients were enrolled. Blood samples were obtained from 171 patients for measurement of inflammatory mediators upon presentation, and day 2, 3, 5, 10 and >30. For the current analysis only patients ≤ 50 and ≥ 80 years were included. Pneumonia Severity Index (PSI) score was calculated at presentation.
Results: This study encompassed 46 CAP patients ≤ 50 years (median 37) and 41 CAP patients ≥ 80 years (median 84). Old patients had more comorbidity, especially chronic obstructive pulmonary disease (49%). In both groups *Streptococcus pneumoniae* was the common causative microorganism. Whereas most young patients had a PSI score of I (54%), 98% of old patients had a PSI score ≥ III ($P < 0.0001$). Four old patients died versus none of the young patients ($P < 0.05$). Old patients demonstrated lower serum C-reactive protein levels on admission and during the course of their disease ($P < 0.001$). The overall serum IL-8 response was higher in older patients ($P < 0.001$), whereas the overall serum IL-1RA response was lower in this group ($P < 0.001$). Serum IL-6 was not discriminative.
Conclusions: Cytokine biomarkers of CAP severity are differentially influenced by a high age. Old CAP patients have lower CRP levels than young CAP patients in spite of more severe disease.

P2.05.23
Age-related changes in cell surface markers of BALB/C mice: A flow cytometric analysis

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Introduction: Age-related phenotypic alterations have been indentified in lymphocytes. However, the effect of aging on lymphocyte surface markers in BALB/c mice was not studied inclusively and systematically in a single study.The aim of this study is to analyze and identify the cell surface markers of lymphocyte populations from various lymphoid organs of young and aged mice.
Material and Methods: Spleen, lymph nodes, mesenteric lymph nodes, thymus, and intraepithelial T lymphocytes were isolated from BALB/c mice. Flow cytometric analysis of lymphocytes was performed.
Results: The CD4:CD8 ratio has declined with age in thymus. In spleen, while the number of $CD4^+CD45R^+$, $CD4^+CD28^+$, $CD4^+CTLA4^+$, and $CD8^+CD28^+$, $NKpan^+$ cell populations have increased with age, TCRαβ+ and $CD62L^+$ cell counts have decreased. In lymph nodes, $CD4^+CTLA4^+$, $CD8^+CD45RB^+$, $CD8^+CD45R^+$, $CD11b^+$, and $CD11c^+$ cell numbers have increased with age but the number of $CD8^+CD28^+$, $CD4^+CD45RB^+$ cells have declined. Partially different results were seen in mesenteric lymph nodes: $CD4^+CD8^-$, $CD4^+CD25^+$, $CD8^+CD25^+$, $CD8^+CD28^+$, $CD62L^+$, and $CD11b^+$ cell counts were less in older mice. In intestinal intraepithelial lymphocytes, the number of $TCR\gamma\delta^+$ cells has decreased with increasing age while $CD4^+CD8\alpha^+$ cell count has increased.
Conclusions: The changes in cell surface markers of T cells of lymphoid organs and mucosal lymphoid tissues in BALB/c mice are crucial during aging. The number of innate immune system cells also differs between young and old BALB/c mice. These results might be important to understand the physiological process of immune senescence and immune regulatory mechanisms in the aging immune system.

P2.05.24
irradiation indeces immune modification in splenocytes

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It is reported that irradiation induces shortened lifespan in mice with dose-dependent. We also previously reported that young-age irradiated mice induces shorten lifespan compared to non-irradiated mice. In addition, the number of lymphocytes in the S- and G2/M-phases was significantly increased in the irradiated mice. However, the detail mechanism is still not fully understood. In the present study, we demonstrated that irradiation at young age induces immune-modulation of splencytes in old age mice. The $p53^{+/-}$ mice were exposed to 3 Gy at 8 weeks (young age) of whole-body irradiation and were sacrificed at 54 weeks of age. Irradiation at young age but not at old age enhanced CDK2 and p21expression in the splenocytes. Western blotting demonstrated phosphorylation of IKKα, IκBα and p65 was induced by irradiation at young age. Furthermore, electrophoretic mobility shift assay demonstrated increased activated-NF-κB in the splenocytes of the mice irradiated at young age but not at old age mice. The expression of IL-6 production was increased in the splenocytes of the mice irradiated at young age. Finally, ATP level in splenocytes was measured, and high level of ATP was observed in the mice irradiated at young age both female and male mice compared to non-irradiated mice. Taken together, these results suggest that young age irradiation induced immune-modulated response in the second lymphoid organ, which relates to be activation of NF-κB, and this effect sustained until old age.

P2.05.25
Cytomegalovirus infection impacts the development of CD4 memory T cells in young individuals

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With increasing age the immune system accumulates memory lymphocytes some of which are senescent on the expense of naive cells. Such a tendency renders the immune system incapable of optimal immune responses. Latent infections fasten this process significantly. Cytomegalovirus (CMV) has been identified as one “master” of this phenomenon. Mechanism of such an impact on the immune system remains unknown, however it has been postulated that this is simply a result of constant immune responses against the virus. We have compared the immune profile of more than 300 young individuals. Employing staining for markers of different stages of memory cells development, i.e. CD4, CD8, CD27, CD28, CD57 and CD45RA, we have found significant differences in the distribution of CD4 subpopulations between CMV+ and CMV- individuals. Surprisingly no significant differences were found in the CD8 subpopulations. In elderly, the immune system inclines towards late-stage memory cells, i.e. CD27-CD28-CD57+CD45RA+/-. We have found this is also true for young individuals infected with CMV. This suggests that CMV directly affect memory/senescent T cell generation. Understanding the mechanism could be essential. Comparing the CD4/CD8 ratio shows no difference between CMV+ and CMV-. Analysing complex data of plasma molecules will soon give us more clues about the impact of CMV on the immune system in aging.

P2.06 Leukocyte trafficking

P2.06.01
CCL17 deficiency is associated with beneficial CNS immune responses and prevents cognitive decline in a mouse model of Alzheimer's disease

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The C-C chemokine CCL17 regulates leukocyte trafficking during inflammation, however, its role in Alzheimer's disease (AD) pathogenesis remains undefined. This study demonstrates that CCL17 controls amyloid β (Aβ) deposition, neuroinflammation, and cognitive decline in a mouse model for AD. CCL17 deficient APP/PS1 mice (APP/PS1-CCL17$^{E/E}$) showed reduced Aβ brain levels, and were protected against neuronal loss and cognitive deficits. Enhanced microgliosis and brain recruitment of Ly6C^{+}CCR2^{+} macrophages expressing mannose receptors associated with elevated brain IL-10 levels pointed to beneficial immune responses in these mice. In the absence of CCL17 we observed accelerated uptake of Aβ, enhanced IL-10 release by activated microglia, reduced expression of the receptor for advanced glycation end products (RAGE) and upregulated Aβ-degrading enzyme neprilysin (NEP) in the brains of APP/PS1 mice. These newly identified roles for CCL17 in regulating microglia function and memory loss suggest that targeting this chemokine may harbor therapeutic potential for the treatment of AD.

P2.06.02
T-cell immunoglobulin- and mucin-domain-containing molecule (Tim)-1 is a novel physiological ligand for P-selectin controlling activated T cell trafficking under inflammatory conditions

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T-cell immunoglobulin- and mucin-domain (Tim) molecules are transmembrane glycoproteins involved in a variety of immune processes. Tim-1 regulates T cell-mediated responses, and has structural similarities with mucosal addressin MAdCAM-1 and mucin PSGL-1. The aim of our study was to investigate a role for Tim-1 in lymphocyte trafficking under inflammatory conditions.
RESULTS. Tim-1 bound P-, E- and L-selectin *in vitro*, and Tim-1 covered microbeads rolled on P- and E-selectin under flow. T_H1 and T_H17 cells from Tim-1 mutant (Tim-1$^{\Delta mucin}$) mice lacking Tim-1 mucin domain had a reduced ability to roll on P-selectin, but not E-selectin, when compared to WT cells, suggesting that Tim-1 is a rolling receptor on T cells. Intravital microscopy studies shown that Tim-1$^{\Delta mucin}$ T_H1 and T_H17 cells had a reduced capability to interact with P-selectin *in vivo*, in thrombin-activated mesenteric venules, and to migrate in the inflamed skin in a model of contact hypersensitivity. Furthermore, in inflamed cerebral venules expressing P-selectin, that Tim-1$^{\Delta mucin}$ activated T cells displayed reduced rolling and arrest, when compared to WT cells, suggesting a role for Tim-1 in the control of primary adhesion also on activated brain endothelium. Finally, Tim-1$^{\Delta mucin}$ T_H1 cells had a reduced ability to migrate in the inflamed CNS, and induce a less sever experimental autoimmune encephalomyelitis compared to WT cells.
In conclusion, our data suggest that Tim-1 represents a novel physiological ligand for P-selectin controlling trafficking of T cells under inflammatory conditions.
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P2.06.03
Expression and function of selectins during human development in utero

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Continuous recirculation of lymphocytes between the blood and lymphoid organs is vital for adequate immune surveillance. Recirculation is mediated by molecular interactions between adhesion molecules on endothelium and their counterparts on lymphocytes. Selectins are a family of adhesion molecules responsible for initial interactions of leukocytes with endothelium. However, relatively little is known about the role of selectins before and at the birth in humans. Here we have studied the expression and function of selectins and their ligands during human ontogeny. We examined cord blood mononuclear cell rolling on and adhesion to stimulated human umbilical vein endothelial cell (HUVEC) monolayer under flow-conditions in vitro in the presence of antibodies against selectins and their counter-receptors. The expression levels of L-selectin, PSGL-1 and cutaneous lymphocyte-associated antigen (CLA) were analyzed by flow cytometry. Immunohistological stainings for endothelial selectins in different tissues during ontogeny were made. We found that the rolling of cord blood mononuclear cells was mediated by L-, E- and P-selectins like in adults. The expression levels of L-selectin, PSGL-1 and CLA were similar on adult and cord blood mononuclear cells. Endothelial selectins were expressed in fetal tissues. P-selectin was expressed already during the first trimester and E-selectin at least from the gestational week 32 onwards. We conclude that selectins are synthetized before birth and are functional thus mediating leukocyte rolling. These results have significance in understanding the mechanisms of inflammatory responses during fetal development and potentially in the use of anti-adhesive therapies.

P2.06.04
Susceptibility of Mycobacterium tuberculosis-infected CCR4 deficient mice is associated with a balance between lymphocytes and Foxp3+ regulatory cells

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Introduction: The control of M. tuberculosis infection requires granuloma formation as consequence of the leukocyte recruitment, partially dependent on chemokine and chemokine receptor. The pattern of chemokine and chemokine receptor expression drives the recruitment of specific leukocyte populations, including lymphocyte subsets. Chemokine receptor 4 (CCR4) is mostly expressed in Th2 CD4+ cells and CD4+Foxp3+ cells. The aim of this study was to assessment the participation of CCR4 in response to pulmonary infection with M. tuberculosis. Methods and Results: CCR4 deficient (CCR4-/-) and wild type (WT) mice were infected with 1x105 bacilli by intra-tracheal route. Seventy days post-infection, Colony-Forming Unit (CFU) number in the lung and spleen, cell subsets and cytokine production were evaluated in the lungs of CCR4-/- and WT mice. At the chronic phase (70 days), CCR4-/- (n=14) mice were more susceptible to M. tuberculosis infection compared to WT (n=14) mice ($p<0.05$). In addition, 70-day infected CCR4-/- mice showed an increase in the total number of CD4+ and CD8+ cells, an increase in the frequency of CD8+ cells, followed by a decrease in the frequency of NK and CD4+Foxp3+ cells compared with WT mice. The lungs of infected CCR4-/- mice also exhibited a significant reduction of IL-17 and production of IFN-γ higher than those observed in infected WT mice. Conclusion: These data show that CCR4 plays a key role at the chronic phase of the infection and suggest that the balance between T lymphocytes and Foxp3+ regulatory cells may drive the resistance for tuberculosis.

P2.06.05

Pivotal role of Ccr1 in murine lupus nephritis

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Systemic lupus erythematosus is a chronic, inflammatory autoimmune disease, the development of which is characterized by a progressive loss of renal function. Such dysfunction is associated with leukocyte infiltration in the glomerular and tubulointerstitial compartments in both human and experimental lupus nephritis. Here, we investigated the role of the Ccr1 chemokine receptor in this infiltration process during the progression of nephritis in the lupus-prone New Zealand Black/New Zealand White (NZB/W) mouse model. We found that peripheral T cells, macrophages, and neutrophils but not B cells from nephritic NZB/W mice were more responsive to Ccr1 ligands than the leukocytes from younger prenephritic NZB/W mice. Acute treatment of nephritic NZB/W mice with the orally available Ccr1 antagonist BL5923 decreased renal infiltration by myeloid cells and T cells, whereas late onset BL5923 treatment reduced tubulointerstitial and glomerular injuries, delayed fatal proteinuria and prolonged animal lifespan. In contrast, systemic and renal humoral autoimmunity was unaffected in BL5923-treated mice, which likely reflected the unchanged numbers of infiltrated B cells in the kidneys. Altogether, these findings define a pivotal role for Ccr1 in the recruitment of T and myeloid cells to inflamed kidneys of NZB/W mice, which in turn contributes to the progression of renal injury.

P2.06.06
Local and systemic effects of chronic peritonitis induced by pristane in the absence of galectin-3

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Recent studies of our group show that galectin-3-/- (gal-3-/-) animals present larger amount of plasma cells in lymphoid organs. Furthermore, in B cell neoplasms, high concentrations of gal-3 provide greater tumor resistance and progression. An important model for induction of plasmacytomas is the injection of pristane, leading to chronic granulomatous inflammation rich in macrophages, plasma cells and peritoneal B cells. Thus, the objectives of this study were to evaluate quantitative and qualitatively peritoneal cavity and lymphoid organs, and analyze formation of oil granulomas due to stimulus of pristane. Animals were intraperitoneally injected with pristane or maintained under physiological conditions. We observed a significant increase of total cell number in peritoneal cavity of injected WT animals, in contrast to reduced cellularity of gal-3-/- animals. Kinetics of cell cycle from peritoneal cavity cells was modified and annexin-V+ cells were increased in gal-3-/- animals. Quantification of peritoneal subpopulations showed an increase in neutrophils, and decreased numbers of both B and T lymphocytes. The spleen presented large amounts of lipid droplets and CD138+ plasma cells abnormally distributed in gal-3-/- animals, forming cell clusters in splenic parenchyma. Tertiary lymphoid organs were formed in both groups, but in gal-3-/- mice less or none lymphoid structures were present in oil granulomas and annexin-V+ cells were increased compared to WT animal. The results obtained allow us to conclude that the kinetics of inflammatory cells occurs differently in the absence of galectin-3, modulating lymphoid and myeloid populations, both in peripheral regions, as peritoneal cavity, spleen and oil granulomas.

P2.06.07
CCR5-dependent T cell migration contribute to squamous cell carcinoma development

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Squamous cell carcinoma (SCC) is one of the most common human cancers worldwide. Recent studies show that Treg cells have a critical role in modulating the antitumor immune responses and consequently the SCC development. Since the accumulation of Tregs at the tumor site is in part due to selective recruitment through the CCR5 and the CCR5-associated chemokines, we investigated the role of CCR5 in the SCC development. The results showed that CCR5 deficient mice are more efficient in controlling papilloma incidence than wild-type (WT) mice. In the absence of CCR5, the percentage of leukocytes in the lymph nodes and tumor was significantly increased. Moreover, the adoptive transfer of CD4+CD25+CCR5+, CD4+CD25−CCR5+ and CD8+CCR5+ T cells to CCR5−/− mice resulted in an increase of papilloma incidence. Interestingly, adoptive transfer of Treg cells induced a SCC lesion more undifferentiated than all the other groups with a higher infiltration of macrophage, dendritic cells and myeloid suppressive cells. Overall, CCR5 is a key receptor for the migration of T cells to the SCC lesions. Thus, a tight control of CCR5+ T cells migration, especially Treg cells, to the SCC lesions could be an important mechanism for avoiding tumor progression.

P2.06.08
The absence of chemokine signalling via CCR5 impairs sepsis outcome

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Sepsis is a systemic inflammatory response that is consequence of the immune system's inability to control an infection. The survival rate during sepsis is dependent on neutrophil recruitment to the infection site, which is mediated by chemokine receptor signaling. In physiological conditions neutrophils express the CXCR family of chemokine receptors. However, it has been showed that chemokine receptors expression profile can be altered under sepsis. In this context, we investigated the possible expression of CCR5 on neutrophils and its role on sepsis. C57BL/6 and CCR5 deficient mice (CCR5-/-) were used to induce sepsis using cecal ligation and puncture (CLP) model. We showed that neutrophils from naïve C57BL/6 mice express high levels of CXCR2 and low levels of CCR5. However, after CLP, in parallel with CXCR2 internalization, neutrophils express higher levels of CCR5. Interestingly, CCR5-/- mice subject to CLP show reduced neutrophil migration to the infection site, increased neutrophil infiltration in lung, increased levels of injury markers and, as consequence, decreased survival rate when compared to C57BL/6 mice. Moreover, we demonstrated that CCR5 has an important role during neutrophil adhesion to the vascular endothelium before transmigration. In summary, we showed that chemokine receptors are modulated on neutrophils during sepsis, whereas the increase in CCR5 expression occurs at the same time that CXCR2 is internalized. Together, our data indicate that CCR5 expression during CLP may represent a protective compensatory mechanism of CXCR2 down-regulation, in order to maintains neutrophil migration to the infection focus and consequently to restrict the infection locally.

P2.06.09
Melatonin regulates the migration of granulocytes into mouse peritoneal cavity via MT2 receptor

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The production of melatonin occurs during the night in response to the darkness and it is inhibited by the light. The aim of our study was to investigate the effect of suppressed (caused by constant lighting) synthesis of melatonin on the migration of granulocytes into mouse peritoneal cavity. BALB/c mice were kept under normal light/dark conditions (LD) and under constant exposure to the light (LL). N-formyl-Met-Leu-Phe (fMCP) was injected into mouse peritoneum to induce migration of granulocytes. The mice were sacrifice 4 hours later and the numbers of granulocytes in peritoneal cavity were counted. The mice kept at LL had significant more granulocytes in the peritoneal cavity than LD mice. This difference was observed during the dark time only. The amount of granulocytes in peritoneum of LD and LL mice did not differ during the light time. LD mice had more granulocytes in the peritoneum in the morning than in the night (when the concentration of melatonin is higher). The injection of MT2 antagonist 4P-PDOT three hours prior fMCP caused increased numbers of granulocytes in peritoneum. rtPCR results showed that melatonin MT2 receptors are expressed in granulocytes and endothelial cells.
Our results demonstrate that melatonin via MT2 receptor negatively regulates fMCP induced granulocyte migration to peritoneal cavity.

P2.06.10
CLEVER-1 as a cell trafficking molecule

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CLEVER-1/Stabilin-1/FEEL-1 is a molecule with two known niches in the host's immune response. It is expressed by a subpopulation of macrophages, where it acts as a scavenger receptor. It is additionally expressed by lymphatic endothelial cells (LEC), inflamed blood vascular endothelial cells (BEC) and high endothelial venules (HEV), where it binds leukocytes in the transmigration phase of the extravasation cascade. Here we explored the significance of CLEVER-1 in homeostatic lymphocyte trafficking to the lymph nodes (LN) with a newly-generated CLEVER-1 knock-out mouse.
WT and KO mice were injected with poly(I:C), a synthetic TLR-3 agonist, to induce lymphopenia. Lymphocyte homing rate was evaluated by sampling peripheral blood. Both the WT and CLEVER-1 KO mice reached the most marked lymphopenia 6 hours after the poly(I:C) injection. However, earlier sampling points revealed that lymphocytes disappear from the blood at a slower rate in the CLEVER-1 KO, indicating that the lymphocyte recirculation rate is potentially slower in the absence of CLEVER-1. These findings were further confirmed with short-term homing experiments using adoptive cell transfer of labeled splenocytes intravenously. In these experiments, mice were sacrificed 2-3 hours after the transfer, LNs were collected and homed cells were quantified by flow cytometry and immunofluorescence microscopy.
These results indicate that CLEVER-1 on HEV is functionally important in regulating lymphocyte entrance into the lymph nodes.

P2.06.11
Collective organization confers chemotactic prowess to lymphoid cells

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Whether lymphoid cells undergo collective migration, a biological process governing tissue morphogenesis and solid cancer invasion, is unknown. We show that both tumoral and non-tumoral lymphoid cell populations can simultaneously adopt collective and individual modes of directed migration when exposed to stable chemokine gradients. In particular, we demonstrate that, when organized as clusters, malignant B cells display a wider chemotactic sensitivity to CCL19 than individual cells. Physical modeling, considering clusters as solid objects, recapitulates cluster motility statistics and predicts that intra-cluster cell cohesion reduces noise and enhances directionality. Strikingly, lymphocyte clusters are resistant to chemorepulsion, a process observed in individual lymphocytes exposed to high chemokine concentrations. We further identify endocytosis and ERK activity as central rheostats to set directional sensing and in particular decision between attraction and repulsion in individual lymphocytes. Our data strongly suggest that clusters resist endocytosis/ERK-mediated directional switch at saturating chemokine concentration by alternating protrusive lymphocytes at the leading edge. In support of intra-cluster dynamics, high-resolution automated tracking reveals complex coordination of lymphocyte orientation, reminiscent of fish school models in which forward migration runs are periodically interrupted by transitory rotation and random phases. Thus, coordinated cluster dynamics confers distinct chemotactic properties, highlighting unexpected features of lymphoid cell migration that are potentially relevant for tissue homing and dissemination.

P2.06.12
Phenotypic characterization of CLEVER-1 knock-out mice

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Common Lymphatic Endothelial and Vascular Endothelial Receptor-1 (CLEVER-1; also known as Stabilin-1 /FEEL-1) is a large cell surface glycoprotein composed of distinct repetitive elements including several Fas-domains, EGF-like repeats, two RGD-motives, and a Link-domain. CLEVER-1 is constitutively expressed on lymphatic and sinusoidal endothelium as well as on type 2 immunosuppressive macrophages. Upon inflammation it is also induced on continuous flat-walled vascular endothelium. CLEVER-1 has been shown to support leukocyte adhesion and transmigration and to function as a scavenging receptor. In order to study the role of CLEVER-1 in vivo we have generated a knock-out mouse line for this protein. In addition, to be able to address the importance of CLEVER-1 in macrophages, we have also made a conditional knock-out mouse line for CLEVER-1 and crossed the line with mice carrying the gene for Cre-recombinase under a myeloid cell lineage specific promoter. Studies using several experimental models for inflammation and cancer are now in progress. The results obtained thus far confirm the role of this multifunctional protein in leukocyte and cancer cell trafficking and corroborate the results obtained, when using anti-Clever-1 antibodies in similar models with wild-type mice. Furthermore, during characterization of the two genetically modified mouse lines also new functions for CLEVER-1 were discovered.

P2.06.13
Studies on the human primary amine oxidase/sialic acid binding Ig-like lectin -9 and -10 interactions

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Leukocyte trafficking from blood vessels into inflamed tissues is essential in maintaining health. Human primary amine oxidase (hAOC3) on the endothelium is a significant player in the trafficking with a still unknown mechanism. The first hAOC3 counter molecules have been identified being sialic acid binding Ig-like lectins -9 and -10 [SIGLEC-9 and -10; Kivi et al. (2009) Blood 114:5385; Aalto et al. (2011) Blood 118: 3725]. Our objective is to characterize the

mechanisms of hAOC3 in the leukocyte trafficking by studying its interaction with SIGLEC-9 and -10.
For the interaction studies we have put up an insect cell production system and purified the protein by chromatographic methods. The binding constants for hAOC3/SIGLEC interaction were determined by a surface plasmon resonance. The mutagenesis of specific arginine residues of SIGLECs was performed to study the importance of these residues in the interaction. The effect of active site residues of hAOC3 on the SIGLEC binding is being addressed. We are also studying the activity of hAOC3 towards the recombinant SIGLECs. We will aim for detailed characterization of the interaction via the co-crystallization of hAOC3/SIGLEC complex. Finally, we will test if hAOC3/SIGLEC interaction influences SIGLEC signaling in neutrophils.
We have been able to produce a soluble extracellular part of SIGLEC-9 as well as a C2-C3-fragment of SIGLEC-10 in the insect cells. The SPR studies indicate specific binding of both SIGLECs to the immobilized hAOC3. The current status of the activity and signaling studies will be presented.

P2.06.14
A novel role for GIRK channels in the immune system

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The physiological role of G protein coupled potassium channels (GIRK) is to translate chemical transmission to electrical signaling, mainly in excitable tissues. Using real-time PCR, electrophysiological measurements and western blot analysis we can now show novel expression of GIRK4 channels in immunological tissue from the spleen, bone marrow, lymph nodes and the peritoneal cavity. FACS analysis reveals that GIRK4 channels are expressed in B but not T lymphocytes. In B lymphocytes, GIRK4 was found to be differentially expressed in naïve mature lineages including the natural antibody secreting B-1 cells and regulatory B-10 cells. In GIRK4 null mice, relative B cell populations in the spleen, inguinal lymph nodes and the peritoneum have been found to be affected. In addition, *in vitro* CXCL13-dependant chemotactic migration assays revealed a two-fold increase in migration of B cells in GIRK4 null mice as well as in wt cells in the presence of Tertiapin-Q, a GIRK-specific channel blocker. This increase was found to be dependent on B cell trans-membrane potential. These findings are the first example of the involvement of G protein coupled ion channels in B cell function, and thus serves as a novel mechanism that translates chemical information into electrical activity in the immune system.

P2.06.15
Integrin alpha 4 differentially affect the homing of effector and regulatory T cell subsets

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Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS), mediated by autoreactive myelin-specific T cells. The immune system disposes of different ways to control effector T cell populations such as myelin specific Th1 and Th17 cells. Regulatory T cells (Tregs) expressing the transcription factor Foxp3 represent the major part of this control. The neutralization of alpha 4 integrin (via Tysabri) is currently used as a second line of treatment for MS and it is thought to prevent the entry of most immune cells in target tissues. Recently, we showed that Itga4 differentially affect the homing of effector T cells and was required for the stability and the homing of the Th1 cells. Our study was designed to understand how Itga4 is controlling regulatory T cells homing and functions during EAE. We used different approaches *in vivo* to evaluate the role of Itag4 on $Foxp3^+$ regulatory T cells. We show that Itga4 is not required for the homing of regulatory T cells in the CNS during EAE and that Itga4 deficient regulatory T cells can limit disease development.
This study reveals that Itga4 differentially affects the homing effector and regulatory T cell subsets during EAE. In conclusions, our work suggests that the development of an anti-Itga4 antibody targeting T cells in particular can be beneficial to increase the suppressive functions of Tregs, to block the homing of Th1 cells and limit side effects associated with Tysabri therapy.
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P2.06.16
Contribution of L-selectin to activated CD4 and CD8 T cell migration into dermal inflammation

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The selectins and α_4 integrins are key mediators of lymphocyte migration out of the blood. The role of L-selectin in lymphocyte homing to LNs is well established, and neutrophil migration to inflammation can be mediated partly by L-selectin. The contribution of L-selectin to T cell migration to inflammation is unknown, as is its role compared to E-and P-selectins and $\alpha_4\beta_1$. To investigate the involvement of L-selectin in migration *in vivo*, T cells were activated with anti-TCR and anti-CD28 and their receptor expression and migration to dermal inflammatory sites determined. L-selectin expression declined in the first 72 h of activation, but by 120 h increased with ~85% CD4 cells positive, and 50-80% of cells expressing E-and P-selectin ligands. L-selectin and α_4 blockade each partially inhibited homing to peripheral and mesenteric LNs, and together abolished it. Anti-L-selectin and anti-α_4 each inhibited 30-50% of the migration to interferon-γ, TNF, LPS, poly I:C and to DTH, and the combination reduced it by 80-90%. Anti-E-selectin was most effective at blocking migration to DTH, and in combination with anti-L-selectin and anti-α_4 mAb CD4 cell migration was virtually eliminated. P-selectin blockade had little effect, but further deccreased migration in combination with anti-L-selectin and abolished migration in the presence of E-selectin blockade. CD8 cell was much less inhibited than CD4 cell migration to most inflammatory sites.
Thus, L-selectin is up-regulated on activated CD4 cells, is an important mediator of migration to dermal inflammation, and together with $\alpha_4\beta_1$ and E-selectin facilitates this migration. (Supported by CIHR)

P2.06.17
Podoplanin (Aggrus)-bound CCL21 regulates adhesive migration of T lymphocytes on the cell surfaces of lymphatic endothelium

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The surface-immobilized form of the chemokine CCL21 causes random migration of CCR7-positive lymphocytes by activating integrins in order to induce cell adhesion. In this study, we focused on adhesive migration of lymphocytes on lymphatic endothelium. First, we detected CCL21 on cell surfaces of lymphatics in murine ears and on those of cultured human dermal lymphatic endothelial cells (LEC). We found that the distribution pattern of CCL21 on the apical surfaces of LEC was "dot-shaped". Further morphological analysis revealed that CCL21 colocalized with podoplanin (also known as aggrus), a lymphatic-specific transmembrane glycoprotein, at microvilli on the apical surfaces of LEC. We confirmed the binding ability of podoplanin to CCL21, using surface plasmon resonance analysis and in situ PLA analysis. We also confirmed the contribution of podoplanin to immobilization of CCL21 on the cell surfaces, by overexpression and knockdown of podoplanin in blood endothelial cells (BEC) and LEC, respectively. Next, we investigated the significance of podoplanin-expression on microvilli formation. We found that microvilli and phosphorylated ERM (exrin-radixin-moesin) proteins were reduced by knockdown of podoplanin in LEC. We also found that mutant form of podoplanin, lacking the cytoplasmic ERM- binding domain, was not accumulated to microvilli. Finally, we examined the stimulatory role of podoplanin-bound CCL21 on the adhesive migration of T lymphocytes on lymphatic endothelium in vitro, using time-lapse image analyses.

P2.06.18
TCR-Induced Activation of LFA-1 Involves Signaling through Tiam1

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Binding of leukocytes to cells and surrounding molecules, leukocyte adhesion, is of critical importance for immune system and has to be strictly regulated. The most important molecule for leukocyte adhesion is the leukocyte specific ß2 integrins. The integrins need activation to become functional, but the molecular events resulting in adhesion have remain a mystery or incompletely understood. T cell activation leads to the phosphorylation of numerous proteins in the cell. In human T cells, activation through the TCR (T cell receptor) results in specific phosphorylation of the T758 on the ß2 chain of LFA-1. We now show that this phosphorylation leads to downstream binding of 14-3-3 proteins, followed by engagement of the guanine nucleotide exchange factor protein, Tiam1 and Rac1 activation. Down regulation of the signaling molecules inhibits LFA-1 activity. Activation by the chemokine stromal cell-derived factor-1a also results in T758 phosphorylation and integrin activation. Thus, TCR and chemokine activation converges on LFA-1 phosphorylation, followed by similar downstream events affecting adhesion. Ultimately this will be of importance for understanding diseases like cancers and autoimmune diseases, and for the development of methods to control inflammation.

P2.06.19
17-parameter flow cytometry: immunophenotyping cellular infiltration during flavivirus encephalitis in the mouse

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Background: Viral encephalitis is a major cause of morbidity and mortality worldwide. The pathogenesis of flaviviral encephalitis is a complex process, where the immune response elicited by the virus contributes substantially to pathological damage in the brain. As this process is still incompletely understood, there is a critical need for a detailed analysis and characterization of cellular infiltration in the infected brain, to elucidate the critical mediators of immunopathology and viral clearance.
Aims and outcomes: The aim of this investigation was to design, evaluate, implement, and standardize a 17-parameter flow cytometry panel to immunophenotype cellular infiltration in virally infected brain and lymphoid tissues.
Method: Brains, lymph nodes, and spleens were isolated from C57BL/6 mice on day-7, following intranasal inoculation with West Nile virus (WNV). Tissue was processed and stained with a mixture of 12-15 antibodies. Cells were analyzed on a 5-laser Becton-Dickinson (BD) FortessaTM, and the data analyzed using FlowJo software.
Results: The panel allowed for simultaneous identification of B cells (CD19, B220), CD4 and CD8 T cells (CD3, CD4, CD8), NK cells (NK1.1), NKT cells (NK1.1, CD3), neutrophils and other granulocytes (Ly6G, Ly6C, CD11b, SSc), monocytes, microglia, and macrophages (CD45, CD11b, CD11c, Ly6C, F4/80, MHCII), dendritic cells subsets (CD11c, MHCII, CD11b, CD8, CD4, CD103), and plasmacytoid dendritic cells (CD11c, MHCII, B220).
Conclusion: We have developed an immunophenotyping panel that allows simultaneous investigation of all major subsets of leukocytes that are relevant to immune infiltration during viral encephalitis. This will provide an excellent research tool for the future.

P2.06.20
CD69 expression and leukocyte distribution in CD69-BAC transgenic mice

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Expressed on almost every leukocyte, CD69 function has been related to co-stimulation in lymphocytes, inhibition of pro-inflammatory responses and distribution of leukocytes. Here we give new clues on the complex regulation of CD69 expression and its effects in the immune system. We observed alterations in several leukocyte populations' distribution in CD69-overexpressing mice generated by BAC transgenesis. These transgenic mice exhibit higher expression of CD69 than the wt littermates in all leukocytes though the CD69 expression of the transgenic mice correlates with the endogenous levels of cell type involved. The resulting phenotype observed consists in an enhanced presence of single-positive thymocytes and reduced presence of T-cells in spleen and LNs. Regulatory T cells are also reduced respect to the wt mice in periphery, although the proportions between Tregs/non Tregs were augmented, showing a different influence of CD69 expression on the distribution between regulatory and effector T cells. B cell expression of CD69 is high in BM of tg mice, though the B subpopulations are similar to wt mice, indicating the B cell development is not affected. However, the numbers of B cells are reduced in spleen but surprisingly not in lymph nodes. In addition, cDC numbers are reduced in spleen and LNs, whereas granulocytes and NK cells numbers were not significantly altered. In sum, this CD69-overexpressing mice conforms a good model to study the effects of CD69 on leukocyte migration and distribution.

P2.06.21
Induction of cytosolic phospholipase $A_2\alpha$ is required for adipose neutrophil infiltration and hepatic insulin resistance early in the course of high fat feeding

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While in established obesity adipose tissue inflammation likely contributes to dysfunctional fat-liver crosstalk, whether this occurs in short-term adaptation to dietary surplus is unclear. Here we set to explore if the adipose tissue infiltration by neutrophils that occurs 3-7d after initiating high-fat diet (HFD) could contribute to the early occurrence of hepatic insulin resistance and to determine the role of cytosolic phospholipase$A_2\alpha$ ($cPLA_2\alpha$) in this process. HFD for 3 days (3dHFD) caused a significant upregulation of $cPLA_2\alpha$ in peri-epididymal fat and the liver. A specific antisense oligonucleotide (AS) effectively prevented $cPLA_2\alpha$ induction and neutrophil infiltration into adipose tissue, an effect likely involving MIP-2. AS also protected against 3dHFD-induced hepatic insulin resistance. To sort out the role of adipose neutrophil infiltration in hepatic insulin resistance (independent of $cPLA_2\alpha$ induction in the liver), mice were intraperitoneally injected with anti-ICAM-1 antibodies. This effectively prevented neutrophil infiltration without affecting $cPLA_2\alpha$ or MIP-2 expression. Nevertheless, similarly to AS, 3dHFD-induced hepatic insulin resistance was prevented. Adipose tissue secretion of TNFα was increased by 3dHFD and was prevented by AS or ICAM-1 antibodies treatment. We propose that an acute, $cPLA_2\alpha$ dependent neutrophil-dominated inflammatory response of adipose tissue contributes to hepatic insulin resistance in short-term hyper-nutrition.

P2.06.22
Deficiency of the leukocyte integrin inhibitor, Del-1 exacerbates experimental autoimmune encephalomyelitis through increased Th17 cells infiltration

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Del-1 is an endogenous endothelial-derived inhibitor of LFA-1-mediated leukocyte adhesion (Choi et al. Science 2008). As Del-1 has high expression in brain, we assessed here its role in

neuroinflammation in experimental autoimmune encephalomyelitis (EAE), the rodent model of multiple sclerosis. We found decreased Del-1 expression in the inflamed spinal cord of mice subjected to the EAE protocol. Moreover, Del-1-deficiency in mice resulted in increased EAE severity, associated with increased inflammatory cell recruitment to the inflamed spinal cord. Immunohistochemistry showed that demyelination and axonal damage of the spinal cord was higher due to Del-1-deficiency. Recently, we proved that Del-1 suppressed IL-17-triggered inflammation (Eskan et al., Nat Immunol, 2012). Consistent with this finding, we found that the infiltration of IL-17 producing $CD4^+$ T cells (Th17 cells) was significantly increased at the onset of the disease. This resulted in enhanced infiltration of $CD45^{high}CD11b^+$ monocytes/macrophages that was up to the effector phase of EAE. Subsequent studies revealed that $IL17R^{-/-}$ deficiency reversed the aggravated EAE phenotype of Del-1-deficiency, accompanied by a decrease of leukocyte infiltration into the spinal cord. Our results suggest that Del-1 deficiency exacerbates EAE disease severity by elevating Th17 cell infiltration to the inflamed spinal cord, which implies that Del-1 could be used as a therapeutic approach for neuro-inflammatory disorders.

P2.06.23
Possible role of Myo1g in vesicular traffic of different molecules of B lymphocytes

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Eukaryotic cells are compartmentalized into membrane structures that establish and maintain defined microenvironments. These structures are connected and interchange components trough a complex mechanism named vesicular traffic. The regulation of this process involves coordinated function of different molecules including members of the myosin superfamily. Class I myosins are proteins with a conserved structure that consists of three regions: head, neck and tail. There are 8 class I myosins in mammals, ranging from myosin 1a to 1h, which are molecular motors that interact both with actin fibers and plasma membrane phosphoinositides. Myosin 1g (Myo1g) binds primarily to PIP2 and PIP3, these phosphoinositides are significantly present in several types of vesicles. We detected the presence of Myo1g in membrane protrusions in B lymphocytes such as microvilli and dendrites-like structures. Also we evaluated phagocytic index of Myo1g deficient B lymphocytes, finding that Myo1g deficiency result in an increased phagocytic activity. Myo1g deficient B lymphocytes show a decrease in the surface expression of CD44 and LFA-1 during activation conditions with LPS plus IL-4 for 48 h. Myo1g deficient B lymphocytes also adhere and spread less to different substrates. As these adhesion molecules are recycled in caveolin-dependent vesicles with lipid rafts, we investigated the presence of Myo1g in lipid rafts, finding an enrichment of myo1g on these microdomains of the plasma membrane. In conclusion, we showed that Myo1g has a role in vesicular traffic in murine B lymphocytes, specifically in caveolin-dependent vesicle recycling.

P2.06.24
AChR-specific T cell trafficking in the thymus of chronic Experimental Autoimmune Myasthenia Gravis

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The immune effector mechanisms and the target autoantigen(s) have been delineated in Myasthenia Gravis (MG), however the events that lead to loss of self-tolerance are not fully understood. The involvement of the thymus has long been reported in MG but its role remains elusive also because limited information has been obtained from experimental MG (EAMG), where thymus has been investigated without conclusive data.

We observed an enlargement of the thymus, suggestive of tissue hyperplasia, in EAMG animals at disease manifestation (wk=4) and in chronic phase (wk=10) as compared to age-matched PBS/CFA or acute EAE animals (wk=2). To better investigate this observation, from GFP+ Lewis rats we generated CD4+ T cells, specific for the immunodominant 97-116 epitope of the rat AChR, and T cell blasts were i.v. injected in EAMG animals. We showed an accumulation of GFP+ T cells in thymic medulla, but not in the cortex; this in vivo migratory pattern, observed selectively in EAMG animals, was specifically associated to AChR-responsive T cells, since OVA-specific GFP+ T cells injected in EAMG animals failed to re-circulate to the thymus.
Although thymocyte trafficking is generally considered to be unidirectional, we demonstrated that immunocompetent T cells migrate to the thymus in a pathological situation. Specific lymphocyte re-circulation to the thymus might be an important step during the onset phase of EAMG, when numerous peripheral T cells acquire an activated phenotype. Our data point to a direct link between auto-antigen activation and thymic involvement in the animal model of Myasthenia Gravis.

P2.06.25
Role of CD38 in regulating the migration of B cells in Chronic Lymphocytic Leukemia

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Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in adults and it is characterized by the proliferation of a malignant clone of B-cells. Progressive disease is defined by the infiltration of leukemic cells into lymphoid tissues where they receive pro-tumour signalling. Understanding CLL cell migration and retention within lymphoid tissues will be essential to devise new treatment strategies. High expression of the surface molecule CD38 on CLL cells is an adverse prognostic marker. Although CD38 has been linked to migratory function in CLL, its role in the migration remains unclear. In this study, we have focussed on analysing leukocyte migration using CLL cells (Mec-1 cell line) over-expressing CD38 compared to control cells. CD38 over-expression enhanced CLL cell spreading on VCAM-1 in response to the chemokine CCL21. Time-lapse microscopy analysis showed that CD38 over-expression reduced the ability of CLL cells to crawl on endothelial cells and transmigrate through an endothelial cell monolayer towards CCL21. In contrast, CLL cells over-expressing CD38 showed markedly enhanced chemotaxis towards CCL21, which correlated with increased Rac1 activity and increased calcium mobilization in response to CCL21. Taken together, our data suggest that CD38 expression alters key steps in CLL cell motility. Current studies aim to identify the critical signalling molecules regulating CD38-dependent responses in CLL.

P2.06.26
The autotaxin/lysophosphatidic acid is a regulator of lymphocyte extravasation at the high endothelial venules of lymph nodes

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While lymphocyte extravasation from the high endothelial venules (HEVs) of lymph nodes is crucial for the maintenance of immune homeostasis, its molecular mechanism remains unclear. Here we report that lymphocyte transmigration across the basal lamina of the HEVs is regulated at least in part by autotaxin (ATX) and its end-product, lysophosphatidic acid (LPA); ATX is a HEV-associated

ectoenzyme that produces LPA from lysophosphatidylcholine (LPC), which is abundant in the systemic circulation. In agreement with selective expression of ATX in HEVs, LPA was constitutively and specifically detected on HEVs by MALDI imaging mass spectrometry. In vivo, inhibition of ATX impaired the lymphocyte extravasation from HEVs without affecting lymphocyte rolling and adhesion at HEVs, and induced lymphocyte accumulation within the endothelial cells (ECs) and sub-EC compartment; this impairment was abrogated by LPA. In vitro, both LPA and LPC induced marked increase in the motility of HEV ECs; LPC's effect was abrogated by ATX inhibition, whereas LPA's effect was abrogated by ATX/LPA receptor inhibition. In in vitro transmigration assay, ATX inhibition impaired release of lymphocytes that had been migrated underneath HEV ECs, and these defects were abrogated by LPA. Collectively, these results strongly suggest that HEV-associated ATX generates LPA locally, and LPA in turn acts on HEV ECs to increase their motility, promoting dynamic lymphocyte-HEV interactions and subsequent lymphocyte transmigration across the basal lamina of HEVs at steady state.
Drs. A. Kubo, M. Suematsu, H. Hayasaka, S. Okudaira, J. Aoki, T. Tanaka, H. Albers, and H. Ovaa have collaborated in this work.

P2.06.27
Langerhans cell penetration through the epidermal- dermal barrier

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Upon inflammation Dendritic cells (DCs) translocate from peripheral sites of antigen uptake into the draining lymph node where they present the processed antigens to T cells. Most skin DCs reside in the dermis from where they crawl into the afferent lymphatic vessels. Langerhans cells (LCs), as they colonize the epidermis, need to cross the basement membrane (BM) of the epidermal-dermal junction before proceeding towards the lymph node. BM penetration is the prototype of a tissue invasion event, which has been extensively studied in culture but rarely addressed in a physiological setting. We establish LC invasion as a novel experimentally tractable model to study the cell biology of BM penetration, allowing us to explore physical and molecular interactions.
By inference from other cell systems DCs might require chemotactic signals, substrate adhesion via integrin receptors, actin cytoskeletal dynamics and pericellular proteolysis to act in concert in order to erode and infiltrate the BM. We explore these different aspects by employing intravital live cell imaging in conjunction with fluorescent reporters in mice harboring DCs conditionally deleted for different candidate genes to dynamically visualize invasion *in situ*. To complement the *in vivo* approach we further developed a reductionist *ex vivo* setup where bone marrow derived DCs invade decellularized skin. Such a reductionist setting provides a much higher level of experimental accessibility.
The live imaging of LCs transmigration *in vivo* and *ex vivo* allows us to uncover basic principles of cell migration that are equally relevant for immunology, embryonic development and cancer biology.

P2.06.28
Identification and Characterization of a Monocyte CXCL4 Receptor

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Activated platelets release micromolar concentrations of the chemokine CXCL4/Platelet Factor-4. The deposition of CXCL4 is implicated in the pathogenesis of atherosclerosis, inducing signaling cascades in monocytes and a distinct transcriptional profile of monocyte maturation via an unknown receptor.
Using the monocytic line THP-1 we observed that CXCL4 could induce pertussis toxin-sensitive intracellular calcium flux and migration, implicating a G protein coupled receptor (GPCR) in the transduction of the CXCL4 signal. Although CXCR3 has been reported to function as a T-cell CXCL4 receptor, THP-1 cells were unresponsive to CXCR3 ligands and CXCL4-mediated migration was also insensitive to a CXCR3-specific antagonist. By interrogating a panel of GPCR transfectants, we identified a cDNA that when expressed in a murine Pre-B cell line could confer sensitivity to CXCL4 in chemotaxis assays and confer the ability to bind a CXCL4-Fc fusion protein generated in house. Using a novel assay of real-time cell migration we observed that CXCL4 could drive the migration of freshly-isolated monocytes which was sensitive to blockade with a specific antagonist.
Identification of the monocyte CXCL4 receptor should provide a platform for the further characterization of this enigmatic chemokine.

P2.06.29
Involvement of CXCR3 and CXCR4 in natural killer cell trafficking into bone marrow during multiple myeloma growth

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Natural Killer (NK) cells represent an important weapon for the immune system in the defense against hematologic malignancies. In particular, NK cells have been shown to be effective against Multiple Myeloma (MM), and are thus under investigation as potential tool for cellular immunotherapy.
In this study we analyzed how NK cell trafficking capacities and effector functions are modulated during tumor growth in the BM, using a MM mouse model.
Initially, we highlighted a differential distribution of NK cell subsets within the BM, which were defined according to the expression of the maturation markers CD11b and KLRG1: at an early stage of tumor growth, the frequency of KLRG1+ NK cells increased, while the total NK cell number was slightly reduced. Furthermore, the pattern of chemokine receptor expression on NK cells was altered during MM development: indeed, when tumor growth was low, CXCR3 was downmodulated, while at later times both CXCR3 and CXCR4 were upregulated as compared to control mice.
By competitive adoptive transfer experiments, we found that while KLRG1+ NK cells similarly migrated to BM of control and tumor-bearing mice, KLRG1- NK cell homing to BM in tumor-bearing mice was impaired.
Moreover, changes in NK cells trafficking behavior were associated with alterations in CXCR3 and CXCR4 ligands expression in BM tumor microenvironment.
Taken together, our data indicate that selected NK cell subsets are retained in the BM depending on the stage of MM growth, and that this differential trafficking might be caused by alterations of chemokine receptor/ligand pairs in tumor microenvironment.

P2.06.30
Natural posttranslational modification of CXC chemokines modifies their in vitro and in vivo activity

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Purification of natural CXC chemokines from conditioned medium of stimulated PBMC and fibroblasts revealed multiple posttranslationally modified forms. Several modifications, i.e. proteolytic truncation and deimination of arginine to citrulline were identified on CXCL5, CXCL8 (IL-8) and CXCL10 (IP-10). The enhanced in vitro activity of N-terminally truncated neutrophil chemotactic proteins CXCL5 and CXCL8 was confirmed. Surprisingly, although intact CXCL5 had potent in vitro chemotactic activity, it failed to recruit neutrophils to the peritoneal cavity in mice. Also truncation of CXCL8 enhanced its in vivo chemotactic properties. In contrast, limited truncation of the lymphocyte chemotactic CXC chemokines CXCL9 - CXCL12 abolished their chemotactic activity. Citrullination of structural proteins by peptidylarginine deiminases (PAD) has been associated with auto-immune diseases such as rheumatoid arthritis and multiple sclerosis. Chemokines were identified as the first PAD substrates that signal in a receptor-dependent manner. PAD2 and PAD4 rapidly and site-specifically deiminate CXCL5, CXCL8 and CXCL10. Although

citrullination hardly affected the in vitro activity of CXCL8, it rendered this chemokine resistant to proteolytic cleavage and reduced its glycosaminoglycan binding properties. More strikingly, intraperitoneal injection of citrullinated CXCL5 or CXCL8 failed to attract neutrophils to the peritoneal pocket. Using a citrulline-specific chemical modification in combination with immunological detection, we observed that neutrophils rather than PBMC produce significant amounts of citrullinated CXCL8. Overall, chemokines are subject to various modifications affecting their activity. These data provide new structure-function information for chemokines in leukocyte mobilization and suggest a role for PAD in the downregulation of acute inflammation.

P2.06.31
Non-hematopoietic endothelial LSP1 orchestrates CXCL2-sensitive motile neutrophil functions in vivo

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The Ca2+- and F-actin-binding leukocyte-specific protein 1 (LSP1) is expressed not only in leukocytes but also in non-hematopoietic endothelial cells. Endothelial LSP1 was recently shown to participate in microvascular permeability increases and transendothelial migration of leukocytes during inflammation. However, the cell-specific functions of LSP1 remain elusive. Using intravital and time-lapsed video microscopy we demonstrate MIP-2/CXCL2-elicited motile neutrophil dynamics in the cremasteric microvasculature of LSP1-deficient chimeric mice. Absence of endothelial LSP1 (Lsp1−/−) resulted in significantly impaired neutrophil chemotactic directionality (chemotaxis index) in CXCL2-elicited intraluminal crawling and extravascular migration as compared to these responses in wild type (Lsp1+/+) endothelial cells. The velocity of migration of Lsp1 neutrophils in extravascular tissue was significantly lower in both Lsp1+/+ and Lsp1−/− chimeric mice as compared to that of Lsp1+/+ neutrophils in these mice. Determination of integrin expression on transmigrated neutrophils from CXCL2-triggered peritonitis in chimeric mice showed that deficiency of endothelial LSP1 mitigated the expression of α6 and β1 on neutrophils irrespective of neutrophil LSP1 expression. Furthermore, immunoblotting of lysates from whole cremaster muscles revealed blunted expression of CD31/PECAM-1 in Lsp1−/− mice as compared to Lsp1+/+ mice. In conclusion, non-hematopoietic endothelial LSP1 is a novel regulator of dynamic neutrophil motile functions during leukocyte recruitment in vivo.

P2.06.32
Sphingosine-1-phosfate receptors are involved in cell migration in T-cell acute lymphoblastic leukemia and lymphoma

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T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are malignant proliferations of T-cell precursors at different stages of normal development. As T-ALL/LBL lymphoblasts present similar characteristics of normal T-cell precursors, molecules involved on the migration of these cells might also be associated with migration and homing of T-ALL/LBL. In this context, the sphingosine-1-phosphate receptor 1 (S1P1), has been described as essential for mouse thymocyte migration and thymic egress. We evaluated the expression and role of S1P1 in T-ALL cell lines in response to its physiological ligand, sphingosine-1-phophate (S1P). At first, we observed that the migratory response of the cells towards S1P was directly related with their expression levels of S1P1. The cell line which express very low levels of S1P1 mRNA (HPB-ALL) do not migrate towards S1P while MOLT-4, JURKAT and CEM cells which express higher levels of S1P1 mRNA migrate towards S1P. S1P induced migration of the cell lines in different concentrations up to 100nM and inhibited cell migration at higher concentrations (500-1000nM). Interestingly, when S1P1 was blocked by W146 (S1P1 specific inhibitor), S1P-induced migration at lower concentrations was barely reduced. Conversely, S1P higher concentrations induced cell migration when S1P1 was blocked, suggesting the involvement of other S1P receptor, in the absence of S1P1 signaling. We observed that the responding cell lines also express S1P3 mRNA suggesting that this receptor may be involved, and this issue is under study. In summary, our results suggest that interactions mediated by S1P/S1P receptors can modulate cell migration of T-ALL/LBL blasts.

P2.06.33
A new mTOR-dependent, Akt-independent pathway differentiates the migratory behavior of regulatory and conventional T cells

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The vitamin A metabolite all-trans retinoic acid (ATRA) is known to induce a gut-homing phenotype in activated CD4+ conventional T cells (Tconv) by upregulating the integrin α4β7 and the chemokine receptor CCR9. We report that in contrast to mouse Tconv, only about half of regulatory T cells (Treg) upregulate CCR9 when stimulated in presence of physiological levels of ATRA, even though Tconv and Treg express identical levels of retinoic acid receptor (RAR). The bimodal distribution of CCR9+ versus CCR9- Treg subsets is not associated with differences in the extent of proliferation, level of FoxP3 expression, or segregation between the populations of thymic-derived Treg (nTreg) and induced Treg (iTreg) that constitute the pool of circulating Treg. Furthermore, we report that exposure to the mTOR inhibitor rapamycin suppresses upregulation of both CCR9+ and α4β7 on Treg, an inhibitory effect that is not evident in Tconv. This suggests that ATRA-induced upregulation of CCR9 and α4β7 on Treg is dependent on activation of an mTOR signaling pathway. This mTOR involvement is independent of Akt activity, since specific inhibition of Akt or its downstream target GSK-3B did not prevent CCR9 expression. Additionally, Rictor-/- Treg (that lack mTORC2) showed unaltered ability to express CCR9 compared to WT control, suggesting the selective participation of mTORC1 or another mTOR complex. These findings reveal a novel difference between ATRA signaling and chemokine receptor induction in Treg versus Tconv, and provide a framework via which the migratory behavior of Treg versus Tconv can be differentially influenced for therapeutic purposes.

P2.06.34
Role of oxysterol EBI2 ligand pathway in inflammation

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In order to mount a rapid and efficient antibody response, B cells undergo a series of dynamic movements within secondary lymphoid organs. The oxysterol 7a,25-dihydroxycholesterol (7a,25-OHC) has been recently identified as a ligand for EBI2, a G protein-coupled receptor, in regulating B cell movement along the B-T boundary and later to inter- and outer-follicular regions. 7a,25-OHC has been historically described as an intermediate in the alternate pathway of hepatic bile acid synthesis. 7a,25-OHC is synthesized from cholesterol by the stepwise actions of two enzymes, CH25H and CYP7B1: CH25H mediates hydroxylation at the 25 position, whereas CYP7B1 mediates the subsequent hydroxylation at the 7a position. 7a,25-OHC can be further metabolized by the enzyme HSD3B7, inactivating it as an EBI2 ligand. The enzymes needed for the generation and degradation of EBI2 ligand are expressed in a wide range of tissues, and in the case of CH25H, are strongly inducible by inflammatory stimuli, but their extra-hepatic function(s) remain poorly understood. Similarly, EBI2 is widely expressed across the hematopoietic lineage but its role outside the B cell compartment has been little investigated. We are currently investigating how the enzymes involved in the production of 7a,25-OHC are regulated in steady-state and inflammation in lymphoid and non-lymphoid organs, and how oxysterol abundance impacts trafficking and activation of EBI2-expressing immune cells under inflammatory conditions.

P2.06.35
CR3 and CR4 differently mediate the adherence to fibrinogen of human dendritic cells

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CR3 and CR4 are members of the family of β2 integrins, both expressed by human monocytes, neutrophil granulocytes (PMN), macrophages and dendritic cells. They consist of a common β-chain (CD18) and a unique α chain (CD11b in CR3 and CD11c in CR4). Their similarity is very high regarding their extracellular domain; 87% respectively. This is why ligand specificity of CR3 and CR4 is very much overlapping, and their main ligands - such as iC3b, fibrinogen, ICAM-1 - are the same. They differ however, in their intracellular domain, which suggests fundamental differences between the two receptors. So far however, very little is known about the function of CD11c. We recently demonstrated that CD11b dominates iC3b mediated phagocytosis over CD11c, the latter having only a supportive role in this process. In our present work we analyzed the role of CD11b/CD18 and CD11c/CD18 in another important cellular function of β2 integrins, namely adherence to fibrinogen, a common ligand of both receptors. Fibrinogen is an acute phase protein present on inflamed endothelium as well as a component of the extracellular matrix. We studied the adhesion of primary human monocytes and monocyte-derived dendritic cells and macrophages to this protein in the presence or absence of blocking antibodies binding to CD11b or CD11c. Interestingly we found that in contrast to phagocytosis, adherence is mainly mediated through CD11c ligation. These results provide further evidence that CD11b/CD18 and CD11c/CD18 have different roles despite their structural similarites.

P2.06.36
Angiotensin II-induced vascular dysfunction depends on aortic infiltration and mutual activation of NK cells and monocytes

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The recruitment of immune cells into the aortic vessel wall is crucial to angiotensin II (ATII) induced vascular dysfunction. But how the different immune cell subsets contribute to this inflammatory process is incompletely understood. Comparing vascular endothelial and smooth muscle function, superoxide formation and expression of NADPH oxidase subunits in C57BL/6, T-bet deficient Tbx21$^{-/-}$ mice, IFN-γ$^{-/-}$ mice and IFN-γ transgenic IFN-SAP mice, we found that ATII-induced vascular inflammation and dysfunction depends on the recruitment of IFN-γ producing immune cells into the aortic vessel wall. Analyzing the role of different immune cell subsets by adoptive transfer and depletion experiments identified NK cells as main IFN-γ producers in the aortic endothelium and their mutual interaction with vascular infiltrating monocytes as crucial events in ATII-induced aortic inflammation.
These results disclose IFN-γ production by vascular infiltrating NK cells as an important factor in ATII-induced vascular dysfunction and suggest NK cells and IFN-γ as a molecular target in the treatment of cardiovascular disease.

P2.06.37
Morphometric analysis of the distribution of decidua NK cells and macrophages in the neighborhood of the trophoblast invasion front

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Trafficking of leukocytes towards and accumulation in the vicinity of immobile tissue structures may be objectified by using algorithms for automated morphometric analysis of the relative densities of wandering cells. We applied a new method of quantitative image analysis to answer the question whether NK cells or macrophages accumulate in the neighborhood of the trophoblast invading the maternal decidua. We used tissue fragments from placentation sites of 1st trimester pregnancy and co-cultures of 1st trimester decidua parietalis with villous explants of the same pregnancy. Cryostat sections from those tissues were double-stained by immunohistochemistry for CD56 or CD163 versus HLA-G and analyzed by automatic quantitative image processing. Distinct cell compartments as well as cell neighborhood areas were defined and relative areal cell densities were calculated. At 1st trimester placentation sites we found a higher density of NK cells as well as of macrophages in close proximity to the invasive trophoblast. In the three-dimensional co-culture model enrichment in the neighbourhood of the trophoblast was observed for macrophages but not for NK cells. As the presented method of analysis of distribution of mobile cells in relation of immobile tissue structures not only replaces laborious counting of cells but also excludes an observer bias, we envisage further applications in other fields such as tumor immunology and inflammation.

P2.06.38
Endothelial- specific gene ablation of CD99L2 impairs leukocyte extravasation in vivo

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Leukocyte extravasation depends on a tightly regulated cascade involving receptors and adhesion molecules on leukocytes and on endothelial cells. One of the most recently identified proteins participating in this process is CD99L2, a small, highly O-glycosylated transmembrane protein with no resemblance to any known protein family.
CD99L2 is expressed on neutrophils, lymphocytes and endothelium, where it locates to the intercellular junctions. With the help of polyclonal antibodies, we could recently show that it is involved in the transmigration of neutrophils through endothelial cells in vitro as well as into inflamed peritoneum and cremaster tissue. Despite of its high expression on lymphocytes, CD99L2 was dispensable for lymphocyte extravasation.
To further elucidate the role of CD99L2 in leukocyte diapedesis and to evaluate its function independent of antibody effects, we generated CD99L2 conditional knock-out mice. By using intravital microscopy in the inflamed cremaster muscle, we were able to clarify that CD99L2 deficiency does not affect adhesion or rolling of leukocytes, but only the actual diapedesis step. Combining techniques of conditional gene deletion and bone marrow transplantation, we found that neutrophil extravasation was only decreased when CD99L2 was missing in endothelial cells, but not on neutrophils. In an in vivo model for allergic reactions, we could for the first time also reveal a role for CD99L2 during T cell transmigration. Similar to the situation for neutrophils, CD99L2 was only required for efficient T cell transmigration on the endothelial, but not the lymphocyte, site.

P2.06.39

PI3Kgamma confers resistance against Trypanosoma cruzi infection due to its important role in microbicidal activity of macrophages

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The phosphatidylinositol-3-kinases (PI3Ks) are a family of lipid kinases that play a crucial role in several cellular processes including survival and proliferation. PI3Kgamma is involved in the signaling of chemotactic factors and leukocytes migration and activation. Here, we evaluated the role of PI3Kgamma during experimental infection by the protozoan parasite *T. cruzi* in C57BL/6 WT mice. We observed that *T. cruzi* infection causes an increase in the PI3Kgamma expression and activation in the mice's heart. Although there is no difference in the blood parasitemia between WT and PI3Kgamma$^{-/-}$ mice, all PI3Kgamma deficient mice died until day 25 after infection, whereas the WT remained alive after 30 days. PI3Kgamma$^{-/-}$ mice also showed greater inflammation, parasitism and lesion in the heart tissue. Interestingly, in the absence of PI3Kgamma, the heart tissue express higher levels of iNOS enzyme after infection compared with WT mice, but the expression of arginase I, that also consume the amino acid arginine thus impairing the nitric oxide (NO) production, is also higher compared with WT mice. PI3K$^{-/-}$ macrophages stimulated *in vitro* with *T. cruzi* plus IFN-gamma fail to produce NO and to kill the parasite compared with WT macrophages. Corroborating these data, the addition of ARG1 inhibitor (BEC) to macrophage culture restores the ability of PI3Kgamma$^{-/-}$ macrophages to produce NO. These results indicate that PI3Kgamma is not involved in the leukocyte migration during *T. cruzi* infection, but it helps in the microbicidal mechanisms of macrophage by mediation of NO production, which is important to kill the intracellular parasites.

P2.06.40

***Nr4a1*-dependent Ly6C^{low} monocytes monitor endothelial cells and orchestrate their disposal in the presence of nucleic acids**

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The functions of *Nr4a1*-dependent Ly6C^{low} monocytes have remained enigmatic. We show herein that they are highly enriched within capillary vessels and scavenge microparticles from their luminal side in a steady state. Induction of inflammation by a TLR7-dependent nucleic acid signal in the kidney cortex triggers G[[Unsupported Character - Symbol Font ]]*i*-dependent intravascular retention of Ly6C^{low} monocytes, which recruit neutrophils in a TLR7-dependent manner to mediate focal necrotic death of endothelial cells, while the monocytes remove cellular debris. Prevention of Ly6C^{low} monocyte development, crawling, or retention in *Nr4a1*$^{-/-}$, *Itgal*$^{-/-}$, and *Tlr7*$^{host-/-BM+/+}$ and *Cx3cr1*$^{-/-}$ mice, respectively, abolished neutrophil recruitment and endothelial killing. Prevention of neutrophil recruitment by monocytes in *Tlr7*$^{host+/+BM-/-}$ bone-marrow chimeras or by antibody-mediated neutrophil depletion also abolished endothelial cell necrosis. *Nr4a1*-dependent Ly6C^{low} monocytes are therefore intravascular housekeepers that orchestrate the necrosis by neutrophils of endothelial cells that signal a local threat sensed via TLR7, and the disposal of cellular debris.

P2.06.41

Simultaneous analysis of multiple fluorescent proteins and fluorochromes by a novel spectral flow cytomete

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We have been revealing immune system based of spatiotemporal regulation of immune cells in the entire body by using photoconvertible fluorescent protein (FP), Kaede and KikGR mice. Flow cytometric analysis of green and red signals of non-photoconverted and photoconverted Kaede or KikGR expressing cells with multiple fluorochrome-conjugated mAbs have been difficult and unable to increase color panels, because of the fluorescence signal overlap between FPs and fluorochromes.
We have newly developed a spectral flow cytometer (FCM) based on a novel measurement principle. Unlike a polychromatic FCM, the spectral FCM with 32 channel linear array PMT detects the fluorescence derived from every fluorescent probe. The data of acquired spectra is analyzed with a unique algorithm.
We detected spectral changes of KikGR color from green to red during photoconversion. We separated EGFP and Venus. One of the feature advantages of this instrument is that it can recognize spectral shape of each fluorescent probes and we could separate EGFP and FITC, their peaks of wavelength are almost same and only spectral shape of GFP is slightly broader than FITC. Finally, we successfully separated 11-colors including kikGR-green and -red simultaneously.
Taken together, spectral FCM allow us to detection of FPs with multicolor fluorochromes, and thus, the system which uses KikGR mice with spectral FCM is a powerful tool to investigate spatiotemporal regulation of immune cells in the entire body.

P2.06.42

The atypical chemokine receptor D6 is mobilized from recycling endosome for adaptive up-regulation and chemokine degradation through a β-arrestin1/myosinVb-dependent mechanism

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The atypical chemokine receptor D6 is a chemokine scavenger with a non-redundant role in the control of inflammation. D6 scavenging activity relies on its unique trafficking properties. The peculiar D6 intracellular distribution is ensured by its constitutive internalization and recycling through Rab4/Rab11-positive endosomes. Ligands induce D6 up-regulation on plasma membrane through a rapid mobilization of receptor from recycling endosomes but the mechanisms by which this adaptive up-regulation is achieved are still undefined. Here we show that in basal conditions D6/myosinVb complexes are differently associated to actin and microtubules, and perturbations of cytoskeletal dynamics dramatically affect receptor constitutive endocytosis and its correct sorting to recycling compartments. Ligand recognition per se rapidly mobilizes the intracellular pool of D6 through activation of a G protein-independent β arrestin1-dependent signaling pathway leading to actin and microtubules rearrangements at plasma membrane via myosinVb, thus upregulating receptor membrane expression and optimizing its scavenging properties. In conclusion, here we provide further evidences that the atypical chemokine receptor D6 is a β arrestin-biased signaling receptor, exerting its scavenger function through activation of a G protein-indipendent signaling pathway aimed at regulating its trafficking properties via myosinVb-dependent control of cytoskeletal dynamics.

P2.06.43
LFA-1 is critical for T-cell mediated protective immunity elicited by heterologous prime-boost vaccination strategy against a systemic parasitic infection

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INTRODUCTION: Heterologous prime-boost vaccination strategy using recombinant plasmid DNA followed by replication-defective human recombinant adenovirus 5 (AdHu5) is a powerful strategy to elicit specific $CD4^+$ and $CD8^+$ T cells which play a key role in protective immunity against intracellular pathogens as for example *Trypanosoma cruzi*, the causative agent of the neglected Chagas' disease. Based on evidences that recirculation after challenge is critical for T-cell mediated protective immunity, herein we tested whether certain integrins, cell adhesion molecules (CAM) and chemokines were key mediators of this process playing a critical role during immunity against infection.
RESULTS: To study the role of integrins, A/Sn mice were immunized with heterologous prime-boost vaccine (rec. plasmid DNA/AdHu5), challenged with parasites (Y strain) and treated with blocking antibodies to LFA-1 and/or VLA-4. Immunized mice treated with control Rat IgG or anti-VLA-4 antibodies controlled the parasitemia and survived the lethal challenge. In contrast, immunized mice treated with anti-LFA-1 or anti-LFA-1 and anti-VLA-4 displayed high parasitemia and all of them died after challenge. LFA-1 blockage neither diminished the frequency nor compromised the capacity of specific $CD8^+$ T cells to respond *in vitro* (IFN-γ and TNF). We performed similar experiments by vaccinating *icam-1*$^{-/-}$ or *cccr5*$^{-/-}$ mice. These mice controlled the infection at similar extension as wild type animals. CONCLUSION: We concluded that LFA-1 integrin, but not VLA-4, ICAM-1 or CCR5 play a critical role in the protective immune response generated by the heterologous prime-boost vaccination possibly by blocking T cell recirculation after challenge.
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P2.06.44
TNFR2 induces IRF-1 dependent IFNβ autocrine signaling in endothelial cells to promote monocyte recruitment

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Endothelial-dependent mechanisms of mononuclear cell influx are not well understood. We discovered that acute stimulation of murine microvascular endothelial cells expressing TNFR1 and TNFR2 with soluble TNF, led to mononuclear CXCR3 chemokine generation through Interferon regulatory factor-1 (IRF-1) induced IFNβ synthesis and subsequent autocrine signaling via the IFNα/β -receptor and STAT1. Both TNFR2 and TNFR1 were required for IRF-1-IFNβ signaling. TNFR2's role in this pathway and monocyte recruitment was also evident following its overexpression and ligand independent activation in human endothelial cells. In vivo, TNFR1 was required for acute renal neutrophil and monocyte recruitment following systemic TNF treatment, while the TNFR2-IRF-1-IFNβ autocrine loop was essential only for monocyte/macrophage influx. In a chronic model of proliferative nephritis, IRF-1 and renal expressed TNFR2, but not TNFR1, were essential for sustained macrophage accumulation. Thus, our data identify a previously unrecognized pathway in endothelial cells that selectively recruits monocytes during a TNF-induced inflammatory response

P2.06.45
Role of CBAP in chemokine-induced zap70-mediated T-cell trafficking

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Activated chemokine receptor initiates inside-out signaling to transiently trigger activation of integrins, a process involving multiple components that have not been fully characterized. Here we report that GM-CSF/IL-3/IL-5 receptor common beta-chain-associated protein (CBAP) is required to optimize this inside-out signaling and activation of integrins. First, knockdown of CBAP expression in human Jurkat T cells caused attenuated CXC chemokine ligand-12 (CXCL12)-induced cell migration and integrin α4β1- and αLβ2-mediated cell adhesion *in vitro*, which could be rescued sufficiently upon expression of murine CBAP proteins. Freshly isolated CBAP-deficient primary T cells also exhibited diminution of chemotaxis toward CC chemokine ligand-21 (CCL21) and CXCL12, and these chemokines-induced T-cell adhesions *in vitro*. Adoptive transfer of isolated naive T cells demonstrated that CBAP deficiency significantly reduced lymph node homing ability *in vivo*. Finally, migration of T cell-receptor-activated T cells induced by inflammatory chemokines was also attenuated in CBAP-deficient cells. Further analyses revealed that CBAP constitutively associated with both integrin β1 and ZAP70 and that CBAP is required for chemokine-induced initial binding of the talin-Vav1 complex to integrin β1 and to facilitate subsequent ZAP70-mediated dissociation of the talin-Vav1 complex and Vav1 phosphorylation. Within such an integrin signaling complex, CBAP likely functions as an adaptor and ultimately leads to activation of both integrin α4β1 and Rac1. Taken together, our data suggest that CBAP indeed can function as a novel signaling component within the ZAP70/Vav1/talin complex and plays an important role in regulating chemokine-promoted T-cell trafficking.

P2.07 Peripheral tolerance and lymphocyte anergy

P2.07.01
Anergy in CLL B lymphocytes: implications for the biology and the therapy of the disease

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Chronic Lymphocytic Leukemia (CLL) is due to the clonal expansion of $CD5^+$ B lymphocytes. For many years CLL has been considered a leukemia of functionally inactive B lymphocytes. In apparent contrast, more recently it has been shown that a portion of cases is actually responsive to external stimuli including the activation though the BCR. That notwithstanding, it holds true that a sizable fraction of CLL patients are indeed characterized by the expansion of clonal B cells endowed with anergic features.
In particular, our studies demonstrated that lack of in vitro signaling capacity upon BCR triggering in a subset of CLL patients, was associated with the anergic features previously described in murine

models of B cell anergy (constitutive activation of ERK1/2 and NF-ATc1, higher basal levels of intracellular calcium, reduced expression of surface IgM and increased expression of the negative regulator receptors CD5 and CD22).
In this group of patients, chronic BCR engagement in vivo leads to the induction of a reversible biochemical anergic program that promotes survival of leukemic cells.
Constitutive ERK and NF-ATc1 activation can be efficiently targeted with small chemical compounds and cells first regain antigen responsiveness and then undergo apoptosis, thus further confirming the pro-survival role of anergy induction and possibly opening new perspectives in the clinical management of this subset of patients.

P2.07.02
Immune regulation mediated by soluble MHC-II targeting CD4+ T-cells

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Soluble MHC-II (sMHCII) molecules are present in body fluids of healthy individuals and are considered to be involved in the maintenance of self tolerance, while they are also related to various diseases. Their concentration increases during in vivo antigen-specific tolerogenic stimulation and it was recently shown that exosome-mediated tolerance is MHC-II dependent. At the cellular level, sMHCII proteins compete with membrane MHCII for TCR binding. Soluble MHC-II proteins were isolated from serum of HSA-tolerant mice and purity was verified through ELISA, SDS-PAGE, Western blot analysis and acoustic biosensor assays using specific a-class II antibodies. SDS-PAGE revealed one band at ~60kDa which was found to be highly glycozylated. Soluble MHCII suppressed an antigen-specific immune response by reducing the number of active cells as tested by 3HTdR incorporation assays. Furthermore, sMHC-II molecules appeared to target purified CD4+ cells in contrast to CD8+ cells and K562 cells used as control in acoustic biosensor assays. In fact, they increased CTLA+/CD4+ numbers while reducing CD28+/CD4+ T-cells as tested by FACS analysis. ELISA experiments showed that sMHCII induced secretion of IL-10, while increasing the expression of CD25 surface marker in control CD4+ T-cells. Evaluation of TCR signaling in CD4+ cells indicated differential pathway activation in the presence or not of sMHCII. Finally, the results showed that sMHCII could negatively regulate antigen-specific immune response in vivo as well as in vitro by inducing immunosuppressive CTLA4+ and CD25+ T-cell populations and immunosuppressive mediators, playing thus an important role in immune homeostasis.

P2.07.03
Hepatocyte growth factor attenuates in vitro murine cytotoxic T-lymphocyte activity

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Background: Hepatocyte growth factor (HGF) is a multifunctional pleiotropic cytokine recognized as a key factor in the prevention and attenuation of disease progression. We have recently reported that HGF limits CD4+ T cell-mediated autoimmune inflammation by promoting tolerogenic dendritic cells (DC) and subsequent regulatory T cells. Whether HGF modulates cell-mediated immunity driven by MHC class I-restricted CD8+ T cells remains to be determined.
Objective: Here we examined whether HGF regulates antigen (Ag)-specific CD8+ T cell responses using an established in vitro model of murine cytotoxic T lymphocyte (CTL)-mediated killing.
Methods: Pmel-1 transgenic gp100-specific CD8+ T cells were activated using Ag-pulsed Ag-presenting cells (APC) in the absence or presence of the HGF (30 ng/ml). After 5 days, cytolytic activity against EL4 or peptide-pulsed EL4 was measured in a 4-h DELFIA® release assay. Cytolitic pathways were quantified by FACS analysis.
Results: HGF treatment reduced MHC class I-restricted CTL-mediated cytolysis. FACS analysis revealed decreased levels of IFN-gamma, TNF, FAS-L, granzyme B, perforin and the degranulation marker CD107a among CD8+ T cells co-cultured with HGF-treated APC. Moreover, HGF suppressed the frequency of the CÐ62L effector memory CTL population.
Conclusion: Our findings indicate that HGF treatment limits both the generation of effector CTL from naive CD8+ T cells and their cytotoxic functions, such as granule exocytosis mediated by perforin/granzymes and the Fas/Fas ligand system. Complementary to its impact on CD4+ T-cell immunity, our findings further suggest that HGF treatment could be exploited to control CD8+ T-cell-mediated, MHC I-restricted dysfunctions.

P2.07.04
Characterisation of a Novel Transmembrane Protein (NTP) in primary human CD4 positive T-Cells

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The gene encoding a transmembrane spanning protein (NTP) was originally discovered in neuronal cells and subsequent studies have identified NTP gene expression in a range of different primary cells and cell lines, but with no reference to its function. Functional roles have been suggested in cellular interactions and regulation of cellular ion fluxes, based on homology to other proteins, yet no experimental data have developed this hypothesis in T-cells. Analysis of early gene expression in anergic, activated and resting CD4+ T-cell clones identified NTP to be differentially expressed between the different states. Expression was stable in the anergic state and fell upon activation. These data suggest that NTP may regulate CD4+ T-cell activation.
The aim of this study was to identify the role of NTP expression in immune cell subsets, focusing primarily on the CD4+ T-cells. NTP expression was investigated in the resting and activated states of CD4+ T-cells in a bid to understand how NTP may be regulated and the effects it may exert on different CD4+ T-cell states.
NTP protein expression has been identified on the surface of a variety of immune cell subsets. Culturing CD4+ T-cells *in vitro* resulted in the internalisation of NTP, creating a challenge to determine NTP function. Interestingly, CD4+ T-cell activation induced elevated NTP expression and whole blood cultures also maintained the expression of NTP. Over-expression studies in primary human CD4+ T-cells has also revealed a potential role for NTP in the regulation of T-cell function, opening up many further research avenues.

P2.07.05
T cell receptor modulation in peripheral CD8 T cells to influenza infection

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Our understanding of complementary T cell receptor (TCR) α and β chain utilization and modulation is very limited for pathogen-specific immunity. We recently reported a method for amplifying paired T cell receptor (TCR) αβ CDR3 regions from single cells in mice and human. Using this technique, we found surprisingly, 25% of influenza-epitope-specific CD8+ cytotoxic T lymphocytes recovered directly from the pneumonic lung expressed a dominant, non-productively rearranged TCRα transcript, an effect that is never seen for TCRβ. T cells with these out-of-frame TCRα mRNAs also express an alternate, in-frame TCRα, while approximately 10% have two productive TCRα transcripts. Taking TCRα out-of-frame mRNA as a surrogate for the alternate allele expression, we found that the proportion of cells with biallelic transcription increases over the course of a response, stayed high in the memory compartment, and decreased in the recall response. However, these biallelic cells appear to expand with low efficiency. Further, cells with out-of-frame TCRα mRNA expression appear to be specifically excluded from the recall response but upregulated to the level seen in primary response in highly inflammatory conditions. Finally, mice deficient in a single alpha locus, thus lacking modulation in alpha chain, showed delayed onset

and decreased severity of Experimental autoimmune encephalomyelitis (EAE) compared to that of the WT littermate controls. Thus, allelic modulation may play a role in down regulation of TCR expression for survival advantage in highly inflammatory condition by down modulating the TCRα mRNA, a finding that has implications for immune memory and for autoimmunity.

P2.07.06
Activation induced split anergy: a mechanism for persistence of autoimmune T cells

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T cell anergy is considered to be one of the major mechanisms for maintaining self-tolerance and restricting autoimmunity. Self-reactive T-cells from patients with autoimmune diseases such as systemic lupus erythematosus (SLE), Sjogren's syndrome and rheumatoid arthritis are believed to avoid anergy. Instead, we found that T-cells from animal models of autoimmune diseases exhibit anergy rather than resistance to it. Autoimmune T-cells, however, also display features of chronic in vivo activation characterized by enhanced activation markers (CD44hiCD62LlowCD69+), IFN-γ production and constitutive phosphorylation of multiple T-cell receptor (TCR) signalling proteins. Those autoimmune T-cells that exhibit such split responsiveness acquire an anergic phenotype but retain effector functions and evade AICD upon TCR stimulation. Thus, persistent activation of T-cells by self-antigens in autoimmune-prone subjects culminates as "activation-induced split anergy" that promotes the development, rather than avoidance, of autoimmunity. The seemingly paradoxical or split phenotype of autoimmune T-cells described above may represent different CD4+ T-cell subsets, with one subset manifesting anergy and the other subset exhibiting activation phenotype. The localization of split T-cell phenotype to one subpopulation indicates a link between anergy and activation and leads us to propose that anergy in autoimmune T-cells might occur as a consequence of chronic in vivo activation with self-antigens.

P2.07.07
Ectopic expression of IL-35Ig by dendritic cells induces IDO-related tolerance in vivo

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Dendritic cells (DCs) are specialized APCs playing a central role in activating, but also in regulating, T-cell responses. In previous studies, we demonstrated that their functional plasticity can be exploited to trigger and amplify a tolerogenic state ('infectious tolerance'), here we focused on the possible modulation of immune responses by ectopic expression of IL-35 in DCs, a potent suppressive cytokine produced by T regulatory cells. We transfected immunogenic CD8- DCs with a single-chain IL-35-Ig gene construct and we found that, after P815AB antigen peptide pulsing, even a minority fraction of transfected cells inhibited the immunogenic presentation of the peptide in a skin test assay, triggering the onset of a long-lasting, specific tolerance subverting the otherwise immunogenic priming to the peptide. This suppressive effect was attributable to changes occurring in vivo in total splenocytes and in their CD11c+ DC fraction after DC/IL-35-Ig/P815AB injection, including induction of tolerogenic indoleamine 2,3-dioxygenase-1 (IDO1) and production of mediators sustaining the mechanism of infectious tolerance (via the IDO1 metabolite kynurenine, TGF-β, and IL-10). On the basis of these findings, we attempted a tolerogenic vaccination protocol to prevent autoimmune diabetes. Interestingly, on treating prediabetic NOD mice with DCs overexpressing IL-35 and presenting the IGRP peptide (one of the most relevant type 1 diabetes self-antigens), we observed delayed and less severe onset of hyperglycemia. In conclusion, DCs overexpressing ectopic IL-35Ig exert an important suppression in vivo, which, on combination with specific antigen, might represent a powerful, selective means of negative vaccination in autoimmune diseases.

P2.07.08
Abnormal transcriptional regulation of the E3 ligase Cbl-b by early growth response genes 2 and 3 in systemic lupus erythematosus patients

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T cells from Systemic Lupus Erythematosus (SLE) patients display resistance to anergy induction, which has been related to the deficiency of the E3 ligase Cbl-b. This abnormal peripheral tolerance is characterized by increased IL-2 production, proliferation and expression of activation and costimulatory markers upon anergy-inducing conditions. The transcription factors Early Growth Response (Egr) 2 and 3 have been implicated in the transcriptional regulation of Cbl-b in murine models. However their role in human T cell responses has not been fully addressed. The aim of this study was to evaluate the expression of the transcription factors Egr-2 and 3 and its relationship to Cbl-b in T cells from SLE patients. We included 16 SLE patients (8 in remission and 8 with active untreated disease) and 16 healthy controls. PBMCs were isolated and $CD4^+$ cells were purified by negative selection.The expression of Egr-2, 3 and Cbl-b was analysed by qPCR and Western blotting. $CD4^+$ cells from SLE patients show increased proliferation and IL-2 synthesis after anergy induction. Moreover, upon anergy induction with ionomycin, decreased expression of *Egr-2* (0.46 vs 1.89, $p<0.001$); *Egr-3* (0.80 vs 2.55,$p<0.001$) and Cbl-b (0.52 vs 0.87, $p=0.028$) was found in lupus T cells in comparison to healthy controls. Our data suggest that the deficiency in Cbl-b expression in $CD4^+$ T cells from SLE patients, without regarding disease activity, is related to transcriptional defects, mainly, decreased Egr-2 and 3 expression, which is related to the breach in peripheral tolerance.

P2.07.09
Olfactory exposure to Tokishakuyaku-san (TJ-23) prolonged cardiac allograft survival and generated regulatory cells

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Background. Herbal medicines have unique odors, and the act of smelling may have modulatory effects on the immune system. We investigated the effect of olfactory exposure to Tokishakuyaku-san (TJ-23), a Japanese herbal medicine, on alloimmune responses in a murine model of cardiac allograft transplantation.
Methods. Naïve or olfactory-dysfunctional CBA mice underwent transplantation of a C57BL/6 heart and were exposed to the odor of TJ-23 until rejection. Some naïve CBA recipients of an allograft were given olfactory exposure to Sairei-to (TJ-114), trimethylthiazoline (TMT), individual components of TJ-23, or a TJ-23 preparation lacking one component. Adoptive transfer studies were performed to determine whether regulatory cells were generated.
Results. Untreated CBA mice rejected their C57BL/6 allografts acutely, as did olfactory-dysfunctional CBA mice exposed to the odor of TJ-23 (median survival times [MSTs], 7 and 7 days, respectively). CBA recipients of a C57BL/6 heart given olfactory exposure to TJ-23 had significantly prolonged allograft survival, whereas those exposed to the odor of TJ-114, TMT (MSTs, 48, 8.5 and 8 days), one component of TJ-23, or TJ-23 lacking a component did not. Secondary allograft recipients that were given, at 30 days after transplantation, either whole splenocytes, $CD4^+$ cells, or $CD4^+CD25^+$ cells from primary recipients exposed to the odor of TJ-23 had indefinitely prolonged allograft survival.
Conclusions. Prolonged survival of cardiac allografts and generation of regulatory cells was associated with exposure to the odor of TJ-23 in our model. The olfactory area of the brain may have a role in the modulation of immune responses.

P2.07.10
Combination of anti-BTLA antibody (6B2) and anti-PD-1 antibody (PIM-2) induced indefinite survival of fully MHC-mismatched murine cardiac allograft

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Background: Programmed death (PD)-1 and B and T lymphocyte attenuator (BTLA) had been implicated in the regulation of autoimmune and may potentially play an important role in alloimmune responses. In this study, we investigated the effect of anti-PD-1 monoclonal antibody (mAb) (PIM-2) and anti-BTLA mAb (6B2) in fully MHC-mismatched murine model of cardiac allograft transplantation.
Methods: CBA mice underwent transplantation of C57BL/6 hearts and received a single dose (100μg) of 6B2 on the day of transplantation (day 0) or four doses on day 0, 3, 6 and 9. Moreover, CBA recipients were also given one dose of combination of 6B2 and PIM-2 on day 0. Adoptive transfer was performed to determine whether regulatory cells were generated. Cell-proliferation and cytokine assessments were also performed.
Result: Untreated CBA mice rejected C57BL/6 allografts acutely (median survival time [MST], 7 days). When CBA recipients were given one and four doses of 6B2, the allograft survivals were significantly prolonged (MSTs, 46 and >100 days, respectively). Moreover, when CBA mice were given with combination for one dose of 6B2 and PIM-2, the allograft survival was indefinitely prolonged (MST, >100 days). Secondary CBA recipients given whole splenocytes from primary combination-treated CBA recipients with C57BL/6 cardiac allografts 30 days after grafting had prolonged C57BL/6 allograft survival (MST, >20 days). Proliferation of splenocytes and interferon-γ production were suppressed and interleukin-4 production was increased in combination-treated mice.
Conclusion: Combination of anti-BTLA mAb (6B2) and anti-PD-1 mAb (PIM-2) could induce hyporesponsiveness of fully MHC-mismatched cardiac allografts and may generate regulatory cells.

P2.07.11
Tolerogenic human IL-10-modulated dendritic cells as inducers of regulatory T cells: analysis of mature CD83highCCR7highHLA-DRhigh and immature CD83lowCCR7negativeHLA-DRlow DC subpopulations

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Human IL-10-modulated, tolerogenic dendritic cells (IL-10DC), which are capable to induce anergic regulatory $CD4^+$ T cells (iTregs), consists of two subpopulations, mature $CD83^{high}CCR7^{high}HLA\text{-}DR^{high}$ and immature $CD83^{low}CCR7^{negative}HLA\text{-}DR^{low}$. Here, we investigated both IL-10DC subsets with regard to their phenotype and tolerogenic capacity in detail. As compared to fully mature DC (mDC), the $CD83^{low}$ IL-10DC subset revealed a significantly diminished expression of CD80, CD86, ICOS-L, and CD40 accompanied by a slight upregulation of PD-L2, ILT3, and ILT4. In contrast, we observed minor changes in expression of costimulatory molecules but significantly increased levels of inhibitory molecules on $CD83^{high}$ IL-10DC, demonstrating significant differences in expression of costimulatory and inhibitory molecules between the two IL-10DC subsets. Notably, primary stimulation of naïve T cells and restimulation experiments demonstrated that both IL-10DC subpopulations, in contrast to mDC and regardless of their maturation state, induced anergic $CD4^+$ T cells as evaluated by a significantly reduced T cell proliferation and diminished Th1 and Th2 responses. In addition, both iTreg subpopulations exhibited regulatory activities and suppressed the activation of responder T cells but lost their anergic state during suppressor experiments. In conclusion, mature $CD83^{high}CCR7^{high}HLA\text{-}DR^{high}$ and immature $CD83^{low}CCR7^{negative}HLA\text{-}DR^{low}$ IL-10DC display properties of tolerogenic human DC, in particular of inducers of iTregs, which may be used as targets for the development of novel therapeutic approaches for allergies, autoimmune disease or transplant rejections.

P2.07.12
A humanized mouse model for the study of B cell tolerance in human lymphocytes

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In most autoimmune diseases, autoreactive B cells fail to undergo negative selection (i.e., tolerance), and thus promote immune responses against self. B cell tolerance mechanisms of receptor editing, deletion and anergy have been established using genetically modified mice. The mechanisms by which human B cells undergo tolerance, however, are currently unknown because of the challenges associated with studying the human immune system. We are using hematopoietic humanized mice (hu-mice) to study mechanisms of human B cell tolerance. Hu-mice are immunodeficient mice transplanted with human hematopoietic stem cells that generate a human immune system within the mouse host. To investigate human B cell tolerance in hu-mice we generated a ubiquitous membrane-tethered *human* Ck-reactive synthetic Ag (hCk-Ag) that allows studies of central B cell tolerance of kappa-bearing human B cells. We find two versions of tolerance in hu-mice: a central tolerance phenotype in which kappa-positive B cells are eliminated from the bone marrow and a second phenotype in which a population of kappa-low, CD19-low B cells are observed in the bone marrow and spleen. The phenotype observed segregates largely with cord blood donor, and is thus likely genetically determined. We are currently investigating the mechanisms of these tolerance phenotypes using intracellular staining for kappa, BrdU incorporation analysis, and molecular analysis of Rag expression and kappa deleting element in developing B cells. Hu-mice represent an ideal model to study the human immune system using the powerful genetic and cell biological methodologies developed in mice.

P2.07.13
Serial transfere of anergy status induced by TSST-1 into newly stimulated T cells

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The term “infectious tolerance” was referred to the suppression process in which anergy/unresponsiveness-induced status is transferred from one to another cell population. In this study, we induced anergy status in vivo by administration of superantigen/toxic shock syndrome toxin-1 (TSST-1) into OVA-TCR Tg mouse T cells, and analyzed how the anergy T cells conduct a suppressive potential in naïve T cells receiving TCR-mediated signaling. When the anergy T cells were co-cultured with naïve T cells in the presence of OVA, anergy status appeared in naïve T cells, which are distinguished from the 1st anergy cells by using (OVAxGFP) Tg mice. The 2ndry generated anergy cells could further lead the appearance of antigen specific unresponsiveness in newly stimulated naïve T cells by the co-culture with 3rd new naïve T cells. In the induced anergy T cells of three generations showing reduced IL-2, IL-10 expression, but not TGF-β or Foxp3, was highly detectable and accelerated during 24 hrs. Although APCs is known to produce IL-10, they don't seem to play a main role for transfer of anergy status into newly stimulated T cells because deletion of such APC didn't provide largely effect of production of IL-2 and IL-10. This assumption is compatible with another experimental result showing that naïve T cells cultured with OVA in the presence of IL-10 did not lead anegy T cells. These results suggest that TSST-1 driven anergy T cells, but not APCs, trigger the “infectious tolerance” in naïve T cells in an IL-10 less dependent manner.

P2.07.14
The role of GRAIL in anti-cancer immune responses

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Severely suppressed immune system is a major risk factor in the tumor development. Strategies to improve immune cell survival and function are needed, and one potential approach is to manipulate the intrinsic properties of T lymphocytes by genetic ablation of tolerance/anergy associated genes. In recent years, the E3 ubiquitin ligases (Cbl-b, Itch and GRAIL) have been acknowledged as key molecules implicated in T cell tolerance. We as well as other groups have reported that $CD4^+$ and $CD8^+$ T cells activated in the absence of both CD28 and ICOS costimulation developed into tolerant T cells, associated with markedly upregulated expression of the E3 ubiquitin ligase GRAIL. Remarkably, genetic inactivation of GRAIL led to $CD4^+$ T cell hyper-responsiveness to TCR/CD3 signaling and their independency to costimulation. In addition, GRAIL deficiency abrogates the suppressive function of regulatory T cells, which are known to contribute to tumoral immune tolerance. There are growing evidences about the negative role of E3 ubiquitin ligases, Cbl-b and Itch, in a variety of cancers including solid tumors and hematopoietic malignancies. However, until now there is no knowledge on the role of GRAIL in tumor progression. Our data indicates that GRAIL controls proliferation and function of $CD8^+$ cytotoxic T lymphocytes. Moreover, ablation of GRAIL results in regression of lymphoma development. Thus, modulation of GRAIL activity might be an important approach to improve T-cell mediated anti-tumor responses.

P2.07.15
The soluble cytoplasmic tail of CD45 (ct-CD45) induces a non-canonical form of anergy in human T cells

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The cytoplasmic tail of CD45 (ct-CD45) is proteolytically cleaved and released upon activation of human phagocytes. The soluble ct-CD45 was found to act on T cells as an inhibitory, cytokine-like factor that reduces T cell proliferation.
In this study, we aimed to elucidate the molecular mechanisms acting within T cells, upon ct-CD45 binding. We demonstrate that ct-CD45 induces a novel form of anergy in human peripheral blood T cells. Ct-CD45 inhibited the proliferation of purified T cells and their cytokine production. Co-stimulation failed to prevent this inhibitory signal. Moreover, we found that T cells activated via CD3/CD28 or CD3/CD63 in the presence of ct-CD45 failed to proliferate in response to restimulation which was reversible by exogenous IL-2 or IL-7. Recent studies have clearly demonstrated that such a hypo-proliferative or anergic state of T cells is an active process where “anergy factors” (e.g. EGR2, EGR3, CBL-b, DGK-α) are being induced to establish and maintain the unresponsive state. However, when we analyzed the gene expression profile of ct-CD45-induced anergic T cells we did not observe induction of any of these anergy factors. Characterizing the expression patterns of cell cycle regulatory factors, we found inhibition in the induction of cyclin D1 while other cyclins were unaltered.
In summary, ct-CD45 triggers an anergy program in T cells which is reversible by exogenous IL-2, acting independently of classical anergy factors. Inhibition of cyclin D1 suggests a cell cycle arrest in the early G1 phase, thus making it distinct from canonical T cell anergy.

P2.07.16
Characterization of the molecular regulatory pathways involved in MDSC-mediated tolerance

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Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of immature myeloid cells mediating immunosuppression in the tumor microenvironment. We recently demonstrated that GM-CSF and G-CSF allow a rapid generation of MDSCs from precursors present in human bone marrow (BM) aspirates, named BM-MDSCs. This cell population is heterogeneous but an immature subset resembling to promyelocytes and defined i-BM-MDSCs (immature BM-MDSCs), is responsible for the whole immune suppression mediated by BM-MDSCs; of note, i-BM-MDSCS are equivalent to MDSCs present in the blood of breast and colorectal cancer patients. When we compared miRNA expression of promyelocytes isolated from fresh BM cells with that of i-BM-MDSCs, we observed differentially expressed miRNAs involved in myeloid differentiation and proliferation, by suggesting a block in the differentiation of these immature cells, coupled to signalling pathways that we are presently investigating. We characterized the mechanisms by which MDSCs are able to interfere with T cell proliferation by culturing activated T cells with i-BM-MDSCs. After cell culture, the number of vital T cells is significantly reduced and part of the lymphocytes undergo apoptosis; moreover, the expression of some immunoregulatory receptors belonging to the B7 family is increased on i-BM-MDSCs only after contact with activated T cells. Additionally, we demonstrated that IL-10 is involved in B7-dependent MDSC-immunosuppression. Our results indicate that the interaction between activated T cells and MDSCs involves the activation of suppressive pathways mediated by inhibitory receptors and thus highlight molecules that could be potential targets of intervention for cancer therapy.

P2.07.17
PD-L1 expression induced by the 2009 pandemic influenza A (H1N1) virus impairs the human T cell response

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PD-L1 expression plays a critical role in the impairment of T cell responses during chronic infections; nevertheless, the expression of PD-L1 on T cells during acute viral infections, particularly with the pandemic influenza virus (A(H1N1)pdm09), as well as the consequences of PD-L1 expression during infection, have not been explored. We found that A(H1N1)pdm09 induced PD-L1 expression on human dendritic cells (DCs) and T cells. PD-L1 expression impaired the T cell response against A(H1N1)pdm09 by slightly decreasing CD4+ T cell proliferation, promoting CD8+ T cell death and reducing cytokine production. Furthermore, we found increased PD-L1 expression on DCs and T cells from influenza-infected patients from the first and second pandemic waves in Mexico City. PD-L1 expression on CD8+ T cells correlated inversely with T cell proportions in patients infected with A(H1N1)pdm09 but not in those with influenza-like illness. Therefore, PD-L1 expression on DCs and T cells could be associated with an impaired T cell response during acute infection with A(H1N1)pdm09 virus.

P2.07.18

Induction of allogeneic immune tolerance by simultaneously blocking ICOS and OX40 costimulatory pathways

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Aim: The aim of the present study was to investigate the effects of induced immune tolerance by simultaneously blocking ICOS/ICOSL and OX40/OX40L costimulatory pathways, as well as potential underlying mechanisms. **Methods:** A eukaryotic expression vector containing both of the CTLA4Ig and OX40Ig fusion protein genes, pEGFP-ICOSIg-OX40Ig, was constructed by homologous recombination. The induction of donor-specific hyporeactivity was evaluated through allogeneic mixed lymphocyte reaction (MLR) using the isolated Wistar rat splenocytes as stimulator cells and the Lewis rat splenocytes as responder cells incubated with ICOSIg, OX40Ig or ICOSIg and OX40Ig combined. ELISA and flow cytometry were also used to evaluate immune reactions. **Results:** Allogeneic MLR, lymphocyte proliferation was significantly inhibited in the three costimulatory blockade groups compared with that in control. Combined ICOSIg and OX40Ig fusion protein synergistically induced superior effect on inhibiting lymphocyte proliferation, and decreasing Th1/Th2-like cell cytokine production, compared with singular blockade of either ICOSIg or OX40Ig. The percentage of $CD4^+CD25^+$ regulatory T cells significantly increased when allogeneic lymphocyte was co-cultured with ICOSIg and OX40Ig combined. **Conclusion:** Combined ex vivo blocking ICOS/ICOSL and OX40/OX40L costimulatory pathways synergistically provided superior benefits on induction of immune tolerance.

P2.08 Antigen receptor signaling

P2.08.01
Functional relevance of NTAL adaptor cleavage

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Non-T-cell activation linker (NTAL) is a transmembrane adaptor protein involved in the proximal signaling associated to immune receptors expressed by B lymphocytes, NK cells, macrophages and mast cells. In B lymphocytes, engagement of the B cell antigen receptor (BCR) induces phosphorylation of its tyrosine residues, allowing the recruitment of several cytosolic mediators for downstream signaling pathways. Very recently, our group has demonstrated that the adaptor LAT, an adaptor protein structurally and evolutionarily related to NTAL and expressed by T cells, is proteolytically cleaved upon Fas engagement in a tyrosine phosphorylation dependent fashion. Here, we analyze NTAL adaptor proteolytic cleavage in mature B lymphocytes, monocytes and also in several cell lines. Upon Fas crosslinking NTAL is cleaved producing a N-terminal fragment of about 22 kDa, and this cleavage is abrogated in the presence of caspase 8/granzyme B inhibitor. Moreover, we identify the aspartic acid residue at which NTAL is cleaved, and, similar to LAT, this aspartic acid residue is located in proximity to a tyrosine residue. Consistently, phosphorylation induction of NTAL by pervanadate inhibits its Fas-mediated cleavage, suggesting that transmembrane adaptors cleavage constitute a general mechanism to negatively regulate intracellular signaling cascades associated to immune receptors.

P2.08.02
Analysis of TCR-Vβ repertoire in lung and blood lymphocytes of pulmonary sarcoidosis patients reveals presence of $CD4^+$ and $CD8^+$ T cell expansions

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Sarcoidosis is a multisystem inflammatory disorder that can affect virtually any organ. The disease is characterized by granuloma formation and an accumulation of activated T cells in the affected organs. The etiology of sarcoidosis is not clear. Most evidence supports a genesis including environmental triggering by one or more still unknown antigens in the lungs, and an aberrant immune response in genetically susceptible individuals.
T cell receptor (TCR) Vβ-chain usage has been incompletely characterized in sarcoidosis patients. We surveyed the TCR Vβ usage in bronchoalveolar lavage (BAL) cells and peripheral blood from 15 HLA-typed Scandinavian sarcoidosis patients. In addition, PBMC from 9 healthy volunteers and BAL cells from three of them were examined. Using flow cytometry and 21 Vβ family-specific antibodies, we covered approximately 70 % of all Vβ chains.
In sarcoidosis patients, we identified 16 $CD4^+$T cell expansions in 271 analyses (5.9 %) in BAL cells and 9 $CD4^+$ T cell expansions in 276 analyses (3.3 %) in PBMC. The corresponding results in $CD8^+$ T lymphocytes were 21 expansions out of 240 analyses (8.7 %) of BAL cells and 12 expansions out of 263 analyses (4.6%) of PBMC. We found no difference in number of expansions between patients and controls in $CD4^+$ cells. However, expansions of T cells expressing Vβ5.1, Vβ7.1, Vβ18, Vβ21.3 and Vβ22 were found only in BAL from patients. This indicates qualitative rather than quantitative differences in Vβ-usage between patients and controls, and strengthens the hypothesis of specific antigen recognition in the lungs of sarcoidosis patients.

P2.08.03
The contribution of ligand affinity of the inhibitory coreceptor ILT2 to differential patterning of TCR at the immune synapse.

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How activating and inhibitory signals integrate at the T cell immune synapse remains poorly understood. The inhibitory coreceptor Ig-like transcript 2 (ILT2) is expressed on T cells and binds MHC I (Kd=2-10 μM). ILT2 also binds the HCMV MHC homologue UL18 with 1000x higher affinity than MHC I. We have previously shown that binding of ILT2 to MHC I confined antigen-induced TCR accumulation to the periphery of the immune synapse and prevented TCR migration to the synapse centre, where ILT2 accumulated. This TCR exclusion is not observed when ILT2 is stimulated by UL18, where TCR and ILT2 cocluster.
We here show that this differential patterning of the immune synapse by viral and host ligands for ILT2 induces qualitatively different TCR signal transduction, but does not alter the dynamics of TCR recycling. Furthermore, we use model T cells expressing ILT2 variants with high-affinity for MHC I (Kd=40nM and 2.5nM) to investigate whether the differential pattering of TCR at the immune synapse results from the greater affinity of the UL18/ILT2 interaction compared to the MHC/ILT2 interaction. We observe that engagement of high-affinity ILT2 by MHC I induces peripheral localisation of the TCR. This suggests that the receptor-ligand affinity does not account for the observed differences in synaptic distribution of the TCR when ILT2 is engaged by MHC compared to UL18. Consequently, factors other than affinity are responsible for the ability of UL18 to differentially affect TCR patterning at the immune synapse and so enhance inhibitory signals which may aid viral immune evasion.

P2.08.04
Tollip-induced down-regulation of MARCH1

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In addition to their classical antigen presenting functions, MHC class II molecules potentiate the TLR-triggered production of pro-inflammatory cytokines. Here, we have addressed the effect of Tollip and MARCH1 on the regulation of MHC II trafficking and TLR signaling. Our results show that MARCH1-deficient mice splenocytes are impaired in their capacity to produce pro-inflammatory cytokines in response to poly(I:C) and that TLR3 and MHC II molecules interact

in the endocytic pathway. Knocking down Tollip expression in human CIITA^{+} HeLa cells increased expression of HLA-DR but reduced the proportion of MHC II molecules associated with the CLIP peptide. Truncation of the HLA-DR cytoplasmic tails abrogated the effect of Tollip on MHC class II expression. While overexpression of Tollip did not affect HLA-DR levels, it antagonized the function of co-transfected MARCH1. We found that Tollip strongly reduced MARCH1 protein levels and that the two molecules appear to compete for binding to MHC II molecules. Altogether, our results demonstrate that Tollip regulates MHC class II trafficking and that MARCH1 may represent a new Tollip target.

P2.08.05
Intrinsic binding of V genes to autoantigens may contribute to BCR repertoire

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B-cell maturation occurs in several steps and it needs a constant stimulus for continuing its development. From the emergence of the pre-B-cell receptor, the signal transduction stimulates and supports B-cell development. Current viewpoints support that both a positive selection pressure for autoantigens and a tonic signaling constitutively stimulate B-cell maturation. In this work, we tested for the presence of a putative DNA binding site in a variable gene segment in its germline configuration, independent of VDJ recombination. After a survey in public antibody data bases, we chose a single mouse heavy variable gene segment highly represented in anti-nucleic acid antibodies and tested it for ssDNA binding in vitro. A phage display approach was used to search for an intrinsic binding to oligo deoxythimidine. The results revealed that binding to antigen can be biased by the use of specific DNA binding VH gene segment. The data shown here support the idea that some variable genes may have intrinsic reactivity towards endogenous autoantigens, and this property may contribute to the establishment of the immature B-cell repertoire.

P2.08.06
PAG (Cbp) as a positive reulator of mast cell signaling

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Antigen-immunoglobulin E (IgE) complexes-mediated triggering of the high-affinity IgE receptor (FcepsilonRI) initiates activation of mast cells and basophiles. Early activation events include, among others, tyrosine phosphorylation of transmembrane adaptor proteins LAT (LAT1), NTAL (LAT2) and PAG (Cbp). Although the role of LAT and NTAL in mast cell signaling has been extensively documented, the role of PAG in mast cells remains elusive. To contribute to elucidating the role of PAG in antigen- or thapsigargin-mediated activation of mast cells, we compared activation events in mouse bone marrow-derived mast cells (BMMCs) with normal or reduced levels of PAG. To this aim we used lentiviruses as efficient vectors for transfer of four different shRNAs which lowered the expression levels of PAG by 70% to 55%. Although activation-induced tyrosine phosphorylation of whole cell lysate was not dramatically affected, PAG knockdowns strongly inhibited beta-glucuronidase release in both, antigen and thapsigargin activated cells. Calcium ions mobilization and extracellular uptake of radioactively labeled $^{45}Ca^{2+}$ was also reduced after antigen triggering. The combined data indicate that PAG is a positive regulator of mast cell signaling.

P2.08.07
The phosphatase JKAP inhibits T-cell receptor signaling and autoimmunity by inactivating Lck

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JNK pathway-associated phosphatase (JKAP, also named DUSP22) is a JNK activator. JKAP also acts as a tyrosine phosphatase to dephosphorylate and inactivate focal adhesion kinase, leading to the suppression of cell motility. However, the physiological role of JKAP in T-cell receptor (TCR) signaling and immune regulation remains unknown. Here we report that JKAP directly interacts with, dephosphorylates and inactivates Lck at Y394, which in turn shuts off TCR signaling. JKAP-knockout T cells display enhanced cell proliferation and cytokine production upon anti-CD3 antibody stimulation. Lymphocyte development is normal in JKAP-knockout mice. JKAP-knockout mice show enhanced T-cell-mediated immune responses and are more susceptible to experimental autoimmune encephalomyelitis. Interestingly, aged JKAP-KO mice spontaneously develop inflammation and autoimmunity. Data from human patients with autoimmune diseases will be presented. Thus, our results indicate that JKAP is the major phosphatase that inactivates Lck in the TCR signaling turn-off stage, leading to suppression of T-cell immunity and autoimmunity. (Supported by NHRI-98A1-IMPP01-014)

P2.08.08
Downregulation of IRF3-IFNa/b pathway is crucial for the differentiation block induced by the leukemogenic fusion protein TEL/AML1

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With an incidence of 20-25%, TEL/AML1 fusion gene is the most common specific genetic rearrangement in acute lymphoblastic leukemia (ALL). ALL lead to an uncontrolled proliferation and spread out from their seat of undifferentiated B cells. Most of the genetic studies on these specific translocation provide the use of patient samples, thereby limited material and from people already presenting ALL. Also results from mouse models,the most failed in showing a phenotype since ALL require second and more changes. In this optic we first stabilized an hematopoietic cell-line (EML) expressing TEL-AML1 (EML-TA) in a stable way. This cell line is able to differentiate until B cells offering the opportunity for create a clear and complete panorama of genomics alterations along the hematopoietic steps and when expressing TEL-AML1 its differentiation is blocked as expected. In order to obtain a profile of total mRNA we run a complete gene profiling of EML at different steps of differentiation and compare it with the EML-TA cell line. From gene and subsequent protein analysis we identify IRF3-IFNα/β pathway as a crucial candidate altered by TEL-AML1. Infect by restoring its activity in EML-TA, cells were able to fully differentiate demonstrating it important role. Even more his disruption by siRNA was sufficient to block cell differentiation. We also identified IL7-mTOR as the activator pathway of IRF3-IFNα/β. This results are confirmed both in mouse (Lin-) and human (Reh cell line and CD34+ from PBMC) cells. This new inside the ALL differentiation block can offer new targets for ALL therapies.

P2.08.09
Higher sensitivity of restored human CD8 T-cells to virus- and tumor-derived antigens

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Research in human immunobiology is mainly based on working with peripheral blood mononuclear cells (PBMC). However, recent investigations have shown that circulating CD4 T-cells are less sensitive to several T-cell activating monoclonal antibodies and recall antigens as compared to tissue-resident cells. Interestingly, the impaired responsiveness of circulating T-cells could be restored by a short-term preculture step of PBMC at a high cell density (HDC PBMC, Römer P. et al. 2011).
Here we report that interferon-gamma ELISpot assays of HDC PBMC can be used as a sensitive in-vitro test for detection and characterization of antigen-specific CD8 T-cell responses in clinical and basic research. Recall responses to various virus- and tumor-derived antigens were significantly increased in sensitivity if CD8 T-cells were allowed to interact with monocytes during HD preculture of PBMC. Depletion of monocytes from PBMC before HDC completely abrogated the increased sensitivity of CD8 T-cells. Under defined long-term culture conditions, initial HD preculture even positively affected the responsiveness of expanded antigen-specific CD8 T-cells, whereas CD8 T-cells of unrelated specificity were unaffected.
Investigations on a molecular level showed significant differences in the RNA expression pattern of precultured CD8 T-cells compared to circulating CD8 T-cells. Changes in the gene expression of cell adhesion molecules, co-stimulatory molecules and metabolism might facilitate cell survival and frequent cell-cell interactions. This result supports the hypothesis that cell-cell interactions, especially between T-cells and matured monocytes, induce sub-threshold T-cell signals, which are responsible for an increase in sensitivity of T-cells towards antigens.

P2.08.10
Regulation of AP-1 activation in T cells by EGR-2

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EGR-2 is a transcription factor that controls inflammatory responses and proliferation of T cells. Deficiency in Egr-2 and -3 in T cells results in impaired proliferation in response to antigen receptor stimulation. Electrophoretic Mobility Assay (EMSA) revealed that the impaired T cell receptor signalling is due to a defect in Activator Protein-1 (AP-1) activity. AP-1 is a heterodimeric protein composed ofAP-1 family members including c-Jun, JunB, c-Fos. Our data shows that EGR-2 directly bind with Batf, which prevents Batf's inhibitory function on AP-1 activation. In addition to the interaction with Batf, we found that EGR-2 can also bind with the AP-1 proteins c-Jun and JunB through the leucine zipper domain. Immunohistochemistry data revealed that EGR-2 and c-Jun are co-localised in the nucleus. Functional analysis using AP-1 luciferase report assay revealed that EGR-2 can enhance AP-1 activation. Our results demonstrate an intracellular regulatory mechanism mediated by Egr-2 in T cells for the control of antigen receptor mediated activation.

P2.08.11
VAV1 controls inflammation in Experimental Autoimmune Encephalomyelitis (EAE)

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Vav1, a guanine nucleotide exchange factors for the Rho family of GTPases, plays an important role in T-cell and B-cell development, activation, tissue homing and effector functions. *Vav1* was found within a rat QTL that regulates the MS animal model EAE, and overlaps with several QTLs for immune-mediated diseases. Analysis of a human cohort showed association of certain haplotypes of the gene with MS. In this work we attempt to further characterize the function of Vav1 by using congenic rats that express different levels of Vav1 and are differentially susceptible to EAE.
In vitro stimulated naïve T cells of the EAE-protected strain (low Vav1) performed poorly in terms of activation, proliferation and cytokine secretion (especially IL-2) in response to TCR triggering. This defect was compensated by the addition of exogenous IL-2. Vav1 is also expressed by APCs, but only macrophages and dendritic cells showed any overt impairment in T cell stimulatory capacity, whereas the defect in B cells seemed restricted to TLR-mediated activation. Analysis of peripheral lymphoid tissue as well as CNS-infiltrating cells in autoantigen immunized animals showed only a minimal defect in all these parameters pointing to compensatory mechanisms in vivo. In fact the EAE-protected strain developed a Th17-skewed response, whereas the susceptible strain preferentially primed for Th1 cells. This Th17 skewing, together with a subsequent impairment in proliferation inside the CNS might account for a strong onset of disease, but decreased inflammatory parameters in later disease stages in the protected strain.

P2.08.12
Phage mini-antibodies and their use for detection of microbial cells by using electro-optical sensor

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Nowadays, cell detection in molecular biology is performed with the help of genetic engineering technologies for cloning recognizing fragments (hypervariable immunoglobulin domains). These technologies are cheaper and can be competitive in selectivity with hybridoma technologies. One such method is antibody phage display. Obtaining the phage miniantibodies to Azospirillum brasilense Sp245 as example, and their use for detection of microbial cells by using electro-optical sensor. Measurements of cellular orientational spectra (OS) of the cells were measured with an ELUS EO analyzer at a wavelength of 670 nm.
The preparation of miniantibodies to Azospirillum brasilense Sp245 surface antigens was made by using a combinatorial phage library of sheep antibodies. The prepared phage antibodies were used for cell detection by dot assay, electro-optical analysis of cell suspensions, and transmission electron microscopy. The results demonstrate that when A. brasilense Sp245 interacted with anti-LPS and antiflagellin mini-Abs, the magnitude of the EO signal changed considerably. It has been also found that the detection of cell A. brasilense Sp245 using the mini-antibodies is possible even in the presence of other cultures.
The findings from this study can be used to develop a rapid test for the detection of bacterial cells and for the assessment of exposure of cell-surface antigenic determinants.
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P2.08.13
Cdc42 controls cytokine secretion at the immune synapse

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Remodeling of the T lymphocyte cytoskeleton at the immune synapse is characterized by polarization of the microtubule organizing center and of the polymerized actin of the T cell toward the antigen presenting cell, and "opening" of the actin meshwork in the central zone of the immune synapse. This cytoskeleton remodeling has been shown to plays a key role in TCR signaling, T cell activation and polarized delivery of cytotoxic granules. Since little is known on how newly synthesized cytokines, once produced, are routed within T cells and on the mechanisms involved in regulating their secretions, we

investigated the role of actin remodeling at the immune synapse in this process. To do so, we silenced the expression of the RhoGTPase Cdc42, a key regulator of cytoskeleton remodeling, in human primary CD4+ T lymphocytes and investigated how this silencing affected formation of the immune synapse and cytokine secretion. We showed that MTOC polarity at the IS, which does not depend on Cdc42, is not required for cytokine secretion by T lymphocytes, whereas microtubule polymerization is. In contrast, actin remodeling at the IS, which depends on Cdc42, controls - the formation of the polymerized actin-ring at the IS, - the dynamic concentration of IFN-γ containing vesicles inside this ring and - the secretion of these vesicles. These results reveal a previously unidentified role of Cdc42-dependent actin remodeling in cytokine exocytosis at the IS.
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P2.08.14
OCILRP2 costimulates T cell activation by activating MAPK signaling pathway

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OCILRP2 is a typical Type-II transmembrane protein that is selectively expressed in the activated T lymphocyte, DC cell and B cells. It has been found to be a novel co-stimulator of T cell activation. However, the signaling pathways underlying OCILRP2 in the T cell activation are still not unclear. In this paper we found that shRNA silence OCILRP2 expression can inhibit the EL4 T cells' proliferation and IL2 production and reduce the MAPK3 and MAPK8 activation and Lck's tyrosine phosphorylation but not PI3K-AKT pathway. The antagonist OP2-antibody can partially impair the CD3/CD28-costimulated EL4 T cell activation, and inhibit the transcription factors NFAT, AP-1 and NF-kB activation. Furthermore, the immunoprecipitation results indicated that OCILRP2 could interact with DAP12 protein, an adaptor containing a intracellular ITAM motif that can transuduct the signal to the MAP kinase activation for T cell activation. Our data demonstrate that after binding to its ligands OCILRP2 and DAP12 forms heterodimer, which then activate the LCK-MAP kinases pathways and the transcription factors NFAT, AP-1 and NF-kB activation resulting in T cell activation.

P2.08.15
Generation of LYPLA1 knockout Jurkat T cells by transcription activator-like effector nuclease (TALEN) technology

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Jurkat T cells are a well-established model system to study TCR signaling. Several mutants have been generated, and the molecular bases for their TCR signaling defects have been identified. We wanted to use a gene-specific approach to generate LYPLA1 knockout Jurkat cells. LYPLA1 is a protein-palmitoyl thioesterase, and its pharmacological inhibition reduces IL-2 secretion after CD3/CD28 stimulation of Jurkat cells.
A TALEN target site within the first coding exon of the LYPLA1 gene was identified, and plasmids encoding for left and right TALENs were generated. Jurkat cells were co-electroporated with the TALEN plasmids and a plasmid coding for GFP to allow selection of transfected cells by fluorescence activated cell sorting.
The functionality of these TALENs in GFP positive Jurkat cells was first verified by enzyme mismatch cleavage. Thereafter, single GFP positive Jurkat cells were sorted, and clonally expanded. These clones were then screened by digestion with a restriction endonuclease that cuts within the wild type target site. Two out of 48 clones were identified as having DNA double-strand breaks repaired by error prone non-homologous end joining on both alleles. The genomic DNA of these clones was then verified to contain insertions and deletions in the targeted region of the first coding exon of LYPLA1 that induced frame shifts.
In conclusion, with the TALEN technology we generated gene-specifically altered Jurkat cells. Variation of the TALEN approach with homologous recombination to introduce a point mutation in the LYPLA1 gene and functional characterization of the LYPLA1 knockout clones are ongoing.

P2.08.16
B cells in patients with end-stage renal disease

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End-stage renal disease (ESRD) is the 5th stage of chronic renal disease which currently affects 873 million people in the world. One of the major problems, besides abnormalities associated with decreased glomerular filtration rate, is the higher prevalence of infections among hemodialyzed patients compared to healthy controls. Up to 36% of ESRD patients die of infection, which is the second main cause of death in this group. Earlier research focused mainly on T cells, leaving a gap in understanding B cells' biology in ESRD. The aim of this research was to evaluate: the expression of CD40, a key B cell surface antigen,, proliferation kinetics and immunoglobulin production. Peripheral blood mononuclear cells were isolated from full blood of ESRD patients and healthy volunteers. Cells were stained with CFSE and cultured in the presence of anti-CD3 as stimulator. Culture consisted of various erythropoietin (Epo) concentrations due to Epo intake by some patients. With the use of flow cytometry it was assessed that B cells of ESRD patients have significantly higher expression of CD40 ex vivo and IgG production, but are not able to maintain those levels after 5 days of cell culture as contrasted with healthy people. Non-Epo patients have higher percentage of apoptotic B cells than Epo patients and healthy people. High concentration of Epo in vitro helps patient's B cells to produce IgM. Research discovered definite influence of Epo on B cells and described certain differences between ESRD patients and healthy population.

P2.08.17
The human CD8β M-4 isoform dominant in effector memory CD8 T cells has distinct cytoplasmic motifs that confer unique properties

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The CD8 co-receptor influences T cell recognition and responses to pathogen infected and tumor cells. During evolution the CD8B gene in the ancestor of humans and chimpanzees, acquired two additional exons. As a result, in humans, there are four CD8β splice variants (M1 to M4) that differ in their cytoplasmic tails. The M-1 isoform which is the equivalent of murine CD8β, is predominantly expressed in naïve T cells, whereas, the M-4 isoform is predominantly expressed in effector memory T cells. The characteristics of the M-4 isoform conferred by its unique 36 amino acid cytoplasmic tail are not known. In this study, we identified a dihydrophobic leucine-based receptor internalization motif in the cytoplasmic tail of M-4 that binds the clathrin adaptor protein AP-2 and regulates M-4 cell surface expression and downregulation. The M-4 cytoplasmic tail was able to associate with ubiquitinated targets in 293T cells and mutations in the amino acids NPW, a potential EH domain binding site, either enhanced or inhibited the interaction. In addition, the M-4 intracellular domain was itself mono-ubiquitinated in both 293T cells and a human T cell line. When peripheral blood human T cells expressed CD8αβ M-4, the frequency of MIP-1β secreting cells responding to antigen presenting cells was two-fold higher as compared to CD8αβ M-1 expressing T cells. Thus, the cytoplasmic tail of the CD8β M-4 isoform has unique characteristics, which likely contributed to its selective expression and function in human effector memory T cells. (Supported by NIH RO1CA048511 to PBK, RO1CA147795 to JLR)

P2.08.18

A novel mechanism for the autonomous termination of pre-B cell receptor expression via induction of lysosomal-associated protein transmembrane 5

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The expression of pre-B cell receptor (pre-BCR) is confined to the early stage of B cell development, and its dysregulation is associated with anomalies of B-lineage cells, including leukemogenesis. Previous studies suggested that the pre-BCR signal might trigger the autonomous termination of pre-BCR expression even before the silencing of the pre-BCR gene expression to prevent the sustained pre-BCR expression. However, the underlying mechanism remains ill defined. Here we demonstrate that the pre-BCR signal induces the expression of lysosomal-associated protein transmembrane 5 (LAPTM5) which leads to the prompt pre-BCR down-modulation. While the LAPTM5 induction had no significant impact on the internalization of cell surface pre-BCR, it elicited the translocation of a large pool of intracellular pre-BCR from the endoplasmic reticulum to the lysosomal compartment, concomitantly with the drastic reduction of intracellular pre-BCR proteins. This reduction was inhibited by lysosomal inhibitors, indicating the lysosomal degradation of pre-BCR. Notably, the LAPTM5 deficiency in pre-B cells led to the augmented expression of surface pre-BCR. Collectively, the pre-BCR induces the prompt down-modulation of its own expression through induction of LAPTM5 that promotes the lysosomal transport and degradation of the intracellular pre-BCR pool, and hence limits the supply of pre-BCR to the cell surface.

P2.08.19
Detection of antigen-stimulated cytokine-secretion in human T-cells at single cell levels on a live cell chip

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Previously we established “on chip single cell analysis method” called ISAAC (immune-spot array assay on a chip), which enabled us to detect secretion of antigen-specific antibody (Ab) from single Ab-producing cells and provided us a very efficient and rapid method to produce antigen-specific Ab. In this study, we report that we could apply the ISAAC to detect antigen-specific human T-cells. We used microwell array chips that have an array of 45,000 to 234,000 microwells whose shape and size are just fit to accommodate single lymphocytes. To evaluate the T-cell ISAAC system, we analyzed the EBV-specific $CD8^+$ T-cells derived from HLA-$A24^+$ latent healthy donors. To this end, we first selected volunteers whose $CD8^+$ T-cells expressed TCR that bound HLA-A*2402 restricted tetramer of the EB virus epitope mixture (EB/A24 tetramer). We purified $CD8^+$ human T-cells and applied the cells onto the chip whose surface was coated with IFN-γ-specific Ab and stimulated them with EB/A24 tetramer on the chip. Six hours after the stimulation, we detected secreted IFN-γ using fluorophore-conjugated IFN-γ-specific Ab on the chip. It was detected on the chip as doughnut-like spots. Without the stimulation, we did not detect any spots. The results show that T-cell ISAAC system enables us to detect antigen-specific human T-cells. Human T-cell ISAAC system might provide a very efficient and rapid method of antigen-specific T-cell detection in human primary T-cell populations and facilitate antigen-specific T-cell therapy in the future.

P2.08.20
Recombinant P. aeruginosa outer membrane proteins and immune response

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There is an urgent need to develop new vaccines for prevention and immunotherapy of infections caused by *P. aeruginosa* since the agent has a high level of antimicrobial resistance, and the clinical course of disease is characterized by multiple organ damage. Induction of immune response under the influence of *P. aeruginosa* antigens is still poorly characterized.
To study the cytokine-mediated immune response in mice immunized with recombinant antigens of *P. aeruginosa*.
Recombinant *P. aeruginosa* outer membrane proteins OprL, OprF, hybrid protein OprF-I, which consists of of OprF and OprI sequences and recombinant toxoid (aTox) were produced. To obtain the expression of these proteins the BL21(DE3) and M15 *Escherichia coli* were used. Mice were injected intraperitoneally with 50 mg of *P. aeruginosa* antigenic preparations. Cytokine levels were determined in mice serum with test system FlowCytomixMouse Thl/Th2 10 plex.
We found that injection of antigenic preparations a wide spectrum of cytokines in mice was induced. The highest levels were observed in the ratio of IL-1 and IL-6 when oprF-I, oprL, aTox, oprF antigens were administered. OprFI actively stimulated the production of IL-2, and IL-5, IL-10, TNF-α and IFN-γ. OprF-I antigen contributed to the induction of IL-6, IL-17, TNF-a and IFNγ, while aTox – to the expression of IL-1, IL-2, IFN-γ. OprL antigen induced IL-17 and TNF-α to greatest extent, while IL-10 – to lesser extent.
The antigens of *P. aeruginosa* intraperitoneally administered to mice favored the formation of the immune response to induce both Th-1 and Th-2 pathways.

P2.08.21
Lck Mediates Signal Transmission from CD59 to the TCR/CD3 Pathway in Jurkat T Cells

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Functional heterogeneity has been considered as a principle mechanism of the adaptive immune response. Here we developed an imaging-based method for studying calcium signaling at the single-cell level as a function of time and stimulus. Jurkat T cells were exposed to stimulatory anti-CD3ε- or anti-CD59-coated surfaces and individual calcium time traces were analyzed via unsupervised clustering. This analysis revealed a heterogeneous calcium response of the cell population in a stimulus-dependent manner. Interestingly, an unresponsive subpopulation identified upon anti-CD59 stimulation coincided with a low CD3 surface expressing subpopulation observed in flow cytometry measurements. Further analysis of TCR/CD3 deficient or overexpressing cells showed that CD59-mediated signaling is strongly dependent on TCR/CD3 surface expression. However, in protein co-patterning and fluorescence recovery after photobleaching experiments no direct physical interaction was observed between CD59 and CD3 at the plasma membrane upon anti-CD59 stimulation. Nonetheless, siRNA-mediated protein knock-downs of downstream signaling molecules revealed that the Src family kinase Lck is essential for both signaling pathways. Moreover, physically linking Lck to CD3ζ completely abolished CD59-triggered calcium signaling, while signaling was still functional upon direct TCR/CD3 stimulation. Altogether, we demonstrate that Lck mediates signal transmission from CD59 to the TCR/CD3 pathway in Jurkat T

cells, and propose that CD59 may act via Lck to modulate T cell responses.

P2.08.22
The initiation of B cell receptor signaling is sensitive to the stiffness properties of the substrate presenting the antigens

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B lymphocytes are activated upon antigen sensing by B cell receptors (BCRs). The substrate presenting the antigen can show different degrees of stiffness. It is not clear if B cells can respond to changes in substrate stiffness. Here we use high resolution, high speed live cell imaging techniques to capture the molecular events in B cell activation after the recognition of antigens tethered to polyacrylamide gel substrates with variable degrees of stiffness as quantified by Young's modulus (2.6-22.1 kPa). We show that the initiation of B cell activation is extremely sensitive to substrate stiffness. B cells exhibit much stronger activation responses when encountering antigens tethered to substrates with a high degree of stiffness as measured by the accumulation of BCR, pSyk and pTyr molecules into the B cell immunological synapse. Antigens tethered to stiff substrates induce the formation of more prominent BCR and pSyk microclusters with significantly enhanced colocalization as compared to antigens tethered to soft substrates. Moreover, the expression of the B cell activation marker CD69 is greatly enhanced in B cells encountering antigens on stiffer substrates. Through time lapse live cell imaging, we find that the different responses of B cells to substrate stiffness are only demonstrated 5 min after BCR and antigen recognition. Using a series of cytoskeleton inhibitors, we determine that the mechanosensing ability of B cells is dependent on microtubules, and only mildly linked to the actin cytoskeleton. These results suggest the importance of the mechanical properties mediated by substrate stiffness in B cell activation.

P2.08.23
C-type lectin related protein OCILRP2 enhances development and functional differentiation of mouse bone marrow-derived dendritic cells

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Osteoclast Inhibitory Lectin Related Protein 2 (OCILRP2) is a typical type II membrane protein, which selectively expresses in the immune tissues (e.g. dendritic cells (DC), B lymphocytes and activated T lymphocytes). Binding to its ligand NKRP1f, OCILRP2 can upregulate the T cell activation mediated by B7.1/CD28 co-stimulation. However, its roles in DC cells are still unclear. Here we found that a soluble OCILRP2-Ig protein, which works as an antagonist of OCILRP2, can significantly suppress the expression of CD80, CD86 and MHC-II molecules on the mouse bone marrow derived immature DC and LPS-induced mature DC. It also can inhibit DC cells' Antigen uptaking and allogeneic T lymphocytes stimulation and the production of inflammatory cytokines of IL-6, IL-12 and TNF-α but not IL-10 in vitro. Further study indicates that OCILRP2-Ig protein can block the OCILRP2-mediated NF-κB activation, which may result in the defective DC generation. Our studies reveal OCILRP2 plays an important role in the DC development and functional differentiation.

P2.08.24
Inhibition of PI3K/Akt pathway in antigen-stimulated B-lymphocytes and hybridoma cells induces their apoptotic death

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Interplay between signaling pathways in stimulated murine B-lymphocytes and Ig-secreting hybridomas was here studied. The in vivo immune response was induced in Balb/c mice by injection of serum-derived protein antigens such as bovine serum albumin or human apo-B100, or human fibronectin. Peripheral blood lymphocytes and splenocytes were isolated from the mice and subpopulations of the antigen-binding B-lymphocytes were then assorted using a FACS and the FITC-labeled antigens. Those subpopulations of B-cells stimulated by the labeled antigen binding to the cell surface immunoglobulin receptors were treated with certain inhibitors of cellular signaling. It was found that the stimulated murine B-lymphocytes become extremely sensitive to inhibitors of the PI3K/Akt pathway such as LY294002, wortmannin, Akti-X and 17AAG whose very low concentrations induced massive apoptosis in subpopulations of the treated B-cells which were pre-stimulated by the antigens. In contrast, the non-stimulated B-lymphocytes from the same cell fractions exhibited normal tolerance to low doses of those inhibitors (i.e. only slight levels of apoptosis). Analogous effects were observed with murine B-hybridoma cells stimulated by antigen binding. Importantly, when a constitutively activated (mutant) form of Akt was transiently overexpressed in the hybridoma cells, this significantly impaired their apoptotic death following the joint action of antigens and PI3K/Akt pathway inhibitors. We conclude that the Akt activation in B-cells stimulated by antigen-binding is necessary for their survival and subsequent propagation. Alternatively, blockade of the Akt activation may causally be realized for apoptotic elimination of autoreactive B-cells in the process of clonal selection in vivo.

P2.08.25
The actin and tetraspanin networks organize receptor nanoclusters to regulate B cell receptor-mediated signaling

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The actin cytoskeleton is involved in numerous cellular events. Thus not surprisingly, increasing evidence also demonstrates the role for the cytoskeleton in various stages of lymphocyte activation. We recently observed that a mere transient alteration of the actin network leads to B cell activation. Here we show that the disruption of the cytoskeleton triggers signaling that not only requires the B cell receptor (BCR), but also the co-receptor CD19. In addition to the cytoskeleton, the co-operation of these two receptors is further coordinated by the CD81-tetraspanin web, which organizes CD19 in the plasma membrane. New insights into the initiation of the BCR signaling are supported by super-resolution microscopy, which demonstrates that endogenous IgM, IgD and CD19 exhibit distinct nano-scale organization within the plasma membrane of naïve B cells. These nanoclusters do not change in character during BCR signaling. Thus, we postulate that cytoskeleton reorganization releases pre-existing BCR nanoclusters, which can interact with CD19 held in place by the tetraspanin network. Our results not only suggest that receptor compartmentalization regulates B cell activation by surface-bound antigen, but also imply a potential role for CD19 in mediating ligand-independent BCR signaling necessary for B cell survival.

P2.08.26
CD22 ligand-binding and signalling domains reciprocally regulate B-cell Ca2+ signalling

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The transmembrane protein CD22 mediates the inhibition of calcium signal after BCR crosslinking. The extracellular domain of CD22 can bind to α2,6-linked sialic acids, while the cytoplasmic domain contains inhibitory motifs (ITIMs). In this study we wanted to analyse the

ligand-binding and signalling domain of CD22 independently and thereby determine their functional interplay.
We generated CD22-R130E knockin mice, in which CD22 is no longer able to bind sialic acids. The CD22-Y5,6F and CD22-Y2,5,6F mice with mutated CD22 ITIM-domains can´t recruit SHP-1 phosphatases to the CD22 cytoplasmic tail anymore.
In the bone marrow we found a reduction of recirculating B cells only in the CD22-ITIM knockin mice, but not in CD22-R130E knockin mice. In the spleen all mice had reduced marginal zone B cells. Only CD22-Y2,5,6F B cells showed also increased turnover in vivo and spontaneous cell death in vitro. Ca2+ measurements revealed a decreased flux after BCR stimulation in CD22-R130E B cells and higher flux in CD22-ITIM mutant B cells. The CD22-R130E mutation affected the CD22/BCR association, because less total, but more phosphorylated CD22 was co-precipitated with IgM.
These data indicate that the cis-ligand binding of CD22 is crucial for regulation of the B cell Ca2+ signalling. This control is mediated by ligand-dependent regulation of the association to the BCR. In contrast, the CD22-ITIM motifs are important for a direct inhibition of Ca2+ signalling. The loss of these inhibitory motifs changes the B cell turnover, the survival of the B cells and thereby affects the homeostasis of B cell populations.

P2.08.27
Germline TSC1 mutations are permissive for T lymphocyte development and homeostasis in Tuberous Sclerosis individuals

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Antigen recognition on the surface of antigen presenting cells initiate a variety of intracellular signals critical for naïve T cells to commit to proliferate and differentiate. Others and we have shown the TSC/mTOR axis to be critical in coordinating cell quiescence, proliferation and fate determination. Blocking mTOR by Rapamycin, or genetic inactivation of components of the mTOR Complex (mTORC) or TSC1, unbalances mTORC1 and mTORC2 regulation, impacting on cell quiescence, proliferation and differentiation. In humans, germline mutations of TSC1 predispose to Tuberous Sclerosis Complex disease, an autosomal dominant disorder characterized by multifocal hamartomatosis. Whether such mutations impact on T cell functions in these individuals was unknown. To this aim we analyzed subset representation, phenotype and function of T cells from two independent families with characterized mutations of TSC1 in parallel to T cells from healthy volunteers and to mouse T cells with mono- or bilallelic deletion of Tsc1 (from T-lineage restricted Tsc1+/- and Tsc1-/- mice). We found that heterozygosis of Tsc1/TSC1 is sufficient for proper hamartin expression and both homeostatic and TCR-induced mTORC1 and mTORC2-dependent signaling in mice and human T cells. In contrast, loss of Tsc1/TSC1 following biallelic genetic inactivation and shRNA-induced down-regulation unbalanced mTORC-dependent FOXO1/3 regulation, leading to loss of mitochondrial potential and apoptotic cell death. Thus, while heterozygosis of Tsc1/TSC1 is sufficient for proper T lymphocyte development and homeostasis, its more severe loss is incompatible with both mouse and human T cell survival.

P2.08.28
Ligation of human Fc receptor like-2 (FCRL2) by specific monoclonal antibodies downregulates B cell receptor mediated signaling

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Introduction: Regulation of the B cell antigen receptor (BCR) signaling is critical for balancing the B cell response and function. Human Fc receptor like-2 (FCRL2) could influence B cell signaling due to possession of both activating and inhibitory tyrosine-based immunoregulatory motifs. Since FCRL2 natural ligand has not been identified yet, we generated FCRL2-specific monoclonal antibodies (mAbs) and employed them to investigate influence of FCRL2 stimulation on BCR signaling in FCRL2 expressing B cell line. Methods: Anti-FCRL2 secreting hybridomas were produced using hybridoma technology and screened with the immunizing protein by ELISA and a stable CHO cell line expressing FCRL2 protein by FACS. Different concentrations of these mAbs and anti-IgM antibodies were used to stimulate FCRL2 expressing tumor cell line (CA46). Phosphorylation of some signaling molecules was analyzed by FACS and immunoblotting. Results: Two hybridoma clones producing mAbs specific for FCRL2 were selected (5A7-E7 and 3D8-G8). Both mAbs displayed no cross-reactivity with the other members of the FCRL family as tested by FACS and ELISA techniques. Engagement of FCRL2 by these mAbs resulted in significant inhibition of BCR signaling mediators such as calcium mobilization, phosphorylation of Erk, p38 and Jnk MAP kinases. A minor or no inhibition of the phosphorylation of whole protein tyrosine, Syk, Vav and Akt was observed in B cells treated with a cocktail of anti-FCRL2 and anti-BCR Abs. Conclusion: These findings indicate that FCRL2 ITIM motifs are functional and anti-FCRL2 mAbs may mimic the FCRL2 natural ligand by induction of inhibitory signals in B cells.

P2.08.29
Cell membrane permeable phosphopeptides of Gab1 inhibit signaling in B cells by interacting with SHP-2 phosphatase

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The multisite docking protein Gab1 plays essential is role in growth factor, cytokine- and antigen receptor signaling. Gab1 has several tyrosine motifs that get phosphorylated upon cell activation and serves as a platform for SH2 domain molecules. One of the main binding partners of Gab1 is SHP-2 tyrosine phosphatase. The intimate interaction between phosphorylated Gab1 motifs and SHP-2 activates the phosphatase. SHP-2 by activating the Erk pathway may play a positive role in signaling, additionally, its role is crucial in many hematological malignancies and cancer.
Our purpose is to develop small molecule modulators based on the sequence of Gab1 to interfere with SHP-2 activity.
First we identified the proteins that bind to Gab1 N terminal phosphopeptide, GDKQVEY(p)LDLDLD from B cell lysate. This phosphopeptide of Gab1 binds PLCγ and SHP-2. We have analyzed the interaction between phosphopeptides and the SH2 domains of SHP-2 and found that the N terminal SH2 domain binds GDKQVEY(p)LDLDLD with one order of magnitude higher affinity as compared to the C terminal domain. In the contrary, the C terminal peptide of Gab1, DERVDY(p)VVVDQQK peptide binds only to the C terminal but not to N terminal SH2 domain.
Comparison of the phosphopeptide unbound and bound crystal structures of N terminal SH2 domain suggests that Tyr66 have a critical role in the allosteric activation of the phosphatase domain. Binding of GDKQVEY(p)LDLDLD to the N terminal SH2 domain enhances SHP-2 phosphatase activity in vitro, however, when administered as cell membrane permeable phosphopeptide into B cells, reduces Erk activity.

P2.09 MHC and other polymorphic genes in health and disease

P2.08.30
Perturbing human TCR signaling pathway through superantigen from Staphylococcus aureus: Multi objective optimization under FBA

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It is necessary to understand the dynamics of a cellular system to evaluate the capacity of an immune response at the time of infection. An integrated system was constructed that included Superantigen (SAg) expression regulatory and TCR signaling pathways of Homo sapiens to compare the pathogen (Staphylococcus aureus) perturbed and unperturbed system. A multi-objective optimization (MOO) formalism under Flux Balance Analysis (FBA) was developed, and analyzed in four ways: (1) Implemented on an unperturbed TCR signaling pathway of H. sapiens, with the objective to maximize the quantity of signal flow for inhibitory molecules (CBL and SHP1), and observed their signal flow values, which were later used as a base line for comparison. (2) The pathogen perturbed TCR signaling pathway (an integration of SAg expression regulatory pathway of S. aureus and TCR signaling pathway of H. sapiens), was studied with the objective of maximizing the quantity of signal flow for stimulatory molecules (ZAP70, LCK and FYN). (3) The same integrated pathway was studied on optimization of two conflicting objectives (minimization of SAg expression and maximization of the quantity of signal flow for TCR:CD3 complex and ZAP70). (4) Perturbation of the signal flow for some of the molecules (ZAP70, LCK, FYN) was performed, under pathogen perturbed and unperturbed conditions to check the effects of perturbation on CBL, SHP1 and Interleukins. Here, we figured out a relation between the TCR signaling inhibitory and stimulatory molecules - balance between these two keeps the system into resting/stimulating stage. Model predictions were validated through experimental evidences.

P2.08.31
Suppression of T cells proliferation by A novel generated monoclonal antibody, COS3A

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Leukocytes surface molecules play crucial role in cell-cell interaction and communication. Some of them have been identified; still some of them are waiting to be identified. Present study, a novel generated monoclonal antibody (mAb) named COS3A was of interest. The COS3A recognizing molecule is expressed on surface of hematopoietic cells but not red blood cells. High expression of this molecule was induced upon T cell activation. Suppression of CD3-mediated T cell proliferation has been observed in the present of the mAb COS3A. This phenomenon was corresponding to inhibition of IL-2 and IL-4 production. Biochemical analysis revealed that the COS3A antigen has a molecular weight of about 30-70 kDa under non-reducing conditions. N-glycosidase F treatment reduced its size to 25 kDa. Similar results were endorsed by pretreatment the cells with tunicamycin, an inhibitor of N-glycosylation, before cell lysates preparation. In conclusion, COS3A molecule is a high N-glycosylated glycoprotein, which might play a critical role in regulation of T cell responses. Further biochemical identification and immunological function of this molecule are underway.

P2.09.01
New genetic variants of the *CCR5* gene in the Omani population

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Background: A number of polymorphisms were identified within the promoter and coding regions of the *CCR5* gene; some of which have been found to affect the CCR5 protein expression and function, consequently affecting HIV-1 binding to cells that normally express the CCR5 protein.
Aims: To identify all the polymorphic sites that exist within the *CCR5* gene as well as the *CCR2V64I* in Omani adult individuals.
Methods:We examined the distribution of the detected variants and compared them with other populations documented in the literature. Blood
samples were collected from 89 Omani adult individuals, and genomic DNA was amplified by polymerase chain reaction and sequenced to identify all the polymorphic sites that exist within a continuous region [of around 4.67 kb] of the *CCR5* gene and to detect the known V64I mutation in the *CCR2* gene.
Results and Conclusion: Out of 32 variable positions detected, four were new indels -2973A/-, -2894A/-, -2827TA/- and -2769T/-, all were located in the 5'UTR. Two new mutations were described for the first time, -2248G/A was detected in one subject in the Intron 1 region of the *CCR5*, whereas +658A/G was found in the coding region. These novel variations in the 5'UTR have shown to exhibit potential effects in the TF binding sites by *in silico* analysis that merit further investigation by experiments to validate our results. Moreover, we identified two novel haplotypes and eight known *CCR2-CCR5* haplotypes, those hapoltypes were found to be at different frequency pattern compared to that documented worldwide.

P2.09.02
TNF-α gene promoter polymorphisms in Algerian patients with ankylosing spondylitis

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Tumor necrosis factor TNF-α is an important pro-inflammatory cytokine that has been implicated in the pathogenesis of ankylosing spondylitis (AS).To evaluate the role of TNF-α gene as susceptibility marker in Algerian AS patients, four single nucleotide polymorphisms (SNPs) (-238G/A, -308G/A, -857C/T and -1031 T/C) were analyzed in 341 patients with AS, and 357 healthy control subjects. The TNF-α SNPs were genotyped by Taqman technology; and lifecodes cytokines sso typing. The analysis of genotype frequency was performed with Phase data.
A statistically significant difference in the frequency of TNF -857 C/C (54.03% vs 77.94%; c2=43.67; P=3.9 x 10-11; OR=0.33) and TNF -857 C/T (42.99% vs 20.34%; c2= 40.65; P= 1.8 x 10-10; OR= 2.95) between patients and controls was found. Conversely, other SNPs (-238, -308, and -1031) did not show a significant allelic and genotypes frequency difference between patients and controls.
The haplotype analysis reveals six common haplotypes in our population (TCGG, TCAG, TCGA, TTGG, CCGG and CCGA). In comparison with healthy control subjects, the analysis of TTGG haplotype in the AS group showed a significantly correlation (p=1.3×10-8, OR = 2.28) as well as the TCGG haplotype in healthy control subjects (p=0.005, OR=0.74).In contrast, no significant difference for other TNF haplotypes was found. These results suggest that the -857 SNP plays a dominant role in susceptibility or resistance to AS in our population.

P2.09.03

Possible involvement of polymorphisms in OAS2, OAS3, and CD209 genes, associated with severe forms of tick-borne encephalitis, in predisposition to hepatitis C in Russian population

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Hepatitis C (HC) is a severe liver disease caused by single-stranded RNA virus of the Flaviviridae family. Previously, we reported an association of several single nucleotide polymorphisms (SNPs) in three innate immunity genes (OAS2, OAS3, and CD209) with human predisposition to severe forms of tick-borne encephalitis (caused by a virus from the same family) in Russian population (Barkhash et al., 2010, 2012). In this study, genotype and allele frequencies for five of these SNPs (OAS3 rs2285932 and rs2072136, OAS2 rs15895 and rs1732778, CD209 rs2287886) were analyzed in 75 HC patients and compared with previously studied population control (265 Novosibirsk citizens) (Barkhash et al., 2010, 2012). For all OAS gene SNPs, no significant differences between HC patients and the control group in genotype or allele frequencies were found. However, highly significant differences were found for the CD209 rs2287886 SNP: an increase in the frequency of A allele (46.6%) ($P = 0.001$), G/A genotype (60.3%) ($P = 0.001$), and a decrease in the frequency of G/G genotype (23.3%) ($P < 0.001$) were detected among HC patients as compared with the control group (31.5%, 39.0%, and 49.0%, respectively). Although an increase in A allele frequency is similar to data on predisposition to tick-borne encephalitis, the genotype distribution in patients and controls is different in case of HC and tick-borne encephalitis (Barkhash et al., 2012). Thus, we found that CD209 rs2287886 SNP is associated with predisposition to HC in Russian population. This work was supported by Russian Foundation for Basic Research (grant 11-04-01206a).

P2.09.04
Role of paired immunoglobulin-like receptor B (PirB) and T CD8 lymphocytes during axonal regeneration following peripheral axotomy

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Major histocompatibility complex class I (MHC-I) molecules, mostly known to present endogenous antigens to TCD8 cells, have been suggested to participate in the CNS synaptic plasticity process during development and after neuronal damage in the adult. Among the MHC-I ligands, PirB may be a key inhibitory molecule related to axonal refinement in response to reinervation of the target muscle. Here we evaluated the immunoreactivity against TCD8 cells, MHC-I, PirB and neurofilaments (axonal marker) following crushing of the sciatic nerve in WT, MHC-I-KO and RAG-KO mice two, four and eight weeks post injury (wpi). Additionally, the sciatic functional index (SFI) was obtained with an automated walking track test up to eight wpi. The SFI showed that all mice strains fully recovered motor function within four wpi, either in a progressive fashion, as observed in MHC-I-KO and RAG-KO mice, or presenting a subtle regression, as seen in WT mice (2-3wpi). Particularly at this stage TCD8 cells were in contact with axons, as revealed by confocal microscopy. MHC-I and PirB immunolabeling increased in WT concurrently with the axonal regeneration ($p<0.05$). In the absence of MHC-I, a higher amount of neurofilaments and PirB was found 2wpi ($p<0.05$), reaching normal levels thereafter. Also, in RAG-KO, MHC-I immunolabeling increased only at 2wpi ($p<0.01$), PirB decreased 4wpi ($p<0.01$) and neurofilament levels remained constant. Overall, the present results suggest that both TCD8 cells and PirB could be involved in the elimination of axonal sprouts during regeneration and following neuromuscular reconnection. Financial Support: FAPESP and CNPq.

P2.09.05
Possible roles of MICA and NKG2D and their interaction in the pathogenesis of autoimmune Addison's disease

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Autoimmune Addison's disease (AAD) is a classic organ-specific autoimmune disorder. Although the disease is characterized by antigen-specific antibody and T cell responses against the adrenal cortex, the exact mechanisms underlying tissue damage and immunopathology are largely unknown. Genetic risk for AAD has been associated with several loci in the HLA complex, including polymorphisms in the gene encoding the MHC class I polypeptide-related sequence A (MICA) protein. MICA is a stress-inducible antigen implicated in tumor surveillance and transplant rejection, but also in autoimmune disorders such as type 1 diabetes. The ligand for MICA, the NK cell activating receptor NKG2D is expressed on NK cells, γδ T cells and a subset of cytotoxic CD8+ T cells. There are thus numerous pathways involving MICA/NKG2D interactions that could play a significant part in the pathogenesis of AAD. The aim of the present study has been to investigate these pathways in the context of adrenal autoimmunity. Initially, we detected abundant expression of MICA on the adrenocortical carcinoma cell-line NCI-H295R (using immunofluorescence), but also on healthy adrenal tissue using immunohistochemistry. Furthermore, short-term IL-15 activated polyclonal T cell lines were able to kill NCI-H295R cells in a partly NKG2D dependent manner. Finally, antigen-specific T cell responses against defined peptides of 21-hydroxylase, the main antigen of AAD, were augmented in the presence of stimulating antibodies against NKG2D. We suggest that MICA/NKG2D interactions could contribute to adrenal autoimmunity in manners both dependent and independent of antigen-specific T cell recognition.

P2.09.06
Cross-presentation in renal transplant recipients

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Extracellular antigens are mostly presented in the context of MHC class II, whereas endogenous antigens are presented in class I. Both pathways can influence the recognition of alloantigens in the context of transplantation, and contribute to indirect and direct recognition respectively. However, exogenous antigens can also be cross-presented in the context of MHC class I. The contribution of cross-presentation to allorecognition in organ transplantation is currently not known.
Therefore, HLA-A2 tetramers were created with different HLA-A1/A3 derived peptides. Peptides were selected based on their binding capacity to HLA-A*0201 and their polymorphism when compared to the recipient HLA profile, and were calculated using HLA epitope prediction programs (SYFPEITHI, The Immune Epitope Database and Analysis Resource (IEDB), Bimas, IMGT/HLA Sequence Database, MAPPP, NetMHC). The HLA-A2 tetramers were labeled with PE and the five consistently highest scoring tetramer-peptide constructs were used to stain cross-reactive recipient CD8 T-cells in a renal transplantation cohort. Peripheral blood mononuclear cells (PBMC) from 20 renal transplant recipients (HLA-A2+) that received a HLA-A1/A3 mismatched kidney were stained. Stains were preformed on samples from before and 12-24 months after transplantation. Cross-reactive CD8 T-cells were found in one renal transplant recipient at 12 and 24months after transplantation. Further characterization of these cells indicated they were mainly present in the CCR7-/CD45RA+ effector memory population.
In conclusion, we have demonstrated that also cross-presented donor alloantigen may contribute to the recognition of transplanted organs. Current efforts are taking place to further isolate and clone the T-cells to asses their specificity and function.

P2.09.07
MHC Class I expression in hematopoietic and non-hematopoietic cells regulates NK cell licensing and viral control

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Inhibitory Ly49G2 receptor-expressing NK cells contribute are needed to control murine (M)CMV infection in MA/My and MHC class I Dk-expressing C57L mice. In bone marrow transplant (BMT) recipient mice, highly effective NK cell-mediated viral control requires Dk expression in both hematopoietic and non-hematopoietic cells. Because Dk is a licensing ligand for Ly49G2, we reasoned that Dk expression in discrete cell lineages modulates NK responsiveness and viral control. To address the question, we generated additional BMT mice and measured the effect of Dk expression on NK effector competency and virus control. As expected, G2+ NK cells in Dk-transgenic (Dk-Tg) mice reconstituted with Dk-Tg bone marrow were fully licensed and conferred MCMV resistance. Conversely, in BMT recipients with Dk expression restricted to only hematopoietic or non-hematopoietic cells, G2+ NK cells displayed reduced activation receptor stimulation and significantly less viral control. These data demonstrate that NK cell licensing is regulated by MHC class I expression in hematopoietic and non-hematopoietic cells and this corresponds to the extent of G2+ NK cell-mediated viral control. Whereas recent data has shown that licensing of adoptively transferred mature NK cells is sensitive to MHC I expression on non-hematopoietic recipient cells, ongoing adoptive transfer experiments with Dk-Tg and non-Tg mature NK cells further examines the relationship between NK cell licensing and their capacity for viral control.

P2.09.08
KIR-HLA interactions during pregnancy impact their population frequencies

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The interaction between embryonic trophoblast cells and uterine Natural Killer (uNK) cells is known to stimulate blood vessel formation to supply nutrients to the placenta. If the embryonic cells express an allogenic HLA genotype that is more different from the mothers HLA genotype, the interaction with uNK cells can be stronger. To detect if an embryonic cell expresses allogenic HLA molecules, the uNK cell uses various Killer Immunoglobulin-like Receptors (KIRs). Different KIRs recognize different subsets of HLA molecules as ligands. In this study we tested whether the recognition of allogenicity during pregnancy influences the co-evolution of HLA and KIR genes. To this end, we first developed a simple model to calculate the chance of allogenic recognition as a function of the KIR ligand frequency in a population. This analysis revealed that the allogenic recognition would be optimal if a KIR molecule recognizes one-third of the HLA haplotypes. Second, for KIR2DL1, KIR2DL2/3, KIR3DL1 and KIR3DL2, the ligand frequency was examined in different populations. In agreement with our model, a ligand frequency of one-third was often observed. Moreover, for KIRs that are more frequent, KIR ligand frequencies were closer to the optimum (of one-third). In addition, we show that the frequencies of KIR2DL2 and KIR2DL3, that are located on the same locus, correlate with the optimality of their respective ligand frequencies. We conclude that the detection of allogenicity, which increases the chance of successful reproduction, has a profound impact on the co-evolution of HLA and KIR in the human population.

P2.09.09
HLA-B*46:01 associated with oncogenic HPV persistence

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Background: Human papillomavirus (HPV) is the major cause of cervical cancer. Persistence of oncogenic HPV is the pivotal step in the natural history of cervical carcinogenesis. We studied HLA class I with oncogenic HPV persistence.
Method: There were 11,923 women participated in this Community-Based Cervical neoplasia Screening Project (CBCSP), which provided two health examinations in 1991-1993 and 1993-1995. Deep-frozen cervical cells were used for HPV DNA amplification by polymerase chain reaction and genotyping by HPV Blot to identify 39 types of HPV. Genomic DNA from Buffy coat was extracted and identified HLA Class I polymorphism using the commercial kit, SeCore.
Result: There were 10,602 women with available Pap smear and cervical cells at enrollment. Among 909 women infected with oncogenic HPV, 580 women attended the follow-up visit, including 242 women persisted infection and 338 women cleared the virus. The allele frequency of HLA-B*46:01 was higher in persistence group than in clearance group (18.6% vs. 13.1%, p value = 0.009). Compare to 23.7% of women in clearance group harbored one or two allele of HLA-B*46:01, 34.7% of women in persistence group harbored it; the odds ratio was 1.7 (1.2-2.5, p value=0.004). A furtherconfirmation was made between women with cervical HSIL+ (almost oncogenic HPV persistent) and clearance group, a two-fold risk (1.3-3.3, p value=0.003) was obtained.
Conclusion: Our results suggested women harbored HLA-B*46:01 had impaired susceptibility to clear oncogenic HPV infection. The HLA pathway clues a possible mechanism for therapy development to clear HPV infection to replace life-long repeated Pap screening.

P2.09.10
Haplotypes of the HLA-G gene are associated TD1

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The TD1 (Type 1 Diabetes) is a multifactorial disease in which the autoimmune destruction of pancreatic cells. A recent study mapping the MHC region, performed on families with TD1 patients, identified the region of the HLA-G as an independent susceptibility gene for the disease A major function of HLA-G is the inhibition of Natural Killer cells and cytotoxic T cells. A relatively higher degree of variation is observed in the 5'URR and 3' UTR, wich may be in linkage disequilibrium. 29 SNPs have been identified in the HLA-G 5'URR , implicated in the regulation of HLA-G expression. 3'UTR region contains 3 polymorphisms related with several posttranscriptional regulatory elements. For the evaluation of the HLA-G 5'URR region variation and the 3'UTR fragments were amplified using PCR and sequenced, for 3'UTR 120 patients with TD1 and 120 controls, and the extended haplotypes 100 patients and 100 controls. The PHASE method and EM algorithm were used to infer haplotypes. Only the +3010 CC was significantly associated with protection, our group has identified the +3010C/G site as a putative target for microRNAs, suggesting a possible posttranscriptional control. We observed a significantly decreased frequency of the UTR-3 haplotype in patients. For extended haplotypes the HG 0104 haplotypes group, this haplotype is associated with the group , which have been considered as high-producing allele HLA-G. The results here presented indicate that the HLA-G gene may play a role in T1DM susceptibility, pathogenesis or both.

P2.09.11
Regulation of MHC class II expression in cancer cell lines involves the RNA binding protein EBP1

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Many tumors including melanoma, glioblastoma, colorectal and breast carcinomas express MHC Class II molecules. The correlation between expression and clinical outcome is an open question and new findings could be reached through the study of CD4+ T cells MHCII tumor specific. Our interest is focused on MHCII post transcriptional regulation, and in M14 human melanoma cell line we demonstrated the existence of a mechanism that, coupling mRNAs

transcription and processing, guarantees the balanced synthesis of mRNAs and the regulated surface expression of the heterodimers. These events occur inside a ribonucleoprotein complex in which we identified EBP1, a characterized RNA binding protein, involved in the regulation of stability and translation of several transcripts, including MHCII.
EBP1, identified as ErbB3 binding protein, presents two isoforms in normal and cancer cells: p48 and p42. Literature data showed that EBP1 isoforms can act as oncogene or tumor suppressor and the different role could be related to the cell type and differentiation stages. In order to analyze the EBP1 function on MHCII expression, we knockdown or overexpress EBP1 isoforms in U87 glioblastoma, MCF7 breast cancer and murine melanoma B16. We determine MHCII expression and mRNA half-life by RT-qPCR and investigate on cancer phenotype by cell cycle analysis. Additionally we will study if EBP1 modulation influences the ability of B16-OVA cell line to present OVA antigens in ex vivo experiments.
In particular our aim is to verify whether EBP1 and other RNA binding proteins of complex regulating MHCII at post transcriptional level affect tumor antigens presentation.

P2.09.12
Major Histocompatibility Complex related LEI0258 allelic segregation in chicken

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Background:The chicken Major Histocompatibility Complex (MHC) has a strong association to disease resistance/susceptibility, production and reproduction traits.Therefore, identifying its polymorphism in populations under selective breeding could be used for selection of disease resistant populations and development of effective vaccines.
Objectives:The aim of the present study was evaluating the MHC polymorphism in two populations of Khorasan indigenous chickens and commercial leghorn breed using microsatellite marker LEI0258 and investigating its segregation. Methods:335 specimens from Khorasan indigenous chickens and commercial Leghorn population (including parents (P) and offsprings (F1)) were analyzed.The MHC genotypes were determined using LEI0258 microsatellite.Allelic segregation analysis from parents to F1 and Hardy-Weinberg equilibrium were conducted using Chi-square test. Results:In Khorasan indigenous chickens 20 different alleles were identified for LEI0258 microsatellite.The allele 321 bp had the highest (22.88%) and the allele 182 bp had the lowest (0.16%) frequency respectively.In the commercial population (Leghorn breed) 3 alleles were found for this marker of which the allele 261 bp had the highest (50%) and alleles 487 bp had the lowest (6 %) frequency respectively.In segregation analysis and Hardy-Weinberg equilibrium of the Khorasan population, no significant differences were observed between parents and F1 progenies in terms of allelic frequencies.
Conclusion:These results indicate a higher genetic variation in indigenous chickens compared to commercial breed.There was not any preference for a particular allele in indigenous chickens and higher frequency of some alleles in F1 population, is due to the high frequency of the same alleles in parents population, their gametes make the population gene pool.

P2.09.13
Motifs of the naturally processed peptides presented by HLA-A*24:07

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Class I HLA molecules mediate immune responses by presenting pathogen-derived peptides to CD8+ T-cells. HLA-A*24:07 is a predominant HLA allele of the Indonesian and South East Asian population. At the moment, there is only one T-cell epitope in the Immune Epitope Database reportedly restricted by A*24:07. HLA-A*24:02 is in the A24 supertype and, although HLA-A*24:07 is closely related to HLA-A*24:02, A*24:07 is not a member of the HLA-A*24 supertype. The two molecules share high homology except for amino acid residue 70, which is a histidine in A*24:02 and a glutamine in A*24:07. The side chain of residue 70 lies in the peptide-binding groove, positioned to impact primary and auxiliary peptide anchors. HLA disease association studies show that these two alleles behave differently in dengue infection as well as in type-1 diabetes. Using proteomic approaches, we systematically compared the peptides presented by A*24:07 and A*24:02. These two A24 were harvested from a matched cell line, immunoaffinity purified, and the A24 ligands were compared by Edman sequencing and tandem mass spectroscopy. Edman analysis indicated slight variations in binding pocket preferences and mass spectroscopy confirmed subtle but clear changes in peptide ligand presentation. This dataset of ligands can be used to improve A*24 predictive algorithms while the A*24:07 reagent can be developed as a tool for characterizing Indonesian and South East Asian immune responses.

P2.09.14
Superior detection of antigen specific CD4+ T-cells using pMHC-II tetramers with altered C-terminal peptide flanking residues

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Background: The importance of T-cells to human health is immense. T-cells are essential for immunity to pathogens and cancer, and they hold the key to vaccination. T-cells also cause a large number of autoimmune diseases and play an important role in organ transplant rejection. The devastating effects of low numbers of just one subset of T-cells, $CD4^+$ T-cells, are all too evident in HIV/AIDS. T-cell receptor (TCR) recognition of peptide epitopes presented by major histocompatibility complex class II (MHC-II) governs $CD4^+$ T-cell activation. The use of MHC-II multimers to identify antigen-specific populations has so far not replicated the success seen with MHC class I multimers. We have previously found modifying $CD4^+$ T-cell epitopes by the substitution of basic residues in the C-terminus enhances T-cell activation.
Aim: To explore the use of C-terminally modified epitopes to generate superior MHC-II tetramers.
Results: Substitution of Arginine into the C-terminus markedly increased the TCR binding affinity by up to two fold. Thermodynamic analysis and structural modelling suggests new electrostatic interactions between TCR and altered C-terminal peptide flank produced the observed TCR binding affinity increase. Furthermore, HLA-DR1 tetramers, with altered C-terminal flanks, could dramatically improve the staining of $HA_{306–318}$ specific $CD4^+$ T-cell clones and allow direct *ex vivo* staining of $HA_{306–318}$ specific $CD4^+$ T-cells from healthy, HLA-DR1 restricted, patients after vaccination.
Conclusion: C-terminally modified MHC-II epitopes can significantly improve TCR binding, offering a route to generate high affinity tetramers with no loss in antigen specificity, with possible attendant implications for vaccination strategies.

P2.09.15
Genetic variants of suppressor molecule BTLA in susceptibility to prostate cancer

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Prostate cancer has become the most common cancers in men and is third men's cancer in terms of mortality in Poland. T cells play a key role in antitumor immunity; therefore expression of inhibitory molecules such as the B- and T-lymphocyte attenuator (BTLA) that inhibits lymphocyte activation could influence cancer susceptibility. An abnormal expression or dysfuncion may be caused by polymorphisms in gene encoding this molecule.
The aim of this study was to evaluate possible associations between *BTLAc.800G>A* (rs9288952), *BTLAc.590A>C* (rs76844316),

BTLAc.88+384C>T (rs1844089) and rs2705535C>T SNPs and susceptibility to prostate cancer.
Genotyping was done using allelic discrimination method with the TaqMan^{O}SNP Genotyping Assays in 162 prostate cancer's patients (pts)and in 260 healthy men originating from the same region in Poland.
The significant difference in distribution of genotypes was observed for rs1844089 SNP. The frequency of genotype [TT] was higher in patients than in healthy men as compare to carriers of C allele (CT+CC) genotypes, p=0,04). The distributions of alleles and genotypes for rs9288952, rs2705535 SNP were similar in patient's and control's groups. The *BTLAc.590A>C* site seems not to be polymorphic in the Polish population, since all typed individuals (100 pts and 100 controls) were AA homozygous.
The haplotype analysis showed that the haplotype rs9288952[G], rs1844089[T], rs2705535[G] was associated with about 2-fold increased risk of prostate cancer (6,3% vs 3,2%, chi^2= 4,6, p=0,03, OR= 2.116, 95%CI [1.053~4.251]).
Our data indicated possible role of polymorphisms in gene encoding suppressor molecule BTLA in susceptibility to prostate cancer.

P2.09.16
Association of HLA-G polymorphisms with gastric adenocarcinoma risk and clinical outcome

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Background: Gastric cancer (GC) is the fourth most common gastrointestinal malignancy and the second leading cause of cancer death worldwide. Human leukocyte antigen-G (HLA-G) molecules are nonclassical HLA-class I molecules that play a crucial role in immuno-tolerance mechanisms. To study the involvement of the HLA-G gene polymorphisms in gastric cancer risk and severity, we investigated the HLA-G genotyping in Iranian Gastric adenocarcinoma patients.
Methods: This case-control study included 100 Gasteric cancer and 102 randomly selected healthy, unrelated Iranian individual's samples as a control.Of 100 cases, 21 (21%) were stage I/II and 79(79%) were stage III/IV. Genomic DNA was isolated from the whole blood using salting-out technique followed by PCR amplification of the exons 2 and 3 of HLA-G gene and performance of PCR-RFLP method. Also Concentrations of sHLA-G in serum were determined with the sHLA-G-specific enzyme linkedimmunosorbent assay (ELISA) kit.
Result:The obtained results indicated frequency of 9 alleles of HLA-G in Iranian individuals including G*01011 (4%), G*01012 (29.86%), G*01013 (10.8%), G*01015 (1.47%), G*01017 (1.96%), G*01018 (2.45%), G*01041 (29.4%), G*01043 (1.96%).The alleles G*01012 and G*01041 had the highest frequencies whereas the alleles G*01015, G*01017, and G*01043 were low frequent. As well data revealed that levels of sHLA-G in serum of GC patients were significantly higher than that in normal controls ($p < 0.0001$).
Conclusion: Our study reveals that HLA-G expression is more frequently observed in patients with advanced disease stage. Moreover, increased sHLA-G levels in plasma might be a useful preoperative biomarker for diagnosis.

P2.09.17
Frequencies of insertion/deletion polymorphism in HLA-G gene in non-small cell lung cancer patients and healthy controls in a Polish population sample

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Lung cancer is a major cause of cancer mortality worldwide. It is a most common neoplasm in males and second one in females in developed countries, including Poland. Non-small cell lung cancer (NSCLC) represents majority of cases. HLA-G is a non-classical MHC molecule expressed predominantly by the trophoblast, but its expression is observed also on some tumor cells, including NSCLC, where HLA-G+ patients survive shorter than HLA-G- ones. Although non-classical and not as polymorphic as classical HLA molecules, nevertheless its gene exhibits some degree of polymorphism, including 14 bp insertion or deletion (ins/del). HLA-G mRNA in del/del homozygotes is expressed on higher level than in individuals with insertion. The aim of this study was to determine the potential association of HLA-G ins/del polymorphism with a prevalence of NSCLC in our Polish population. Venous blood DNA samples from 324 NSCLC patients and 288 ethnically matched controls were typed by agarose gel electrophoresis of PCR products. No significant differences were found between patients and controls in distribution of genotypes: del/del, 35.5% in patients vs 33.3% in controls; ins/del, 50.0% in patients vs 51.1% in controls; ins/ins, 14.5% in patients vs 15.6% in controls. We conclude that other factors than ins/del polymorphism may be responsible for HLA-G expression on some NSCLC tumors.

P2.09.18
Association study among HLA, KIR, MICA, PARK2, and cytokine genes in patients with leprosy from Southern Brazil

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Polymorphisms of HLA, KIR, MICA, PARK and cytokine genes were analysed in patients with leprosy and healthy volunteers from Brazil. HLA, KIR and MICA genes were genotyped by PCR-SSO. PARK2-2599 polymorphism was investigated by real time PCR. PCR-SSP was performed for: TNF-308/-238; IL2-330/+166; IL6-174; IFNG+874; TGFB1+869/+915; and IL10-592/-819/-1082. HLA-DRB1*16 was associated to per se leprosy (P=0.0059), tuberculoid and dimorph forms (P=0.016 and P=0.001, respectivelly), while the HLA-DRB1*16:02 was associated to lepromatous form (P=0.023). KIR2DS2 and KIR2DS3 frequencies were higher in tuberculoid leprosy when compared to lepromatous leprosy. Analysis of KIR-ligands reveled KIR2DL1-C2/C2 elevated in tuberculoid in comparison to all other leprosy subgroups and controls. A negative association between KIR2DL3-C1 and KIR2DL3-C1/C1 and the tuberculoid group was identified. Borderline patients exhibited a higher frequency of KIR3DL2-A3/11 than the controls and lepromatous patients, and a lower frequency of KIR2DL1-C2 than the controls and tuberculoid subgroup. MICA*027 allele was decreased (P= 0.01) in leprosy patients and MICA*010 (P = 0.05) and MICA*027 alleles (P = 0.01) in multibacillary leprosy patients compared to the control group. No significant difference of genotype frequencies among the groups for PARK2 gene was observed. For TNF-308, a higher frequency of GG genotype (P = 0.009), along with a decreased frequency of GA/AA genotypes was observed among leprosy patients as compared to the control group (P = 0.009). Analysis of IL10 genotypes revealed lower frequency of GCC/GCC haplotype in lepromatous leprosy patients (6.2%) in comparison to controls (15.4%). The study suggests the involvement of immune response genes in leprosy.

P2.09.19
Association of MICA gene with the development of chronic chagasic cardiopathy

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Chagas' disease, caused by the protozoan Trypanosoma cruzi is an anthropozoonosis common in Latin America. Chronic chagasic cardiopathy (CCC) is the most serious clinical manifestation of disease; which has been found in approximately 30% of individuals infected. The aim of this study was to investigate the influence of MICA gene on the development of CCC in a population of southern Brazil. The study enrolled 171 serologically-diagnosed Chagas disease patients treated at the University Hospital of Londrina and the Chagas Disease Laboratory of the State University of Maringá. From 171 patients, it was possible to obtain the cardiological diagnosis of

only 129, and those who had an abnormal electrocardiogram were classified as patients with CCC (N=44) and those with normal electrocardiogram in patients without CCC (N=85). A control group was formed of 159 healthy individuals - spouses of patients or blood donors from the Regional Blood Bank in Maringá. Genotyping of MICA alleles was performed by PCR-SSOP. MICA*007 was more frequent in controls than in patients (0.3 vs. 2.8%, P=0.015, OR=0.10, 95% CI=0.002-0.74). In patients with CCC, MICA*008 was more frequent compared to patients without CCC (35.2 vs. 21.8%, P=0.029, OR=1.95, 95% CI = 1.10-3.46) and the controls (35.2 vs. 23.0%, P=0.028, OR=1.82, 95% CI=1.09-3.03). The haplotype MICA*008_HLA-B*08 showed a higher frequency in patients compared to controls (7.0 vs. 1.6%, P=0.028, OR=4.69, 95% CI=1.14-30.25). In conclusion, MICA*007 is linked to protection against Chagas disease, while HLA-B*008_MICA*08 is associated with susceptibility and MICA*008 has influence on the development of chronic chagasic cardiopathy.

P2.09.20
MIC and NKG2D genes polymorphism in Mexican population

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Nowadays, the knowledge about polymorphism in genes involved in the immune response helps to identify the susceptibility or risk in the development of different human diseases. Therefore, the objective was to determine the distribution of MHC class I chain related genes (MIC) polymorphisms and Single Nucleotide Polymorphisms (SNP) rs1049174, rs2255336 and rs2617160 of KLRK1 (NKG2D) gene in the Mexican population by their interaction, MICA/MICB-NKG2D. The polymorphism of *MICA* and *MICB* genes and the SNPs of *NKG2D* were analyzed by Reference Strand Conformation Analysis (RSCA) and Sequence Specific Oligonucleotide Probes (SSOP)-Single Specific Primer (SSP) and allelic discrimination, respectively. We genotyped 336 samples of persons clinically healthy and MICA, MICB and NKG2D polymorphisms were identified by RSCA, SSOP-SSP and allelic discrimination, techniques, respectively. The most frequent allele were MICA *002:01 (33.28%), MICB *005:02 (63.09%), NKG2D rs1049174 *T (61.31%), NKG2D rs2255336 *C (61.31%) and NKG2D rs2617160 *G (93.30%). We also found that the most frequent combination of genotypes in the genes MICA/MICB was *002:01-*002:01/*005:02-*005:02 with 7.44%, while the combination of genotypes at SNPs NKG2D in positions rs1049174/rs2255336/rs2617160 was *A-*T/*C-*G/*G-*G with 45.53%. The data obtained provide other genetic markers involved in the immune innate response that can be considered for the study of susceptibility or protection in different diseases of public health and participation in transplants in Mexican individuals. FOFOI 2005/1/I/016, CONACYT 14019.

P2.09.21
Association between the HLA-G molecule and lymph node metastasis in papillary thyroid cancer

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Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer and can present as lymph node metastasis in 30 to 65% of cases when initially diagnosed. High frequency recurrence, distant metastasis and treatment resistance can be found in cases of PTC so early diagnosis and treatment are critical for improved prognosis and better survival rates. The characterization of new biomarkers has proved useful for the diagnosis and follow-up of these patients. HLA-G is a non-classical HLA class I molecule whose expression in cancer cells has been associated with tumor evasion of immune response. Therefore, the aim of this study was to investigate the HLA-G expression and its clinical significance in PTC. Paraffinembedded thyroid biopsies of 70 PTC patients (40 of whom had presented with metastasis) were evaluated. HLA-G-staining was observed in tumor cells in PTC, and the HLA-G expression was significantly associated with an increased occurrence of lymph node metastasis (p=0.0006) and capsular invasion (p=0.02) This preliminary data shows the HLA-G expression in thyroid carcinoma specimens for the first time and suggest that this expression could impair efficient anti-tumor immunity in PTC. This would indicate that HLA-G could have an independent prognostic value in PTC, principally for tumor recurrence.

P2.09.22
MHC class I chain related gene A polymorphism and gastroduodenal lesions in Mexican population infected by *Helicobacter pylori*

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MHC class I chain related gene A (*MICA*) is a highly polymorphic gene expressed in gastrointestinal epithelial cells. MICA activates NK and gamma delta T cells trough the binding of NKG2D receptor under cellular stress situations (infection or transformation). *Helicobacter pylori* (*H. pylori*) colonization of the gastric mucosa is a major cause for chronic gastritis and predisposition for peptic ulcer and gastric cancer. We analyze the associations between allelic and microsatellite polymorphism of *MICA*, and gastroduodenal lesions related to *H. pylori* infection in Mexican Mestizo patients.
We genotyped by RSCA technique 282 *H. pylori*-positive patient samples diagnosed by endoscopy and histology (non-atrophic gastritis=139, intestinal metaplasia=67, gastric cancer=34 and duodenal ulcer=42), and 96 *H. pylori* seropositive asymptomatic persons without gastric lesions. The allelic and microsatellital frequencies in the different groups of patients were statistically compared by chi-square and Fisher test (*pc*<0.05). We found in the asymptomatic group five microsatellites: A4 (5.8%), A5 (25.2%), A5.1 (10.7%), A6 (18.0%) and A9 (40.3%) and six *MICA* alleles: *001 (5.2%), *00201 (40.6%), *004 (17.2%), *00801 (10.9%), *010 (25.0%) and *011 (1.1%). In patients we observed a decrease of the 00201/00201 genotype frequency in duodenal ulcer (9.5%) when compared with non-atrophic gastritis (20.0%) and asymptomatics (23.0%), but it was not statistically significant. We did not find any association with gastric cancer. These results strongly suggest that the *MICA* polymorphism may not be associated with the gastroduodenal diseases provoked by *H. pylori* infection in Mexican population. (CONACyT 6957, FOFOI 2001/007 and 2002/110).

P2.09.23
***CTLA-4* and *CD28* gene polymorphisms in renal cancer in the Polish population**

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Renal cancer is one of the fastest growing cancers in Poland and number of cases has increased about 10% in the last decade. Since T cells play a key role in antitumor immunity, abnormal expression of co-stimulatory molecules CTLA-4, CD28 that regulate T-cell activity, could influence cancer susceptibility. An aberrant expression may be caused by polymorphisms in genes encoding those molecules.
The aim of this study was to evaluate the association between polymorphisms of *CTLA-4* and *CD28* genes and susceptibility to renal cancer in the Polish population.
Altogether 305 renal cancer patients and 380 healthy controls were genotyped by PCR-RFLP method for the following single nucleotide polymorphisms (SNP): *CTLA-4*g.319C>T (rs5742909), *CTLA-4*c.+49A>G (rs231775) and CT60A>G (rs3087243). Genotyping of the *CTLA-4* Jo31G>T (rs11571302), *CD28*c.17+3T>C (rs3116496) and *CD28*g.54779795G>A (rs3181098) SNPs was done using allelic discrimination methods with the TaqMan SNP Genotyping Assay.
We found a trend to a higher frequency of patients carrying the A allele in *CTLA-4*c.+49A>G polymorphism (AA + AG genotypes) compared to the GG homozygotes than in controls. Moreover CT60AA and Jo31TT genotypes we noticed rarely in renal cancer patients than in controls, compared to their heterozygotes

counterparts CT60GA and Jo31GT (p=0.04 and p=0.09, respectively). In addition we demonstrated a trend toward a lower frequency of AA homozygous in *CD28* rs3181098 polymorphism in patients compared to controls (p=0.09).
Our results indicates that polymorphisms in *CTLA-4* and *CD28* genes might be considered as potentially low-penetrating risk factor for renal cancer, but the results are required to be elucidated in further studies.

P2.09.24
Optimization of T-cell antigens for vaccine development

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A single T-cell can recognize upward to a million different peptides expressed at the surface of the Major Histocompatibility Complex (MHC) molecule. Unfortunately, T-cells may not always have the optimum specificity for their target antigens, thus preventing the clearance of infected or tumour cells. Therefore, improving the antigenicity and immunogenicity of T-cell epitopes is a major challenge for therapeutic vaccination. We have developed different technologies to modify peptide sequences in order to enhance their affinity for the T-cell receptor (TCR) such as combinatorial peptide libraries (CPLs).
We produced a T-cell clone (MEL5) restricted to the well characterized HLA-A*0201 and specific to a melanoma epitope. Combinatorial libraries were used to design peptides 1,000 fold more potent than the MEL 5 wild-type epitope. The priming of peripheral blood mononuclear cells from healthy donors with these agonists shows a high expansion of different clonotypic melanoma specific $CD8^+$ T-cells capable for the majority of donors to kill melanoma tumour cells. Using the same CPLs technology we have designed high affinity peptide for a *Mycobacterium tuberculosis* T-cell clone. Future work will allow us to determine if these agonists can be used for the development of potential mTB vaccines. These *in vitro* studies demonstrate the possibility to use these technologies to elicit high cytotoxic T-cell response against wild-type epitope using agonists in vaccination setting.

P2.09.25
The HLA-DRB1*01 allele confers genetic susceptibility to lepromatous leprosy in Mexicans

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Background: Despite multidrug therapy had reduced the overall prevalence of leprosy in México, it remains endemic in some regions of the country. Some genetic basis for the immune susceptibility towards Mycobacterium leprae had been already established in different populations around the globe. Methods:
52 patients diagnosed with leprosy were classified according to the international criteria establish by Ridley and Jopling. 99 healthy individuals, unrelated to the patients and matched by ethnicity, were included as control group. Genomic DNA was purified according to Miller's method, and HLA-DRB1 locus was genotyped. Data was analyzed with Genosearch Typing software. Statistical analysis included $\chi 2$ test or Fisher's exact test and the odds ratio (OR). Significant p values ≤ 0.05) were corrected taking into account the number of alleles observed (pc).
Results: Here we report that HLA-DRB1*01 allele in a Mexican Mestizo population with leprosy is significantly more frequent (23.00%, pc < 0.001, OR = 5.60) than in healthy individuals (5.00%). The analysis of groups based on clinical classification confirmed the strong association of HLA-DRB1*01 (p<0.001, OR=4.6, n=41); and (p=0.03, OR=6.2, n=6), for lepromatous and dimorphic leprosy, respectively.
Conclusions: This is the first time the association of HLA-DRB1*01 with immune susceptibly to lepromatous leprosy is found on this population. The study of leprosy patients from known endemic regions may give more insights into the pathogenesis of the disease as well as its heterogeneous distribution in México.

P2.09.26
Victory model; Simulation of structures and functions of Antibody and MHC by innovative hands, forehand and arms modeling approaches

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Introduction: Hand, forehand and arms (forelimb) molecular modeling for showing of Antibody and MHC structures and functions named "Victory model" in this article, that encompasses immunoanatomical models used to mimic the behaviour of biological molecules. "Victory model" name come from "Y"-shaped of antibody when we showing it by forelimb.
Method: The study consists of the three major phases: (a) conceptualization of the model based on the literature reviews in field of immunology, biochemistry, anatomy and computer science, (b) visualization the models by forelimb, and (c) refinement of the models and checking their quality of displaying concepts.
Results: About 72 different models obtained for showing different antibody structures and Isotypes (IgM, IgG and etc.), Ig variable and constant regions, disulfide bonds, Immunoglobulin diversity, Somatic hypermutation and affinity maturation, Activation of complement and interaction with other molecules and cells. Also, 25 forelimb models obtained for MHC class I and II structure and functions. In this study some hidden aspects of antibody interactions and functions, cause touchability of models with eyes, were defined that never have been understood.
Conclusion: The findings of the study presents a models for designing innovative training projects that enable teachers to better transfer concepts to student in immunology classes and students can simply exercise and have deep, accurate and touchable insight to structure and functions of antibody and MHC. Besides, it may raising discussions that forelimb may be takes some evolutions patterns and shaping forms from specific antigen recognizing proteins as both have responsibility in Control of Objects.

P2.09.27
HLA-DRB1*11 is associated with Papillon-Lefevre Syndrome in Mexican patients carrying a novel cathepsin C mutation.

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Background: Papillon-Lefèvre Syndrome (PLS) is characterized by aggressive periodontitis and palmoplantar hyperkeratosis. This autosomal recessive disorder, caused by mutations in cathepsin C (CTSC), has a worldwide prevalence of 1-4 cases per million in the general population. In México, the prevalence of this syndrome is unknown. The diagnosis of twenty patients in the state of Sinaloa highlights the need to characterize this syndrome in Mexicans.
Methods: HLA were typed in nine PLS patients and their relatives. Gene expression, enzymatic activity and mutational analysis of CTSC were assayed in these individuals. Frequencies of CTSC gene polymorphisms and HLA alleles were also determined in healthy individuals.
Results: HLA-DRB1*11 was found significantly more frequent (p = 0.0071) in patients than controls (33.33% vs. 7.32%), with an estimated relative risk of 6.33. Patients showed normal CTSC gene expression, but a deep reduction (up to 85%) in enzymatic activity in

comparison to unrelated healthy individuals. A novel loss-of-function mutation, c.203T>G (p.Leu68Arg), was found in all patients, and some carried the polymorphism c.458C > T (p.Thr153Ile). Allelic frequencies in patients, relatives and controls were 88.89%, 38.24% and 0.25% for G (c.203T>G); and 11.11%, 8.82% and 9.00% for T (c.458C>T).
Conclusions: This is the first report on the association of HLA-DRB1*11 with PLS. The novel loss-of-function mutation of CTSC gene (c.203 T > G) found in patients correlated with their diminished enzymatic activity. The study of additional PLS patients may give more insights into the etiology of the disease as well as its prevalence in México.

P2.09.28
Influence of TIM-1 haplotypes on CD4 counts in HIV seroprevalent North Indians

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Introduction: T cell immunoglobulin and mucin domain family member-1 (TIM-1) molecules are expressed on Th2 $CD4^+$ cells and regulate Th1/Th2 immune responses. The mucin domain (exon 4) of this molecule is characterized by high degree of variability. However, the distribution of TIM-1 haplotypes and their population specific influence on HIV/AIDS is largely unknown.
Material & Methods: Sequencing analysis of TIM-1 exon 4 was performed in 227 HIV +ve and 288 healthy individuals (North Indians). Haplotypes were compared among the groups by the chi square test. Continuous variables with the haplotypic background were compared by Wilcoxon rank-sum test.
Results: Seven TIM-1 haplotypes W-A, W-C, D1, D1*, D3-A, D3-C and D4 were observed. D3-A was observed predominantly (>0.465) followed by D3-C (0.219), D4 (0.214), W-A (0.057), D1 (0.021) and W-C (0.007) haplotypes. Differences among the healthy vs HIV +ve individuals were statistically insignificant, suggesting lack of influence on HIV susceptibility/resistance. Additionally, a novel D1 related haplotype D1* was observed (0.007). Further, significantly higher CD4 counts were observed in D3-A carrying HIV patients, however in subgroups of ART naïve and on ART patients, moderate influence was observed. These findings suggest a possible link of D3A with low levels of TIM-1 expression and hence lower Th2 promotion and enhanced Th1 responses. Incidentally, Th1 to Th2 shift is well associated with poor HIV prognosis.
Conclusion: We represent the first report on distribution of TIM-1 haplotypes in the North Indian population in context of HIV susceptibility/resistance and disease status.
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P2.09.29
Association studies of functional *ICOS* gene polymorphisms with schizophrenia disease in the Polish population

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Several reports indicate a possible role of the immune system dysregulation in the pathogenesis of schizophrenia. The inducible co-stimulatory molecule (ICOS) which is expressed on the T-cell surface after activation enhances all the basic T-cell responses proliferation, secretion of lymphokines, the up-regulation of molecules that mediate cell-cell interaction
The study was undertaken to evaluate the association between three *ICOS* polymorphisms (which were recently described as functional ones) and susceptibility to schizophrenia in a Polish population.
One hundred and fifty patients with schizophrenia and 170 healthy subjects were genotyped for the following polymorphisms: *ICOSc.1624C>T* (rs10932037), *ICOSc.602A>C* (rs10183087) and *ICOSc.2373G>C* (rs4675379) using allelic discrimination methods with the TaqMan® SNP Genotyping Assay.
We have noted that frequencies of alleles *ICOSc.1624C>T* [C], *ICOSc.602A>C* [A], and *ICOSc. 2373G>C* [G] were higher in patients of schizophrenia than in healthy controls, but differences did not reach statistical significance (0.930 vs. 0.898, p=0.16; 0.817 vs. 0.777, p=0.21 and 0.889 vs 0.852, p=0.19, respectively).
The result of the study although do not confirm the association of investigated polymorphisms with susceptibility to schizophrenia, while present weak trend to overpresence of wild-type alleles of *ICOS* gene and are warrant further investigation through extended studies.

P2.09.30
Change of C/A at the SNP −592 of human IL-10 gene increase serum IL-10 levels in patients with cervical lesions and cervical cancer

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An immunosuppressive state had been identified in women with human papillomavirus (HPV) persistent infection, characterized by high levels of interleukin (IL)-10 at cervix level. We have demonstrated that the -592 SNP in IL-10 gene promoter is associated with increased risk of cervical lesions (CL) and cervical cancer (CC). We analyzed whether exist significative differences of serum IL-10 protein between CL and CC cases and HPV negatives controls. Additionally, whether exchange in this SNP results in increased IL-10 gene promoter activity in HPV transformed cells.
Using a cross-sectional design, peripheral blood samples of CL patients (n=204), with CC (n=80) and without CL HPV positives (n=66) and HPV negatives (n=100) were used to evaluate seric concentrations of IL-10 by ELISA. Transient transfection assay was performed in C33 and HeLa cells with two luciferase constructs containing the IL-10 promoter (C allele or A allele). The serum IL-10 protein, were significantly higher in CL and CC cases (p<0.01), being higher in patients carrying the risk allele A. The mean levels of IL-10 increased in consecutive clinical stages of the CC. The C to A nucleotide exchange results in increased IL-10 gene promoter activity in C33 an HPV transformed cell line.
In conclusion, we demonstrated that the C to A nucleotide exchange in this SNP results in increased IL-10 gene promoter activity in HPV transformed cells, supporting its role as an activator element and that be associated with the expression regulation of IL-10 at systemic level, which was progressively higher in CL and CC cases.

P2.09.31
Comparison of the distribution of two promoter single nucleotide polymorphisms, -725C>G>T and -716T>G, in non-small cell lung cancer and healthy population sample

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HLA-G, a human non-classical MHC molecule, is not expressed in pulmonary structural cells, but may appear in activated macrophages and dendritic cells infiltrating lung carcinomas, as well as in tumor cells themselves. Several polymorphisms, potentially affecting HLA-G gene expression, were described in its promoter region. We attempted to check whether these polymorphisms might be associated with susceptibility to non-small cell lung cancer (NSCLC). Here, we present our results on -725C>G>T and -716T>G single nucleotide polymorphisms (SNPs) distribution in 324 patients diagnosed with NSCLC and in 288 healthy control individuals, both groups of ethnical Poles inhabiting the Lower Silesia region. In -725 SNP, five different genotypes were found (CC, CG, CT, GG, and GT). Their frequencies in patients and controls did not differ (p=0.67).

Three genotypes were found in -716 SNP (TT, TG, and GG). Similarly to above mentioned SNP, also here no statistically significant differences were observed (p=0.16). We are planning to type for other polymorphisms in HLA-G genes in order to examine whether some of them, or haplotypes created by them, may influence the prevalence of NSCLC in our population.

P2.09.32
Associations of IFN-alpha genetic variation with the functional phenotype of T subsets

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Interferon-alpha (IFN- alpha) functions in viral defense and plays a key role between the innate and adaptive immune systems. This cytokine has numerous effects on the immune system including up-regulating the expressions of MHC molecules, cytokine and chemokines, inducing the maturation of dendritic cells, activating natural killer cells, and stimulating CD8+ T cell proliferation. Single nucleotide polymorphism (SNP) marker has been widely used for genetic investigations and thus can be an effective tool for determining the associations between genetic characteristics on immune system and disease susceptibility. In the present study, we characterized the IFN-alpha SNPs, T cell proliferation and phenotypic analysis of cell surface markers on porcine peripheral blood mononuclear cells (PBMC). Results showed that one SNP was found in -235 of the IFN-alpha promoter region. The PBMC with IFN-alpha -235 G/G genotype has significantly less CD4+- but higher CD8+-positive T cells than those with the -235 A/G and -235 A/A genotypes ($p<0.05$). The significantly increased cell proliferation of ConA-stimulated PBMC was found in those with the IFN-alpha -235 A/A genotype as compared with the -235 A/G and -235 G/G genotypes ($p<0.05$). Results indicated that IFN-alpha SNPs may be associated with the functional phenotypes and cell proliferation of T subsets.

P2.10 Autophagy and cell death in the immune system

P2.10.01
Elucidating the role of UVRAG in T cell biology

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Autophagy is a well-conserved intracellular degradation process that plays important roles in the regulation of cell survival and metabolism. Recently, there is an expanded role for autophagy in the adaptive immune system where it contributes to MHCII cross-presentation of endogenous antigens, and cell survival. Autophagosome formation has been observed in proliferating T cells and it is necessary for the regulation of T cell survival and proliferation. UVRAG, initially identified through its ability to partially complement UV sensitivity in xeroderma pigmentosum cells, has been shown to positively regulate the activity of the class III PI3 Kinase complex in autophagy. Using the conditional UVRAG deletion in T cells, we examined the role of UVRAG in T cell biology and autophagy. A thorough phenotypic analysis of these mice has shown a critical role for UVRAG in T cell development, homeostasis and function.

P2.10.02
Galectin-1 is a novel component of cytotoxic granules and contributes to in vivo CTL killing

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The mechanism of cytotoxicity mediated by Natural Killer (NK) cells and Cytotoxic T Lymphocytes (CTL) is involved in the control of cancer progression, virus mediated diseases and many other intracellular related pathologies. These cells are particularly adapted to promote contact mediated cell death due to their ability to produce a special type of organelles, namely secretory lysosomes, aka cytotoxic granules. By proteomic analysis, we identified dozens of putative novel cytotoxic granule proteins in NK and CTL granule preparations, as well as most of the already known granule constituents. Using confocal as well as electron microscopy we show that galectin-1, a protein previously described to have immunomodulatory activity, including regulation of apoptosis, is indeed present inside the cytotoxic granules. Moreover, using an adenoviral system to intramuscularly deliver antigens, we show that galectin-1 deficient mice display an increased frequency of specific CD8 T cells but an impaired in vivo CTL activity against antigen-specific targets. Taken together, our results show that galectin-1 is a novel component of the CTL granules and participate in in vivo cytotoxic T cell killing. Financial support: This research was partially supported by CNPq and FAPESP (Brazil) and NSF (Ireland).

P2.10.03
Autocrine and paracrine interferon-γ directly enhances CD8 T cell motility and contact-dependent cytotoxicity

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Many epithelial cancers, such as bowel and cervical cancer, are infiltrated by large numbers of activated CD8 T cells that are unable to effect tumour killing. We examine the basic mechanisms by which primary cytotoxic T cells kill primary epithelial cells presenting cognate antigen. We show that *in vivo*, IFN-gamma production by CD8 T cells is necessary for rejection of skin grafts expressing Ova. The ability of CD8 T cells to migrate to inflamed tissue was enhanced by paracrine IFN-gamma. We developed a novel quantitative and qualitative *in vitro* killing assay that enables assessment of cell-to-cell interactions and the mechanisms involved in cytotoxic killing, using live-cell microscopy of primary cells. Primary keratinocytes presenting Ova were co-cultured with antigen-specific CD8 T cells. CD8 T cells kill KC by caspase-3 dependent mechanisms following prolonged attachment times that were specific to target cell type. The kinetics of CD8 T cell migration, speed and travel distances, were closely related to their cytotoxic function. IFN-gamma, whether produced in autocrine fashion from antigen primed effector CD8 cells, or provided exogenously, enhanced CD8 T cell motility and cytotoxity. Significantly, inhibition of the autocrine activity of IFN-gamma on effector T cells for four hours resulted in dysfunctional cells that did not survive. We conclude that IFN-gamma is necessary locally to facilitate the effector functions of effector T cells even post-priming. The absolute need for local IFN-gamma to enable effector T cell cytotoxic function is critical in the development of immunotherapies for cancer and chronic viral infections.

P2.10.04
SLAMF-1 is a signaling receptor modulating autophagy in a subset of chronic lymphocytic leukemia patients with a good prognosis

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Human SLAMF-1 (signaling lymphocytic activation molecule) is expressed on hematopoietic cells where it acts as a co-activator through self-interactions. Moreover, it performs as a microbial sensor, regulating bacterial phagosome functions through an ubiquitous cellular autophagic machinery. We investigated the role of SLAMF-1 in B cells, exploiting chronic lymphocytic leukemia (CLL) as a model. CLL is characterized by the expansion of a monoclonal population of mature B lymphocytes, with a highly variable clinical course. In a cohort of 300 clinically and molecularly characterized CLL patients, the expression of SLAMF-1 correlates with a good prognosis. Functional experiments demonstrated that SLAMF-1 initiates a signaling pathway through a direct interaction with the adaptor molecule Eat-2 with the consequent activation of Vav-1, p38 and Jnk1/2. Furthermore, a prolonged engagement of SLAMF-1 led to the appearance of autophagic vesicles, as confirmed by biochemical analyses, confocal and transmission electron microscopy. The modulation of autophagy was mediated by reactive oxygen species and by the sequential phosphorylation of Jnk1/2 and Bcl-2: the final result is the activation of Bcl-2 and the release of Beclin-1, an essential member of the autophagic complex. The addition of ROS scavengers (SOD and catalase) and the Jnk1/2 inhibitor SP600125 led to a decrease in autophagy, validating the model. Taken together, these results suggest that SLAMF-1 could represent a novel marker for the subset of CLL patients with an indolent clinical course and highlight a hypothetical link between the activation of the autophagic process and a better clinical outcome in CLL, with potential translational implications.

P2.10.05
LPS induces intrinsic apoptosis of 6-sulfo LacNAc+ dendritic cells (slanDCs)

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slanDCs are the most abundant myeloid DCs in human blood and are characterized by a high capacity to produce pro-inflammatory cytokines upon TLR-stimulation. While slanDCs, also known as a non-classical monocyte subset/CD16^{+}-DCs, are susceptible to spontaneous and oxidant-induced apoptosis, how they respond to LPS in terms of viability is unknown. Hence, our aim was to investigate the molecular mechanisms whereby LPS influences slanDC survival. slanDCs, isolated from buffy coats of healthy donors, were cultured in the presence or absence of death receptor agonists, H_2O_2 or LPS, in combination or not with apoptosis blockers or N-acetyl-cysteine. Cellular viability and caspase activities were determined by flow cytometry and luminescent assays, respectively. Reactive oxygen species (ROS) production was established by either the cytochrome C reduction assay or the nitroblue tetrazolium test. LPS accelerated spontaneous slanDC death independently from endogenous FasL-, TRAIL- and TNFα-involvement. These findings were in agreement with increases in caspase-9 and -3, but not caspase-8, activities. Furthermore, LPS-stimulated slanDCs produced ROS and were susceptible to oxidant-induced cell death upon incubation with H_2O_2, with the addition of N-acetyl-cysteine partially reducing the apoptotic cell percentage upon LPS-treatment. In conclusion, LPS negatively affects slanDC survival, suggesting the existence of self-regulatory mechanisms that limit slanDC activation. This effect is independent on death receptor-mediated extrinsic pathways. On the other hand, an oxidative process induced by LPS is involved in its pro-apoptotic effect on slanDCs.
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P2.10.06
Autophagy as a link between immunity and inflammation in idiopathic inflammatory myopathies

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Idiopathic inflammatory myopathies (IIMs), a heterogeneous group of immune-mediated muscle disorders characterized by muscle inflammation and weakness, comprise inclusion body myositis (IBM), polymyositis (PM), juvenile dermatomyositis (JDM), and adult dermatomyositis (DM). An impairment in the autophagic pathway has been well documented in sporadic IBM, where it has been suggested to be responsible for the accumulation of multiple-protein aggregates, typical of the myopathy. The main candidates responsible for this impairment are suggested to be TLRs.
We evaluated the autophagic process also in PM and DM, in particular the interaction between autophagosome maturation and innate immune system.
LC3 and other autophagic molecules, together with TLR3, TLR4, HSP60 and HMGB1/2 were analysed in IIM and control muscles by qPCR, immunohistochemistry and immunoblot. Myoblasts and myotubes from PM, DM and control muscle biopsies were analyzed to evaluate changes in TLR and HMGB1/2 expression after induction or inhibition of autophagy.
Gene expression analysis showed a dysregulation of autophagy in all IIM subgroups. A tight correlation between autophagy and innate immunity in myopathic muscles compared to controls was highlighted by confocal microscopy; activation of stress response and the presence of bacterial infection were observed connected with an abnormal accumulation of autophagosomes in IIM myofibers.
These findings demonstrate the involvement of the autophagic process in the pathogenesis of all subtypes of IIMs; it is not clear if this mechanism represents a consequence or a cause of the induction of immune response and inflammation. Autophagic machinery components might represent a possible target for new therapeutic approaches for IIM.

P2.10.07
O-linked N-acetylglucosaminyl transferase (Ogt) promotes the survival of mature B cells

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O-linked β-N-acetylglucosamine (*O*-GlcNAc) modification (O-GlcNAcylation) is a process of adding GlcNAc to protein serine or threonine residues. This posttranslational modification is triggered by *O*-linked N-acetylglucosaminyl transferase (Ogt) and is involved in the regulation of many functions of proteins including protein stability, localization and transcriptional activities. *O*-GlcNAcylation plays important roles in many cell types and in many diseases such as diabetes, neuron degenerative diseases and cancer. In B cells, it was reported that accumulation of *O*-GlcNAcylation resulted from overexpression of Ogt led to enhanced B cell activation, but whether Ogt involves in the regulation of development, differentiation or survival of B cells was largely unknown. Thus, we created a mouse line in which *Ogt* was specifically deleted in B cells by crossing *Ogt*-floxed mouse to CD19-*cre* mouse. Deletion of *Ogt* in mice results in reduced B cell populations in bone marrow, spleens and lymph nodes. Further analysis of B cell subsets revealed that B cell development was normal but the numbers of mature B cells were decreased in *Ogt* deficient mice, which was linked with the enhanced apoptosis of mature B cells in *Ogt* deficient mice. These data suggest that Ogt may play a positive role in the survival of mature B cells. However, further studies are needed to elucidate the molecular mechanisms contributing this phenotype.

P2.10.08
Selective MHCII-mediated cell death as a potential mechanism for reversing inflammation in a chemically-induced mouse model of colitis

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Dysregulation of immune cell apoptosis is implicated in the pathogenesis of inflammatory bowel disease and selective depletion of pro-inflammatory, non-antigen specific B and T cells may be a rational therapeutic approach. We recently demonstrated that non-antigenspecific activation of resting B cells via Toll-like receptor (TLR) ligation leads to increased cell surface expression of MHC class II invariant chain peptide (CLIP) in the MHCII groove, thereby providing protection from T cell dependent MHCII-mediated cell death. Using a computational approach, we designed small peptides that reverse inflammatory symptoms in several animal models of chronic inflammation including TLR-induced preeclampsia and autoimmune myocarditis, by depleting CLIP+ immune cells. In the current study, we examined the in vitro effects of dextran sodium sulphate (DSS) or TNBS, classical chemical agents used in an animal model of colitis, on CLIP expression on murine B cells and gamma delta T cells isolated from spleen and Peyer's patches. Our results demonstrated that DSS/TNBS caused a significant expansion of B cell and gamma delta T cell populations. Moreover, flow cytometric analysis shows that in both live cell sub-populations there is increased expression of ectopic CLIP/MHCII complexes. Furthermore, our data demonstrate that peptide treatment of chemically-activated cells followed by treatment with anti-MHCII antibody resulted in increased MHC class II-mediated cell death.
These novel findings suggest increased CLIP expression on pro-inflammatory immune cells may contribute to DSS/TNBS-mediated colitis and predict an innovative therapeutic approach whereby peptide depletion of CLIP may reverse inflammation by selective cell death of pro-inflammatory MHCII cells.

P2.10.09
Low-density lipoprotein receptor-related protein-1 (LRP-1) mediates autophagy and apoptosis caused by Helicobacter pylori VacA

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In *Helicobacter pylori* infection, vacuolating cytotoxin (VacA) induces mitochondrial damage leading to eventually apoptotic cell death. It has also been proposed that VacA-induced autophagy serves as a host mechanism to limit toxin-induced cellular damage. Apoptosis and autophagy are two dynamic and opposing processes that must be balanced to regulate cell death and survival. The VacA receptor(s) responsible for apoptotic cell death and autophagy has not been identified during intoxication.
Here we identify the low-density lipoprotein receptor-related protein-1 (LRP-1) as the VacA receptor for toxin-induced autophagy in the human gastric epithelial cell line AZ-521, and show that VacA internalization through binding to LRP1 specifically regulates the autophagic process including generation of LC3-II from LC3-I, which is involved in formation of autophagosomes and autolysosomes. Knockdown of LRP1 and Agt5 inhibited generation of LC3-II as well as cleavage of PARP, a marker of apoptosis, in response to VacA, whereas caspase inhibitor, Z-VAD-FMK, and necroptosis inhibitor, Necrostatin-1, did not inhibit VacA-induced autophagy, suggesting that VacA-induced autophagy via LRP1 binding precedes apoptosis. In addition, Both NPPB and DIDS inhibited VacA-induced LC3-II generation in AZ-521 cells suggesting that channel activity may be required for LRP1-dependent autophagy. Other VacA receptors such as RPTP[[Unsupported Character - Symbol Font ]], RPTP[[Unsupported Character - Symbol Font ]], and fibronectin did not affect VacA-induced autophagy or apoptosis. Therefore, we propose that a single cell surface receptor, LRP1, mediates VacA-induced autophagy as well as apoptosis in gastric epithelial cell line AZ-521 cells.

P2.10.10
Suppression of *Mcl-1* via RNA interference sensitizes human U937cells towards apoptosis induction

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Human leukemia is one of the common hematological malignant diseases and a major leading cause of human death. The current primary treatment for leukemia is anticancer drug-based chemotherapy that uses one or more drugs to destroy cancer cells, often accompanied by the development of drug resistance and severe side effects. Therefore, it is imperative to develop other potential therapeutic agents for the treatment of this disease. Defects in apoptosis signaling contribute to this resistance. Myeloid cell leukemia-1 (Mcl-1) is an anti-apoptotic member of the Bcl-2 protein family which interferes with mitochondrial activation. Previous studies have shown that Mcl-1 is highly expressed in Human leukemia. In this study, we manipulated expression of the Mcl-1 protein in U937cells by RNA interference and analyzed its impact on apoptosis sensitivity of U937 cells *in vitro*.
RNA interference was performed by transfecting siRNA to specifically knock down Mcl-1 expression in U937 cells. Mcl-1 expression was measured by quantitative real-time PCR and Western blot. Induction of apoptosis after treatment with Mcl-1 siRNA was measured by MTT assay.
RNA interference efficiently downregulated Mcl-1 expression in U937 cells. Mcl-1 downregulation sensitized U937 cells to apoptosis. Sensitization was accompanied by profound activation of caspase-3. Mcl-1 knockdown significantly augmented apoptosis sensitivity of U937 cells.
Our data suggest that specific downregulation of Mcl-1 by RNA interference is a promising approach to sensitize U937 cells towards molecularly targeted therapies.

P2.10.11
Bcl-2 antagonist as an immunomodulatory drug for transplantation

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The role of the Bcl-2 family members in cell death and survival appear to be ubiquitous, so that their iatrogenic perturbation might at first glance be expected to be non-specific. Nevertheless, the expression levels of pro- and anti-apoptotic molecules differ among tissues and indeed, as we have found among immune cells. We examined the effects of the drug ABT-737, a mimetic of the killer BH3 domain of the Bcl-2 family of proteins that induces apoptosis by antagonizing Bcl-2, Bcl-XL and Bcl-W (without any effect on A1 and Mcl-1) on the mouse immune system. Treatment with ABT-737 reduced the numbers of selected immunocyte sub-populations, most markedly in lymph nodes. It inhibited de novo immune responses but had little effect on pre-existing ones. ABT-737 was sufficiently immuno-modulatory to allow long-term survival of pancreatic allografts, reversing established diabetes in this model. Our findings provide an insight into the selective mechanisms of immune cell survival and how this selectivity enables a novel strategy for immune modulation.

P2.10.12
CD95 (Fas) and CD178 (Fasl) induce apoptosis in CD4+ and CD8+ cells from peripheral blood and spleen in dogs naturally infected by Leishmania spp

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Infected dogs are urban reservoirs of Leishmania chagasi, causative agent of visceral leishmaniasis, a public health problem in many countries including Brazil. The dogs have immune suppression and apoptosis of lymphocytes is involved in this process. To investigate apoptosis in CD4 + and CD8 + lymphocytes and the expression and function of major receptors involved in apoptosis, FAS (CD95) / FASL (CD178) and TRAIL (CD253) were evaluated by flow cytometry on

peripheral blood cells and spleen of 38 symptomatic dogs with moderate visceral leishmaniasis and 25 healthy dogs. Apoptosis of CD4 + cell and CD8 + cell from spleen and blood was greater in infected dogs than in healthy. In the CD4+ cell from blood and spleen of infected dogs was lower than observed in healthy dogs. In the CD8+ cell from infected dogs the expression of FAS increase whereas FASL decrease compared to healthy dogs. The apoptosis observed in CD4 + cells from blood and spleen decreased in the presence of blocking antibodies to FAS and FASL, the reduction of apoptosis in CD8 + cells by the presence of blocking antibodies was only observed in the spleen. Our data together show that apoptosis of CD4 + and CD8 + is a mechanism that is involved in the infectious process dogs Leishmania spp. with the participation of molecules FAS and FASL. Our results could help to improve the current understanding of the immune response in dogs infected with L. chagasi allowing future therapeutic interventions to reduce the depletion of lymphocytes.

P2.10.13
FASL −844 T/C polymorphism: A biomarker of good prognosis of breast cancer

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FAS/FASL system plays a crucial role in modulating apoptosis. The single nucleotide polymorphism of the FASL gene (FASL −844 T/C), is located within a putative binding motif of CAAT/enhancer-binding protein ß transcription factor. Higher basal expression of FASL is significantly associated with the FASL−844C allele. Transformed cells carrying the FASL-844CC genotype may create an immunoprivileged site by killing cytotoxic immune cells and thus escaping host immune-surveillance.
FASL −844 T/C polymorphism was examined in 438 patients with breast cancer using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.
FASL −844CC genotype was significantly associated with SBR 1-2 tumour grade (P = 0.007). In patients with diagnosis age ≤ 50 years, FASL −844CC genotype showed significant associations with T1-T2 clinical tumour size (P = 0.01) and SBR grade 1-2 (P = 0.02). A marginally significant association was also found with negative nodal status (P = 0.06).
Thus, it is possible that at younger ages, high expression of FASL in T lymphocytes carrying CC genotype may increase their ability to eliminate FAS-sensitive tumor cells. This aspect can probably contribute to the efficiency of the anti-tumor response of young patients and likely confers an advantage of a good prognosis.

P2.10.14
The effect of Z36 on Human T-cell Leukemia

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Human T-cell Leukemia is aggressive and malignant blood disease. Above all, Adult T-cell Leukemia (ATL) has a very poor prognosis even with intensive chemotherapy, indicating the need for development for new drugs to treat the disease. Z36 which is BCL-XL inhibitor have been known induces autophagy. But the effect in Human T-cell Leukemia of Z36 is not reported.In this study, we demonstrate that the effect of Z36 on cell viability of Human T-cell Leukemia cell lines such as Hut 78, Hut 102, jurkat, MT-1, MT-2 and MT-4 in vitro. Z36 inhibited proliferation of all Human T-cell Leukemia cell lines with different sensitivity at 24 hr after the treatment. The effect of Z36 on Human T-cell Leukemia was restored by bafilomycin-A but not by 3-MA and choloroqine, which are known to be autophagy inhibitors. Western blot showed that Z36 induced LC3-II, which is known as a marker for autophagy. Proliferation of SiATG5-treated cells did not inhibit by Z36. These results suggest that Z36 induced autophagic cell death through ATG5 in human T-cell leukemia.

P2.10.15
An innovative method for assessing autophagy using the FlowSight imaging flow cytometer

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Autophagy is a process in which normal cellular components that accumulate during growth and differentiation are degraded via the lysosome; it is a survival mechanism that reallocates nutrients from unnecessary processes to more vital processes in the cell. Basal levels of autophagy are usually low but can be up-regulated by numerous stimuli including starvation, physiological stress, pharmacological agents and infections. In addition, suppression of autophagy has been associated with cancer, neurodegenerative disorders, infectious diseases and inflammation. During autophagy, cytoplasmic LC3 is processed and recruited to the autophagosomal membranes; therefore, cells undergoing autophagy can be identified by visualizing LC3 puncta using immunofluorescence microscopy. While manual microscopy allows visual identification of autophagy on a per-cell basis, an objective and statistically rigorous assessment is difficult to obtain. To overcome these problems, we used the FlowSight imaging cytometry platform to collect imagery of large numbers of cells and assess autophagy in an objective, quantitative, and statistically robust manner. In this study, we demonstrate a method for determining image-based parameters for assessing the LC3 puncta in starved and non-starved U2OS RFP-LC3 human osteosarcoma reporter cells.

P2.10.16
Accumulation of autophagic adaptor protein, p62/sequestosome 1, in the brains of Alzheimer's disease

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p62/sequestosome 1 interacts with autophagy-related gene 8 family, which plays a critical role in the initial step of macroautophagy. p62 structurally contains an ubiquitin-associated domain at the carboxyl terminus, thereby binds with ubiquitinated and misfolded proteins. Based on these properties, p62 can selectively sequestrate substrates, and serve to degrade them. Also the increased level of p62 implies that autophagic flux is disrupted, because p62 is one of autophagic substrates. We investigated whether p62 expression level is altered in the brains with Alzheimer's disease (AD). Immunoblot analyses showed that p62 level is significantly increased in the brains with AD relative to controls. Further analyses showed that several stress-responsive genes are induced in AD brains. To explore the relationship between p62 and stress-responsive genes, we focused on the Keap1/Nrf2 system, which highly contributes to stress response. Immunoblotting revealed that total and p62-immunoprecipitated Keap1 levels are not altered between AD and controls. Interestingly, however, it was evident that the level of phosphorylated p62 at the serine residue 349 is significantly increased in AD brains. Considering this phosphorylated serine corresponds to the Keap1-binding region of p62, these findings suggest that the phosphorylation affects Keap1/Nrf2 system, leading to expression of stress responsive genes.
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P2.10.17
Interference of modified vaccinia virus Ankara with autophagy: implications for innate and adaptive immunity

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Introduction: Modified vaccinia virus Ankara (MVA) represents a highly attenuated
strain of vaccinia virus that has been used as a vaccine delivery vector and recently suggested to be also effective as an adjuvant. MVA's high immunogenicity has been explained by its ability to target dendritic cells and its viral cytotoxicity leading to cross-presentation by bystander cells. Recently, immunogenicity of dying cells has been linked to the level of cellular autophagic activity. Therefore, we hypothesised that MVA infection interferes with target cell autophagic homeostasis leading to immunogenic cell death.
Methods: We analysed MVA effects on eukaryotic cell autophagy using HUH7 cells stably expressing LC3-GFP protein, fluorescence microscopy and flow cytometry. MVA effect on murine bone marrow-derived dendritic cells (BMDCs) was analysed using Cyto-ID® Autophagy Detection Kit and flow cytometry. Effects of MVA on cell viability and autophagy were correlated to viral effects on BMDCs capacity to induce T cell proliferation.
Results: MVA infection resulted in an enhanced autophagic flux in both HUH7 cells and BMDCs. Viral infection was accompanied by increased apoptosis. This combination of dying with autophagy appeared highly immunogenic and resulted in a substantial increase in CD8+ T cell proliferation. Importantly, raised CD8+ T cell proliferation was abgrogated upon autophagy inhibition.
Conclusion: Our data suggest that MVA's ability to induce cell death with autophagy in BMDCs contributes to a higher immunogenicity and provides a potential mechanism to explain MVA's potential effects as an adjuvant.

P2.10.18
Mechanisms of cytotoxic effects of different monoclonal antibody to tumor-associated ganglioside GD2

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Targets for cancer immunotherapy can be not just proteins, but also glycosphingolipids, e.g. gangliosides. GD2 is the most interesting tumor-associated ganglioside. GD2 expression on normal cells is restricted, but it abundantly expressed on the surface of neuroblastoma, glioma and several lymphomas. Recently was shown that GD2-mAb has direct cytotoxic effect on tumor cells. The role of GD2 in the mechanisms of the cytotoxic effects is unclear.
We have shown that GD2 on the surface of tumor cells can be not just target but also effector molecule. We found predominant role of GD2 in the reception and transduction of cytotoxic signal. To prove that GD2 is the single molecule for accepting of cell death signals the following experiments was made:
- investigation of cross-reactivity of different GD2-mAb to proteins and other gangliosides;
- analysis of ganglioside composition of GD2-positive tumor cells;
- comparison between cytotoxic effects and GD2 expression level on the surface of tumor cells. We used inhibitor of ganglioside biosynthesis PDMP and siRNA GM2/GD2 GM2/GD2 synthase to decrease GD2 expression level.
To determine whether GD2-mAb-induced cytotoxicity is apoptosis or necrosis we used different methodological approaches. In our work was investigated participations of lipid rafts, changes of cell volume, involvements of caspases, ATP, ROS, also was made analysis of mitochondria depolarization. The signal transduction of GD2-mAb-induced cytotoxity in tumor cell has both apoptosis and necrosis features. The results of our work open a new point of view to tumor-associated ganglioside GD2 as new receptor of cell death of tumor cells.

P2.10.19
Transcriptional control of FAS-ligand regulates the differential activation induced cell death of human Thelper cells

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T cell immune response results from activation of naive CD4 T cells in functionally distinct Thelper (Th) subsets. The control of the homeostasis of the inflammatory Th cells, and in particular of the Th17 cells, is crucial to avoid autoimmune inflammation.
Limited information is available on the homeostatic signals that regulate survival and cell death of human Th cell subsets to control their expansion.
We analysed apoptosis induced by T cell receptor (TCR) stimulation of different Th subsets. We found that human Th17 clones are the most resistant to apoptosis compared to other Th subsets. Th1 clones are particularly sensitive to apoptosis and they express high levels of cleaved Caspase-8 and Caspase-3, which correlates with the cleavage of its substrate, poly(ADP-ribose) polymerase-1. We found that FAS receptor is expressed by all Th profiles. Interestingly, Th1 cells upon TCR stimulation specifically express FAS-ligand, whereas in Th17 cells this expression is impaired. Thus, we analysed the mechanisms involved in the differential expression of FAS-ligand in human Th1 and Th17 cells.
In summary, our results indicate that FAS-FAS-ligand interaction leads to Caspases' cleavage and cell death. In contrast, the lack of FAS-ligand induction in resistant Th17 cells impairs the Caspase 8 activation and the resulting cell death. We defined the mechanisms regulating FAS-ligand expression and their modulation may provide new targets to prevent persistent Th17-mediated inflammation in autoimmune disease.

P2.10.20
BCL3, induced by Tax and HTLV-1, inhibits NF-κB activation and promotes autophagy

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The human T cell leukemia virus type 1 (HTLV-1) is a complex human retrovirus that causes an aggressive leukemia known as adult T cell leukemia (ATL).The HTLV-1-encoded oncoprotein Tax induces persistent activation of the nuclear factor-κB (NF-κB) pathway, which is perceived as the primary cause of ATL. BCL3, a member of the NF-κB inhibitor (IκB) family, is highly expressed in many HTLV-1-infected T cell lines and ATL cells. However, the role of BCL3 in Tax-induced NF-κB activation has not been fully elucidated. Here, we show that Tax induces BCL3 expression, which in turn negatively regulates the Tax-induced NF-κB activation. Interestingly, both BCL3 up-regulation and NF-κB inhibition promote the autophagy process in HTLV-1-infected cells. Consistent with this, over-expression of BCL3 also results in enhancement of rapamycin-, pifithrin-α- or starvation-induced autophagy in control cells. Together, these data demonstrate that BCL3 acts as a negative regulator of NF-κB activation and promotes autophagy in HTLV-1-infected cells

P2.10.21
A protective role of autophagy as an anti-oxidant system in human primary T cells: a potential therapeutic strategy for autoimmune diseases

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Background: Autophagy is an intracellular degradation system that plays an important role in T cell survival. However, the precise mechanistic link between autophagy and cell death in human primary T cells is unclear because monitoring autophagy in small numbers of human primary cells remains controversial.
Methods: Human naïve and effector memory (EM) $CD4^+$ T cells were isolated from the peripheral blood samples of healthy donors and stimulated with anti-CD3/CD28-coated beads. Two days after

stimulation, GFP-LC3 fusion protein which can function as an autophagy sensor was overexpressed in human activated T cells using a retroviral vector system.
Results: We showed that autophagy was induced after T cell receptor stimulation. We also found that autophagy-defective naïve $CD4^+$ T cells were susceptible to apoptosis via the intrinsic apoptotic pathway, which resulted from the accumulation of reactive oxygen species (ROS) due to defective mitophagy. EMT cells had lower autophagic activity than naïve T cells, which contributed to their enhanced apoptotic cell death due to increased ROS. Moreover, a combination of an autophagy blocker and an inhibitor of the mitochondrial electron transport chain (mETC) synergistically increased intracellular ROS levels and enhanced apoptotic death of activated T cells.
Conclusions: These results suggest a protective role of autophagy as an anti-oxidant system in human activated T cells. A combination of an autophagy blocker and an inhibitor of the mETC could be a novel therapeutic strategy for autoimmune diseases.

P2.10.22
Restoration of ASC expression sensitizes colorectal cancer cells to genotoxic stress-induced caspase-independent cell death

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Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), an essential component of the inflammasome complex, is frequently silenced by abberant methylation of its CpG islands in many tumor cells. However, the molecular function of ASC in cancer cells is not well characterized. Here, we investigated the effect of ASC expression in human colorectal cancer cell DLD-1, in which the *ASC* gene is epigenetically silenced by methylation. Restoration of ASC expression in DLD-1 cells by demethylating agent or by stable transfection potentiated cell death mediated by DNA damaging agent. Contrarily, ASC knockdown in HT-29 cells rendered cells less susceptible to etoposide toxicity. The increased susceptibility of ASC-expressing DLD-1 cells to genotoxic stress was independent of inflammasome or caspase activation, but partially dependent on mitochondrial ROS production and JNK activation. Taken together, our data suggest that ASC expression in cancer cells is an important factor in determining their susceptibility to chemotherapy.

P2.10.23
Lactobacillus rhamnosus GG modulates autophagy function in T cells and myeloid cells as mechanism for adjuvant effect in rotavirus vaccine induced effector T cell responses

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Autophagy has direct effects in the regulation of adaptive immunity through limiting the duration of synapse formation between T cell and antigen presenting cell (APC) and magnitude of the ensuring T-cell activation. *Lactobacillus rhamnosus* GG (LGG) suppressed rotavirus-induced autophagy in ileal epithelium and significantly enhanced intestinal virus-specific effector T-cell response to a human rotavirus (HRV) vaccine in gnotobiotic (Gn) pigs, which led to our postulation that LGG promotes effector T-cell responses by down-regulating autophagy in T cells and APCs to stabilize immunologic synapse. To test this hypothesis, we studied the autophagy function in sort-purified ileal CD3+ T cells and SWC3+ myeloid cells (as APCs) from Gn pigs primed by rotavirus infection. Cells were stimulated *in vitro* with live LGG and/or purified HRV antigen with or without lysosomal inhibitor, bafilomycin A_1. In CD3+ T cells, LGG and HRV alone significantly reduced the expression of VPS34, Becline 1 and LC3-II, as well as LC3 turnover, and increased p62 accumulation compared to mock control. LGG+HRV significantly enhanced VPS34, Beclin 1, and Atg12 expression, whereas down-regulated LC3-II expression and LC3 turnover, and significantly increased p62 accumulation. In SWC3+ APCs, LGG+HRV significantly increased VPS34, Beclin 1 and Atg12 expression, whereas significantly reduced LC3-II expression and reduced LC3 turnover. In conclusion, LGG and HRV both down-regulate autophagy activity in T cells and APCs. LGG+HRV synergistically further reduced autophagy function. Reducing autophagy in T cells and APCs is potentially a novel mechanism for the adjuvant effect of LGG in enhancing effector T-cell responses to rotavirus vaccines.

P2.10.24
Rapid Dynamic Exchange of LC3 in Intracellular Protein Aggregates but not in Autophagosomes

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The microtubule-associated protein light chain 3 (LC3) is a marker protein that is involved in the formation of autophagosomes and autolysosomes, which are usually characterized and monitored by fluorescence microscopy using fluorescent protein-tagged LC3 probes (FP-LC3). FP-LC3 and endogenous LC3 can also be incorporated into intracellular protein aggregates in an autophagy-independent manner. However, the dynamic process of LC3 associated with autophagosomes/autolysosomes or protein aggregates in living cells remains unclear. Here, the dynamic properties of the two types of FP-LC3-containing puncta were explored. The data reveal that the fluorescent signals of the FP-LC3 attached to phagophores or in mature autolysosomes do not recover after photobleaching, indicating that the dissociation of LC3 from the autophagosome membranes may be very slow or not constitutive. In contrast, the FP-LC3 in the protein aggregates exhibits fast kinetics of association and dissociation with the fluorescent puncta, indicating that a rapid exchange occurs between the aggregates and cytoplasmic pool. The later is mainly due to the transient interaction of LC3 and the ubiquitin-associated protein p62. Thus, we find the different dynamic properties of LC3 in two types of punctate structures using the quantitative imaging and analyzing approaches. Because of this finding, we provide a convenient and useful approach to distinguish autophagosomes from LC3-involed protein aggregates in living cells. Using this approach, we also find that the FP-LC3 puncta that localized adjacent to the phagophore marker Atg16L1 were protein aggregates, that exhibited different kinetics compared to the phagophores alone.

P2.12 Signalling in immune cells

P2.12.01
Symmetry breaking in reconstituted actin cortices

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The actin cytoskeleton plays a central role in many cellular processes. In particular, the dynamic actin cortex plays a pivotal role in cell division, in polarity, in motility and in the immune response. In all these contexts, the cortical actin network has to break symmetry to generate polar cytoskeletal dynamics. Despite extensive research, the mechanisms responsible for regulating cortical dynamics in vivo and inducing symmetry breaking are still unclear. We developed a reconstituted model system that self-organizes into dynamic actin cortices at the inner interface of water-in-oil emulsions. This artificial system undergoes spontaneous symmetry breaking, driven by myosin-induced cortical actin flows, which appear remarkably similar to the cortical flows involved in T-cell receptor organization in the immunological synapse. The contractile behaviour of the reconstituted cortices exhibits a sharp temperature-dependent transition, facilitating the use of temperature as an external parameter to control the onset of symmetry breaking. The reconstituted cortices further display occasional local detachment of the contractile actin network from the interface as seen in cellular blebs, and polar force generation, which can lead to deformation of the interface. Our in vitro model system recapitulates the rich dynamics seen in actin cortices in vivo, allowing us to reveal the basic requirements for actin cortex formation and symmetry breaking. Moreover, this synthetic system paves the way for further exploration of artificial cells towards the

realization of a minimal model system of the immunological synapse that can shed light on the mechanisms underlying the diverse cellular responses following T-cell activation.

P2.12.02
The study of nicotinic acetylcholine receptor α7 subunit expression in human lymphocytes using toxins and antibodies

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Nicotinic acetylcholine receptors (nAChRs) are transmembrane proteins assembled from α subunits (α1-α10) either alone or together with non-α subunits. Alpha 7 nAChR is neuronal subtype naturally expressed by hippocampus and DRG neurons however its expression is also shown for lymphoid cells. Non neuronal nAChR expression often is identified by anti-nAChRs antibodies which in some cases generate false positive results. For α7, antibody staining was found similar in wild-type and subunit specific knockout mice. The aim of the present study was to analyze α7 expression by human lymphocytes and various cell lines using antibody against α7 and a highly specific to α7 α-bungarotoxin (α-Bng). To control the specificity of α-Bng we generated HEK293 cells transiently expressing recombinant α7 in chimeric glycine receptor. Specificity of α-Bng binding to transfected cells was confirmed by cobratoxin competition at 100x excess. We demonstrated that anti-α7 antibodies bound both blood lymphocytes and various lymphoid and epithelial cell lines as was shown by flow cytometry and confocal microscopy. On the contrary α-Bng bound only transfected HEK293 cells and neurons from mouse ganglia but neither blood lymphocytes nor cell lines. These results do not correspond to earlier published data.

P2.12.03
Identification of signalling cascades during the inflammatory activation of phagocytes induced by S100A8 and S100A9

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This project focuses on the biological relevance of potential receptors for the inflammatory response in monocytes induced by the Damage Associated Molecular Pattern (DAMP) proteins S100A8 and S100A9. These phagocyte-derived molecules activate innate immune cells via toll-like-receptor 4 (TLR4). The receptor for advanced glycation end products (RAGE) has been suggested as alternative receptor.
Human monocytes were stimulated with S100A8 and S100A9 or LPS for 4 h. RNA was isolated and used for genome-wide gene expression arrays. Activated pathways were analyzed by using DAVID bioinformatics resources.
The gene expression arrays show differences in expression pattern of about 1000 genes for monocytes which are stimulated with LPS, S100A8 or S100A9 in comparison to non-stimulated cells. We found no prominent differences in up or down regulated genes between LPS and S100A8 or S100A9 stimulated cells. The majority of regulated genes are involved in TLR4 signalling and many are known targets of TLR4-dependent NF-kB-induced transactivation. This leads to the assumption that TLR4 is the main receptor involved in the activation of monocytes by S100A8 and S100A9, whereas RAGE seems to be not involved. However, a substantial number of genes induced by S100A8, S100A9 or LPS have not been described in the context of TLR4-signalling so far. Interestingly, stimulation of monocytes with S100A8 or S100A9 results in up-regulation of anti-apoptotic proteins on both mRNA and protein level. These findings were confirmed using functional apoptosis assays. Beside direct stimulatory effects S100A8 and S100A9 promote survival of a pro-inflammatory phenotype of monocytes.

P2.12.04
Type-1 serine/threonine phosphatases and PI3K signaling contribute for the TLR antagonist effect of Natterins

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Studies carried out previously by our group have shown that the venom of *Thalassophryne nattereri* develops an inappropriate cellular inflammatory response characterized by a reduced number of leukocytes in the tissue injured. We identified in the venom a new proteases family named Natterins responsible to the inhibition of the leukocyte rolling in cremaster endothelial cells in a TLR2- or TLR4-MyD88-mediated signals dependent mechanism. Here, we investigated the involvement of phosphoinositide 3-kinase (PI3K) and type-1 protein serine/threonine phosphatase (PP1) in the Natterins antagonist effect. Using intravital microscopy we pretreated (i.p) Swiss mice for 30 minutes with MG132 (26S proteassome inhibitor at 10 μM); PP1 inhibitor (IPP-1 10 μM) or wortmannin (0.005 μM) a specific phosphatidylinositol 3-kinase inhibitor. Then, we investigated the number of leukocytes rolling in cremaster venules after topical application of 20 μL of LPS (0.02 μg/mL) in mice previously pretreated (6 h) with intraescrotal injection of Natterins (0.02 μg/mL). Control experiments were performed in saline- or Natterins-treated mice applied with 20 μL of saline or LPS. The number of rolling leukocyte in LPS-mice was higher compared with control-mice, but Natterins virtually abrogated the rolling of leukocytes in venules after topical application of LPS. Also, the early treatment with both IPP-1 or PI3K inhibitors blocked the inhibitory effect of Natterins in the rolling leukocytes induced by LPS, in contrast with MG132. These data provide a molecular level explanation for the antagonist function of Natterins that is dependent on serine/threonine phosphatase and PI3K signaling. S**upported by:** FAPESP and CNPq.

P2.12.05
T cell proliferation defect of DiGeorge syndrome patients is associated with impaired CrKL signaling

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DiGeorge Syndrome is a primary immunodeficiency that is caused by the heterozygous deletion of 22q11.2, characterized by conotruncal abnormalities, thymic hypoplasia, and bacterial or viral recurrent infections. The features of DiGeorge vary widely, even among patients of the same family, but the most common chromosomal deletion observed in DiGeorge patients comprises the *CRKL* gene which encodes for an adaptor protein involved in intracellular signaling.
We investigated T-cell functions in 4 patients with DiGeorge Syndrome and 22q11.2 deletion. In particular, we studied proliferation, apoptosis and CD25, CD69 expression on CD4, stimulated with IL-2 plus anti-CD3 and anti-CD28. In addition, we evaluated the CrKL expression and phosphorylation in patients and controls. PHA-activated T cells under IL-2 stimulation, we evaluated ERK1/2, STAT3 and STAT5 phosphorylation. In DiGeorge syndrome patients we observed a marked reduction of T cell proliferation as compared to control subjects and low level expression of both CrKL protein and phospho-CrKL. While, in control subjects, CrKL was strongly phosphorylated after stimulation with IL-2.
We show that CrKL is involved in IL-2 signaling, the main T cell proliferation factor. CrKL protein levels and its phosphorylation are markedly reduced in DiGeorge patients, suggesting that CrKL might play a role in the functional defects of T cells.

P2.12.06
CD38/CD203a/CD73 runs in human T lymphocytes a novel adenosinergic loop independent from CD39

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Tumor microenvironment is marked by increased levels of extracellular NAD+ as well as by selective up-regulation of the ectoenzymes involved in its metabolism. The final consequence is an increase of local concentrations of the nucleoside adenosine (ADO), a powerful pleiotropic molecule involved in the modulation of immunity and inflammation.
CD203a (pyrophosphatase, PC-1) and CD38 (NADase) are two members of a larger family of nucleotide-metabolizing ectoenzymes. Substrates of CD203a are ATP as well as NAD+ and ADPR produced by CD38. The final product is AMP which can be further metabolized by CD73 (5'-nucleotidase) to ADO.
The aim of our work was to demonstrate the presence of an adenosinergic axis connecting CD38, CD203a and CD73 in lymphocytes and likely in other cells of different lineage. This axis may lead to the production of extracellular ADO in a CD39 (diphosphohydrolase, NTPDase)-independent way. The working hypothesis was confirmed in a human Jurkat T cell line. The components of the pathway were induced to be surface expressed upon activation and the products of their enzymatic activities analyzed by HPLC assays. The results indicate that PMA-activated Jurkat T cells, expressing CD38/CD203a at high levels, metabolize extracellular NAD+ originating AMP. In turn, this is converted to ADO by Jurkat cells transfected with the CD73 gene. Our data suggests that the CD38/CD203a/CD73 signalling pathway may constitute a new adenosinergic axis which bypasses CD39 in the formation of AMP. This pathway may play significant roles in physiology (activation, differentiation) and in the regulating of immune response against tumors.

P2.12.07
Genetically engineered chimeric molecules targeting Influenza A viral antigen

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Objectives: The use of live and attenuated strains of viruses for vaccination comprises the risk of evoking epitopes possessing undesirable characteristics. DNA vaccination using naked DNA encoding viral antigens has the potential to induce both humoral and cellular immune responses as live attenuated viral vaccines do. We hypothesized that sequences encoding an epitope of virus hemagglutinin (IP) attached to sequences encoding a scFv antibody fragment against co-stimulatory cell surface receptors (CR1/2 on mouse B cells or FcγRI on human monocytes) would result in the in vivo expression of a chimeric viral peptide with increased immunogenicity.
Methods: Gene and protein engineering; DNA immunization; ELISA; Cytotoxic assay;
Results: The DNA constructs were engineered by linking of sequences encoding the scFv antibody fragment against the appropriate receptor and IP peptide representing a conserved epitope of influenza A virus hemagglutinin. We have inserted the DNA constructs into protein expression vector system and used them as naked DNA vaccine. Serum antibodies against the viral peptide were measured with ELISA. The cell-mediated immune response induced in the animals was evaluated using a cytotoxic assay.
An immunization with a DNA plasmid containing the described construct induced a strong anti-influenza cytotoxic response lasting for more than 6 months. After prime-boosting with protein chimeric molecule we obtained anti-influenza cytotoxic and antibody response.
Conclusion: Immunization of mice with pure DNA, encoding the antigen of interest attached to a scFv antibody fragment to positive receptors, followed by prime-boosting has been successfully used to induce protective immunity against a model pathogen.

P2.12.08
Experimental and computational study of ROS and RNS in endothelial and leukocyte interactions

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Endothelial oxidative stress increases leukocyte-endothelial cell interactions, which is accompanied by leukocyte activation and increased oxidative and nitrosative stress in the vasculature. Studies related to leukocyte-endothelial cell interactions have questioned whether leukocyte recruitment, adhesion and activation are sequential events. We developed parallel experimental and computational strategies to study the process of leukocyte-endothelial cell interactions in details. The experimental method involved determination of adhesion effect of THP-1 cells to HUVEC cells cultured under normal/high glucose conditions for 24 hours. The computational method involved a biotransport model for an isolated microvascular blood vessel segment to quantify the changes in the local NO, O2•- and peroxynitrite levels at different regions of the microvasculature during leukocyte-endothelial cell interactions. The key results include (i) A significant increase in adhesion of THP-1 cells to HUVEC cells was observed under high glucose conditions compared to normal glucose, (ii) High glucose treated HUVEC cells showed significant increase in reactive oxygen species (ROS) concentration and (iii) endothelial oxidative stress increased O2•- and peroxynitrite levels in the lumen by 4 fold. The results show that leukocyte adhesion and activation can be independent events. Supported by NIH R01 HL084337.

P2.12.09
Arhgap12 as a novel binding partner of GULP regulates Rac1 activity during Stablin-2-mediated cell corpse engulfment

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Stabilin-2 is a phosphatidylserine receptor that mediates the clearance of apoptotic cells. An adaptor protein, GULP, directly interacts with stabilin-2 via its PTB domain and plays a key role in stabilin-2-mediated phagocytosis. However, mechanisms that control the stabilin-2-mediated cell corpse engulfment through actin rearrangement are largely unknown. Herein, we identified ArhGAP12 as a novel partner of GULP. ArhGAP12 interacted with the proline-rich motif of GULP via its Src homology 3 (SH3) domain and dissociated from GULP upon stimulation of stabilin-2. Knockdown of ArhGAP12 increased stabilin-2-mediated phagocytosis, while overexpressionof ArhGAP12 decreased phagocytosis. ArhGAP12 specifically controlled the activity of Rac1 but of not RhoA. Therefore, inhibition of Rac1 activity by ArhGAP12 led to the decrease of stabilin-2-mediated phagocytosis. Taken together, these results indicate that ArhGAP12 is a novel partner of GULP and plays an important role in stablin-2-mediated cell corpse engulfment by the regulation of rac1 activity.

P2.12.10
Acetylation modulates Interleukin-2 receptor signaling

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It is well known that interleukin-2 (IL-2) is a cytokine to modulate activation and proliferation of T cells. Furthermore, recent studies have revealed that an intact IL-2 receptor signaling pathway is crucial for regulatory T cell function and survival. IL-2 has an important role in immune responses. However, the precise molecular mechanisms underlying IL-2-mediated signaling in supportive or regulatory function of T cells has not been elucidated. Murine T cell line CTLL-2 was stimulated with IL-2, and examined the kinetics of intracellular signaling. As similar to the previous data, phosphorylation of janus

kinases 3 (jak3) and stat5 was detected. Furthermore, acetylated form of these molecules was also detected. To investigate IL-2-mediated acetylation of STAT5 in CTLL-2 cell, we examined the subcellular localization of lysine acetyltranferase (KAT) before and after IL-2 treatment. Activity of KAT was coprecipitated with IL-2 receptor beta chain and gamma chain. CBP was recovered from anti-IL-2R gamma chain immunoprecipitates in a ligand stimulation dependent manner. These findings prompted us to explore whether IL-2 could induce CBP nuclear export. In CTLL-2 cells, most of CBP was detected in nuclei. CBP started to increase its level in cytoplasm by receiving IL-2 treatment. These results suggest that acetylation plays a critical role from IL-2 receptor to its downstream Stat5 activation.

P2.12.11
Galphai2 and Galphai3 differentially regulate arrest and chemotaxis in neutrophils

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Leukocyte recruitment to inflammation sites progresses in a multistep cascade. Chemokine stimulation is one of the most important stimuli and regulates multiple steps including arrest, transmigration and chemotaxis. The most important chemokine receptor in mouse neutrophils is CXCR2, which couples through Galphai2 and Galphai3 conforming heterotremeric G proteins. Neutrophils arrest in response to CXCR2 stimulation. This is defective in Galphai2 deficient neutrophils. Hear, we show that Galphai3 deficient neutrophils showed inhibited transmigration and normal arrest. We also tested Galphai2 or Galphai3 deficient neutrophils in a CXCL1 gradient generated by a microfluidic device. Galphai3, but not Galphai2, deficient neutrophils showed significantly reduced migration and directionality. This defect in Galphai3 deficient neutrophils were also confirmed in vivo. Galphai2, but not Galphai3, deficient neutrophils showed decreased Ca influx with CXCR2 stimulation. Conversely, Galphai3, but not Galphai2, deficient neutrophils exhibited reduced AKT phosphorylation with CXCR2 stimulation. We conclude that Galphai2 and Galphai3 differentially regulate signaling for arrest, transmigration and chemotaxis.

P2.12.12
RUNX transcription factors regulate the transcriptional activity of CD69

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CD69 receptor is considered a universally marker of leukocyte activation, however it is immediately expressed after lymphocyte activation and appears on other leukocyte surfaces at later times. It has been also related with the expression of the migration controller molecule S1P1, revealing also an expression dependent on the localization of the cells involved. This extremely fine-tune regulation requires several mechanisms to control CD69 expression. Through *in silico* studies, here we analyze the putative conserved transcription factor binding sites located within the previously described conserved non-coding sequences 1, 2, 3 & 4 (CNS1, CNS2, CNS3 & CNS4). These studies reveal the importance of CNS2 which binds the majority of transcription factors studied by prediction software and ChIP-seq data from ENCODE consortium. They also reflect the specificity of CNS2, CNS1 and promoter regulatory features for hematopoietic lineages. Also we show new evidences on the transcriptional regulation of CD69, defining a minimal core sequence of the *cis* acting element CNS2, responsible for the main enhancer activity of this region. This core region is controlled by different transcription factors, and among them RUNX binding site shows the most important contribution to transcriptional enhancement activity. In addition, silencing of RUNX1 in Jurkat cell line in basal state and under PKC stimulation showed a reduction in CD69 expression. This new data suggest a main role of RUNX1 in the transcriptional regulation of CD69, according to the previous studies that showed the reduction of CD69 expression in the thymocytes of CD4+ T cell-conditional KO mice.

P2.12.13
Identification of a novel immunoregulatory signaling pathway exploited by M. tuberculosis in dendritic cells

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The causative agent of tuberculosis, M. tuberculosis, has infected over a third of the world's population and the persistence of latent infections poses a massive burden to health care systems and human well-being. The dendritic cell (DC) plays a crucial role in shaping the nature of the adaptive immune response after exposure to pathogens, and the interaction between M. tuberculosis and the dendritic cell is of profound importance for the course of infection. During their interaction, the DC is exposed to multiple M. tuberculosis-derived ligands recognized by a range of pattern recognition receptors, but the result is typically an immune response that is not very effective at clearing the bacteria from the host. The reason why the induced immune response is ineffective at clearing the bacteria is not fully understood, but clues may be given in the signaling pathways induced in DCs upon M. tuberculosis-exposure.
High resolution LC-MS/MS was used for a global analysis of the proteome and the phospho-proteome in human DCs upon stimulation with intact M. tuberculosis or purified lipopolysaccharide (LPS). Data were analyzed using MaxQuant and Python, and the algorithm NetworKIN was used for prediction of kinases responsible for the observed phosphorylation sites.
Multiple phosphorylation sites and protein kinases were identified that validate previously identified intracellular signaling structures induced in DCs by M. tuberculosis. Importantly, from the MS data analysis, FMS-related tyrosine kinase 3 (FLT3), that signals through JAK2 and STAT3, was identified as a novel protein kinase potentially activated in DCs by M. tuberculosis.

P2.12.14
The late endosomal transporter CD222 spatially regulates the distribution of proximal T cell signaling molecules

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The complex regulation of T cell activation is not fully understood, but endosomal transport pathways are more and more recognized to play an important role in T cell signaling cascades. CD222, also known as the cation-independent mannose 6 phospate/insulin-like growth factor 2 receptor, is one of the central components of endosomal pathways - CD222 transports its cargo proteins both from the Golgi apparatus and from the cell surface to lysosomes. Further CD222 is thought to be involved in endosomal recycling pathways and protein transport from the Golgi to the plasma membrane. Upon T cell activation CD222 expression is subsequently upregulated on the cell surface, yet the biological relevance of this membrane accumulation remains elusive. Here, we aimed to investigate the impact of CD222 on T cell activation using lentiviral knock-down and zinc-finger nuclease knock-out approaches, mass spectrometry, co-immunoprecipitation and confocal laser scanning microscopy. We found that the silencing of CD222 in T cells resulted both in abrogated T cell effector functions, like cytokine secretion, and down-regulated calcium flux, whereas T cell proliferation remained unaffected. Via mass spectrometric analysis we identified several interaction partner candidates for CD222 known to be involved in T cell activation, which we confirmed by co-immunoprecipitation. Our data uncover a yet undescribed function of CD222 in T cell signaling and suggest a model for the mechanism of CD222-driven regulation of T cell activation.

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P2.12.15

The activation and co-stimulatory functions of Fc receptor like 1 protein (FcRL1) in human B cells depend on phosphatidylinositol 3-kinase

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Fc receptor like protein 1 (FcRL1) is expressed on the surface of all B cells starting from the pro-B stage. Our previous study has identified the activation and co-stimulatory activities of FcRL1 but the molecular mechanism by which FcRL1 activates B cells remains unknown. Here we report that cross-linking of FcRL1 induced its tyrosine phosphorylation, which recruited the association of Grb2 adaptor protein and phosphatidylinositol 3-kinase (PI3-K), and subsequently stimulated the activation of downstream ERK and Akt kinases. The tyrosine 369 (Y369) of FcRL1 was the major phosphorylation site after cross-linking and this residue was essential for the activation of Grb2/ERK and PI3-K/Akt pathways. The PI3-K-Akt pathway was required for FcRL1-mediated B cell proliferation. Interestingly, FcRL1 ligation induced modest tyrosine phosphorylation of CD19. Co-ligation of FcRL1 with surface IgM significantly increased the tyrosine phosphorylation of CD19 and the association of PI3-K to CD19. Altogether, our data suggests that the activation and co-stimulatory functions of FcRL1 are related to the PI3-K-Akt pathway.

P2.12.16

Sterile alpha motif (SAM) and individual H5 alpha helix mediates SLP-76 oligomer formation for microclusters and T-cell activation

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Despite the importance of the immune adaptor SLP-76 in T cell immunity, it has been unclear whether SLP-76 directly selfassociates to form higher order oligomers for T-cell activation. In this study, we show that SLP-76 self-associates in response to TCR ligation as mediated by the N-terminal sterile alpha motif (SAM) domain. SLP-76 coprecipitatedalternately tagged SLP-76 in response to anti-CD3 ligation. Dynamic light scattering (DLS) and fluorescent microscale thermophoresis (MST) of the isolated SAM domain (residues 1-78) revealed evidence of dimers and tetramers. Consistently, deletion of the SAM region eliminated SLP-76 coprecipitation of itself, concurrent with a loss of microcluster formation, NFAT transcription and interleukin 2 production in Jurkat or primary T-cells. Further, different regions in the SAM domain independently contributed to self-association such that retention of the conserved single SAM H5 alpha helix sufficed to support SLP-76 binding to SLP-76 concurrent with smaller microclusters, but nevertheless, enhanced anti-CD3 driven NFAT transcription and IL-2 production. Our data identifies for the first time that anti-CD3 ligation induces SLP-76 oligomerization asmediated by the SAM domain.

P2.12.17

Functionally Relevant phoshorylatable Serine Clusters targeted by Casein Kinase 2 on the Cytoplasmic region of the human CD6 Lymphocyte Surface Receptor

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CD6 is a transmembrane lymphocyte glycoprotein mainly expressed on T cells and a subset of mature B cells (B1a), where it physically associates with the antigen-specific receptor present on their surface. Accordingly, CD6 is typically involved in lymphocyte activation and differentiation through its interaction with CD166/ALCAM, an adhesion molecule from the Ig superfamily. More recently, it has also been implicated in sensing the presence of conserved bacterial cell wall components. Cross-linking of CD6 by either endogenous or exogenous ligands transduces intracellular signals of still incompletely understood nature through its long cytoplasmic tail, which lacks intrinsic catalytic activity but presents several consensus motifs suitable for phosphorylation and signal transduction. In the present work, mapping of constitutive and cell activation-induced serine phosphorylation sites has been achieved by deletion and/or point mutation analysis of the CD6 cytoplasmic tail. Two serine clusters (S480/482/484 and S560/562/565/567/568), which are embedded into Casein Kinase 2 (CK2) consensus motifs, are shown to be involved in the constitutive phosphorylation of CD6. Moreover, disruption of these serine clusters interferes with MAPK activation following CD6 binding to either specifc mAbs or previously reported endogenous (ALCAM/CD166) or exogenous (bacterial LPS) ligands. Interestingly, several alternatively spliced CD6 cytoplasmic isoforms devoid of the above mentioned serine clusters were isolated following lymphocyte activation. In conclusion, the data point to the regulation of CD6 function by intracytoplasmic CK2-phosporylation sites, which is either complementary or alternative to that of the extracellular ALCAM-binding site.

P2.12.18

Targeted mitochondrial antioxidant SkQ has anti-inflammatory properties in vitro and in vivo

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Reactive oxygen species (ROS) have versatile roles in inflammation as both signal mediators and effector molecules. Two distinct kinds of ROS are those produced in mitochondria as a byproduct of respiratory chain and on the plasma membrane of immune effector cells by NADPH oxidase. Distinguishing the relative impact of mitochondrial and cellular ROS is difficult because in inflammation the latter are produced in vast excess, and common antioxidants target ROS from any source. SkQ1 (plastoquinonyl-decyl-triphenylphosphonium bromide) is a representative of the family of mitochondria-targeted antioxidants. Triphenylphosphonium group combines positive charge and hydrophobic properties, providing highly specific accumulation of the quinone in the internal membrane of mitochondria. As a result, the concentration of SkQ1 in mitochondria is up to seven orders of magnitude higher. Stabilization of mitochondria results in multiple biological effects of SkQ1 that are observed in in vivo and in vitro in nanomolar range. Importantly, the window between antioxidant and prooxidant concentrations is about 1000-fold, providing high flexibility in experimental and potential clinical applications. This makes SkQ1 a promising tool to explore the role of ROS and the relative impact of endogenous (mitochondrial) and plasma membrane ROS in inflammation signaling pathways. Immunomodulating properties of SkQ1 have been shown in a number of in vivo models. It limits neutrophil infiltration and excessive inflammation in models of hepatitis (induced by D-Gal+LPS), bacterial pyelonephritis, autoimmune arthritis and wound healing. The molecular mechanisms of these effects are currently under investigation.

P2.12.19

SAP-mediated inhibition of Diacylglycerol kinase alpha regulates TCR-induced diacylglycerol signaling and restimulation induced cell death in XLP patients

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X-linked lymphoproliferative disease (XLP) is a rare congenital immunodeficiency leading to a usually fatal lymphocytosis following EBV infection. It is characterized by loss of expression of SLAM-associated protein (SAP), which results in defective SLAM family receptors and TCR signaling. Recent evidences showed that SAP deficiency causes resistance to TCR re-stimulation induced cell death (RICD), a process that physiologically constrains T cell expansion during immune responses (Snow, JCI 2009).
We recently showed that, upon TCR stimulation, SAP mediates negative regulation of the enzymatic activity of Diacylglycerol kinase alpha (DGKα) (Baldanzi, J. Immunol. 2011). DGKα, phosphorylating diacylglcyerol to phosphatidic acid, is a well-known negative regulator of TCR signaling. In SAP-deficient Jurkat cells, inhibition of DGKα partially rescues defective TCR/CD28 signaling, including Ras and ERK-1/2 activation, PKCθ membrane recruitment, induction of NF-AT transcriptional activity and IL-2 production.
Here we show that both DGKα silencing and pharmacological inhibition restore diacylglycerol signaling in SAP deficient primary T cells, thereby enhancing T cell activation (ERK phosphorylation and cytokines production). Moreover, DGKα knockdown efficiently restores FASL and BIM expression, reestablishing TCR-induced apoptosis of SAP deficient cells. Intriguingly, DGKα silencing/inhibition fully restore RICD in SAP knockdown T cells, as well as in T cells from XLP patients. Thus, DGKα inhibition is sufficient to correct the defective TCR signaling and RICD in SAP defective cells ex-vivo, indicating a key role of the SAP-DGKα pathway in RICD control. These data also suggest that DGKα inhibition could be a novel pharmacological strategy for treatment of XLP and others lymphoproliferative diseases.

P2.12.20
Food contaminant mycotoxin Fumonisin B2 regulates the mTOR kinase signaling pathways in brain lymphocytes: implication for neurological disorders

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Mycotoxin Fumonisin B2 (FB2, $C_{34}H_{59}NO_{14}$, *M*705) is produced by fungi *Fusarium moniliforme* and other species contaminated a corn world-wide. Previously we found that FB2 regulated the lymphocyte activation, proliferation, and apoptosis. Our late breaking data concerns the FB2 regulation of mTOR kinase signaling pathways in brain lymphocytes *in vivo*.
Methods: FB2 [0.01 - 1.0 μM] was injected intraperitoneally to FVB male mice 12 weeks old. Each 15 minutes to 2h and then each 1h to 6h three animals were killed by guillotine and brains were removed. One part of brain was frozen and stained with mTOR-related protein antibodies and for morphological examination. Other part was homogenized to obtain the isolated lymphocytes stained and analyzed by flow cytometry. Statistical analysis from three experiments was performed using ANOVA.
Results: FB2 regulates the key mTOR signaling proteins in brain lymphocytes: upstream inhibitors TSC1/TSC2 (hamartin and tuberin proteins), adapter protein 14-3-3, mTOR-associated proteins Raptor, Rheb, and HSP70, downstream effectors p70S6K1 and 4E-BP1 as well as some mTORC2-associated proteins. FB2 effects have been found to be irreversible concerning some proteins followed by the specific morphological changes in brain tissues. Brain B cells have been found to be activated under FB2 exposure and produced a large level of immunoglobulins. Activated CD8+ formed the clusters in cortex and white matter followed by neuronal cell death and oligodendrocyte apoptosis. Activated CD4+ T cells underwent an apoptosis.
Conclusion: food contaminant fumonisin B2 possesses the brain lesion due to the specific regulation of the mTOR signaling proteins.

P2.12.21
The influence of calpain-calpastatin system in human lymphocyte - assessment of lymphocyte signaling process of centenarians and elderly people

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The calpain-calpastatin system (CCS) in the lymphocytes and many other cell types contains two neutral, cytoplasmic proteases (1- and 2-calpain) and their common endogenous inhibitor - calpastatin. Their property of cleaving only relatively few specific sites in the proteins and peptides, as well as their list of substrates, makes them potentially important regulators of the immune response. Thus it is feasible that the system may be involved (beneficially or otherwise) in the functioning of lymphocytes and its deregulation may impact on or participate in the process of immune system ageing; consequently, a hypothesis of different activity of the CCS in the immune cells of centenarians (as compared to younger healthy elderly) is valid and attractive.
Thus, the aim of current project is to establish the role of CCS system function for the effective immune response and of its malfunction as a potential participant in the mechanisms of the immune cells' ageing and cells signaling.
We assess the number status and activation of lymphocytes population and the amount and the activities of CCS in the various subpopulations of peripheral blood cells. We compare lymphocytes from Polish healthy centenarians and elderly. Our preliminary results suggest the differences in the expression of CCS members between the different lymphocyte populations (including the CD4+, CD8+, CD19+)and their activation in the age groups. Project funded by the Polish National Science Center grant DEC-2011/01/M/NZ3/02948 CALPACENT

P2.12.22
CD157-extracellular matrix proteins interactions enhance integrin-mediated signalling cascade in monocytes

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CD157 is a GPI-anchored ectoenzyme belonging to the NADase/ADP-ribosyl cyclase gene family involved in the control of human neutrophils and monocytes adhesion, migration and diapedesis. We demonstrated that CD157 physically interacts with β1 and β2 integrins in monocytes and its cross-linking by means of a monoclonal antibody (mimicking the natural ligand) recruits integrins to lipid rafts, promoting the transduction of optimal intracellular signals converging on the MAPK and PI3K pathways.
Here, we demonstrate that CD157 binds selected extracellular matrix proteins. Using solid phase binding assays and surface plasmon resonance analysis, we found that human recombinant CD157 binds to fibronectin, fibrinogen, laminin and collagen type I but not to vitronectin or to the polysaccharide components of extracellular matrix (such as heparin and hyaluronan). CD157 binding is concentration-dependent and is prevented by selected anti-CD157 monoclonal antibodies. Using recombinant fibronectin fragments, CD157 binding sites were mapped within the N-terminal and C-terminal heparin binding regions of fibronectin, which lack integrin-binding motifs.
Co-immunoprecipitation experiments using monocytic THP-1 cell line confirmed that: i) CD157 physically associates with fibronectin and ii) CD157 binding to fibronectin is required for promoting the association between CD157 and integrins.
We hypothesize that the simultaneous engagement of CD157 and integrins by distinct domains of fibronectin represents the physiological mechanism promoting the interaction between CD157

and integrins, leading to the optimal activation of the signalling cascade which controls crucial aspects of monocytes extravasation.

P2.12.23
IL-21 isoform is a membrane-bound ligand and activates directly interacted cells

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IL-21 is a pleiotropic cytokine that regulates the function of T cells, B cells, natural killer cells, and myeloid cells. We previously identified an IL-21 isoform, IL-21iso, in humans and mice, and found that IL-21iso was secreted in much smaller amounts than conventional IL-21. In this study, we determined that secreted IL-21iso also has less signaling activity than IL-21. However, the amounts of intracellular IL-21 or IL-21iso, and the level of STAT3 phosphorylation induced by the two IL-21 forms, were similar. IL-21-sensitive reporter cells co-cultured with cells producing IL-21iso showed STAT3 activation, apoptosis, and proliferation. However, when IL-21iso-producing cells were cultured in a transwell chamber, which prevented direct contact with the IL-21-sensitive cells, no IL-21iso-induced signaling was observed. Though IL-21iso is secreted in smaller amounts and has less potent signaling activity than IL-21, IL-21iso acts both on IL-21iso-bearing cells and other IL-21-sensitive cells through direct interactions probably without being secreted. Thus, IL-21iso's regulation of immune cells may be limited to the immediate proximity around the IL-21iso-producing cells, in regions such as immune organs or inflammation sites. Cytokine 61, 656-663, 2013.

P2.12.24
Differential TLR1/2 pro-inflammatory signaling during the first 9 months of life

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Newborns are particularly susceptible to infections and generally respond poorly to vaccination. The underlying cause may be immaturity of innate immunity. Despite evidence that toll-like receptor (TLR) sensor function in newborns is well developed, we and others have shown lower mycobacteria-mediated production of pro-inflammatory cytokines in monocytes from newborns compared to older infants.
We investigated whether differential ligand activation of key pro-inflammatory signaling pathways downstream of TLR1/2, a major pathogen recognition receptor of mycobacteria, underlies age-related differences in innate cell responses.
We analysed cryopreserved mononuclear cells from cord (n=10) and peripheral blood collected from 10 week (n=7) and 36 week old (n=7) healthy infants. Degradation (D-) of NFκB-inhibitor IκB and phosphorylation (P-) of MAP kinases p38 and erk in monocytes stimulated with TLR1/2 ligand PAM3 was measured by flow cytometry.
Higher basal proportions of P-erk and D-IκB monocytes were observed in 10-week-old infants, compared with newborns. Upon stimulation infants of all ages showed increased IκB degradation and phosphorylation of p38 and erk. However, newborns displayed higher induction of P-erk and lower induction of D-IκB than older infants. Simultaneous activation of all three signal transduction molecules was observed predominantly in 10 and 36-week-old infants, while monocytes from newborns primarily exhibited activation of only one molecule at a time. In particular, induction of P-Erk, in the absence of P-p38 and D-IκB, was observed only in newborns.
Greater co-activation of pro-inflammatory signaling cascades, such as this TLR1/2 pathway, may underlie higher innate pro-inflammatory cytokine responses to mycobacteria in older infants, compared with newborns.

P2.12.25
Dasatinib leads to a reduced number of B cells in mice and promotes apoptosis of human CD19+ peripheral blood B cells

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Dasatinib is a highly potent BCR-ABL and Src family kinase inhibitor which also inhibits a variety of other tyrosine kinases. It is currently approved for the treatment of imatinib-resistant chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia, and there are ongoing clinical trials to evaluate its use in several other cancer types. Here we show that Dasatinib leads to a markedly reduced number of lymphocytes in mice treated for 2 and 4 weeks. The reduction in lymphocytes was due to a specific loss of $CD19^+$ B cells, since T cells numbers were not affected. A reduced level of $CD19^+$ B lineage cells was detected in bone marrow, blood and spleen. The effect on spleen was reflected by a clear reduction in organ size. In contrast, Dasatinib induced an increase in the number of myeloid cells. When human $CD19^+$ B cells were cultured with Dasatinib [128 nM], we observed a 7-fold increase in apoptotic cells after 5 days. In contrast, no similar effect on T cell viability was observed. However, Dasatinib induced a comparable broad inhibition of the early events of human B and T cell receptor signaling i.e. Src family kinases, Syk, PLCγ2, p70 S6 and ERK1/2 in B cells and CD3ζ, Src family kinases, ZAP70, PLCγ1, p70 S6 and ERK1/2 in T cells. Overall, this study reveals an important effect of Dasatinib with the specific loss of $CD19^+$ B cells, which may have an impact for patients on long-term Dasatinib treatment.

P2.12.26
Regulation of TCR trafficking and immune synapse assembly by Rab29

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Notwithstanding their central implication in vesicular trafficking, the function of several members of the Rab family of small GTPases remains elusive, as exemplified by Rab29/Rab7L1. Recently Rab29 has been shown to be selectively cleaved by GtgE, a type III secretion effector produced by broad-host Salmonella serovars. This finding provides not only insight into the biology of Salmonella but also a new tool to address the role of Rab29 in vesicular trafficking. Here we have addressed the potential implication of Rab29 in TCR trafficking. Rab29 was found to be associated with the endomembrane system, co-localizing with Rab8, which has been implicated in endosome recycling in polarized cells, as well as with the recycling Rabs, Rab4 and Rab11. Rab29 depletion by T cell transfection with a construct encoding GtgE resulted in impaired TCR recycling. Immune synapse (IS) assembly was also impaired in Rab29 depleted cells, as assessed by imaging protein tyrosine phosphorylation and TCR clustering at the IS. This defect resulted from the inability of the TCR to undergo polarized recycling to the IS. Collectively, these results provide the first evidence of a role for Rab29 in vesicular trafficking and identify a new player in TCR recycling and IS assembly.

P2.12.27
A mutation in the moesin gene induces an alteration of T lymphocyte adhesion/migration process in a new combined immunodeficiency syndrome

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In the present study, we report in four non related pediatric patients a new primary immunodeficiency associated with a missense mutation in the moesin gene. The disease is characterized by a profound T, B and NK lymphoid deficiency and neutropenia. Exome analysis of all patients revealed an identical 683C>T missens mutation in the exon 5 of moesin gene located in Xq11.1. The mutation introduces an amino acid change (p.R171W) in the highly conserved FERM domain. Moesin belongs to a group of proteins that link membrane proteins to actin and is implicated in various cellular functions such as survival, adhesion, migration and T-cell activation. Flow cytometric analysis of patients demonstrated a decreased expression of chemokine receptor CXCR4 and CCR7 on CD4+ and CD8+ T cells. In parallel, expression of adhesion molecule LFA1 and VLA-4 was increased on CD8+ T cells. Adhesion and migration assays performed on T lymphocytes of P1 indicated stronger adhesion capacities of CD8+ T cells on VCAM-1 and fibronectin and decreased migration capacities of CD4+ and CD8+ T cells in response to SDF-1 (CXCR4 ligand) or CCL21 (CCR7 ligand). An alteration of the ROCK/MLC pathway may explain these results. Altogether, our results indicate a role of moesin in the migration properties of lymphocytes in human.

P2.12.28
Characterization of THEMIS as a novel attenuator of TCR signalling

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Stimulation of the T cell antigen receptor (TCR) induces formation of phosphorylation-dependent signalling networks, ultimately leading to T cell proliferation and differentiation. Composition and dynamics of the TCR signalosome are still incompletely understood. Others and we have recently identified the T-cell-specific phosphoprotein Thymocyte-expressed molecule involved in selection (THEMIS), as a key player in the double positive thymocyte selection process. The exact role of THEMIS in signalling, especially in peripheral T cells, has remained poorly characterized and controversial. In the present study we show that THEMIS is a new member of the TCR-proximal signalosome. TCR ligation leads to rapid recruitment of THEMIS to the immunological synapse via the transmembrane adapter LAT, accompanied by Lck -and ZAP70-mediated Tyr-phosphorylation of conserved Tyr-residues at the C-terminus of THEMIS. LAT recruitment of THEMIS is dependent on the constitutively associated adapter molecule GRB2. We can further show that the THEMIS-GRB2 complex is indispensable for T cell development in vivo. In mature human T cells, shRNA-mediated knockdown of THEMIS expression leads to enhanced TCR-proximal phosphorylation events and an increase in expression of surface activation markers. Ultimately, in the absence of THEMIS, human peripheral T cells and model T cell lines are significantly more susceptible to activation-induced cell death due to deregulated signalling emanating from the TCR. Our data suggest that THEMIS contributes to a negative feedback that limits cell death during activation of peripheral T cells and have implications for the mechanism that sets the threshold between positive and negative selection during T cell development.

P2.12.29
Dendritic cell CD83 enhances calcium signaling in T lymphocytes by affecting calcium release from intracellular stores

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CD83 is a marker of mature dendritic cells (mDCs) directly related to their limphostimulatory ability, but little is known about the mechanism of action of CD83. We aimed to show the influence of CD83, present in mDCs, upon the calcium signaling in T lymphocytes during the immunological synapse made between them. Monocytes were differentiated into mDCs by culture, for seven days, in the presence of GM-CSF, IL-4, and, in the last two days, TNF-α. In calcium signaling assays, purified T lymphocytes were incubated with the calcium indicator Fluo-4AM and analyzed in the presence of allogeneic mDCs. As expected, blockage of CD83 with a specific antibody reduced the proliferation of lymphocytes in the presence of allogeneic mDCs. CD83 antibodies reduced the calcium signaling amplitude observed in T lymphocytes by flow cytometry, fluorescent and confocal microscopy. The percentage of responding cells also reduced from 58.1% to 34.3% in the presence of antibody. Also, CD83 knockdown mDCs induced lower calcium signal amplitude in T lymphocytes, compared to scrambled siRNA-treated mDCs. In the absence of extracellular calcium, CD83 antibodies completely abrogated T cell signaling, which indicates that CD83 signaling might act upon calcium release from intracellular stores. These data suggest that the presence of CD83 in mDCs enhances T lymphocytes proliferation by inducing greater calcium release from intracellular stores in T lymphocytes. Supported by FAPESP #2009/54599-5 #2011/01082-5.

P2.12.30
Platelets and leukocytes reactivity in patients with ischemic stroke

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The most common cause of stroke is the sudden occlusion of a blood vessel by a thrombus or embolism, associated with platelet and leukocyte dysfunction. The last one determines the induction of pro-inflammatory cascade, cerebral injury progression and complications development.
The aim of this investigation was to estimate the cooperation between platelets and leukocytes in blood of patients with ischemic stroke depending on outcome.
Materials and Methods. The functional state of platelets and leukocytes was estimated in 40 patients with acute ischemic stroke. The 1st group includes 30 stroke survivors, the 2nd - 10 patients with lethal outcome. We assessed platelets aggregation induced by epinephrine (2,5 μM), ADP (5 μM), thrombin (1,5 NIH) and angiotensin II (1 μM). Platelet-leukocyte aggregates (PLA) in peripheral blood were analyzed morphologically.
Results. The total number of leukocytes and segmented neutrophils percentage rates in complete blood count were significantly higher in 2nd group of patients comparing with 1st group ($p=0,005$ and $p=0,026$ respectively). Pro-inflammatory activation of leukocytes was related with PLA formation and lysed leukocytes appearance, which number were significantly higher in 2nd group ($p<0,001$). Comparison of all agonists effects on blood cells activation shown the most prominent effect of thrombin, inducing formation of heterogeneous and homogeneous aggregates, which number was in two times higher in patients with lethal outcome comparing 1st group ($p=0,001$).
Conclusion. Ischemic stroke is associated with pro-inflammatory activation of platelets and leukocytes, accelerated by thrombin and reflected in PLA formation. These findings can be useful in ischemic stroke outcome prognosis.

P2.12.31
The chicken IFN- λ Receptor Complex

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High Pathogenic Avian Influenza(HPAI) viruses, such as H7N9 and H5N1 HPAI, have an immense effect on poultry production and there is a need for new strategies to deal with these infections. Moreover, there is a clear need to understand the processes associated with the host response to virus to help to develop these new strategies. Host antiviral molecules and their receptors, like interferons(IFN), are key players in the antiviral immune response and mainly responsible for a successful resolution of an infection. With this in mind, we investigated IFN-λ and the IFN-λ specific component of the receptor complex, and their role in viral infections. We characterized the chicken IFN-λ receptor and showed that is has 60% and 59% amino acid sequence conservations to the human and mouse IFN- λ receptor, respectively. Analysis of the expression of IFN- λ receptor in chicken tissues showed its presence over a large range of tissues with the highest expression in the lung, spleen, intestine and cecal tonsils. Similarly, a number of chicken cell lines, such as the HD11 chicken macrophage-like cell line, tested positive for this receptor. To investigate the function of the IFN- λ receptor, RNAi mediated gene knock down was used to target the IFN- λ Receptor in HD11 cells. This silencing approach showed a greater than 50% knockdown of IFN- λR mRNA *in vitro*. These receptor gene silencing experiments will be used to assess the role of the IFN- λ receptor complex in the signaling response to viral infections.

P2.12.32
A novel tool to isolate and analyze lipid rafts

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The experimental basis for the lipid raft concept are detergent-resistant membrane domains that contain high levels of cholesterol and sphingolipids and are enriched in glycosylphosphatidylinositol-anchored and palmitoylated proteins as well as a subset of transmembrane proteins, e.g. Lck and LAT in T cells. Various methods exist to isolate lipid rafts for biochemical analysis, with cell lysate fractionation via sucrose density gradient ultracentrifugation still being the gold standard for separation of raft markers. In general, these methods are laborious and time-consuming and need a large number of cells. Here we present a fast and easy method to harvest lipid raft proteins within minutes via passage of cells through a detergent gradient by low g centrifugation, requiring little starting material. Fine-tuning of gradient density and the usage of raft-conserving and -disrupting detergents enable not only the generation of a lipid raft fraction for conventional down-stream analysis as immunoblotting, but also the analysis of lipid raft composition on a single cell level via flow cytometry. Thus it is possible to easily monitor and quantify proteins integral to and associated with lipid raft domains at the cell surface, e.g. prior and post stimulation of the T cell receptor. In conclusion, we describe a method suitable for quick, easy, reproducible and sensitive analysis of lipid raft-associated molecules on a single cell level.

Regulation of soluble IL-15 complexes after irradiation, chemotherapy, and VSV infection

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During the steady state, IL-15 is primarily located either intracellularly or on the cell surface, where it can stimulate lymphocyte development and homeostasis via transpresentation. Conversely, soluble (s) IL-15 complexes have been shown to be generated after irradiation or nonmyeloabablative chemotherapy; however, the mechanisms generating sIL-15 complexes are not clear. This lead us to investigate if sIL-15 complexes are generated in other types of lymphopenia or by other inflammatory events. We report here that while lymphopenia induced by sub-lethal irradiation increased serum sIL-15 complexes, lymphopenic RAG-/- mice do not express increased levels of serum sIL-15 complexes. Additionally, in contrast to other studies, nonmyeloabablative chemotherapy induced by cyclophosphamide (200mg/kg) did not increase sIL-15 complexes whereas antibody-mediated T cell depletion did increase sIL-15 complexes suggesting the manner of cell death dictates the mechanisms generating sIL-15 complexes. We next investigated the role of IFN-a in the induction of sIL-15 complexes and found that treating mice with IFN-a encoding plasmids(via hydrodynamic delivery), Poly I:C, or VSV potently induced serum sIL-15 complexes. Furthermore, similar to Poly I:C or VSV, irradiation increased serum IFN-a levels. Correlating to the lack of sIL-15 complexes, chemotherapy did not induce IFN-a. Type I IFN signaling was important as IFN-R-/- mice displayed impaired production of sIL-15 complexes in response to Poly I:C, VSV infection, or irradiation. Overall, these data provide evidence that IFN-a is an important upstream signal generating sIL-15 complexes.

P2.12.34
IL-10 receptor and endoplasmic reticulum stress impairs STAT3 activation in type 1 diabetes patients and their healthy first degree relatives

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Autoimmune diseases including type 1 diabetes (T1D) result from disordered immune tolerance. We showed that an endoplasmic reticulum (ER) stress signature predicted poor outcome in recent-onset T1D patients, and is observed in a proportion of at-risk first degree relatives (FDR). To determine the relationship of ER stress to immune cell signalling, we compared monocyte and T cell STAT3 phosphorylation in response to IL-6 and IL-10 in 30 T1D, 35 FDR, 11 healthy controls, and 22 rheumatoid arthritis (RA) disease controls. Cytokine-stimulated signalling was detected in peripheral blood monocytes and T cells by flow cytometry. Expression of ER stress genes GRP78 and DDIT3 was quantified from PBMCs by RT-PCR. Induction of phospho (P)-STAT3 by IL-10 but not IL-6 was significantly reduced in T1D and FDR monocytes and T cells relative to healthy or RA controls. Basal levels of total STAT3 were comparable in all groups. This reduction in P-STAT3 response in T1D patients and FDR was associated with reduced IL-10-, but not IL-6-receptor expression by monocytes and T cells. IL-10 receptor expression and P-STAT3 induction in response to IL-6 and IL-10 were negatively associated with GRP78 and DDIT3 ER stress gene expression. IL-10 was shown to block ER stress in gut epithelium. Thus, our data implicate a disease-specific IL-10 receptor defect and its consequences on excess ER stress and disordered peripheral tolerance in T1D pathogenesis.

P2.12.35
Enhanced expression of adenosine-producing ectonucleotidases in activated and memory T cells

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Extracellular adenosine exerts its immunosuppressive effects through activation of cyclic AMP synthesis in target cells. Its accumulation is largely regulated by the activity of the cell-surface enzymes CD39 and CD73 converting pro-inflammatory ATP into immunosuppressive adenosine in a two-step process. It has recently been demonstrated that adenosine production strongly contributes to the immunosuppressive activity of regulatory T cells (Treg). To elucidate

the role of extracellular adenosine in T cell function, we investigated the expression of CD39 and CD73 in various T cell subpopulations in the *ret* transgenic model of melanoma and orthotopic model of pancreatic adenocarcinoma in mice. We confirmed that CD39 and CD73 are expressed at high levels on the vast majority of Treg. Furthermore, we detected both enzymes on $CD4^+FoxP3^-$ conventional T cells (Tcon). Notably, we found that the frequency of $CD39^+$ and $CD73^+$ cells among activated ($CD25^+$) Tcon is significantly higher than that within the $CD25^-FoxP3^-$ subset. Moreover, *in vitro* activation of both $CD4^+$ and $CD8^+$ T cells from healthy mice led to a significant upregulation of the CD39 expression. Studying memory $CD4^+$ and $CD8^+$ T cells *in vivo*, we found that both central and effector memory compartments are highly enriched in $CD39^+$ and $CD73^+$ cells, as compared to naïve T cells. Furthermore, after a short-term restimulation *in vitro*, the IFNγ-producing subpopulation contained a higher proportion of ectonucleotidase-expressing cells than the IFNγ-negative counterpart.
Taken together, our data suggest that CD39 and CD73 expressed on effector T cells might be involved in the regulation of T cell activation.

P2.12.36
Phenotypical analysis of transgenic mice expressing soluble human CD6 supports an immunomodulatory role for this protein

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CD6, a SRCR-SF group B membrane receptor, is a membrane glycoprotein expressed in thymocytes, mature T cells, B1-a cells, NK cells, hematopoietic precursors, and some brain regions. This molecule is physically associated to the TCR, likely modulating its activation and differentiation signals. The ultimate function of CD6 has not yet been deciphered. Although initially considered as a co-stimulatory molecule, a recent report proposes that CD6 may act as a negative modulator, since CD6-negative lymphocytes were hyper-reactive to TCR-mediated stimulation. To date, no CD6 knock-out or knock-in mice have been developed, so the functional relevance of this protein remains unclear.
To gain insight into the function of CD6, we have developed transgenic C57Bl/6 mice expressing a circulating soluble form of human CD6 (shCD6). This shCD6 would supposedly work as a decoy receptor specifically blocking the ligand-receptor interactions mediated by membrane-bound CD6, thus resulting in a functional knocking-down of this protein. In these mice, shCD6 expression is under the transcriptional control of the lymphocyte-specific promoter from the lck tyrosine kinase, as well as of the Ig heavy chain enhancer, so that this molecule will be expressed both in T and B cells. The shCD6 is readily detected in the serum of these mice (at the ng/ml range) and preliminary phenotypical analysis shows significant changes in some specific lymphocyte subpopulations both in central and peripheral lymphoid organs. These support a relevant role of CD6-mediated interactions in lymphocyte development as well as in homeostasis of some peripheral lymphocyte subsets and the anti-tumoral response.

P2.12.37
Understanding signalling requirements of LTi cells in vivo and in vitro

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The diverse family of innate lymphoid cells (ILCs) is comprised of lymphocytes that, unlike T- and B cells, do not utilize antigen receptors generated via somatic recombination. These include cytotoxic NK cells, innate helper cells (also known as nuocytes or ILC2), lymphoid tissue inducer (LTi) cells, and closely related to them $NKp46^+$ IL-22-producing lymphocytes located in the lamina propria of the intestine, jointly referred to as ILC3. ILCs are currently in focus of a number of studies investigating their role in mucosal tissue homeostasis and the interaction of the immune system and gut microbiota. While considerable progress has been made in the understanding of physiological roles of LTi cells in controlling the organogenesis of lymphoid tissues and regulation of intestinal immunity, little is known about molecular mechanisms governing LT cells' (and other ILCs) responses to external stimuli, as well as the exact nature of stimuli themselves. Here, we demonstrate that canonical NFκB signalling can be readily activated in ILC3s by stimulation with IL-1β and TLR agonists, accompanied by activation of p38 and Jnk kinases. Additionally, we report preliminary evidence that canonical NFκB signalling is important for the development of *in vitro* differentiated ILC3 cells. We are currently investigating the role of NFκB and other signalling pathways in ILCs *in vivo*. These preliminary studies will contribute to better understanding of mechanisms of ILC function and development.

P2.12.38
Elucidation of IL-17 signaling pathways

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Proteins that belong to the family of IL-17 ligands coordinate various aspects of inflammatory reactions to provide adequate responses to fungal and bacterial infections. However, their excessive signaling results in various immune-related pathologies such as rheumatoid arthritis, psoriasis, multiple sclerosis, asthma and others. At the same time, the molecular mechanisms of IL-17 signaling are not well understood. Stimulation of IL-17 receptors results in activation of several kinase pathways and stabilization of cytokines and chemokines mRNAs. IL-17 triggers the formation of receptor signaling complex containing several TRAF molecules that mediate activation of NF-kB and MAP kinase signaling pathways. Still, the role of individual TRAF molecules in IL-17 signal transduction pathway remains obscure. To reveal the functional significance of TRAFs for IL-17 receptor stimulation we have examined the composition of IL-17-induced protein signaling complexes in several cell lines. We also determined the functional outcome of individual and combined TRAF absence on IL-17 signaling. Understanding the significance of TRAF proteins for IL-17 signaling is important for the overall understanding of IL-17 biology and for the design of therapeutics that can be used in the treatment of human pathologies that are driven by IL-17 expression and activity.

P2.12.39
Biological evaluation of human PBMC and Th17 cells using MILLIPLEX® MAP cell signaling and cytokine panels

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Peripheral Blood Mononuclear Cells (PBMC) are composed of all blood cells having a round nucleus. This includes lymphocytes, monocytes, and macrophages. The lymphocyte population is made up of T cells, B cells, and Natural Killer cells. Th17 cells are recently discovered T helper cells that play important roles in the establishment and maximization of the capabilities of the immune system. Th17 cells are derived from CD4+ lymphocytes which are prevalent in human PBMC samples. In this study, we used six Milliplex® Cell Signaling panels and the Human Th17 Circulating panel to compose a biochemical profile of PBMC before, during, and after a differentiation process. We also examined the effect of Fetal Bovine Serum treatment on undifferentiated and differentiated PBMC. Our differentiation process consisted of a 7-day treatment of four cytokines, IL-1β, IL-6, IL-23, and TGFβ, as demonstrated in *Zheng et al*. Our results from cell signaling panels demonstrated that several analytes showed increased constitutive expression and phosphorylation after differentiation and stimulation with Fetal Bovine Serum. Additionally, the human Th17 panel showed that cytokine treatments induced significantly increased secretion of many cytokines including Th17 cell specific cytokines from human PBMCs (e.g. IL-17F). In conclusion, the Th17 and cell signaling panels will be useful tools for Th17 cell-related cytokine and signaling profiling in PBMC and various biological samples.

P2.12.40
TRPV1 channel in CD4+ T cells regulates their activation and their inflammatory properties

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TRPV1 is a calcium-permeable channel best studied in peripheral sensory neurons as a pain receptor. Here we demonstrate the functional expression of TRPV1 in CD4+ T cells and its physiological consequences. TRPV1 is activated by TCR ligation and acts as a non-store operated calcium channel. Moreover, TRPV1 is required for the proper transduction of the major TCR-mediated signaling events and is necessary for the subsequent T-cell cytokine production. Genetic and pharmacological inhibition of TRPV1 in human CD4+ T cells resulted in an impaired T cell activation profile similar to that observed in murine Trpv1-/- CD4+ T cells. In vivo, TRPV1 has an indispensible role in the acquisition of inflammatory properties by CD4+ T cells in two models of inflammatory bowel disease (IBD). These findings suggest that the inhibition of TRPV1 might be implemented to restrain pathogenic CD4+ T cell responses as it occurs in chronic inflammatory diseases such as IBD.

P3.01 Lymphoid organogenesis and lineage commitment

P3.01.01
The role of ADAM10 in B cell biology

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A disintegrin and metalloproteinase 10 (ADAM10) is a zinc dependent proteinase related to matrix metalloproteinases. ADAM10 has emerged as a key regulator of cellular processes by cleaving and shedding extracellular domains of multiple transmembrane receptors and ligands. In particular, ADAM10 has been identified as a key regulator of lymphocyte development. Here we report that ADAM10 is dispensable for early B cell development within the bone marrow. However, deletion of ADAM10 from all peripheral B cells or in post-switch cells leads to severe impairments in humoral responses. When ADAM10 was deleted from all peripheral B cells, a decrease in antigen specific IgG production was seen both with respect to serum levels and IgG ASCs, indicating that plasma cell (PC) differentiation is influenced. Cells producing high affinity antigen specific antibodies were particularly affected, consistent with defects in germinal center (GC) reactions. Moreover, changes in lymphoid architecture were also observed. Consistent with these findings, follicular dendritic cell (FDC)-reticula was undetectable following immunization. On the other hand, when ADAM10 was deleted in post-switch B cells, GC formation and lymphoid architecture were not impaired. Despite normal architecture, however, antibody production was still affected, likely due to abnormal gene expression in ADAM10-deficient PCs. Consistent with this hypothesis, PCs isolated from ADAM10Δ/ΔIgG1-cre+/- showed decreased expression of genes that facilitate plasma cell differentiation and function and increased expression of Bcl6, an inhibitor of PC differentiation. Our results thus demonstrate that B cell ADAM10 expression is necessary for optimal humoral responses.

P3.01.02
Roles of Polycomb group proteins in the maintenance of T cell fate

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Cell fate is determined by the combination of lineage-specific gene regulatory programs and epigenetic regulation. We have previously shown that the transcription factor Bcl11b is essential for T cell lineage commitment (Ikawa et al. Science, 2010). However, the mechanisms that maintain the T cell fate after the commitment remain elusive. Here, we show that the Polycomb group (PcG) proteins play a key role in this process. PcG proteins are epigenetic gene silencers that are implicated in the maintenance of embryonic and adult stem cells. We have generated the conditional mutant mice of Ring1A/B (LckCre-Ring1A-/-Ring1Bfl/fl) to study the role of Ring1A/B in T cell development. The number of total thymocytes of Ring1A/B cKO mice was 10-20 times smaller than that of control mice. Most of the cells were blocked at early CD4-CD8- (double negative: DN) stage. The T cell development was only partially rescued by crossing Cdkn2a-/- mice, suggesting that the existence of other target genes of PcG proteins in the T cell differentiation. Interestingly, ectopic expression of B cell lineage-associated genes (CD19, Ebf1, Blnk, and Irf4) were found in DN cells from Cdkn2a, Ring1A/B TKO mice. The ChIP analysis showed that the Ring1B and histon H3K27 trimethylation is highly enriched in the regulatory regions of B lineage-associated genes in normal thymocytes. We are currently examining the lineage promiscuity of these DN cells in TKO mice for the role of PcG proteins in the maintenance of T cell fate after the T cell lineage commitment.

P3.01.03
The repression of myeloid genes by Bach family restricts common lymphoid progenitors fate to B cell lineage

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Cell differentiation in multicellular organisms requires specification (the activation of a lineage-specific program) and commitment (the inhibition of alternative programs), in which the progressive gain and loss of lineage programs are orchestrated by the concerted action of transcription factors. B cell lineage specification and commitment are both dependent on the activity of early B cell factor-1 (EBF1). EBF1 directly promotes the expression of B cell specific genes, and establishes the transcriptional network for pro-B cell development. However, B-lineage commitment mechanism regulated by EBF1 is unclear. While the transcription factor Bach1 is ubiquitously expressed, Bach2 is specifically expressed in B-lineage cells. An analysis of compound Bach1/Bach2-deficiency (DD) suggests that these genes play critical function in the development of early B cells. We found that the expression of Bach2 preceded that of EBF1 in CLPs, and an overexpression of Bach2 in multipotent progenitors (MPPs) induced the differentiation of B220+ B cells and repressed the differentiation of CD11b myeloid in vitro. In DD mice, the numbers of CLPs and all stages of B cells significantly decreased. DD CLPs exhibited a lower frequency of B cell colony formation than WT cells, indicating a cell autonomous defect in the lineage choice. The expression of B cell specific genes decreased in DD CLPs, with concomitant manifestation of myeloid-association genes. We suggest that, in B cell development, specification is carried out by EBF1 and commitment is executed by Bach factors.

P3.01.04
FoxN1 is a Direct Transcriptional Regulator of Delta-like 4 in Thymic Stromal Cells

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Notch signaling is a vital cue provided by thymic stromal cells (TSCs) to direct lymphocyte progenitors to become T-cells. This signaling is critical to enforce the T-cell fate to the progenitors emigrating from the bone marrow. The Notch ligand, Delta-like 4 (Dll4), is expressed by TSCs and is essential for Notch triggering within the thymus. Despite its importance, the molecular regulators of Dll4 expression remain unknown. We showed that Dll4 mRNA is lost in thymic stromal monolayer cultures (TSMCs). Our recent work demonstrates that this loss is quite rapid with only 2-10% of initial Dll4 mRNA levels remaining after 4h of culture. FoxN1 is an important transcription factor for successful TSC differentiation, and a candidate regulator of Dll4 expression. Not only was FoxN1 lost with similar kinetics in TSMCs, when ectopically expressed in the stromal cells, we noted an increase in Dll4 mRNA levels. Furthermore, there are 5 putative FoxN1 binding sites in the proximal promoter of Dll4, and the 3' UTR of Dll4 mRNA contains canonical AU-Rich elements (ARE), suggesting that ARE-mediated transcript degradation might affect Dll4 mRNA maintenance. Our recent results showed that activated p38, a kinase regulating ARE containing mRNA stability, is absent in TSMCs. We are now examining the role of the ARE pathway in Dll4 mRNA maintenance and the identity of the potential p38-dependent signals that control its function. By understanding Dll4 regulation, we will gain insight not only into the biology of the thymus but how structure affects function at the molecular level.

P3.01.05
Features of colony-forming cells-fibroblast precursors circulating in biological fluids

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Blood, transudation and exudate of serous sacs from different laboratory animals were examined to reveal clonogenic cells-fibroblast precursors therein (CFC-F). Guinea pig blood, peritoneal, pleural and pericardial fluids of rabbits, guinea pigs and rats, as well as rat peritoneal exudates demonstrated clonogenic cells that form fibroblast-like cell colonies in primary monolayer cultures. For the most part these belong to the population of inducible osteogenic cells-precursors. Inducible cells-precursors being detected in biological fluids according to their properties that are the degree of dependence on age and strains of animals, on the culture conditions - density of explanted cells, the presence of feeder (irradiated cells of allogenic or syngeneic bone marrow), gaseous medium composition, were found by morphology and diameter of their formed colonies, morphohistochemical peculiarities of their composing elements to be identical to stromal cells-precursors of bone marrow. Highest degree of correlation (over 0,8) was detected between clonogenic cells of bone marrow and peritoneal transudation, peritoneal transudation and exudate from rats with respect to dependence of CFC-F numbers and ECO-F values on animal age and density of explanted cells. Analysis of cell immunophenotype in monolayer cultures of cellular elements from murine peritoneal transudation demonstrates CD80+ cell predominance (63,4%), expression of MHCI (83,3%), some CD34+ cells and macrophages (20,0 and 21,8%, respectively). Guinea pig blood being taken from different sites of cardiovascular system demonstrates that highest level of CFC-F number is observed in blood outflowing from hematopoietic organs. Greatest proliferative activity possess clonogenic precursors being revealed in venous portions of circulatory system.

P3.01.06
Protein kinase CK2beta is an essential regulator of peripheral B cell development

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Serine-threonine kinase CK2 is involved in oncogenesis of B-cell derived tumors chronic lymphocytic leukemia and multiple myeloma. To gain insights into its role in B-lymphocytes, we generated CK2β conditional knockout mice in B-cells. Non B-cell lineages were normal in CD19-CRE CK2βflox/flox mice. In the bone marrow, CD19-CRE CK2βflox/flox mice displayed a reduction of B-cells and the B220high IgMhigh recirculating population was found dramatically reduced. B-cell progenitors were apparently not affected by CK2β loss. On the contrary, B220+ CD19+ B-cells in peripheral blood, lymph-nodes, spleen and peritoneal cavity were markedly reduced. However, splenic IgDlow IgMhigh B-cell subset was reduced whereas we observed an increase of the IgDhigh IgMlow population, indicating an imbalance between the frequency of follicular (FO) and marginal zone (MZ) B-cells. Detailed FO and MZ B-cell populations analysis showed that FO B-cells were reduced by approximately 35-40% (from 72% to 45%), whereas MZ B-cells were increased up to three folds (from 8.5% to 23%). Histological analysis of CD19-CRE CK2βflox/flox spleens revealed a reduction of the size of follicles, absence of spontaneous germinal centers and an expansion of the interfollicular-marginal zone areas. In vitro class switch recombination assays demonstrated a moderate impairment in IgG1 and IgG3 class switch. In vitro cell cycle analysis experiments suggested an impairment in G1-S and S-G2 transition of CD19-CRE CK2βflox/flox B cells. Results of in vivo experiments testing T-cell dependent and T-cell independent responses will be described.
Our results place CK2β as a novel regulator of B-lymphocyte development and survival.

P3.01.07
Poly(ADP-ribose)polymerase-1 (PARP-1) negatively controls regulatory T cell differentiation

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Poly(ADP-ribose)polymerase-1 (PARP-1) plays a relevant role in inflammatory/immune responses by affecting leukocytes activation and differentiation. Our previous findings showed that PARP-1KO mice display increased numbers of regulatory $CD4^+CD25^+/Foxp3^+$ T cells in thymus, spleen and lymph nodes compared with WT controls. We wondered whether PARP-1 affects differentiation, function and stability of Treg cells. Purified PARP-1KO naïve CD4 cells stimulated in the presence of TGFβ1 expressed Foxp3 mRNA at higher level and generated a higher number of $Foxp3^+$ inducible Treg cells (iTregs) than WT control cells. This finding was not due to a higher resistance to apoptosis induction but rather to a higher conversion rate to $Foxp3^+$ iTreg cells. PARP-1KO iTreg cells showed features (anergy, CTLA-4 expression) similar to WT cells. We also analyzed naturally occurring Treg cells and found that lack of PARP-1 did not affect Treg cell phenotype (CTLA-4, GITR) and functions. Indeed PARP-1KO Treg cells inhibited proliferation and cytokine production in freshly isolated $CD4^+CD25^-$, and in Th1- and Th2-polarized cells as efficiently as WT Treg cells. PARP-1KO Treg cells also controlled the graft versus host response in a MHC-mismatched model. We also wondered whether lack of PARP-1 could affect the stability of the Treg cell phenotype. Preliminary results showed that in the presence of inflammatory stimuli a higher percentage of PARP-1KO Treg cells maintained Foxp3 expression compared with WT Treg cells. In conclusion, our findings demonstrate that PARP-1 affects Treg cell differentiation contributing to the regulation of Foxp3 mRNA expression.

P3.01.08

The critical role of NADPH oxidase on inducible bronchus-associated lymphoid tissue formation in newborn mice

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Inducible bronchus-associated lymphoid tissue (iBALT), an ectopic lymphoid tissue in respiratory epithelium, is associated with chronic lung diseases. Previous studies have shown that dendritic cells are crucial for iBALT formation. However, how leukocytes participate in the early iBALT formation remains unclear. Reactive oxygen species (ROS) produced by the activated oxidase (NOX2) of leukocytes have been known to be essential for immune defense, immunoregulation and leukocyte trafficking. In this study, we investigated the role of leukocyte-produced ROS on iBALT formation using a murine model. After repeated neonatal stimulation with lipopolysaccharide (LPS) through the airway, we found that there was well-organized iBALT formation, which was composed of T- and B-cell areas and high endothelial venules in young C57BL/6 mice. When comparing the iBALT induction of wild-type (WT) and neutrophil cytosolic factor 1 (p47phox component of NOX2) knockout ($Ncf1^{-/-}$) mice, we found that the number of well-organized iBALT formation decreased in $Ncf1^{-/-}$ mice. Although there were no significant differences in the number of $CD3^+$ T cell and $B220^+$ B cell in the lung, there were more iNOS-expressing cells within the iBALT increased nitrosylation in the lung tissue of WT mice. In addition, the airway hyperresponsiveness was higher in WT than that in $Ncf1^{-/-}$ mice after toluene diisocyanate stimulation. In conclusion, our study showed that leukocyte-produced ROS are critical for the formation of iBALT in LPS-stimulated newborn animals. The LPS-induced iBALT may exacerbate the airway hyperresponsiveness in chemical-induced asthma later in the development.

P3.01.09
Plasticity of antigen-specific Th17 and Regulatory T Cells in response to environmental cues

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Introduction. Th17 and regulatory T cells (Tregs) have been identified as independent T cell subsets bearing different effector functions. However accumulating evidence suggests that T cell phenotypes are more plastic than previously appreciated and that Treg convert to Th17 under appropriate conditions. In this study we analyzed the environment-induced reprogramming capacity of antigen-specific Th17 and Treg cells.
Materials and Methods. We adoptively transferred CD45.1 antigen-specific induced Tregs (iTreg) or Th17 cells generated in vitro into congenic (CD45.2) mice pre-treated in order to induce either inflammation or oral tolerance. Spleen, lymphoid organs and lamina propria were dissected at different time points and cells were analyzed to assess Foxp3 expression, IL-17 and IFN-γ production on the transferred cells.
Results. Our results indicate that under inflammatory conditions iTreg can be reprogrammed into Th17 or Th1 -like cells in vivo. On the other hand, adoptively transferred Th17 cells partially lost IL-17 production while upregulating Foxp3 expression after induction of oral tolerance, a result that is consistent with the conversion into Treg cells.
Discussion. Our data demonstrates a reciprocal plasticity in Treg and Th17 cell programs under condition of immune response and tolerance induction. A better understanding of the mechanisms controlling reprogramming in these cells should provide important tools for designing immunotherapeutic strategies to control autoimmunity and infections.

P3.02 Role of the stroma in innate and adaptive immunity

P3.02.01
Thymocyte selection regulates the homeostasis of IL-7-expressing thymic cortical epithelial cells in vivo

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Thymic epithelial cells (TECs) help orchestrate thymopoiesis and TEC differentiation relies on bidirectional interactions with thymocytes. While the molecular mediators that stimulate medullary TEC (mTEC) maturation are partially elucidated, the signals that regulate cortical TEC (cTEC) homeostasis remain elusive. Using IL-7 reporter mice, in which YFP expression identifies TECs that co-express high levels of the essential thymopoietin IL-7 (Il7YFP+TECs), we show that Il7YFP+TECs reside within the CD205+Ly51+CD40lo cTEC lineage that co-expresses Dll4, Ccl25, Ctsl, Psmb11, Prss16, Ccrl1 and segregates from CD80+CD40himTECs expressing Tnfrsf11a, Ctss and Aire. Analysis of Ccrl1/IL-7 double reporter mice corroborates that Il7YFP+TECs define a cTEC subset. We find that the frequency of Il7YFP+TECs gradually declines as mTEC development unfolds. Using thymic organotypic cultures, we report that the thymocyte-induced reduction in Il7YFP+TECs dissociates from the RANK-mediated differentiation of CD80+mTECs, suggesting that Il7YFP+TECs are not direct mTEC precursors. Il7YFP+TECs are sustained in Rag2-/- mice, even following in vivo anti-CD3ε treatment that mimics the process of pre-TCR β-selection of thymocytes to the double-positive (DP) stage. Using Marilyn-Rag2-/- TCR transgenic, we find that positive selection into CD4 lineage moderately reduces the frequency of Il7YFP+TECs, while negative selection provokes a striking loss of Il7YFP+TECs. These results infer that the strength of MHC/peptide-TCR interactions between TECs and thymocytes during selection constitutes a novel rheostat that controls the maintenance of the IL-7-expressing cTECs.

P3.02.02
Host-derived osteopontin drives the metastatic spread of breast cancer tuning the immunesuppressive microenvironment of the lung niche

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The matricellular protein osteopontin (OPN) has been involved in malignant transformation, tumor progression and metastasis. Nevertheless, while the pro-tumorigenic and pro-metastatic activity of tumor-derived OPN has been widely reported, the role of host-derived OPN has not been determined yet.
To solve such issue, we have injected into OPN-deficient mice 4T1 mammary tumor cells, spontaneously metastatizing to the lungs when injected into the mammary fat-pad, and found a significant reduction in lung metastases, despite similar primary tumor size. The growth of 4T1, as for the vast majority of tumors, is associated with the expansion of a heterogeneous immature myeloid population that contributes to its progression and metastatization. These cells, collectively referred as myeloid derived suppressor cells (MDSC), comprise a granulocytic (Gr-1high) and a monocytic (Gr-1int/low) subset, endowed with a higher immune suppressive capacity. Analysis of such population showed that the inhibition of lung metastases in OPN-deficient mice goes along with a skewing of MDSC polarization toward the Gr1high subset, whereas the Gr1int/low subset is more expanded in wild-type mice. Moreover, the latter subset, sorted from lungs of tumor-bearing OPN-deficient animals, is less suppressive in vitro toward CD8 T cells than its wild-type counterpart, and associated with a lower expression of arginase-1 and phospho-STAT3. Furthermore, we have also found in the lungs of OPN-deficient tumor bearing mice a reduction in another suppressive population, T regulatory FoxP3+ cells. All together, these data suggest that OPN is a regulator of the local immune response and a gatekeeper for metastatic colonization of the lung niche.

P3.02.03

In vivo evidence on mesenchymal stromal cells modulates the lymphomyeloid lineage commitment

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The development of hematopoietic stem/ progenitor cells (HSC/HPC) to immune lineage cells is regulated within the marrow microenvironment. Mesenchymal stromal cells (MSCs) is known to exhibit two key features that MSCs enhance the graft engraftment and hematopoietic reconstitution, but inhibit the host T cell activation to alloantigen challenged after allogeneic hematopoietic stem cell (HSC) transplantation. However, little is known about how MSCs affect immune cell lineage commitment. Here, we demonstrate that in vivo MSCs treatment could effect on both HSC differentiation and their microenvironment. MSCs obtained from bone marrow, amniotic fluid, and placenta were used in this study. In vitro, all types of MSCs expand HSCs, enhance multiple hematopoietic lineage development, and inhibit CD3 positive T cell functional activities. In a murine model of sub-lethal total body irradiation (TBI) showed that all MSCs reversed TBI-induced apoptotic program and decreased the loss of functional HPCs. In vivo MSCs increased LSK cell number and functional activity without inhibiting their differentiation. Detailed immunophenotyping demonstrated a MSCs-dependent increase in short-term HSCs/multipotent progenitors, as well as, MSCs elevated bone marrow HSCs to differentiate into myeloid progenitors (GMP) and pre-B lymphoid progenitors in the marrow. However, all types of MScs have significantly increased the differentiate outcome to B cells and NK cell lineage populations but inhibit the T cell lineage development and its functional activities in thymus, spleen, and blood. These data strongly suggested that the actions of MSCs on front-end and terminal of immune cells development may mediated by the different regulatory mechanisms.

P3.02.04
Thymic Nurse Cells from diabetic mice present phenotypic and functional alterations and express leptin receptor

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Thymic nurse cells (TNCs) represent a particular model for thymic epithelial cells and thymocyte interactions since they are large epithelial cell that completely envelops about 2-
200 viable thymocytes, mostly CD4+CD8+ thymocytes. Since thymus is under an intense endocrine control, it is not surprising that leptin hormone plays an important role in maintaining the thymus structure and functionality. Previously, we have observed, during the hypoinsulinemia condition, that intrathymic leptin levels were decreased. Severe thymic alterations in both lymphoid and epithelial compartments were also seen. Here, we evaluated phenotypical and morphological alterations in TNCs from experimentally induced-diabetic mice. Phenotypic TNCs analysis, performed by optical and scanning electron microscopy, and by flow cytometry technique, revealed decreased numbers of TNCs isolated from diabetic mice and an increased proportion of small-sized TNCs, which contained few thymocytes. The observation of primary cultures of TNCs has also revealed an alteration in the pattern of thymocyte released by these cells and an increase of annexin positive TNCs isolated from diabetic mice. In addition, by immunohistochemistry, our results demonstrated that TNCs express leptin receptors and that leptin is produced by thymocytes released from the TNC interaction in control and diabetic mice. We conclude that TNCs from diabetic mice present morphological and functional alterations that may compromise both the development and the selection of the repertoire of T cells. Also, to our knowledge, this is the first time that the expression of leptin-receptor in TNCs is reported. This work was supported by FAPESP grants (#2010/19558-3 & #2010/06831-3).

P3.02.05
CD49b regulates the establishment and maintenance of T helper cell memory

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We have demonstrated that professional memory CD4 T cells reside and rest in the bone marrow (BM) adhering to IL-7-expressing stromal niches. Their absence induces a defective generation of high-affinity long-lived plasma cells. However, the molecular mechanisms on their establishment and maintenance remain unclear. We here demonstrate the roles of CD49b, which is also called integrin alpha2 and is expressed on most memory CD4 T cells, in the establishment and maintenance of memory CD4 T cells in the BM. We first counted antigen-specific effector and memory CD4 T cells in immunized CD49b-deficient and wild-type mice. The number of CD49b-deficient memory CD4 T cells was significantly impaired compared to wild-type cells, while the number of effector cells in both mice was comparable. So, to clarify why CD49b-deficient memory CD4 T cells are not generated, we transferred CD49b-deficient effector CD4 T cells into naive mice and evaluated their migration ability into the BM. The loss of CD49b induced a defective migration of effector cells into the BM. Then, to examine a role of CD49b in the maintenance of memory CD4 T cells, we inhibited the integrin during memory phase with injection of Fab fragments of anti-CD49b neutralization antibodies. The inhibition of CD49b reduced the number of memory CD4 T cells in the BM. We suggest that CD49b regulates the establishment and maintenance of memory CD4 T cells controlling the immigration of memory cell precursors into the BM and also the persistence of memory CD4 T cells on their survival niches.

P3.02.06
Effects on vaccine memory after Doxorubicin treatment of healthy Rhesus Macaques

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It has previously been shown that children treated with chemotherapy for leukemia loose immunity they acquired from the childhood vaccination program. In this study, we aim to map the detailed events of the B-cell memory upon immunosuppressive treatment.
Healthy Rhesus Macaques, previously vaccinated against measles, were treated with 3 doses of either high-dose Doxorubicin (n=5) or NaCl solution (n=5), and the cellular and serological effects were evaluated. Interestingly, the peripheral lymphocyte and monocyte levels decreased only transiently after each dose of Doxorubicin, whereas the B-cell counts failed to recover in the treatment-free periods between the doses – in two animals also after cessation of treatment. This occurred in spite of high serum levels of the B-cell homeostasis protein BAFF, showing a pulsatile increase after every dose. The antibody titers remained stable throughout the treatment period, and 5 months after administration of the last dose. Probably, a direct effect on titers is seen only after more repeated dosing for a longer period. Ongoing research is now directed to quantify antigen-specific bone marrow plasma cells and memory B-cells from lymphoid organs, in order to further understand how chemotherapy interferes with vaccine memory. This may lead to a better understanding of how it can be protected during immunosuppressive treatment.

P3.02.07

Expression of IL-4 Receptor alpha on smooth muscle cells is not necessary for development of experimental allergic asthma

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Airflow in the lungs of patients with allergic asthma is impaired by excessive mucus production and airway smooth muscle contractions. Elevated levels of the cytokines IL-4 and IL-13 are associated with this pathology. In vitro studies have suggested that IL-4 receptor alpha signalling on smooth muscle cells is critical for airway inflammation and airway hyperresponsiveness.
In order to define the contribution of IL-4 and IL-13 to the onset of asthmatic pathology the role of their key receptor IL-4Ralpha in smooth muscle cells was examined in vivo.
By using transgenic SMC-MHCcreIL-4Ralpha-/lox mice deficient for IL-4Ralpha in smooth muscle cells, effects of impaired IL-4Ralpha signalling in these cells on the outcome of asthmatic disease were investigated. Allergic asthma was introduced in mice by repeated sensitisation with ovalbumin/alum followed by intranasal allergen challenge. Mice were investigated for the presence of airway hyperresponsiveness, airway inflammation, allergen specific antibody production, Th2 type cytokine responses and lung pathology.
Airway hyperresponsiveness, airway inflammation, mucus production, Th2 cytokine production and specific antibody responses were unaffected in SMC-MHCcreIL-4Ralpha-/lox mice when compared to control animals.
The impairment of IL-4Ralpha on smooth muscle cells had no effect on major aetiological markers of allergic asthma. These findings suggest that IL-4/IL-13 responsiveness in airway smooth muscle cells during the early phase of allergic asthma is not, as suggested, necessary for the outcome of the disease. Therefore, therapies targeting the IL-4Ralpha might have no direct effect on smooth muscle cells in an allergic asthma response. (J Allergy Clin Immunol 2010;126:347-54.)

P3.02.08

Skin-resident fibroblasts create unique niches for regulating ATP-mediated mast cell activation

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Mast cells (MCs) play critical roles in both protective immunity against pathogenic microorganisms and the development of inflammatory responses. We previously reported that colonic MCs highly expressed one of the receptors for extracellular adenosine triphosphate (ATP), called P2X7, which was involved in the ATP-mediated excessive inflammatory symptom. Therefore, the suppression of P2X7-mediated MC-activation led to the inhibition of colitis. Here, we found that expression levels of P2X7 on MCs were different among tissues. Especially, the P2X7 expressions on skin MCs were significantly lower than that of MCs in other tissues. Along with this finding, only in the MCs from skin compartment, ATP-induced cytokine and chemokine productions were not observed. P2X7 expression was decreased when bone-marrow-derived MCs (BMMC) were adoptively transferred into the skin of MC-deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice, indicating that extrinsic inhibitory mechanisms exist specifically in the skin region. In agreement with this finding, co-culture of BMMCs with skin fibroblasts but not with stromal cells from other tissue resulted in the reduction of P2X7
expression in BMMCs. In addition, co-culture system using transwell plate indicated that cell-cell contact was not required for the inhibition of P2X7. Consequently, ATP-mediated cytokines and chemokine production were inhibited in skin co-cultured MCs. Our novel findings demonstrate skin-resident fibroblasts create unique niches for regulating homeostasis network by inhibition of P2X7 expression and function in MCs.

P3.02.09

PI3K p110δ controls stromal cell distribution and is expressed in gp38+ stromal cells in secondary lymphoid organs

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Stromal cells create a network that physically supports lymphocyte displacement and anchorage of antigen-presenting cells and secrete chemokines involved in lymphocyte homing. Lack of PI3K p110δ subunit or catalytic activity in mice provokes structural anomalies in secondary lymphoid organs (SLO), which suggests a role for p110δ in their formation and maintenance. We tested whether lack of p110δ activity affects the immune response, not only through its role in lymphoid cells but also in stromal cells. We used p110δD910A/D910A mice, which express a catalytically inactive p110δ form, for bone marrow reconstitution assays. In lethally irradiated p110δWT/WT and p110δD910A/D910A mice reconstituted with total bone marrow from p110δWT/WT or p110δD910A/D910A donors, we studied total lymphoid cell number and the distribution of lymphoid cell subsets in homeostatic conditions and after antigen stimulation. We also analyzed the distribution and texture of SLO stromal cell populations in p110δWT/WT and p110δD910A/D910A mice and tested whether p110δ is expressed in those cells. Spleen structure in p110δWT/WT mice (organized) was similar to that of p110δWT/WT recipients reconstituted with p110δWT/WT or p110δD910A/D910A bone marrow, whereas that of p110δD910A/D910A mice (disorganized) was similar to that of p110δD910A/D910A mice reconstituted with p110δWT/WT or p110δD910A/D910A bone marrow. These findings suggest a role for p110δ in SLO stroma. p110δD910A/D910A SLO showed severe defects in T cell zone stromal cell distribution and impaired secretion of chemokines involved in T cell homing to SLO. Our data identify p110δ expression in gp38+ SLO stromal cell subsets and highlight the importance of these cells in immune response generation.

P3.02.10

Prostate cancer-associated stroma induces immunosuppressive monocytes

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The presence of monocytes within the tumour microenvironment of prostate cancer (PCa) patients is associated with poor prognosis. Increased numbers of $CD8^+$ T lymphocytes, however, correlate with a more favourable outcome. Although the immune modulatory effects of tumour cells have been studied widely, the role of PCa-associated stroma on the infiltration and the subsequent function of immune cells are largely unknown and are the subjects of this study. Isolating, culturing and characterising PCa-associated primary stromal cells from patients undergoing radical retropubic prostatectomy provided us with a unique and relevant cell type to carry out this study. We demonstrate that stromal cells produce monocyte chemotactic protein-1 (MCP-1) which triggers the migration of undifferentiated monocytes towards PCa-associated stroma. In the presence of tumour stroma, differentiation of monocytes into dendritic cells (DC) is uncharacteristic, consisting of high CD14 expression and elevated levels of immunosuppressive markers, such as programmed cell death ligand-1 (PD-L1) and CD200R, both able to induce T cell dysfunction. Raised levels of the immunosuppressive cytokine IL-10 are also present in monocytes cultured in the presence of PCa-associated stromal cells whilst levels of the stimulatory cytokine IL-12 are dramatically reduced. These monocytes are efficient phagocytes that are able to engulf antigens but their antigen-processing

mechanism is impaired as they are inefficient in cross-presenting these antigens to cytotoxic T cells. Our data provides strong evidence that PCa-associated stroma plays an important role in negatively regulating cellular immune responses in the tumour microenvironment, indicating the need for simultaneous targeting of tumour and stroma alike.

P3.02.11
Analysis of tumor microenvironment and immune responses against cancer stem cells and application to cancer immunotherapy

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Cancer stem cells (CSCs), a small population of cancer cells with stem cell-like phenotype and tumor-initiating capacity, are important targets for cancer therapy because they are considered to be responsible for recurrence after chemotherapy. In order to clarify immunopathological properties of CSCs, CSCs with high capacity of tumor initiation were collected from various cancer cell lines by sorting a side population or ALDH1-positive cells. By comparative gene expression analysis of CSCs derived from human solid cancer cell lines, several CSC genes were identified, including SOX2, SMCP, DNAJB8 and OR7C1. These genes were expressed preferentially in CSCs, but barely detected in normal adult tissues except for testis. RNAi-mediated knockdown of the genes dramatically decreased the tumorigenic capacity, indicating that the genes could be associated with tumor-initiating capacity of CSCs. We could induce CSC-specific cytotoxic T-cells (CTLs) from peripheral blood lymphocytes of cancer patients after in vitro stimulation with CSC antigenic peptides. CSCs were susceptible to cytotoxicity of CTLs, while they were resistant to chemotherapeutic drugs. In addition, adoptive transfer of CSC-specific CTLs showed higher tumor suppressive capacity in vivo in a mouse transplantation model. On the other hand, some CSCs modulated tumor microenvironment through the interaction with stromal fibroblasts. Our findings highlighted novel CSC-specific antigens and important roles of stromal fibroblasts for the antigenicity and immune escape of CSCs.

P3.02.12
Aggregates of B cells and platelet MPs circulate in the human peripheral blood

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Objective: Activated platelets shed microparticles (MPs) in vivo and definitely in vitro upon aging under storage. Like platelets, platelet MPs contribute to hemostatic and inflammatory responses. We sought to determine the interactions between platelet MPs and peripheral B lymphocytes in the healthy blood circulation to propose a possible role for platelet MPs in the regulation of B cell function.
Materials and Methods: Isolated B cells were studied for binding to platelet MPs using flow cytometry technique. Additionally, ELISA method was also used to demonstrate binding of MPs to B cells in the human blood. Moreover, new binding capabilities of B cells for MPs were studied by ELISA.
Results: B cells showed heterogenic populations in flow cytometry due to the formation of different sizes of aggregates between B cells and MPs. Using the ELISA method we also demonstrated the attachment of a percent of B lymphocytes to platelet MPs in the human blood. Moreover, addition of MPs in vitro increased ELISA absorbance.
Conclusion: Binding of platelet MPs to B cells apprears to take normally place in vivo and a percent of B cells circulate in the blood attached with platelet MPs.

P3.03 Thymic selection and T cell development

P3.03.01
Role of sonic hedgehog in T cell development

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The Hedgehog (Hh) family of secreted intracellular signalling molecules plays a major role in many patterning processes and organogenesis during embryonic development and are involved in the homeostasis and renewal of adult tissues. The Hh family member, Sonic Hedgehog (Shh) has been previously found to influence T-cell development and transition at the double negative stage (DN). In this project we have utilized T-cell receptor transgenic mice models (TCR-transgenics) to investigate the role of Shh in T-cell development and repertoire selection. Our data from thymic organ cultures have shown that Shh influence T-cells selection and maturation during the later stages of T-cell development in their transition from double positive (DP) to single positive (SP) cell population.

P3.03.02
Analysing the role of E2F7 and E2F8 in peripheral T cells and during T cell development

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Thymocytes undergo several important steps during their development towards mature T cells. Because of the random nature of the TCR rearrangement the positive selection checkpoint is necessary to ensure that the double positive (DP) cells can interact with the MHC molecule. Negative selection, which is important to eliminate self reactive thymocytes, is controlled by the avidity of the interaction between the TCR and the self antigen. We are analysing transcription factors expressed at the DP stage which may be involved in these processes. We identified E2F7 and E2F8 as potential candidates of our approach which belong to the E2F family that is known to be important for differentiation, apoptosis and function as transcriptional repressors important for the control of cell proliferation. Because the double deficiency is embryonic lethal we have crossed E2F7 fl/fl E2F8 fl/fl mice with Lck-cre and CD4-cre mice and analysed the phenotype of thymocytes and T cells. Astonishingly, we could show that the population sizes of CD4+ and CD8+ T cells, naïve T-cells, T regs and TCRγδ T cells are not altered compared to control mice. Furthermore, we investigated B cells, macrophages, dendritic cells, NK and NK T cells, again without finding any differences in conditional double knockouts compared to controls.

P3.03.03
CCL2 chemokine in central tolerance

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The goal of the project is to study the role of CCL2 on dendritic cell (DC) recruitment and T cell tolerance in thymus.
CCL2 is a chemokine involved in the recruitment of immune cells during inflammation. DC can take up antigens in periphery and migrate into the thymus to mediate tolerance. Three DC subsets have been identified in mouse thymus and they all express CCR2, the main receptor for CCL2.
We separated thymocytes from stromal cells (mainly thymic epithelial cells (TEC) and DC) by percoll gradient centrifugation. We demonstrated by quantitative PCR that CCL2 was induced in stromal cells, mainly TEC (CD45-negative stromal cells), by interaction with autoreactive thymocytes. Analysis of thymic DC subsets in CCL2-deficient mice showed a decrease of the Sirpα+-subset.
To study the role of CCL2 in T cell tolerance, we took advantage of mice that overexpress CCL2 in thymus. Overexpression of CCL2 in

thymus of myelin oligodendrocyte glycoprotein (MOG)-specific TCR transgenic mice (2D2 mice) induced loss of CD4 from MOG-specific T cells in thymus and lymph node (LN). LN T cells from mice overexpressing CCL2 in thymus responded poorly to MOG and these mice were resistant to experimental autoimmune encephalomyelitis.
We conclude that CCL2 induced by interaction between autoreactive thymocytes and stromal cells is involved in regulation of T cell tolerance and we propose that DC recruitment plays a role.

P3.03.04
Does citrullination occur in medullary thymic epithelial cells?

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Introduction:
The presence of antibodies against citrullinated peptide antigens (ACPA) is a hallmark of rheumatoid arthritis. However, it is unclear how these autoantibodies develop. It has been proposed, that peripheral citrullination generates neoepitopes that are favored targets of autoreactive immune cells evading central tolerance induction. We investigated the expression of peptidyl arginine deiminases (PAD) in medullary thymic epithelial cells (mTEC).
Methods:
Thymic epithelial cells were isolated from 4-5 weeks old mice susceptible (DBA/1) or not susceptible (NMRI, C57BL/6) for collagen-induced arthritis (CIA). After TEC-enrichment by percoll density gradient centrifugation and magnetic depletion of CD45+ cells, immature mTEC (CD45-EpCAM+CD80lowLy-51-) and mature mTEC (CD45-EpCAM+CD80highLy-51-) were FACS-sorted. Cortical TEC were identified as CD45-EpCAM+CD80-Ly-51+. mRNA and protein were isolated from sorted mTEC populations for subsequent expression analysis.
Results:
Flow cytometry analysis revealed a significantly decreased number of mTEC in thymi of CIA-susceptible DBA/1 mice in comparison to NMRI and C57BL/6 mice, whereas the number of T cells and cTEC did not show any differences.
Subsequent quantitative PCR confirmed cell identity since mature mTEC showed significantly elevated expression of aire and the tissue restricted gene insulin2 compared to immature ones. Pad expression was higher in mature mTEC compared to immature mTEC and correlated well with the expression of aire. Furthermore, the expression of pad6 was significantly elevated in DBA/1 mature mTEC.
Conclusion:
Pad genes are readily expressed at RNA level in murine mTEC. However, it remains to be determined whether PAD protein and citrullination can also be found in mTEC. This is work in progress.

P3.03.05
Significant involvement of NF-κB-inducing Kinase in shaping mature T cell repertoire

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NF-κB-inducing kinase (NIK) is known to play a critical role for maintaining proper immune functions. This is exemplified in the spontaneous mutant mouse *alymphoplasia* (*aly*), which lacks functional NIK and has been demonstrated to be immune-compromised and also to be autoimmune-prone. In order to investigate the role of NIK in αβ T cell repertoire formation, we analyzed T cell development in *aly*/*aly* mice bearing a transgenic αβ TCR. The results showed that the maturation efficiency of idiotype^{high+} T cells in the TCR-Tg mouse was found to be lower in *aly*/*aly* mice as compared to that in *aly*/+ mice, implying that mature αβ T cell repertoire could be altered by the systemic lack of functional NIK. It was also noted that in one strain of negatively selecting background, the peripheral number of CD8^{low} idiotype^{high+} cells, which are thought to potentially represent γδ lineage of T cells, were markedly decreased in *aly*/*aly* mice. When the γδ T cells in *aly*/*aly* mice without TCR transgene were investigated, the proportion of γδ T cells in the periphery of *aly*/*aly* mice was found to be 1/2 to 1/5 of those in *aly*/+ mice. Analyses of bone marrow chimera mice suggested that NIK in host cells, rather than in donor cells, is important for maintaining normal number of peripheral γδ T cells. These results suggest that NIK may be involved in thymic positive selection of some αβ T cells and that NIK in non-hematopoietic cells is important for optimal development and/or maintenance of γδ T cells.

P3.03.06
Homeostatic properties and phenotypic maturation of murine CD4$^+$ pre-thymic emigrants in the thymus

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After a tightly regulated developmental program in the thymus, “mature” single positive (SP) thymocytes leave the thymus and enter the periphery. These newly arrived recent thymic emigrants (RTEs) are phenotypically and functionally immature, and will complete a dynamic maturation in the peripheral lymphoid organs before being licensed to be resident naïve T cells. To study the early events occurring in the RTE maturation process, we identified the phenotype of CD4$^+$ pre-RTEs, a population of CD4$^+$ SP thymocytes that have acquired the thymus egress capability. Compared to peripheral naïve T cells, CD4$^+$ pre-RTEs displayed superior survival capability in lymphoreplete mice and faster proliferation under lymphopenic condition. The differences in Bcl2/Bim expression and/or heightened IL-7 signaling pathway may account for the pre-RTEs’ better responsiveness to homeostatic signals. Qa2, the expression of which indicates the phenotypic maturation of SPs and RTEs, was found to be upregulated in CD4$^+$ pre-RTEs in thymic perivascular space. Migratory dendritic cells surround this region contribute to Qa2 expression in pre-RTEs. The dendritic cell-driven Qa2 induction of CD4$^+$ pre-RTEs is independent of MHC class II and Aire molecules.

P3.03.07
Transcriptional modification induced in a human epithelial cell line by transfected AIRE

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AIRE gene expression promotes the transcription of TRAs (tissue-restricted antigens) in thymus medullary epithelial cells. TRA expression is directly connected to the negative selection of T cells recognising peripheral antigens in thymus, presumably inducing the generation of peptides from TRAs to be presented by MHC on mTECs. The exact mechanism of AIRE action and the generation of TRA-derived peptides is unclear. To study TRA transcription, synthesis and processing we generated an AIRE+ cell by transfecting the human thyroid epithelial cell line HT93 with the AIRE gene. We performed bioinformatics analyses of microarray data on mRNA transcripts from transfected vs. untransfected HT93, focusing on the gene expression of significant transcripts and the genomic localization of AIRE-induced transcripts. The transcriptional analyses revealed that in response to AIRE, cells significantly triggered the regulation of 776 transcripts ($p<0.01$, t-test), including differentially up-regulated (283) and down-regulated (494) transcripts ($p<0.01$, t-test in whole transcriptomic analysis). Within up-regulated genes, we found 10 genes codifying potential TRAs. Other genes related to apoptosis, cell cycle and cytoskeleton were significantly upregulated. The analysis of the whole transcriptome by localisation of AIRE-regulated clusters showed that AIRE controls the up-regulation of several transcripts linked to canonical cellular process, . The identified transcripts suggest that AIRE regulates changes likely to strengthen the cell remodeling of the transcriptional program in the epithelial cell line and show the up-regulation of specific gene cassettes in all autosomal chromosomes. This pattern suggests that AIRE promotes a wide action on the chromatin transcriptional activation. *co-senior authors

P3.03.08
SOCS1 deficiency results in fatal inflammatory disease in the absence of thymic T cells

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The suppressor of cytokine signaling (SOCS) family controls cytokine signaling and regulates the development of many inflammatory diseases. SOCS1 inhibits signaling of various cytokines, including IL-2, IL-7, IL-15 and IFN-γ. Previous studies revealed that SOCS1 knockout (KO) mice die within three weeks of age with a complex inflammatory disease, which is prevented by generating IFN-γ deficient or lymphocyte deficient SOCS1 KO mice. In addition, SOCS1 deficiency causes abnormal T cell activation and differentiation into IFN-γ-producing cells, suggesting that activated T cells play a key role in the disease.
To further investigate the T cell role in the disease, we crossed SOCS1 KO mice with nude mice to generate thymus-deficient SOCS1 KO mice (nude SOCS1 KO). Unexpectedly, nude SOCS1 KO mice showed growth retardation and early death similarly to SOCS1 KO mice. They also demonstrated IFN-γ signature like SOCS1 KO mice, as characterized by MHC class I up-regulation in bone marrow myeloid cells and STAT1 phosphorylation in liver. Interestingly, despite lacking thymus, T cells were expanded in nude SOCS1 KO mice and most of them were either CD4 or CD8 positive αβT cells. Furthermore, T helper cells differentiated toward Th1 rather than Th17 and Treg development was defective in these mice. These results suggest that T cells are the key cellular mediators of the disease due to SOCS1 deficiency even under athymic condition. Regulation of cytokine signaling by SOCS1 might be involved in thymus-independent T cell development and homeostasis.

P3.03.09
Regulation of CD8 T cell function by the small GTPase Arl4d

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The liver is known to induce tolerance rather than immunity. Liver sinusoidal endothelial cells (LSEC) can induce a non-responsive state in naïve CD8 T cells after antigen-specific stimulation. Members of the ADP-ribosylation factor (ARF) family of guanine-nucleotide-binding (G) proteins, including the ARF-like (ARL) proteins, regulate membrane traffic and organelle structure by recruiting cargo-sorting coat proteins, modulating membrane lipid composition and interacting with regulators of other G proteins. Arl4D is localized to the plasma membrane and can recruit cytohesin-2/ARNO and modulate actin remodeling via regulating ARF6 activity.
We found Arl4d mRNA to be highly induced during CD8 T cell stimulation by LSEC. Using CD8 T cells from Alr4d-/- mice we found the lack of Alr4d to result in the overproduction of IL-2. As the amount of IL-2 produced by naïve CD8 T cells during antigen-specific interaction with LSEC is an important factor for the induction of non-responsive CD8 T cells, the action of Arl4d may be central to dampen IL-2 production and for subsequent abrogation of immediate effector function in naïve CD8 T cells by LSEC.

P3.03.10
Signal integration in thymocytes is crucial for thymic selection

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Thymocytes are generated, selected and mature in the thymus to create a diverse, functional and self-tolerant T cell repertoire. Selection of thymocytes relies on the level of their T cell receptor (TCR) self-reactivity. Self-reactivity of thymocytes is quantified by repeated engagement of their TCRs with a diverse repertoire of self-peptides complexed with self-MHC molecule (spMHC) and may originate from either part of the complex. The role and relative importance of MHC versus self-peptides in the thymic selection is not resolved. The fact that the selected T cell repertoire is MHC-restricted, requires a good mechanistic explanation of how thymocytes are able to quantify their affinity to MHC. In order to analyze how thymocytes quantify their self-reactivity, extract their MHC affinity, and how they use this information for cell fate determination, we developed a signal integration model for thymic selection, mimicking the requirement of repeated TCR signaling for successful selection and cell maturation. In this model, a polyclonal repertoire of thymocytes sequentially interacts with diverse spMHCs and integrate the affinity-dependent TCR ligation signals. The strength and dynamics of the integrated signal is taken as a reference for cell fate determination. Quantification of the integrated signal and discrimination between its sustained and transient dynamics turns out as a successful basis for extracting affinity of thymocytes to MHC, detecting the existence of cognate self-peptide, and selecting a MHC-restricted self-tolerant T cell repertoire. Regulatory T-cell differentiation versus clonal deletion of autoreactive thymocytes is discussed with this model.

P3.03.11
Vascular Endothelial Growth Factor and Semaphorin 3A influence murine thymocyte migration in vitro

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Angiogenic Vascular Endothelial Growth Factor (VEGF) and axon guidance molecule Semaphorin 3A (Sema 3A) are known to be produced by thymic epithelial cells (Corbel C. et al., 2007; Lepelletier Y. et al., 2007) but their function in T-lymphocyte development is not known. Both factors have common receptor - Neuropilin 1 which is abundantly expressed on the surface of murine thymocytes. The aim of the study was to evaluate the role of VEGF and Sema 3A in murine thymocyte migration in vitro. With the help of transwell systems we found that Sema 3A at a concentration of 100 ng/ml possessed chemorepellent activity towards thymocytes while VEGF at the range of 1-100 ng/ml showed no effect. Incubation of thymocytes in the presence of anti-Neuropilin 1 antibodies abolished effect of Sema 3A. We further evaluated thymocyte adhesion to the monolayer of endothelial EA.hy 926 cells, preincubated with Sema 3A or VEGF during 4 hours. VEGF at a concentration of 50 ng/ml increased thymocyte adhesion 1,5 times while Sema 3A had no effect. As a positive control we used 10 ng/ml of TNF-α. Transendothelial thymocyte migration through the monolayer of endothelial cells preincubated with 50-100 ng/ml VEGF during 4 hours was increased 1,9 times as compared to the control cultures while preincubation with Sema 3A did not show any influence. These data suggest that both factors may act on transendothelial migration of thymocytes and their egress to periphery: Sema 3A - directly on thymocytes as chemorepellent while VEGF can modify endothelial cell.

P3.03.12
Shaping of epitope-specific CTL precursor populations by thymic selection

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Cytotoxic CD8+ T cells (CTL) play a significant role in the control and removal of virally infected cells and tumours by recognizing specific viral peptide fragments in complex with class I Major Histocompatibility Complexes (MHCI). Using a tetramer-based magnetic enrichment strategy to identify the extremely small number of epitope-specific CTL precursors (CTLps) from naïve mice, work from our group and others has demonstrated the importance of the naïve CTLp pool in determining CTL immune magnitudes after antigen challenge. The frequencies of naïve epitope-specific CTLps are highly reproducible between MHC-matched individuals for both mice and humans, suggesting that thymic selection plays a key role in their establishment. While the broad consequences of thymic selection are well established (i.e. self-MHC restriction and self-tolerance), the impact of selection events on the frequency and TCR usage within epitope-specific CTLp populations is unknown. Here, we investigate how the nature of the positively selecting MHCI ligand impacts on the emergent population of mature CD8+ T cells and their ability to respond to cognate pMHCI in the periphery. Furthermore, we address the extent to which naive CTLp populations are edited by negative selection, using mice lacking a key regulator of negative selection, the BH3-only protein, Bim. This work will address the fundamental question of whether the pre-immune repertoire is principally defined by TCR gene recombination or thymic selection events. Thus, it may have implications for manipulating CTL responses by defining how the characteristics of the preimmune CTL repertoire, and consequently antigen-driven CTL responses, are determined.

P3.03.13
CD8aa TCRab intraepithelial lymphocytes MHC restriction

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T lymphocytes develop in the thymus into two major lineages characterized by expression of ab and gd TCR. Each lineage can be further subdivided into distinct subsets that differ in TCR specificity, phenotype and function. CD8aa TCRab intraepithelial lymphocytes (T-IEL) located in the epithelium of the small intestine is phenotypically different from conventional T cells. CD8aa T-IEL have been studied for years however the question of their MHC restriction has not been elucidated. Therefore, we decided to clone TCR isolated from naturally arising CD8aa T-IEL and retrovirally express these TCRs in BM chimera. First, we cloned four TCRab that were expressed retrovirally in RagKO BM chimera. Analysis of the chimera showed that all TCR clones gave rise to T cells. The T cell that developed were CD4-CD8b- but CD8a+ and phenotypically identical to CD8aa T-IEL isolated from wild type mice. In addition, they were preferentially found in the gut. In order to define the MHC restriction of these particular TCRs, BM chimeras in various MHC-deficient backgrounds were generated. Subsequent analysis of the chimera for the presence of CD8aaTCRab IEL demonstrated that they are dependent either on Kb, Db MHC I, Qa2 or b2m-dependent MHC I aside from KbDb. In conclusion, our results indicate that CD8aa T-IEL develops from precursors expressing particular TCRs that allow their engagement in this unique T cell lineage. In addition, CD8aa T-IEL can harbor clones selected on different MHC molecules.

P3.03.14
The role of WW domain-containing oxidoreductase in lymphocyte development

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WW domain-containing oxidoreductase (WOX1) is a newly isolated tumor suppressor protein. Downregulation of WOX1 protein expression has been found in many types of human cancers. Previous studies have suggested that WOX1 is involved in many signaling pathways that regulate cell apoptosis. Ectopic WOX1 increases tumor necrosis factor, staurosporine, UV or p53-induced cell death. Conversely, functional suppression of WOX1 by dominant negative mutants or siRNA protects cells from apoptosis. Although many studies have revealed a role of WOX1 in regulating cancer cell apoptosis, whether WOX1 is involved in lymphocyte development is unknown. To explore the functional role of WOX1 in vivo, we have recently generated whole-body knockout murine models by targeting exon 1 or exons 2-4 of the gene. All WOX1-deficient mice died by three weeks of age. Reduced body weights and marked atrophy of the spleen and thymus were observed in WOX1-deficient mice. Significantly reduced cell numbers of splenocytes and thymocytes were determined in WOX1-deficient mice, as compared with the wild-type and heterozygous mice. By TUNEL assay, massive thymocyte apoptosis was found in WOX1-deficient mice. Moreover, we determined that WOX1-deficient mice exhibited a profound defect in B cell development. Together, using gene knockout mice, our study clearly demonstrates that WOX1 is a key regulator in lymphocyte development.

P3.03.15
The investigation of the expression of P2Y1 and P2Y2 nucleotide receptors on thymocytes using monoclonal antibodies

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Extracellular nucleotides are an important mediator of cell-to-cell interactions in the nervous, vascular and immune system. Nucleotides are released by various cell types under physiological and stress conditions. Nucleotides act via wide range of P2X and P2Y nucleotide receptors. We investigated the expression of P2Y1 and P2Y2 receptors on various thymocyte populations using rat monoclonal antibodies. The rats were immunized with primary rat fibroblasts transfected with P2Y1 or P2Y2 nucleotide receptors. The specificity of monoclonal antibodies was tested by flow-cytometry and fluorescence microscopy. The obtained antibodies bind to very small population (1% or less) of all thymocytes. More antibody binding cells were detected on CD4-CD8- double negative and (CD4CD8)low thymocytes. Those thymocyte populations were analyzed according to the expression of CD44 and CD25 cell surface markers. The expression of P2Y1 and P2Y2 receptors were found mostly on CD44-CD25- cells. The majority (18-25%) of P2Y1 and P2Y2 receptor positive cells were from (CD4CD8)lowCD44-CD25- cells. Lower numbers of double negative CD4-CD8- CD44-CD25- thymocytes (3 - 6 %) were stained with antibodies to P2Y1 and P2Y2 receptors. Analyzing (CD4CD8)low thymocytes we found that the Y1-10 and Y2-2 antibodies bind to a small population of TCRβ+, Thy1.1int, CD3int, CD69+ and CD69-, DX5+ and DX5- thymocytes. The antibodies did not bind to TCRγ/δ-, CD3-, Thy1.1- thymocytes. ATP (but neither UTP nor dATP) at the concentration of 0.1mM induce apoptosis in thymocytes culturing them for 1 day. Y2-2 (but not Y1-10) antibodies protect from ATP induced apoptosis CD8+CD4-, DP, CD8-CD4+, (CD8CD4)low, but not DN thymocytes.

P3.03.16
Role of Miz-1 (Myc-Interacting Zinc-finger protein-1) as a regulator of p53 dependent pathways during pre TCR selection

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The Myc-Interacting Zinc-finger protein-1 (Miz-1) was first identified as a binding partner for the proto-oncogene c-Myc. While Miz-1 itself functions as a transcriptional transactivator, it enables c-Myc to act as a transcriptional repressor of genes regulating cell cycle progression, such as the cyclin dependent kinase inhibitors p21Waf1 or p15ink4b. Using mice engineered to express a non-functional form of Miz-1 (Miz-1ΔPOZ mice), we have accumulated evidence that Miz-1 exerts a role in DN3 pre-T cells at the so-called β-selection checkpoint. Our data suggest that cells undergoing V(D)J recombination (i.e. DN3s), require Miz-1 to restrain p53 activity, notably its ability to induce apoptosis through the upregulation of genes such as Bax, Noxa or Puma. This is supported by our finding that in the absence of a functional Miz-1, pre-TCR bearing cells are lost from the thymus by apoptosis, have high levels of Bax, Noxa and Puma and a reduced capacity to progress through the cell cycle, causing a very low thymic cellularity. Moreover, Miz-1ΔPOZ mice lacking p53 have a normal thymic cellularity and in particular, DN3 cells are present at almost normal frequencies and successfully transit through β-selection. These results are intriguing and suggest that activation of p53 has to be controlled when V(D)J recombination occurs and that Miz-1 can exert such a control on p53. We hypothesize therefore that Miz-1 is necessary to protect pre-T cells from premature apoptosis by restricting a p53-mediated DNA damage response that is initiated by double strand breaks that occur during V(D)J recombination.

P3.03.17
Thymic nurse cells play an important role in MHC restriction and express progenitor markers associated with epithelial maintenance

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Thymic nurse cells (TNCs) were first characterized as lympho-epithelial complexes localized within the thymic cortex. Internalized immature thymocytes within specialized vacuoles were shown to undergo MHC restriction resulting in both positive as well as negative selection. In a recent report, we used the TNC-specific monoclonal antibody and also anti-cytokeratin antibodies K5 and K8 to show that a subset of TNCs bearing the phenotype $pH91^{+}K5^{+}K8^{+}$ to be strategically localized within the cortico-medullary junction of the murine thymus. Thymic epithelial cells exhibiting these markers ($K8^{+}K5^{+}$) have been suggested to play a role in the maintenance of thymic cortex and medulla. In this study, we examined the ontogenesis of the TNCs with the aid of pH91. Confocal microscopic analyses of embryonic thymic sections show the onset expression of the pH91-specific antigen to occur at E11.5 stage of development and persist throughout embryological development as well as postnatal mice. Analyses of $pH91^{+}$ cells show the concomitant expression of K5, K8, Foxn1 and P63. At E17.5 stage of development, we observe the formation of the multicellular, lympho-epithelial complexes that exhibit the $pH91^{+}K8^{+}K5^{+}Foxn1^{+}P63^{+}$ phenotype. Using FACs analysis, we show increases in MHC class II expression from E13.5 to E18.5 of thymus development. Taken together, these data show the TNC-specific antigen is expressed early in thymus development and TNCs may be important in the process of MHC restriction as well as maintenance of thymic epithelia.

P3.03.18
TCRβ but not the TCRδ enhancer exerts transcriptional control on the TCRα locus during development

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T lymphocytes develop in the thymus. Thymocytes at CD4-CD8- (DN) stage first recombine and express the T cell receptor beta chain (TCRβ). TCRβ pairs with the pre-T cell receptor alpha (pTα) and CD3 to form the pre-T cell receptor complex whose signalling induces progression to the CD4+CD8+ (DP) stage. At this DP stage, recombination of the TCRα locus and its expression normally take place. However, transgenic TCR mouse models are known to express TCRα prematurely at the DN stage. This suggests either that recombination is sufficient for expression and there is no further transcriptional control, or the transcriptional control in mice with insertion TCR transgenes is aberrant. To investigate these questions, we used a insertion mouse model with the transgenic TCRα exactly mimicking a physiological recombination event. We again found TCRα expression prematurely at the DN stage , and that the expression is highly enhanced by the presence of transgenic TCRβ. This suggests transcriptional control of the TCRα locus by TCRβ, and a possible mechanism could be via signalling through the pre-TCR. We used pTα deficient mice to test this possibility, and indeed found the TCRβ cannot enhance expression of TCRα in pTα knockout mice. This expression-enhancing effect of TCRβ was restored upon anti-CD3ε treatment which is expected to mimic pre-TCR signalling. Thus we show control of TCRα expression by TCRβ mediated through pre-TCR signalling. In addition, we show that in contrast to previous suggestions, TCRα expression does not depend on the presence of this element.

P3.03.19
Pharmacological inhibition of glycogen synthase kinase 3 regulates T cell development *in vitro*

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The development of functional non-autoreactive T cells requires receptor-mediated transition through multiple checkpoints in the thymus. Double negative 3 (DN3) thymocytes are selected for the presence of a rearranged TCR beta chain in a process termed beta-selection which requires signalling via the preTCR and Notch1. Pre-selection DN3 are referred to as DN3a and express low levels of CD27 and CD98, while post-selection DN3 (which are TCR beta positive) express higher levels of CD27 and CD98. Additional signalling from Stromal-Derived Factor 1alpha (SDF1alpha)/CXCl12 ensures optimal proliferative expansion of DN3 thymocytes. Signal integration by these receptors converges on core pathways such as the Phosphatidyinositol-3-kinase (PI3K) pathway. Glycogen Synthase Kinase 3 (GSK3) is generally thought to be negatively regulated by the PI3K pathways.
We have shown that a GSK3-inhibiting drug, CHIR99021, promotes the proliferative expansion of DN3a cultured with recombinant Delta Like4 and SDF1alpha. Here we show that developmental progression of either DN3a or DN3b is promoted by CHIR99021. In the absence of preTCR- or Notch1-mediated signalling differentiation is rescued in a partial or entire manner respectively when CHIR99021 was present. Furthermore, inhibition of GSK3 using CHIR99021 appears to antagonize IL-7-mediated arrest of development at the DN stage. In addition to the effect on T cell development, CHIR99021 increased IL-7 dependent proliferation and caused enhanced cell recovery in these experiments. These experiments indicate a potentially important role for inactivation of GSK3 during the process of beta-selection.

P3.03.20

Role of conserved MHC residues for interaction with T cell antigen receptors

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Most αβ T cell receptors (TCRs) survey the multitude of antigens via peptides presented in the context of major histocompatibilty complex (MHC) molecules displayed on the surface of antigen presenting cells. These TCRs make contact with conserved, solvent-exposed residues on α-helices of the MHC. Previously we have examined the effect of mutating these residues of MHCII IA^b on the response of a bulk T cell population. We have found that many of these mutations cause widespread interference of TCR recognition. To assure that this interference was caused by direct recognition of TCR elements, we examined the peptide repertoires of our most pronounced mutations by mass spectrometry and observed no gross differences. Furthermore, we are going to express these mutants as the only class II MHC in the thymus of a mouse and study their effect on thymic selection. We will either do this by generating a transgenic mouse or attempting to use zinc finger nuclease (ZFN) technology to make knock-in mice expressing these mutations. Using ZFNs designed for IA^b, we have generated many knockout mice. Once we have generated the mutant mouse, we will characterize the selected repertoire of T cells in the periphery. The evolutionary hypothesis suggesting TCRs and MHCs have coevolved would predict a reduction in the number of peripheral T cells, a reduction in the diversity of the repertoire, or a skewing of the V-gene usage.

P3.03.21
Generation of a conditional knock-out mouse for SATB1 to study its importance for CD4+ T cells

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Special AT-rich-binding protein 1 (SATB1) is a nuclear protein, providing higher order chromatin organization and regulation of gene expression. SATB1 is highly important during T-cell development in the thymus and regulates about 2 % of thymocyte genes. Recently, we identified repression of SATB1 in regulatory T cells (Treg cells) as an important feature required to suppress Teffector-cell programs in Treg cells to maintain Treg-cell function.
To further address the functional impact of SATB1 in Teffector-cell programs, we recently generated a conditional SATB1 knock-out mouse to perform spatiotemporally controlled somatic removal of the SATB1 gene since the hitherto available complete knock-out mice lacked peripheral T cells and died from neurological defects after three weeks of age.
For the establishment of this conditional knock-out model we used an approach making use of a Cre-dependent genetic switch (FLEX switch), enabling the disruption of the SATB1 gene by deletion of the ATG-containing exon 2 with concomitant induction of a GFP reporter. Furthermore, gene targeting was performed by a novel approach, in which the recently developed transcription activator-like effector nucleases (TALEN) genome editing enzymes were co-injected with the linearized targeting vector into the nucleus of mouse pronuclear-stage embryos, thereby circumventing gene targeting in ES cell culture. This yielded a founder mouse with recombined 5' and 3' homology regions, which furthermore showed germline transmission of the targeted allele.
Breeding this mouse to different Cre-expressing mice will allow us to clarify the importance of SATB1 for the development and the function of effector T cells.

P3.03.22
Themis CABIT domains exert distinct functions in thymocyte positive selection

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Themis (Gasp) is a newly identified Grb2-binding protein highly expressed in immature thymocytes, that is essential for positive selection, but not for negative selection in the thymus. Despite possible involvement of Themis in TCR-mediated signal transduction, its function is still unknown and controversial. Themis contains a nuclear localization signal, a proline rich sequence, and two uncharacterized domains named CABIT (CABIT1, CABIT2). In the present study, we especially focused on the function of these newly identified CABIT domains.
We established a series of transgenic (Tg) mice expressing mutant Themis molecules including deletion of the highly conserved core region of CABIT1 and/or CABIT2 domains, called delta-Core1 and delta-Core2, respectively. All of the hCD2 promoter-driven mutant Tg mice were bred with Themis-deficient mice to evaluate the biochemical features of the molecules and the effect of each mutation on positive selection. Both Themis delta-Core1 and delta-Core2 mutation abolished association with Grb2 and tyrosine-phosphorylation upon TCR stimulation. Moreover, both Themis mutations resulted in defective positive selection. Interestingly, delta-Core2 Themis showed localization to the nucleus similar to wildtype, whereas delta-Core1 Themis revealed abrogated nuclear translocation. Furthermore, delta-Core1 Tg, but not delta-Core2 Tg, showed impaired T cell development even in the co-existence of wildtype Themis, indicating that Core1 deletion conferred dominant negative inhibition on T cell development.
Collectively, our current results indicate that CABIT1 and CABIT2 are both required for positive selection, and each of the CABIT domains exerts distinct functions during positive selection.

P3.03.23
Background of Primary Immunodeficiencies in Patients with Severe Atopic Dermatitis

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Primary immunodeficiency diseases (PIDs) are a group of heterogeneous inherited disorders, characterized by recurrent infections, autoimmunity and malignancy. Some PIDs such as hyper IgE syndrome (HIES) and Wiskott-Aldrich syndrome (WAS) may be initially presented as atopic dermatisis (AD), especially in its severe form resulting in diagnostic delay and poor prognosis of patients. The aim of this study was to evaluate the frequency of PIDs among patients with severe AD and to determine factors that can help to raise suspicion toward these disorders. Seventy-five patients with a well-established diagnosis of severe AD were enrolled in this study. Initial immunological evaluations, including humoral and cellular investigation were performed in all individuals. Patients underwent further investigations in a case of suspicious to a probable PID.
Among all patients with severe AD, 5 (6.6%) were diagnosed with HIES and 1 (1.3%) with WAS. Family history of PIDs, family history of death in early infancy, positive history of recurrent infections such as skin and respiratory infections, otitis media and sinusitis were observed significantly higher in patients with a diagnosis of PID. The presence of an underlying PID could explain the poor prognosis and refraction to treatment of some patients with severe AD. Several clinical and laboratory findings can help the physicians to focus toward PIDs which are more serious. Delay in diagnosis of PID cases with skin manifestation of AD without proper management may results in lower quality of life and higher morbidity and mortality rates.

P3.03.24

A single cell approach to investigating the role of asymmetric cell division in fate determination of CD8+ T cells

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A hallmark of a successful immune response against infection is the ability to produce a population of effector cells that can fight the infection then be cleared, as well as the generation of long-lasting memory subsets to protect against future encounters. Although many models try to explain the mechanism by which immune cells are capable of differentiating into these lineages, the exact mechanism and history of fate commitment is still debatable. Our lab and others have recently shown the importance of asymmetric cell division in hematopoietic cell function and fate.
Here, we describe a single cell approach to understanding fate commitment of naive CD8 T cells in response to antigen presentation by dendritic cells. By using micro-wells and micromanipulation technologies, we have developed a high throughput time-lapse imaging platform that allows us to follow single cells through activation and subsequent divisions up to 9 rounds. In addition, we have designed a customized imaging analysis toolbox ('TACTICS') to generate lineage trees as cells progress through the division cycles. By correlating times and pattern of division with expression of differentiation markers , we have begun to investigate how behaviors and influences in the first divisions following antigen presentation can affect the propensity of a CD8T cell to adopt a memory or effector cell fate.

P3.03.25
Thymectomy in early childhood: decrease of naive CD4+ T cells and diminished immune response after vaccination

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Our study was aimed to evaluate the influence of thymectomy in early childhood on naive T cell populations, T cell receptor exicion circles (TRECs) and Ki67 expression in thymectomized patients (TP) compared to healthy age matched controls (HC). Further, our study was aimed to compare the immune response to a neo-antigen (tick born encephalitis virus vaccination, TBEV) in TP and HC. Flow cytometry data were used to compare naive T cell (CD4+ CD45RA+CD62L+) and memory T cell(CD28+CD45RO+) counts of 101 TP and 81 HD. 44 TP and 56 HC were vaccinated against TBEV following the Austrian immunization schedule using FSME Immune junior (Baxter, Vienna, Austria). TBEV specific IgG values and IgG specific avidities were measured 4 weeks after the second and third vaccination and two years after the third vaccination using a commercially available ELISA. TP showed significantly lower values according to naive T cells ($p<0.001$) and lower TRECs ($p<0.001$) compared to HC. TREC counts showed a significant correlation with ongoing time after thymectomy ($p<0.001$). TP showed a higher percentage of Ki67+ naive T cells compared to HC ($p<0.01$). Four weeks after the second TBEV vaccination significantly lower TBEV specific IgG values were detected in TP when compared to HC ($p<0.05$). TP showed alterations in the peripheral T cell subpopulations similar to findings in elderly people after physiological thymic involution. TP showed a diminished immune response but a normal memory function of the immune system.

P3.04 Genetic rearrangements of lymphocyte receptors

P3.04.01
Construction and functional activity of fibronectin scaffold containing different chains of hypervariable complementarity determining regions of mouse antibody against human IgG4

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Antigen binding affinity of antibody is determined by 6 chains of hypervariable complementarity determining regions (CRDs). It is not known how many chains mediate specific binding of full antibody. The aim of this work was to construct fibronectin (FN) scaffold of mouse antibody against human IgG4 with different sets of chains. CDRs were cloned from mouse monoclonal antibody 5C7 into 3FN10 scaffold in E.coli system. We constructed variants with one light (L) and one heavy (H) chains; two L chains L1-L3 and two H chains H2-H3; as well as four chains L1-L3-H2-H3; the latter was done by bicistron cloning. Fab fragment was cloned in Saccharomyces cerevisiae yeast system. Single chains constructs did not bind IgG4; two chain constructs recognized IgG4 with low affinity; L1-L3-H2-H3 construct demonstrated the affinity compared with Fab fragments and only slightly less than 5C7 antibody. Thus we demonstrated that four CDRs chains in 3FN10 scaffold can mimic the full-length antibody binding.
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P3.04.02
Bi-polarized function of the 3' regulatory region at the immunoglobulin heavy chain locus

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Expression of complex loci in mammals is developmentally regulated or induced by specific stimuli. Regulation of gene expression is often effected by distant regulatory elements and involves a complex interplay between enhancers, silencers, insulators, locus control regions (LCRs) and target promoters.
An LCR, named the 3' regulatory region (3'RR), is located downstream of the immunoglobulin heavy chain (IgH) locus, and is composed of 4 enhancers that act in synergy to effect a long-range activation of various inducible germ-line (GL) promoters. Enhancer RNAs (eRNAs) have been described at the 3'RR but their function is unknown. Additionally, whether the target promoters compete for the 3'RR activity is presently unknown. It is also unclear whether the 3'RR effect is exclusively targeted towards upstream promoters or whether it is bi-directional.
In an attempt to tackle these topics in vivo, we generated a knock-in mouse line in which the Iα GL promoter was duplicated downstream of the 3'RR, and performed chromatin studies as well as transcription analyses on a single-cell basis.
We will present our most recent data on the effect of this mutation on GL transcription derived from the various GL promoters, on the competition between GL promoters, and on eRNAs' expression.

P3.04.03
Specification of the Vκ gene repertoire at the mouse Igκ gene locus

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The processes of Ig gene monoallelic activation and silencing, as well as locus contraction and looping, are essential for regulating V(D)J-recombination and creating a diverse antibody repertoire. However, the mechansisms for these events currently are poorly understood. We have previously identified six DNase I hypersensitive sites (HS1-

6) within the mouse Igκ gene V-J intervening region and have shown in pre-B cells that HS1-2 specifies locus contraction and looping, and HS3-6 specifies recombination silencing. These sequences have been termed Cer and Sis, respectively. Targeted deletion of either Cer or Sis leads to the preferential usage of Jκ-proximal Vκ genes. Interestingly, each element possesses a pair of functional CTCF binding sites, although Vκ gene germline transcription is only modestly upregulated upon deletion of either element. Here we address the question of whether Cer and Sis may be functionally redundant and act independently as insulator boundaries by preventing the downstream enhancers from activating proximal Vκ gene germline transcription. Targeted deletion of HS1-6 strikingly led to 96% of the Vκ gene usage from the Jκ-proximal 12 Vκ genes, resulted in >20-fold increases in their germline transcription, and led to increased non-lineage-specific Igκ gene rearrangements in T cells. Thus, our studies demonstrate that DNase I hypersensitive sites within the Jκ-Cκ region are essential for generating locus contraction and looping, and for creating a diverse tissue-specific antibody repertoire by preventing inappropriate proximal Vκ gene germline transcription.
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P3.04.04
Reassessing HLA-DRB3, DRB4 and DRB5 expression in different cell types

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The immune response depends on the CD4+ T cell antigen presentation by HLA class II molecules, remaining the DR- region the most studied and variable. While all individual always present DRB1 loci, the presence or absence of DRB3, DRB4 or DRB5 loci is variable in different individuals, defining DR high variability. The continuous presence of DRB1 defines our knowledge about DRB1 (peptide binding capacity, regulation of their expression ,...) as equivalent to DR molecule, extrapolating DRB1 functions to DRB3/B4 / B5 loci.
The present study try to evaluate whether the regulation of the DRB3/4/5 expression is analogous to DRB1, assessing the quantification of DRB3/4/5 expression in relation with the corresponding DRB1 level of each haplotype in several class-II-expressing cells; we have studied both cell-surface expression (flow-cytometry levels) and mRNA transcription (RT-PCR levels). In parallel, a bioinformatic assessment of 5'-region of different DRB3/4/5 loci and alleles was developed to evaluate this region by high throughput sequencing.
Our results define differences in the relative proportions of each locus according the different cell populations and DR haplotypes; it is consistent with the observation that there are minor differences in the promoter 5'-region of DRB3, DRB4 and DRB5, someway defining differential transcriptional mechanisms. It seems logical to suggest that these differences could require reassessment of our knowledge in the DRB3/4/5 functional role, and consequently they could re-define a differentiable peptide repertoire for each DRB3/4/5 loci.
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P3.04.05
Intrinsic bias and public rearrangements in the human immunoglobulin VLambda light chain repertoire

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With the rapid advancement of next-generation sequencing technologies (NGS), study of the immunoglobulin B-cell receptor (BCR) repertoire has become increasingly feasible. Herein, we conducted a deep repertoire study of the BCR lambda light chain (IGL) using Roche 454 NGS to generate ~180k IGL variable gene transcript sequences derived from B cells collected from two healthy human individuals' peripheral blood mononuclear cells and three NOD-*scid-IL2R*$_{\gamma}^{null}$ mice engrafted with human hematopoietic stem cells. In addition, we have included ~500k IGL sequences provided by Rinat-Pfizer derived from two pairs of monozygotic twins. We found that IGL preferential gene segment utilization is common and, surprisingly, public CDR-L3s are shared at a minimal frequency of ~20% across the multiple samples. This included the presence of identical full-length IGLs. Additionally, comparative analysis between public and private CDR-L3 groups highlighted notable differences such as reduction of N/P nucleotide addition within the public group. Overall the data suggest positive selection of BCR IGL chains independent of the IGH chain. In general, BCR repertoire studies present the challenge of managing and analyzing daunting quantities of raw sequence data, so we have begun development of a cloud-based solution (APPSOMA) to provide a universally accessible server for data storage, management, and computational tools to streamline the repertoire analysis process. This service will be made available to the public. As such, the results from our IGL repertoire study will be derived from the cloud-based pipeline to showcase its benefits and convenience to the repertoire community.

P3.04.06
Defects in VDJ recombination and thymocyte development induced by T cell-specific deletion of protein phosphatase 4/PP4

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Thymocyte development is regulated at many dimensions such as gene rearrangement, receptor generation and repertoire selection, of which all are essential for establishing a protective immune system for the host. Protein phosphatase 4 (PP4) is a ubiquitous serine/threonine phosphatase that has been shown to regulate MAP kinase signaling and irradiation-induced DNA repair. Recently we generated mice with T cell specific-ablation of PP4, and observed defects in their thymocyte development. Mixed bone marrow chimera experiment showed that PP4 deficiency induced partial blocks at multiple developmental stages. Further characterizations revealed reduced IL-7 responses and increased apoptosis in PP4-deficient thymocytes. More interestingly, in these thymocytes we also found accumulation of VDJ recombination intermediates via linker-mediated PCR, implicating defects in gene rearrangement; results from an inducible in vitro VDJ recombination assay corroborated this conclusion. The introduction of TCR transgenes rescued PP4-deficient thymocyte numbers to WT levels, suggesting defective TCR generation as the major contributor to the impaired thymocyte development in PP4-deficient mice. Finally, enhanced H2AX phosphorylation and foci formation in PP4-deficient thymocytes suggested altered NHEJ responses as the mechanism of PP4-mediated regulations. Our results thus provide clear evidences that PP4 is an essential component of VDJ recombination and thymocyte development, and further raise the possibility of therapeutic modulation of immune repertoires with PP4-inhibiting compounds.

P3.04.07
XLF is required for the generation of junctional diversity by TdT

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V(D)J recombination takes place during lymphocyte development in order to generate a large repertoire of T and B cell receptors. This process starts with the introduction of DNA double strands breaks by the recombination activating genes (RAG1/RAG2). Subsequently, the breaks are processed by factors of the non-homologous end-joining (NHEJ) pathway and non-templated (N) nucleotides are introduced by terminal deoxynucleotidyl transferase (TdT) to generate junction diversity. Finally, these breaks are repaired. Mutations in the NHEJ factor XLF (Cernunnos) result in an immunodeficiency syndrome characterized by sensitivity for ionizing radiation, microcephaly, and developmental delay. We showed that compared to deficiencies in the NHEJ factors Artemis and DNA-PKcs, XLF deficiency has a more pronounced effect on DNA repair given the extreme radiosensitivity,

whereas the effect on V(D)J recombination was milder. Moreover, we found that XLF deficient patients have a reduced CDR3 length of the immunoglobulin heavy chain and T-cell receptor gamma rearrangements caused by a significant reduction in the number of N-nulceotides. This may imply that XLF is essential for the generation of junction diversity by TdT. We showed that XLF is not necessary for the recruitment of TdT to DNA ends, indicating that XLF might be required for proper positioning of TdT within the repair complex. In conclusion, XLF has a prominent role in DNA repair and during V(D)J recombination XLF is involved in the generation of junctional diversity by TdT.

P3.04.08
High throughput sequencing data reveals the shape of the immunoglobulin repertoire

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VDJ recombination generates such unfathomable diversity among lymphocyte receptors that it has been said that any immunoglobulin heavy chain (IGH) rearrangement is likely to be generated just once in a human lifetime. Deep sequencing now allows us to test this prediction.
Unique features of the IGH gene locus means that HTS data from a single individual can be used to generate empirically-derived distributions of probabilities associated with each part of the rearrangement process. This has allowed us to create large, simulated repertoires of IGH sequences, defining the 'shape' and composition of an individual's IGH repertoire. Simulation results have been cross-validated with observed repertoires from other genotype-matched individuals.
VDJ recombination is not an unconstrained generator of incomprehensible diversity, but rather has evolved to shape the IGH repertoire towards a relatively small pool of high frequency IGHs. Remarkably, around 5% of the possible VDJ rearrangements are carried by 95% of naïve B cells, and these are shared with other individuals of similar genotype. A long 'tail' of low frequency, extremely diverse IGHs are unique to an individual. The highest frequency IGHs are each carried by about a million naïve lymphocytes, while each IGH in the tail is carried by a unique lymphocyte. The core of the B-cell repertoire is genetically hard-wired, and this may ensure that each individual possesses a repertoire of functionally important IGH at high copy number. This 'shape' of the repertoire has important implications for the ontogeny of the immune response, and for the kinetics of immune reactivity.

P3.04.09
Identifying factors responsible for maintenance of immunoglobulin class switch recombination

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Class switch recombination (CSR) in B-cells occurs in response to antigenic stimulation and is responsible for the production of additional antibody isotypes present in an immune response. The mechanism behind this genetic rearrangement requires mutations introduced by the enzyme activation induced cytidine deaminase (AID) and careful interplay of several DNA repair processes in a manner which remains poorly understood.
Our laboratory recently completed a genome-wide shRNA screen in pursuit of uncharacterized factors involved in CSR. We applied a lentiviral shRNA library based knock-down approach with the CSR-inducible murine B-cell line, CH12F3-2. From this screen we identified an extensive list of candidate factors required for maintenance of CSR, which we have validated on an individual basis. We have carried out further studies on a select group of these putative CSR factors based on bioinformatics analysis and literature review.
One of the genes of exceptional interest identified from the screen encodes an evolutionarily conserved protein with putative functions in DNA damage repair, DNA replication, and transcription. Our studies confirm an additional role for maintenance of CSR in B-cells. We demonstrate that the gene of interest exhibits its function following AID mutagenesis and formation of DNA double-stranded breaks, consistent with published reports suggesting a DNA repair function for this protein. We also reveal that a reduced level of this protein leads to cell cycle arrest and a delayed response to genotoxic stress. Our results reveal a novel link between DNA repair and successful CSR.

P3.04.10
Detection Of Interferon Alpha Receptor 2 in Interferon Resistant HCV Patients

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Hepatitis C virus (HCV) infects more than 3 percent of world's population and 10 percent of Pakistan's population. The virus multiplies in the host with novel methods, defending itself from host immune response, leading ultimately to liver cirrhosis and hepatocellular carcinoma (HCC). The approved therapy for the disease is interferon alpha (INF-α) in combination with ribavirin. The disease is incurable/ resistant due to multiple viral and cellular factors. A strong functional host system can minimize the viral count to zero.
In my study, the detection of IFNAR-2 was examined in patients who were resistant to IFN therapy (administered once or twice). Their liver biopsy specimens were then used to investigate the detection of IFNAR2.The significance of this work was based on the fact that IFN has a thousand fold greater affinity to IFNAR-2 as compared to IFNAR-1, making it a key factor in the initiation of IFN signalling pathway. However IFNAR-2 mRNA was detected in 90% of the patients in this study, while all the responders were positive for IFNAR-2. The results of my study also showed that there was no significant difference in the presence of IFNAR-2 in HCV patients responding or showing resistance to IFN therapy. Accordingly, after sequencing the complete coding region of IFNAR-2, DNA sequence revealed a novel transition at nucleotide positions converting amino acid proline into arginine resulting in resistance to IFN therapy in HCV patients

P3.04.11
In vivo analysis of Aicda gene regulation: critical balance between upstream enhancer and intronic repressor for the appropriate expression

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The Aicda gene, encoding activation-induced cytidine deaminase (AID) is strongly transcribed in activated B cells to diversify immunoglobulin genes, yet low-level expressions upon physiological/pathological stimuli in various cells have been reported. Mutagenic nature of AID has shown to be involved in tumor development. Here, by using a transgenic strategy with bacterial artificial chromosomes (BAC), we examined the in vivo function of Aicda regulatory elements which cluster in two regions, namely approximately 8-kb upstream of the transcription start site (region 4) and the first intron (region 2). Deletion of either of regions completely abolished the expression of Aicda-BAC-reporters, indicating critical roles of these elements. Furthermore, we found that the selective deletion of two C/EBP binding sites in region 4 inactivates its enhancer activity in spite of the presence of the intact NF-κB-, STAT6- and Smad-binding-sites. On the other hand, the selective deletion of E2F- and c-Myb-binding-sites in region 2 increased the frequency of the cells with active Aicda promoter in germinal center B cells, indicating that E2F and c-Myb function as silencer in vivo. Interestingly, the silencer deletion did not cause the ectopic activation of Aicda promoter, indicating that specific stimulation of the enhancer is required for Aicda activation. In summary, the precise regulation of the Aicda promoter appears to dependent on a coordinated balance of activities between the enhancer and silencer elements.

P3.04.12
T-cell receptor (TCR) profiling by next-generation sequencing

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Background
T-lymphocytes have the capacity to recognize antigens from millions of pathogens with a remarkable degree of specificity, due to the molecular diversity of the T cell receptor (TCR) repertoire. Because of this diversity, the TCR, arguably the most important molecule in both host defense and autoimmunity has been impossible to study in-depth with conventional sequencing. Next-generation sequencing, by defining individual molecules, now allows the delineation of this repertoire in unprecedented depth.
Objective
To evaluate the accuracy of our innovative TCR profiling protocol.
Experimental Approach
RNA from T cells clones with one defined TCR rearrangement was mixed at different concentrations with samples of normal human CD4+ T cells. A 5'-RACE protocol that we have developed was used for the unbiased amplification of all TCRs, despite the large diversity of their 5' ends. Amplicons were multiplexed and sequenced at a depth of 1,000,000x on the Illumina MiSeq. V, J and D segment choice were assigned by a paired-ends modification of the IMGT algorithm. Copy count of TCR clones was used to calculate the frequency of each clone in every mix.
Results
In our preliminary studies the assay was able to accurately detect the specific clones as well as their concentrations in each mix. It was found to be sensitive, reliable and repeatable.
Conclusions
Accurate profiling of the TCR repertoire using 5' race and next generation sequencing is a promising unbiased and precise method.

P3.04.13
AID expression in pancreas promotes an inflammatory response

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Activation-induced deaminase (AID) is essential for the reactions of antibody secondary diversification in germinal centers, namely somatic hypermutation (SHM) and class switch recombination (CSR), which take place in B cells that have been activated by antigen. However, AID activity is not completely restricted to immunoglobulin genes and can trigger both the accumulation of mutations in numerous loci and the generation of lymphomagenic chromosome translocations. This potentially oncogenic activity of AID may not be limited to the B cell lineage, as it has been shown that AID expression can be induced in epithelial cells through the activation of the NF-κB pathway. Importantly, NF-κB-dependent inflammation is an important player in many neoplasias, including pancreatic adenocarcinoma (PDAC). To analyze the role of AID in the development of PDAC we have generated a conditional mouse model for AID overexpression in pancreas ($ROSA26^{AIDki/+}p48Cre^{ki/+}$). AID activity was confirmed by the detection of mutations by next generation sequencing. Intriguingly, $ROSA26^{AIDki/+}p48Cre^{ki/+}$ mice did not seem to develop PDAC. However, analysis of aged $ROSA26^{AIDki/+}p48Cre^{ki/+}$ mice revealed an inflammatory response in the pancreas. This inflammation was characterized by the appearance of CD3 and Pax5 positive foci and increased levels of TNFα and IL1ra. We propose that AID activity in epithelial pancreatic cells could promote relatively widespread lesions on the DNA leading to the activation of the DNA damage response, which in turn would promote the expression of NKG2D ligands, the recruitment of cells expressing NKG2D receptors and the removal of damaged cells, thereby blocking PDAC development.

P3.04.14
Holy cow - reshaping bovine antibody diversity with disulfide-bonded ultralong CDR H3s

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Cows have limited genome encoded combinatorial diversity potential, yet mount a robust antibody response. Cows have few V-regions but exceptionally long CDR H3 loops, however the mechanism for creating diversity is not understood. Crystal structures of two cow antibodies reveal that these CDR H3s form a very unusual architecture composed of a b-strand "stalk" that supports a structurally diverse, disulfide-bonded, "knob" domain. Deep sequencing revealed that ultralong CDR H3s contain a remarkable complexity of cysteines, suggesting that these disulfide-bonded mini-domains may arise during repertoire development. Sequence analysis indicates that diversity arises from somatic hypermutation of an ultralong DH with a severe codon bias towards mutation to cysteine. These unusual antibodies can be elicited to recognize defined antigens through the knob domain. Thus, the bovine immune system produces an antibody repertoire composed of CDR H3s of unprecedented length that fold into a diversity of mini-domains generated through combinations of somatically generated disulfides.

P3.04.15
T cell receptor repertoire plasticity in peripheral CD8 T cells

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Using a technique for the paired amplification of TCRα and β chains from single cells, we have discovered a surprising plasticity in the peripheral TCR repertoire, particularly under conditions of inflammation. This plasticity includes multiple instances of TCR revision, where changes are made in the TCR coding sequence by de novo rearrangement. Here we describe the induction of this process in murine CD8+ T cells derived from influenza-infected lungs and human T cells derived from CMV-infected individuals ex vivo or stimulated in single-cell cultures in vitro. Taking advantage of the co-expression of productive and non-productive *Tcra* alleles, we demonstrated secondary rearrangement in the TCRβ locus ex vivo, while the in vitro cultures demonstrated a remarkably high rate of revision following strong stimulation. Not surprisingly, revision was found to be RAG-dependent. Utilizing a model of peripheral deletion of RAG2, we showed that mice deficient in revision showed delayed onset and severity of disease in an experimental autoimmune encephalomyelitis (EAE) model, but also produced a less functional T cell response following infectious challenge. Thus, peripheral TCR revision appears to be a robust process that contributes to optimal T cell functionality during infection but may also contribute to the development of autoimmunity.

P3.05 Antigen processing and presentation

P3.05.01
Hypoxia directly enhances dendritic cell antigen presentation

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Danger molecules from ischemic tissues activate innate immunity by increasing dendritic cell (DC) immunogenicity.
To evaluate whether hypoxia directly impacts on DC antigen presentation capacity, we used mouse B6-OVA-DCs, expressing chicken OVA peptides in the context of class I and II MHC, and OVA-specific TCR-transgenic B6 syngeneic CD8 (OTI) and CD4 (OTII) T cells.
Bone-marrow derived OVA-DCs were pre-exposed for 4hr to 1% (hypoxia) or 20% O_2 (normoxia) and then used as stimulators of OTI

or OTII T cells for 2 days. Higher numbers of IFNγ-producing CD8 and CD4 T cell clones emerged ($p<0.05$) in response to hypoxic than to normoxic OVA-DCs (OTI 162±26, OTII 72±20 vs: OTI 115±23, OTII 36±15 spots/50000 cells, n=4). A larger increase of IFNγ-producing clones was found in response to hypoxic OVA-DCs exposed to conditioned medium (cm) from hypoxic OVA-B6 renal tubuli vs normoxic OVA-DC exposed to cm from normoxic tubuli ($p<0.01$).
Similarly, non-transgenic B6-DCs pre-incubated with OVA protein (plus hypoxic tubular cm) during hypoxia, induced higher numbers of IFNγ-producing T cell clones than normoxic B6 DCs (pre-incubated with OVA plus normoxic tubular cm): OTI 60±15, OTII 50±10 vs OTI 31±8, OTII 23±6 spots, n=3, $p<0.05$. This indicates that hypoxia augmented the capacity of DCs to present and cross-present antigens to CD4 and CD8 T cells, respectively.
Altogether these results suggest that hypoxia increases antigen presentation by DCs both by direct action on DCs and through release of soluble factors from injured tissues. Such events eventually translate into an augmented adaptive T cells immune response.

P3.05.02
Synthetic immunoactive fragments of Survivin: selection and application for monomer and dimer detection

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We have selected 15 immunoactive fragments of Survivin and synthesized them. Survivin is overexpressed in tumor tissues and exists in monomeric and dimeric forms. It was shown that the monomer participates in mitosis regulation while the dimer's function is not clear yet. Survivin monomer and dimer detection in normal and tumor cells is necessary for functional studies of survivin state and for tumor differential diagnostics. We proposed that the immunoactive fragments of survivin will be able to induce specific antibodies for selective detection of monomer and dimer. All the selected peptides included B- epitopes of survivin and several of them included predicted T-helper epitopes. Prediction of T-helper epitopes was based on the algorithm where the motif consists of nine amino acid residues with a hydrophobic residue in the 1-st position and positively charged residue at the 9-th position. All the synthetic fragments with T-helper epitopes were able to induce antibody response in experimental animals in a free nonconjugated with a protein carrier form. Peptides without T-helper epitopes were immunoactive only as KLH-conjugates. Monospecific antibodies to different regions of survivin were obtained from rabbit antipeptide sera using an affinity chromatography on sorbents with immobilized peptides. Using western blot of cell lysates from different types of tumor we selected antibodies which were able to differentiate monomeric and dimeric forms of survivin. These antibodies may be used for cancer diagnostics and functional studies of survivin state.
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P3.05.03
The impact of sub-cellular localization, dose, and expression pattern of tumour antigen on cross-presentation efficiency

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Effective cross-presentation mediated anti-tumor immunity requires the identification of suitable target-antigens. Yet, much remains unclear about the significance of tumor-antigen properties such as sub-cellular localization, dose, and expression pattern, in efficient cross-presentation induction. The subcellular localization of an antigen within a tumour cell, may affect how efficiently it is cross-presented in different physiological contexts.
In this study, we compared tumor specific T-cell responses elicited by mouse B16-F10 melanoma tumours expressing a model antigen (CL4 epitope of haemagglutinin), in cytoplasmic, secretory, or nuclear sub-cellular compartments within the tumor-cell. All three tumor cell-lines similarly expressed ~7 fg of antigen per cell.
Our results show that cross-presentation is remarkably sensitive to antigen-dose. Cross-presentation induced proliferation of CL4-TCR transgenic $CD8^+$ T-cells was evident at as little as 0.35 ng, near maximal from 0.70 – 2.45 ng without change, and maximal at 3.5 ng of inoculated antigen-bearing cells. At 0.35 ng of antigen-bearing cells, we found that nuclear localized antigen was less efficiently cross-presented compared to its cytoplasmic and secreted counterparts. Furthermore, gemcitabine (an apoptosis inducing chemotherapy) augmented cross-presentation efficiency when antigen was cytoplasmic and nuclear localized, but intriguingly diminished when antigen was secreted.
Put together, our data suggests that the dose and localization of an antigen within a tumor can influence the robustness of the anti-tumor T-cell response. In addition, gemcitabine can augment cross-presentation by presumably unmasking previously unavailable nuclear antigens via apoptosis. These findings imply that the sub-cellular localisation of an antigen should be taken into account when designing immunotherapy and chemotherapy strategies.

P3.05.04
High resolution mass spectrometry reveals unanticipated depth and diversity of HLA peptidomes

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The HLA peptidome reflects the state of health of each cell to the immune system, and is thought to communicate the changes in the cellular proteome under pathological conditions such as virus infection or tumorigenic transformation. In this study we used a linear quadrupole - Orbitrap mass spectrometer and together with the MaxQuant bioinformatics platforms to acquire the largest HLA peptidome to be identified so far in any single system. The HLA peptidomes purified from the B-cell acute lymphoblastic leukemia isogenic lines SupB15WT and SupB15RT where the latter is resistant to imatinib treatment, revealed multi-level complexity. This complexity was validated in other primary and cancer cell lines. A subset of the cellular proteins representing fundamental tissue specific characteristics are selectively presented in all cell types analyzed, and in addition proliferation, transcription and translation are presented in cancer cells. Interestingly, individual proteins yield many different peptides with epitopes that fit the cellular HLA allele's consensus binding motifs contrary to the 'one epitope per protein' hypothesis. SupB15WT cells were treated with imatinib, and the changes in their HLA repertoires were quantified with label-free or SILAC methods. Interestingly, the expression levels of their proteins of origin did not predict expression levels of their HLA peptides indicating that the cellular mechanisms responsible for the processing of the HLA peptides play a key role in diversifying the repertoire of HLA peptidomes.

P3.05.05
Tumor associated MUC1 carried by microvesicles is cross-processed by Dendritic cells generating immunogenic MUC1 glycoepitopes and is cross-presented to CD8+T cells

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The induction of an efficacious anti-tumor immune response (IR) requires the cross-processing and presentation of tumor antigen by Dendritic Cells (DCs). Block of endocytated tumor associated antigen (TAA) in the early compartments of the intracellular processing machinery shifts the IR towards a Th2 balance. MUC1 is one of the most relevant tumor associated glycoprotein expressed by epithelial cells and its immunogenicity is altered by the glycosylation profile. Moreover soluble MUC1 antigen has shown to be blocked in endolysosomal compartment of DCs thus inducing mostly a Th2 response. Objective of this study was to investigate whether glycosylation pattern and MUC1 bound to microvesicles could influence the antigen processing by DCs. MUC1 as soluble molecule, independently by the glycosylation profile, appears to be blocked in the pre-endosomal compartment. Receptor-mediated endocytosis pushes further the processing in the HLAII compartment. Cross-

processing of MUC1 in HLAI compartment is observed only when MUC1 is carried by microvesicles (MUC1-MVs). Moreover only DCs stimulated with MUC1-MVs are able to induce IFN-γ production by MUC1 specific CD8+T cells. The distinct processing of the MUC1 membrane bound is accompanied by deglycosylation processes thus generating Tn-MUC1 immunogenic glycoepitopes. These results show that MUC1 undergoes to alternative processing pathways depending by its form of release thus modifying MUC1 immunogenicity. Moreover these experimental evidence can be important to design efficacious glycoantigen formulations for DC-based cancer vaccines.

P3.05.06
Differential antigen-dependency of CD4+ and CD8+ T cells

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The presence of cognate antigen is the key requirement for the differentiation and expansion of T cells. Nevertheless, the impact of stimulus duration has only rarely been compared between CD4+ and CD8+ T cells side by side. We followed proliferation of both subsets upon transient or persistent TCR stimulation and found CD4+, but not CD8+ T cells to be more dependent on prolonged antigen presentation for extensive proliferation. The impaired ability of CD4+ T cells to expand in the absence of antigen was not caused by coinhibitory signals or a lack of inflammatory cytokines. The antigen dependency of CD4+ T cells was not affected by Th1/Th2 polarization or memory differentiation and thus is likely to be a lineage intrinsic feature. The prolonged proliferation of CD8+ T cells after ceasing the TCR stimulus resulted in complete effector differentiation and was independent of self-peptide/MHC I complexes. Transcriptome analyses revealed gene expression of CD4+ T cells to be more dependent on persistent TCR stimulation than of CD8+ T cells. These data reflected the biology of MHC molecules, as we could demonstrate that MHC class II, but not class I molecules were stabilized on activated dendritic cells in vivo.

P3.05.07
Immunoproteasome induction and MHC class I molecules presentation in motor neurons of a familial Amyotrophic Lateral Ssclerosis mouse model

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease, characterized by selective death of motor neurons. Growing evidence suggest that the immune system play a role in the pathogenesis and progression of ALS. We recently found in SOD1G93A mice, the most reliable animal model of ALS, a progressive upregulation of the immunoproteasome subunit, LMP7, starting before symptoms until the advanced stage of disease.
Immunoproteasome recognizes and degrades polyubiquitinated protein substrates to generate small protein fragments that can be used by major histocompatibility complex (MHC) class I molecules for the display of antigens to the adaptive immune system. Therefore, we aimed to examine the expression of MHCI and the associated β2 microglobulin (β2m), together with LMP7 in the spinal cord and peripheral nerves of SOD1G93A mice at differenst stages of the disease using immunohostochemical analysis and confocal microscopy.
We report that motor neurons and surrounding glial cells in SOD1G93A mice at early stages of the disease, exhibit a remarkable induction of LMP7, MHC-I and β2 microglobulin. In particular a remarkable overexpression of all these proteins was found in the motor axons and neuromuscular junction of SOD1G03A mice during the disease course.
These data suggests that the activation of the adaptive immune system molecules both at central and peripheral level take part in the pathogenesis and/or progression of ALS. Studies are ongoing to investigate the beneficial or detrimental effect of this immune response.

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P3.05.08
In vitro study of Thyroglobulin peptides associated to HLA-DR3 under thyroid antigen presentation conditions

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Thyroglobulin (Tg) peptides are presented in vivo by HLA-DR molecules in human autoimmune Graves' disease and at least one of these (Tg2098) has been shown to induce autoimmune thyroiditis in HLA-DR3 transgenic mice. Here, we have indentified immunodominant epitopes from Tg that are bound and presented to HLA-DR3, using an new in vitro cell-free system that recreates the MHC class II antigen processing compartment.
We used soluble HLA-DR3, HLA-DM and thyroglobulin to mimick different processing conditions, i.e. (i) dendritic cell processing (Cathepsin B, H and S at pH5) and (ii) predigestion of Tg as in the thyroid colloid (cathepsins B, L and S at pH 7) before antigen uptake. Peptide/HLA-DR3 complexes were immunoprecipitated with the anti-DR monoclonal antibody L243. Bound peptides were eluted with trifluoroacetic acid 0.1% and filtered through a 10-kDa molecular weight cut-off Microcon filter. Samples were analyzed by MALDI and peptides were sequenced using a LTQ Orbitrap. MS/MS fragmentation spectra were searched using SEQUEST against a combined Human UniProt target/decoy database.
Predigestion of Tg significatively increased the number of sequenced peptides, especially those predicted to be high affinity binders, as well as the number of peptides in each nested set. Moreover, 3 peptides previously described in literature were sequenced using this in vitro system. The immunodominant peptide (Tg2098) only appeared when Tg was predigested. Data suggests that degradation in peripheral tissue may condition antigen presentation, allowing the generation of a greater diversity of peptides than those generated by the digestion of full antigens.

P3.05.09
Epitopes of 21-hydroxylase targeted by autoreactive T cells in patients with autoimmune Addison's disease are also targeted by T cells in mice immunized with 21-hydroxylase protein

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Autoimmune Addison's disease, or primary adrenocortical insufficiency, is a prototypic organ-specific autoimmune disorder, caused by immune-mediated destruction of the adrenal cortex and subsequent lack of steroid hormones. The disease is characterized by antigen-specific immune responses against certain enzymes vital for steroid hormone biosynthesis, most specifically 21-hydoxylase (21OH), which is exclusively expressed in the adrenal cortex. Both serum autoantibodies and peripheral blood T cell responses against 21OH can readily be demonstrated in patients with Addison's disease. Although patient T cell responses against 21OH epitopes display a broad pattern (partly reflecting HLA differences), dominant peptide epitopes have also been described, such as a B*0801 restricted epitope towards the C-terminal end of the protein. In the present study we have compared *ex vivo* T cell responses against 21OH peptides in Addison's disease patients and in mice immunized with full-length human 21OH protein. Interestingly, the dominating epitopes in patients are also targeted by T cells in immunized mice, in both the CD4+ and CD8+ compartments. This trend was also noted across several mouse strains carrying different MHC haplotypes. We therefore conclude that these epitopes probably are naturally processed and presented from the full-length 21OH protein.

P3.05.10
Sub-optimal antigen presentation of CD8+ T cell epitopes by dendritic cells infected with Trypanosoma cruzi

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Introduction: The $CD8^+$ T cell mediated immune response observed after infection with the protozoan parasite *Trypanosoma cruzi* is sub-optimal characterized by a low T cell fitness. A possible explanation for the sub-optimal immune response could be related to a defective antigen presentation by dendritic cells (DC). Here, we tested the hypothesis that antigen presentation of $CD8^+$ T cell epitopes by *T. cruzi*-infected DC is sub-optimal.
Results: Murine bone marrow derived cultured DC were infected *in vitro* and used to stimulate IFN-γ secretion by effector $CD8^+$ T cells collected from infected mice. By sorting, we observed that once exposed to *T. cruzi*, both DC infected or not stimulated specific T cells. The activation of T cells was dependent on TAP, ribosomes, proteasomes and *trans*-Golgi network, indicating that epitopes are processed and presented through the canonical cytosolic pathway. Nonetheless, infected DC stimulated significantly less specific $CD8^+$ T cells when compared to DC pulsed with peptide or infected with a recombinant human adenovirus type 5 expressing a *T. cruzi* $CD8^+$ T cell epitope. The difference cannot be attributed to the amount of antigen produced upon infection as it did not change when we used different doses of parasites or adenoviruses. Also, we were unable to find differences on the DC activation markers (CD40, CD80, CD86 and MHC-II).
Conclusion: We concluded that DC infected *in vitro* with *T. cruzi* are sub-optimal to present parasite derived $CD8^+$ T cells epitopes, a fact that may explain the sub-optimal activation of these cells we described.

P3.05.11
Bone marrow endothelial cells of patients with multiple myeloma: antigen presenting functions and T cell regulation

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Immunity against cancer is hampered by local mechanisms promoting tumor evasion from T cell surveillance or tumor-specific T cell tolerance in the tumor microenvironment. We have analyzed the antigen presenting capacities of endothelial cells from bone marrow of patients with multiple myeloma (MM), an inexorably lethal plasma cell malignancy. We have found that bone marrow endothelial cells basally express both class I and class II MHC molecules as well as the costimulatory molecules CD80 and CD86. The expression level of these molecules is slightly lower than that of professional antigen presenting cells, such as dendritic cells, from the same patients. Bone marrow endothelial cells also express the components of the MHC class I antigen processing machinery which include the constitutive proteasome subunits δ (β1), MB1 (β5) and ζ (β2); the interferon-γ (INF-γ)-inducible proteasome (immunoproteasome) subunits LMP2 (β1i), LMP7 (β5i) and LMP10 (β2i); the peptide transporters TAP1 and TAP2 and the endoplasmic reticulum chaperones calnexin, calreticulin, ERp57 and tapasin. When bone marrow endothelial cells are cultured, CD80 and CD86 expression decreases, class II MHC molecule largely disappears and class I MHC molecule does not significantly change. Both class II MHC and costimulatory molecule expression are restored by bone marrow endothelial cell exposure to IFN-γ and IL-6. Cultured bone marrow endothelial cells can activate purified allogeneic and autologous CD4 and CD8 T cells.

P3.05.12
Inhibition of ERAP1 function promotes human NK cell immune responses against tumors: an innovative strategy of anticancer immunotherapy

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Endoplasmic reticulum aminopeptidase 1 (ERAP1) plays a key role in regulating the immune system function by trimming peptides for MHC class I antigen presentation. We have recently demonstrated that inhibition of ERAP1 in a murine T cell lymphoma results in tumor rejection in syngeneic mice by triggering innate and adaptive anti-tumor immune responses. Tumor rejection is mainly due to an immediate NK cell response and depends on the MHC class I-peptide repertoire presented by ERAP1-silenced cells.
Since the engagement of several human NK cell inhibitory receptors is strictly dependent on the composition of MHC class I-bound peptides, it is plausible that all individuals expressing these receptors could be sensitive to ERAP1 inhibition. To identify HLA class I-KIR combinations that are relevant to ERAP1 inhibition, several human cell lines, i.e. the C1R transfectants and tumor cell lines, were pharmacologically and genetically inhibited for ERAP1 function and tested for NK cell recognition. Herein, we show that suppression of peptide trimming affects the engagement of specific KIRs by enhancing NK cell killing. These results suggest a potential role for human ERAP1 in regulating NK cell immunity and establish rules to identify individuals who could benefit from ERAP1 inhibition for NK cell-mediated immunotherapeutic protocol.

P3.05.13
Human mesenchymal stromal cells derived from cervical neoplasisas down-regulate the expression of HLA class I molecules on cervical cancer cells

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Mesenchymal stromal cells (MSCs), have the ability to home to tumors and participate in tumor stroma formation and support the growth of neoplastic cells; however, little is known about the way MSCs favor the immune evasion of tumor cells. Since the expression of HLA class I molecules (HLA-I) on tumor cells is necessary for their immune recognition, in this work we analyse whether MSCs influence the expression of HLA-I on cervical cancer cells (CeCa). For this purpose, tumor cells derived from CaSki cell line (HPV16+) were co-cultured for 96 h with MSCs derived from bone marrow- (BM-), normal cérvix- (NC-) and CeCa at ratio 1:1. In these experiments tumor cells cells were separated from MSCs by transwell chamber. After co-culture, CeCa cells were analyzed for HLA-I expression by flow cytometry and real time PCR. We observed that, HLA-I expression on CaSki cells diminished by 21.44%, 9.7% and 35.45% in the presence of BM- NC- and CeCa-MSCs respectively. Interestingly, the HLA-I downregulation on CeCa cells was strongly associated with a diminished expression of mRNA of the HLA class I heavy chain, mainly when CeCa cells were co-cultuted with CeCa-MSCs. In addition, conditioned media derived from CeCa/CeCa-MSCs cocultures were able to induce a strong HLA-I downregulation on CeCa cells. These results suggest that tumor-infiltrated MSCs may influence the immune evasion of tumor cells through the secretion of soluble factors that induce HLA-I downregulation. Support: CONACYT (84071,106591 and 87183); FIS-IMSS/PROT (762, 800 and 1014; DGAPA-PAPIIT (IN223010 and IN217013) grants.

P3.05.14
Effect of MBA on therapeutic protein immunogenicity

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Antibodies play a central role in prophylaxis and prevention of infectious agents. The advantages of passive immunization with animal antibodies are: rapid adjustment to new forms of pathogens, ability to provide a multi epitope mixture of antibodies, relatively low cost of research and development, simple and inexpensive production due to the very efficient yield of antibodies in large animals' serum or chickens' egg yolk. However, passive vaccination with animal antibodies comprises a major disadvantage: repeated injections from the same cross-species source can evoke a strong immune response in the treated individual against the antibodies themselves. Although conjugation of polyethylene glycol (PEG) to the antibodies can potentially reduce their immunogenicity, the reduction is inconsistent and may significantly harm antibody activity. In previous work we have shown that conjugation of mannosamine-biotin adduct (MBA) molecules to antibodies efficiently decreases their immunogenicity and does not impair antibody activity.
Since the MBA molecule masks the antibodies better than currently used molecules, and to improve the MBA molecule or design additional masking molecules, we are currently investigating the immunological mechanism by which MBA decreases the immune response. The research focused on mechanisms involved in modified protein degradation and presentation by antigen-presenting cells. This could provide a better understanding of immune response regulation/silencing of foreign antigens. In addition, masking antibodies may pave the way to a novel, inexpensive and accessible approach for the prevention and treatment of infectious diseases.

P3.05.15
Identification of gliadin peptides restricted by the HLA Class I B8 encoded by B*0801 allele in linkage with the celiac disease-associated DR3-DQ2 haplotype

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Initial studies associated the HLA Class-I A*01 and B*08 alleles with genetic susceptibility to celiac disease (CD), although further analysis showed a primary association with alleles encoding for HLA DQ2 molecules. Thus, because of the strong linkage disequilibrium of A*01 and B*08 with the DR3-DQ2 haplotype, a potential role of A1 and B8 molecules in CD pathogenesis should be revaluated. We screened 2 alpha-, 2 omega-, and 2 gamma-gliadin proteins using bioinformatic algorithms available from the IEDB (www.iedb.org) for the presence of peptides predicted to bind A*0101 and B*0801. The top 1% scoring 9- and 10-mer peptides were synthesized and tested in MHC-peptide binding assays using purified A*0101 and B*0801 molecules. 23/97 peptides with binding motifs to A*0101 or B*0801 bound purified molecules with high affinity (IC50<500nM). Of these, 20/23 (87%) bound B*0801, whilst 3/23 (13%) bound A*0101. Peptides were assayed singularly by γ-IFN-ELISPOT in fresh PBMCs from 24 celiac patients on gluten free diet, grouped according to the presence or absence of A1 or B8 alleles. Several HLA B8-binding gliadin peptides induced strong IFN-γ responses in at least two HLA B8positive patients. Immunogenicity of selected peptides was further assessed on short-term CTL obtained from PBMC of responsive celiac patients. In conclusion, gliadin contains peptides that activate HLA B8-restricted CD8 T cells, suggesting that HLA Class I alleles may contribute to the increased susceptibility to CD of subjects carrying the HLA-DR3-DQ2 haplotype. Our data support a role of adaptive CD8 T cells in CD pathogenesis.

P3.05.16
Identification of inducible lymphoid structures in the skin: a key site to elicit contact hypersensitivity responses

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Epicutaneous sensitization is established in the draining lymph nodes where dendritic cells (DCs) and T cells colocalize, however it remains unclear how the elicitation of cutaneous immune responses are efficiently induced in the skin.
To address this issue, we used murine contact hypersensitivity (CHS) as a delayed-type hypersensitivity model, which consists of the sensitization and elicitation phases. Initially, using CD11c-DTR (diphtheria toxin receptor) and Langerin-DTR knockin mice, we identified dermal DCs (dDCs), but not Langerhans cells, as a responsible DC subset for elicitation of CHS. This finding suggests that the dermis is the primary site for elicitation. Next, we visualized the dynamics of dDCs and memory T cells in the elicitation phase of CHS using two-photon microscopy, and found that dDCs accumulated in perivascular areas and interacted with skin-infiltrating memory T cells for several hours. This immunological synapse-like interaction was essential for memory T cells proliferation in situ in an antigen and integrin LFA-1 dependent manners. Intriguingly, dDC accumulation was abrogated by depletion of macrophages by means of LysM-DTR chimeric mice. Taken together, we have demonstrated for the first time that lymphoid structures are induced at the post capillary venules in the elicitation phase of CHS, and that this complex consists of perivascular macrophages, dDCs and memory T cells which interact via stable conjugated formation to induce efficient cutaneous acquired immune responses.

P3.05.17
Novel antigenic MHC class I peptides are generated and presented in the absence of tapasin

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Circulating CD8+ T cells recognize target cells by surveying the peptide MHC class I complex (pMHC I) on the surface, however the mechanisms of peptide processing yet remain to be clarified. Here we show that processing inside the endoplasmic reticulum (ER) profoundly affects the pMHC I repertoire formation and consequent T cell responses. In the absence of tapasin, a key component of the peptide loading complex (PLC), the repertoire is quantitatively and qualitatively altered, and the cells present a set of novel antigenic peptides that are never observed in wild type. Both induced antibody responses specific to tapasin-deficient cells and instability on the surface suggest the unique structure of novel antigenic pMHCIs. Indeed, a large-scale peptide sequencing by mass spectrometry reveals that the repertoire contains many unique peptides, which lose conventional anchor motives for MHC I binding. Our findings provide the molecular mechanism by which peptides are processed by PLC, and imply that such anchor-less peptides elicit CD8+ T cell responses to the cells with antigen processing defects.

P3.05.18
On the role of heparan sulfates in antigen cross-presentation

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We have previously shown that heparan sulfates (HS) can play a role in MHC class II restricted presentation of antigens (Ag) endowed with the ability to bind these sulfated polysaccharides. Furthermore, we showed that when such Ag are included in immune complexes, their MHC class II restricted presentation is increased up to 700 fold, demonstrating that HS can also contribute to receptor-mediated Ag class II restricted presentation (Léonetti and al., 2010). Here, we aimed to determine if HS also play a role in receptor-mediated Ag cross-presentation.

To investigate this aspect, we constructed three fusion proteins, which can bind immunoglobulins through their ZZ moiety and thus can target surface immunoglobulins. Two of them, called ZZOvaTat22-57 and ZZOvaDTR-BD can also bind HS while the third, called ZZOva, is devoid of this property. Cell binding experiments show that ZZOvaTat22-57 and ZZOvaDTR-BD can bind the Jaws II dendritic cell line, whereas ZZOva does not. Furthermore, in vitro studies show that ZZOvaTat22-57 and ZZOvaDTR-BD are 27 times more efficiently cross-presented than ZZOva. Then, to investigate whether HS can also influence cross-presentation mediated via other receptors, we complexed the three proteins with different anti-CD antibodies. We observed a synergistic effect when the Ag is targeted to the MHC class II molecule and has the capacity to bind HS.
Our results show that HS may positively influence different pathways of receptor-mediated Ag cross presentation. These findings might pave the way to a new generation of vaccine with improve T-cell stimulating properties.

P3.05.19
Porcine leukocyte antigen-derived peptides that are presented by human MHC molecules

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T cell-mediated xenorejection responses are reported to be mediated primarily by $CD4^+$ T cells, and the activation of xenospecific $CD4^+$ T cells occurs mostly from indirect recognition of xenoantigens. In indirect xenorecognition, human T cells are activated by porcine leukocyte antigen (SLA)-derived peptides that are processed and presented in the context of human MHC molecules on host antigen presenting cells. In an effort to design strategies to mitigate human CD4+ T cell-mediated xenorejection, we firstly identified HLA DR4-restricted immunogenic peptides derived from SLA. The peptide exhibiting the most potent binding capacity to HLA-DRB1*0401 *in vitro* was LRSWTAADTAAQISK. However, the peptide eliciting the most potent $CD4^+$ Th1 response in HLA-DRB1*0401-transgenic mouse was LRSWTAADTAAQISK, which differs only one amino acid at position 14. We also identified three SLA-derived HLA-A2-restricted peptides, and the peptide-specific CTL-inducing activities of the peptides were compared in HLA-A*0201-transgenic mice. Our findings have implications not only for the identification of an immunogenic indirect epitope derived from SLA molecules, but also for the development of epitope-specific immunoregulation strategies.

P3.05.20
A new a high throughput method to assess quantitative and qualitative differences in the antibody response

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The ideal vaccine is able to confer long-term protection, therefore not only it must induce high antibody titers, but these antibodies must be functional, that means able to bind the pathogen with high affinity and to neutralize it. Monitoring and understanding how vaccination or infection can modulate the antibody affinity maturation to bring to a most efficient response to a pathogen still remains a hard task. The quantitative evaluation of the antibody response after infection/vaccination is easily performed by conventional ELISA assays but can require high volume samples not always available. The methodologies currently used to evaluate antibody affinity (e.g Surface Plasmon Resonance; Quartz Crystal Microbalance; Solution Equilibrium Titration) are time consuming, no high-throughput, often not suitable for the analysis and characterization of very high affinity antibodies and polyclonal antibodies. In the present study we explore the possibility to evaluate affinity of monoclonal antibodies to specific antigens by analyzing the antigen-antibody reaction profiles obtained by Gyrolab® system, a technology that allows to perform miniaturized immunoassays in a high throughput manner. We describe the experimental protocol that would allow to determine in a high-throughput manner the affinity/avidity of antibodies specific for antigens of different nature (proteins, carbohydrates, glycoconjugates) or antigen portions (peptides, domains, fusions).

P3.05.21
Cooperative effects of ankylosing spondylitis-associated ERAP1 polymorphisms in the generation and destruction of HLA-B27 ligands

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The endoplasmic reticulum aminopeptidase 1 (ERAP1) is responsible for trimming antigenic peptide precursors before loading onto MHC class I molecules. Single nucleotide polymorphisms in ERAP1 have been associated with ankylosing spondylitis (AS), a disease strongly dependent on HLA-B27. It has been hypothesized that altered processing of the HLA-B27 peptidome by polymorphism in this enzyme contributes to this disease. Since natural ERAP1 variants are complex allotypes carrying multiple AS-associated polymorphisms, the contribution of individual changes and their possible interacting effects remain ill-defined. Thus, we analyzed the influence of two AS associated changes, K528R and D575N, in all possible combinations in the generation and destruction of HLA-B27 ligands from peptide precursors in vitro by mass spectrometry. Hydrolytic activity of the four variants towards a fluorogenic substrate showed that the relative effect of changes at one position was dependent on the nature of the residue at the other position, demonstrating their co-operative effect on ERAP1 activity. The four variants showed significant differences in their trimming of synthetic peptide precursors, and on the destruction of the HLA-B27 ligands generated from them. These differences were both variant and peptide-dependent. Surprisingly, since both generation and destruction of the natural epitopes were similarly affected by ERAP1 polymorphism, variants differing in activity often yielded comparable amounts of a given natural ligand. This suggests that alterations in the epitope generation/destruction balance by ERAP1 polymorphism might be limited to a subset of the HLA-B27 peptidome and the pathogenetic role of ERAP1 in AS is through their functional interaction with HLA-B27

P3.05.22
Citrullination of myelin antigens increases binding to Multiple Sclerosis associated MHC class II molecules and enhances T cell responses

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Our study aims at characterizing the adaptive immune response towards citrullinated myelin proteins in multiple sclerosis (MS). Specific T cell responses towards modified peptides were assessed for a role in disease progression. MS might appear from breakdown of immunological self-tolerance, induced by recognition of self-proteins that have undergone uncontrolled posttranslational modifications (PTM). Citrullination is a catalyzed process, whereby arginine is deiminated to citrulline. In MS, disease severity seems to follow the citrullination degree of myelin basic protein (MBP), T cell responses to citrullinated MBP are detected in MS patients, and citrullination of MBP elicits stronger Th1 responses in vitro. Given the high level of citrullinated MBP protein in MS brain tissue, a response to de novo formed citrullinated MBP-peptides could promote disease.
Stimulating PBMCs with a set of immunodominant citrullinated and non-citrullinated MBP- and MOG-peptide pairs revealed that 4 peptide pairs significantly modulated cytokine responses in MS patients compared to healthy controls. HLA-blocking during stimulation and peptide-binding studies on MHC class II molecules showed that these peptides were preferentially binding to MS associated DR molecules, with a stronger binding affinity to MHC for the citrullinated peptide versions.
Using such modified myelin peptides as antigens, we are able to study MHC class II peptide interactions and T-cell immunity, and we are provided with leads to study fine specificity and regulation of T-

and B-cell responses in different stages of disease development in humans.

P3.05.23
Thromboxane A2 controls primary T cell - DC interactions in vivo

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Thromboxane A2 (TXA2) is a prostanoid formed by thromboxane synthase. Macrophages and activated dendritic cells (DCs) produce TXA2, while the TXA2 receptor TP is expressed by naïve T lymphocytes. In vitro assays have shown that high doses of pharmacological TP agonists induce chemokinetic motility of T cells and thereby decrease non-cognate T cell-DC interactions. Its in vivo function in lymphoid tissue during the control of dynamic T cell-DC interactions with varying peptide-MHC density and affinity has remained elusive. Control and TP-deficient TCR transgenic CD4+ T cells were adoptively transferred into recipient mice prepared for intravital twophoton microscopy (2PM) scanning. We observed that, when activated DCs were presenting cognate peptide, the migration speed of peptide-specific CD4+ T cells was markedly reduced in the absence of TP expression as compared to wild type cells as a function of the antigenic dose. Thus, in presence of low pMHC density on DCs, the decrease of TP-deficient CD4+ T cell speed was more pronounced than at high pMHC densities. This reduction in T cell motility correlated with prolonged interactions times between TP-deficient CD4+ T cells and DCs and increased activation parameters. In contrast, TXA2 secretion by activated DCs without cognate pMHC did not affect T cell motility parameters irrespective of TP expression, indicating that TXA2 acts locally at the T cell-DC immunological synapse. In sum, our data identify TXA2-induced TP signaling as a negative regulator of T cell-DC interactions under conditions of low pMHC abundance, which may contribute to prevent excessive immune reactions and autoimmunity.

P3.05.24
Independent transport of MHC class II and H2-DM to processing compartments and convergence with molecules of distinct trafficking pathways

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$CD4^+$ T cells recognize peptides bound to MHC class II molecules (MHCII). Antigenic peptides presented by MHCII predominantly derive from exogenous proteins and are acquired in an endosomal/lysosomal compartment termed *MIIC*. For efficient peptide binding to MHCII, the lumen of *MIICs* also contain DM, a non-classical MHCII, which upon physical contact with an MHCII, relaxes its structure and releases the invariant chain-derived CLIP. For efficient MHCII-DM interaction certain APCs, such as B cells additionally express another non-classical MHCII, known as DO, which retains DM, preventing its binding to MHCII until the lysosomal pH reaches an optimal low. However, as the majority of dendritic cells (DC) fail to express DO, another mechanism must exist to keep DM from binding MHCII in DC. We examined this issue in mouse bone marrow-derived DCs differentiated for five days with GM-CSF, plus 24h in the presence of LPS. At time 0 (no LPS) most MHCII were intracellularly distributed in small Rab7+ and some Rab7+/Rab5+ vesicles, which upon addition of LPS progressively converged into large, pericentriolar Rab7+/Rab5+/Rab9+/Lamp2+ compartments, to eventually be transported to the periphery, associated only to Rab11 with or without the invariant chain. On the other hand, in early stages, DM was in small Rab5-/Rab7+ vesicles, which eventually converged with MHCII+/Rab7+/Rab5+/Rab9+/Lamp2+ compartments to remain there when MHCII had already departed for the cell surface. These results indicate that in some DC, DM and MHCII are kept physically appart until they arrive in the processing compartments.

P3.05.25
Ultrastructure of human autologous macrophage lymphocyte rosettes in Chagas disease

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The processing and antigen presentation are involved in human macrophage lymphocyte rosette (MLR) and in this phenomenon of multiple immune synapse (IS) the autologous antigens of senescent neutrophils are presented by endocitic way like phagocytosis of cells undergoing apoptosis naturally in the body. In Chagas disease cardiomyopathy *T. cruzi* is the etiologic agent but also autoimmune phenomena have been described and observed an increment in occurrence of this phenomenon and CD4 PAS-positive lymphocytes producing IFN gamma are involved. Material and methods: healthy human blood samples, anticoagulated with heparin (n = 10) and chagasic samples (n=6) (Blood Bank, UNC) Autologous cultures in TC199 medium (SIGMA, St. Louis, MO). Samples: 48, 72, 96 and 120 h. MLR technique. PAS technique. Samples of MLRs underwent electron microscopy. Results: MLRs of chagasic patients involved more lymphocytes. Significant differences were observed between PAS positive lymphocytes from MLRs ($p < 0.01$). In Chagas cultures were observed plasma cells and neutrophils up to 48 h. Ultrastructure: redistribution of mitochondria in lymphocytes of MLRs, to the IS area. We observed multivesicular, multilamellar and tubular structures in spatial organization of MIIC along time culture in macrophages. In lymphocytes were observed differences in arrangement of chromatin between healthy individuals and Chagas patients as in previous works. Is noted that in cytoplasm were observed double-membrane vesicles to a greater extent in Chagas patients, we postulate costimulator B7 molecules carriers and we are preparing further studies to immunostaining. In all cases observed exosomes, IS and MLR phenomenon greater degree in chagasic patients cultures.

P3.05.26
The non-classical class II genes of the chicken, DMA and DMB, are similar to those of mammals, but a second DMB gene (DMB1) is differentially expressed with unusual regulatory and structural features

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Although smaller, simpler and rearranged, the chicken major histocompatibility complex (MHC) contains most of the core antigen presentation genes found in the mammalian MHC. Strong associations of chicken MHC haplotypes with resistance to pathogens and response to vaccines are attributed to single dominantly-expressed MHC class I (BF) and class II (BL) molecules. The single dominant class I is the result of co-evolution with the closely-linked antigen processing genes TAP and tapasin, but the situation for class II is less clear. To facilitate investigation of whether single dominant class II expression could result from similar co-evolution with associated antigen presentation molecules, we characterised the chicken class II DM region. We show that chickens have three DM genes in their MHC, a single alpha chain gene DMA, and, unusually, two beta chain genes DMB1 and DMB2, of which DMB2 is dominantly-expressed. The chicken DM genes encode proteins with high structural and sequence homology to those described in mammals. However, the two beta chains differ substantially at the amino acid level and several features of DMB1 suggest an unusual function. We find DMB1 and DMB2 differentially expressed in tissues at the RNA and protein level, with DMB1 strongly expressed in intestinal epithelial cells, suggestive of a role in gut immunity. In pursuing the functional properties of the DMs and their interaction with the classical class II molecules, we aim to enhance our understanding of chicken MHC associations with immune responses and, more generally, illuminate mechanisms underlying class II evolution.

P3.05.27
Role of the external loops in the formation of antigenic structure of OmpF porin from *Yersinia pseudotuberculosis*

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Porins are the components of Gram-negative bacteria outer membrane (OM) playing a crucial role at the pathogen - host interrelations. Porins are the factors of microbial pathogenicity, participating in the forming of protective immunity at the same time. We have investigated the antigenic structure of the recombinant porin from OM of *Yersinia pseudotuberculosis*, the causative agents of pseudotuberculosis, using polyclonal antibodies against the full -sized OmpF porin (RP) and against the mutant porins with deletions of the some external loops of the protein (del1, del4, del6, del8). As result of refolding conducted by dialysis and ion-exchange chromatography, the recombinant proteins were obtained as trimers and were used for immunization. It is interesting to note the more immunogenicity of del4 and del6 porins for mice, compared with RP. Perhaps, the absence of the loop sites corresponding to L4 and L6 makes some of T-epitopes more "accessible" to form a ternary complex of antigen / T-receptor / MHC receptor. On the contrary, deletion of the loop L1 led to 50% loss of antigenic epitopes involved in the formation of the humoral immune response against the OmpF porin. Lack of loops L6 and L8 was found to have no effect on the structure of B-epitopes formation for both RP and the mutants, as soon as efficiency of antibody - antigens binding was similar. At the same time, deletions of the loops L1 and L4 caused the loss of 10% and 20% of antigenic epitopes, respectively.

P3.05.28
The wild-type hemochromatosis protein HFE inhibits MHC I antigen presentation

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MHC class I (MHC I) antigen presentation is an ubiquitous process by which cells present endogenous proteins to $CD8^+$ T lymphocytes during immune surveillance and response. Hereditary hemochromatosis protein, HFE, is involved in cellular iron uptake but, while structurally homologous to MHC I, is unable to bind peptides. However, increasing evidence points to a role for HFE in the immune system. In this study, we investigated the impact of HFE on MHC I antigen presentation. Using transient HFE transfection assays in a model of antigen presenting cells (293-A2/APC), we show that wild-type HFE, but not C282Y-mutated HFE, inhibits the secretion of macrophage inflammatory protein 1-beta (MIP-1β) from $CD8^+$ T lymphocyte clones generated to specifically-recognize selected antigens. Wild-type HFE expression also resulted in major decreases in $CD8^+$ T lymphocyte activation as measured by 4-1BB expression. We further demonstrate that inhibition of antigen recognition was maintained regardless of MHC I surface levels, β2-microglobulin competition, HFE ability to interact with transferrin receptor, antigen origin, or epitope affinity. We identified the α1-2 domains of HFE_{WT} as being responsible for inhibiting antigen recognition. However, recognition of externally peptide-pulsed 293-A2/APC remained uninhibited in presence of wild-type HFE, indicating that HFE may affect T cell recognition by interfering with intracellular antigen processing. Overall, our data point to a new physiological role for wild-type HFE in the MHC I antigen presentation pathway, which could modulate antigen immunogenicity and the cellular immune response.

P3.05.29
Carbon monoxide decreases endosome-lysosome fusion and inhibits soluble antigen presentation by dendritic cells to T cells

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Heme oxygenase-1 (HO-1) inhibits immune responses and inflammatory reactions via the catabolism of heme into carbon monoxide (CO), Fe^{2+} and biliverdin. We have previously shown that either induction or treatment with exogenous CO inhibits LPS-induced maturation in human dendritic cells (DCs). Here, we have evaluated the capacity of CO, to regulate antigen presentation on MHC-I and MHC-II molecules by murine DCs. We observed that CO treatment significantly inhibited the capacity of DCs to present soluble antigens to T cells on class I and class II MHC molecules. Inhibition was restricted to soluble OVA protein, as no inhibition was observed for antigenic OVA-derived peptides or bead-bound OVA protein. Further, CO failed to inhibit MHC-I presentation when DCs expressed OVA as an endogenous antigen, suggesting that inhibition was probably due to impairment of processing of internalized soluble antigens. Inhibition of antigen presentation was not due to reduced antigen uptake by DCs, as both endocytosis and phagocytosis remained functional after CO treatment. On the contrary, CO significantly reduced the efficiency of fusion between late endosomes and lysosomes and not by phagosomes and lysosomes. These data suggest that CO can inhibit the DC ability to present exogenous soluble antigens to both $CD8^+$ and $CD4^+$ T cells by blocking antigen processing at the level of late endosome-lysosome fusion.

P3.05.30
Brucella spp. lumazine synthase (BLS) vaccine carrier as a model to study antigen processing and persistance

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BLS is a highly immunogenic stable decamer, successfully used as a carrier for several proteins. BLS immunization activates murine dendritic cells (DC) and recruits DC, B cells, CD8+ and CD4+ cells at the draining lymph nodes (LN) via TLR4. BLS induces the cross-presentation of associated peptides and a TLR4-dependent specific cytotoxicity. Here, we show which cells BLS is associated with after immunization and its intracellular localization in vitro. Mice were immunized in the hind footpad with BLS-Fluorophore. At 4hs BLS was found in 30±4% of DC and in 10±2% of B lymphocytes at popliteal LN, and in 14±3% of the DC at the inguinal LN. At 120h BLS was still found within DC. In TLR4-deficient mice similar results were observed until 4hs; at longer times BLS was not observed. These results show that BLS resides for longer periods in DC and suggest that DC carry BLS to the inguinal LN. Subcellular cocalization of BLS was studied by confocal microscopy. Peritoneal macrophages (Mo) and BMDC were incubated with BLS-Fluorophore. Only minor colocalization was observed with classical antigen presentation markers. BLS colocalized with TLR4 at the cell membrane at 5min and with cytoplasmic TLR4 at 6h. BLS was observed at least until 6hs in BALB/c BMDC or Mo; in TLR4-deficient mice it was found only for 1h. These results suggest that BLS binds to TLR4 at the plasma membrane and subsequently enters to the cytoplasm. BLS would escape the endocytic pathway of antigen processing. BLS persistence is dependent on TLR4 signaling.

P3.05.31
Stable antigen is most effective for eliciting CD8+ T cell responses after DNA vaccination and infection with recombinant vaccinia virus in vivo

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The induction of strong CD8+ T-cell responses against infectious diseases and cancer has remained a major challenge. Depending on the source of antigen and the infectious agent, priming of CD8+ T cells requires direct and/or cross-presentation of antigenic peptides on major histocompatibility complex (MHC) class I molecules by professional antigen-presenting cells (APCs). However, both pathways show distinct preferences concerning antigen stability. Whereas direct presentation was shown to efficiently present peptides derived from rapidly degraded proteins, cross-presentation is dependent on long-lived antigen species. Here, we analyzed the impact of antigen stability on DNA vaccination and recombinant vaccinia virus (VV) infection using altered versions of the same antigen. The long-lived nucleoprotein (NP) of lymphocytic choriomeningitis virus (LCMV) can be targeted for degradation by N-terminal fusion to ubiquitin or, as we show here, to the ubiquitin-like modifier FAT10. Direct presentation by cells either transfected with NP-encoding plasmids or infected with recombinant VV in vitro was enhanced in the presence of short-lived antigens. In vivo, however, the highest induction of NP-specific CD8+ T-cell responses was achieved in the presence of long-lived NP. Our experiments provide evidence that targeting antigens for proteasomal degradation does not improve the immunogenicity of DNA vaccines and recombinant VVs. Rather, it is the long-lived antigen that is superior for the efficient activation of MHC class I-restricted immune responses in vivo. Hence, our results suggest a dominant role for antigen cross-priming in DNA vaccination and recombinant VV infection.

P3.05.32
The Mer Receptor Tyrosine Kinase promotes B-cell antigen processing through the IgD B-cell Receptor

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The Mer receptor tyrosine kinase facilitates macrophage and DC apoptotic-cell clearance and regulates immune tolerance. Mer may also be important in B-cell activation, as B cells in Mer-KO mice fail to develop autoantibodies when allo-activated by T cells. In vivo cross-linking of membrane IgD is a well-studied model of T-dependent B-cell activation. BCR cross-linking leads to antigen presentation to T cells, which then get activated and provide cognate help to a new set of B cells, resulting in a large IgG response. To test the role of Mer in the immune response to B-cell membrane IgD cross-linking, Mer-KO mice were given goat anti-mouse IgD intraperitoneally. Mer-KO mice had significantly lower IgE and IgG anti-goat IgG responses compared to B6 WT controls. In vivo cytokine capture assay (IVCCA) studies revealed decreased IL-4 but increased IFN-γ levels in Mer-KO mice. FACS and BrdU incorporation studies revealed comparable initial B-cell activation and proliferation between Mer-KO mice and WT controls, but subsequent T-cell proliferation and activation was arrested in Mer-KO mice. The antigen processing ability of B cells was estimated by using monoclonal anti-IgD antibody labeled with pHrodo-SE, a pH-sensitive fluorogen with increased fluorescence in acidic environments, i.e. lysosomes during antigen processing. Mer-KO B cells showed more than two-fold decreased antigen processing compared to WT B cells subjected to the same treatment. Taken together, Mer expression is required for optimal B-cell antigen processing. The mechanism may involve protein transport and microtubule rearrangement.

P3.05.33
Genetic Regulation of the Bovine Immune System and Implications for Health

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Diseases that affect dairy cattle contribute to economic loss with adverse implications for human and animal health and food safety. The inclusion of immune response (IR) traits in breeding objectives shows promise for improving inherent animal health. Dairy cows classified as High Immune Responders in herds across Canada and one large herd in the US have been found to have improved response to vaccination, increased milk and colostrum quality and a decreased occurrence of disease. The objective of this research was to perform a genome-wide association study to determine genetic profiles associated with enhanced antibody-mediated IR (AMIR). A total of 680 Holstein cows from 58 herds across Canada were immunized with a type 2 test antigen to measure serum antibody. High (n=81) and Low (n=82) AMIR cows were genotyped using the Illumina Bovine SNP50. A total of 198 SNP were significantly associated with AMIR. Of these, the majority (83%) were on chromosome 23, which contains the bovine Major Histocompatability Complex (MHC). Candidate genes within 250,000 base pairs of the significant SNPs included genes in the MHC, complement system and cytokines, including interleukin-17. Based on these genes, various biological pathways associated with AMIR were identified, including the antigen processing and presentation pathway, important in host defense. The significant genetic variation associated with AMIR in this study suggests that including AMIR in genomic selection could help decrease the incidence of disease in the dairy industry thereby minimizing the use of antibiotics, improving animal health, milk quality and food safety.

P3.05.34
The influence of MHCII haplotypes on T cell priming and disease development in a chronic model of rheumatoid arthritis

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The genetic component in rheumatoid arthritis is explained largely by MHCII genes. However, the priming of autoreactive T cells occurs prior to the clinical onset and is therefore poorly characterized. Pristane-induced arthritis (PIA) in the rat is a T cell driven chronic arthritis model, which is free from exogenous antigens and therefore ideal to study the effect of MHC-genes in an autoimmune context. We recently established a panel of five recombinant strains, which differ only in a 0.2 Mb region in the MHCII locus. Here we performed a comparative analysis of the development of PIA in these strains and show that this interval affects onset, progression and severity of early arthritis while it does not regulate chronicity. PIA was RT1-B (HLA-DQ) associated in all strain as indicated by the expression of MHCII and the reduction of acute and chronic PIA by blocking of RT1-B but not RT1-D (HLA-DR). The ligandome of both MHCII molecules was analyzed and multiple peptides from ubiquitous RA-related auto-antigens identified. The presence of haplotype-specific invariant chain-derived peptides on RT1-B correlated with disease protection, suggesting that an unedited MHCII peptide repertoire increases PIA susceptibility. Arthritis protection was further associated with an immature T cell phenotype, a more vigorous T cell proliferation and a Th17 bias. Moreover, neutralization of IFN-g, but not IL-17, before onset ameliorated disease in all strains. In contrast, the arthritis progression was strictly dependent on IL-17 but not IFN-g and neutralization of IL-17 was accompanied by a substantial reduction of neutrophils.

P3.05.35
Stem-cell transplantation restores CD8 T-cell immunity and corrects MHC class I deficiency

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Major Histocompatibility Complex class I (MHC class I) deficiency is a primary immunodeficiency disorder that is associated with aberrant CD8 T cell and natural killer cell (NK) development and function. Clinical presentations consist of recurrent bacterial infections, chronic inflammation and necrotizing, granulomatous skin lesions.
We analyzed a patient who was initially transplanted for an unknown immunodeficiency disorder associated with CD8 lymphopenia to evaluate if a distinctive MHC class I disorder was present.
Extended MHC class I investigations and NK evaluations were undertaken to determine the nature of the primary defect and its consequence upon immune reconstitution.
A marked reduction in MHC C and E alleles was noted in the individual with preservation of MHC class I expression in MHC A and B alleles. Novel flow cytometry for intracellular transporter associated with antigen processing (TAP) proteins demonstrated an absence of TAP1 and TAP2 consistent with MHC class I deficiency. Following a matched unrelated stem cell transplant there was clinical improvement associated with restoration of TAP 1 and TAP 2 expression in the hematopoietic lineage and CD8 T cell reconstitution. Pre transplant NK cells displayed a defect in recognition of MHC class I negative targets and a distinctive over expression of NKG2A and killer immunoglobulin like receptors (KIR). These defects partitioned post transplant with maintenance of hyporesponsiveness to MHC class I negative targets but correction of NKG2A and KIR expression.
Stem cell transplantation was successfully undertaken for MHC class I deficiency, correcting CD8 deficiency and some aspects of NK cell development.

P3.05.36
Systematic analysis of the antigen processing machinery in HPV-positive cell lines

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Clearance of human papillomavirus (HPV)-induced lesions is mediated by a cellular immune response, consisting of both cytotoxic T lymphocyte (CTL) and T helper (Th) cell responses. However, HPV has evolved multiple immune evasion strategies, among which modulation of expression of antigen processing machinery (APM) components directly interferes with T cell recognition of HPV-transformed cells.
Thus, we here aim to systematically analyze the expression of all APM components in a comprehensive collection of HPV-positive cells, including cervical and head and neck squamous carcinoma cell lines, as well as in three HPV-negative controls. Gene expression levels are determined by a quantitative real-time PCR screening, and protein levels by immunoblotting. To address the immunoproteasome and a feasible induction of APM component expression levels, all cells are analyzed with and without interferon (IFN)-gamma treatment.
We observed altered expression levels for some APM components in several HPV-positive cell lines compared to primary keratinocytes. However, transcriptional and translational downregulation of some components was reversed in an IFN-gamma-positive milieu.
Taken together, this study will provide a systematic appraisal of HPV-mediated APM changes, and if these could possibly be counteracted by IFN-gamma in the tumor microenvironment.

P3.06 Costimulation

P3.06.01
The effect of a high intensity interval exercise on the proliferative response of lymphocytes

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The exercise provides a challenge for the whole body homeostasis, and the immune system displays substantial disturbances in response to a single exercise session. In recent years, high intensity interval exercise (HIIE) has stained out as a modality of training. This study evaluated effect of a HIIE session on lymphocyte proliferative response in young sedentary men (n=6, 24.7 ± 3.5 yrs, BMI 21.5 ± 2.3 kg/m2 and VO2 max 34.2 ± 4.1 mLO2.kg-1.min-1). Volunteers performed a HIIE session, in a cycle ergometer, which consisted of 9 series of 1 min at 100% of peak power, with 75 seconds of active recovery, at 30W, between series. Venous blood was collected before, immediately after and 30 minutes after HIIE. Peripheral blood mononuclear cells of individuals were stained with Carboxyfluorescein Succinimidyl Ester (10mM) and stimulated with the Staphylococcus aureus enterotoxin B superantigen (SEB) (100 ng/mL) or PHA (0.001 mg/mL), for 5 days at 37°, 5% CO2. Cell proliferation analysis was performed using flow cytometry. Data was analyzed using One-way Anova, considering a=0.05. The proliferative response of lymphocytes to SEB stimulation was reduced immediately and 30 minutes after HIIE (P = 0.01), compared to rest values, although there was no effect of HIIE on the lymphocyte proliferative response to PHA stimulation (P = 0.25). HIIE reduced the super antigen-stimulated lymphocyte proliferation, what may affect the adaptive arm of the immune response. Mechanisms potentially involved on the modulation of lymphocyte proliferation response by HIIE, like cell activation and cytokine secretion, is under investigation.

P3.06.02
Regulatory T cells control Th1 priming by inhibiting the CD70/CD27 pathway

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Although naturally occurring Tregs are essential to prevent auto-immune disorders, the molecular basis of their suppressive function is still ill defined. Based on several observations showing that Tregs selectively control the development of Th1-type responses, we sought to determine the effect of suppression on the Th1-prone interleukin-12 and CD70 costimulatory molecule.
Our data show both in vivo and in vitro that Tregs inhibited the CD27/CD70 pathway, while sparing IL-12 production. Treg depletion in vivo resulted in increased CD70 expression on dendritic cells (DCs) and enhanced IFNγ production that was prevented by injection of neutralizing mAb to CD70. In vitro, Tregs inhibited CD70 expression on activated splenic DCs in a CD27-dependent manner. Surprisingly, CD70 downregulation correlated with intercellular transfer of intact CD27 from Tregs to DCs. Indeed, when cultured with retrovirally transduced T cells expressing a (intracellular) GFP- and (extracellular) HA- tagged CD27, DCs acquired both GFP and HA. The analysis of the CD27 and CD70 localisation by confocal microscopy using retrovirally transduced BMDCs (mCherry-CD70) and T cells (CD27-GFP) revealed the formation of tunneling nanotubes that spanned from DCs to T cells and supported vesicular traffic of CD27 to DCs in a CD70-dependent process. Whether both molecules are internalized and degraded in DCs remains to be determined.
Our data thus suggest a novel mechanism, in addition to CTLA-4 inhibition of CD80/CD86 costimulation, by which CD27+ Tregs might tune DC function by regulating the surface expression of CD70.

P3.06.03
XCR1 promotes the activation of CD8+ T lymphocytes by optimizing their interaction with CD8a+ dendritic cells.

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The main function of dendritic cells in the immune system is the detection of pathogens and the initiation of adaptive immune responses. Resident CD8α+ dendritic cells are highly efficient for cytotoxic T lymphocytes priming. Indeed, they are major producers of interleukin-12, a cytokine particularly important in the activation of CD8+ T lymphocytes. They are also able to load protein derived from exogenously antigens onto MHC class I molecules; a mechanism called cross-presentation. Moreover, this DC subset expresses specifically the chemokine receptor XCR1. With migrating CD103+ DC, CD8α+ DC are the unique murine cell types that express XCR1. The ligand of XCR1 is the XCL1 chemokine which is highly secreted by activated CD8+ T lymphocytes. CD8+ T cells activation was previously shown to be impaired in XCR1-deficient mice, but the underlying mechanism is unknown. Our results show that XCR1 is not involved in CD8α+ DC maturation but rather stabilizes their contacts with CD8+ T cells. Hence, XCR1 on CD8α+ DC promote the activation of CD8+ T cells by optimizing their interactions with CD8α+ DC. These results open new perspectives on the understanding of the role of XCR1 in the initiation of efficient CD8+ T cell responses by CD8α+ DC. This work received funding from Aix-Marseille Université (scholarship from the University President to S.G.), Association pour la Recherche sur le Cancer (ARC, funding to K.C. and M.D.) and the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013 Grant Agreement no. 281225 to M.D.).

P3.06.04
The Inducible Costimulator (ICOS) facilitates interleukin-4 synthesis through phosphoinositide 3-kinase signaling

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The Inducible Costimulator (ICOS) is a member of the CD28 family of costimulatory receptor that is highly expressed in follicular helper T cells (Tfh), a subset of CD4 T cells that migrate to the B cell zone and facilitate germinal center reaction. Although ICOS is known to play a critical role in shaping the Tfh population during immune reactions its role in the effector function of Tfh cells has been unclear. Here we demonstrate that ICOS assists TCR-mediated signal transduction by potentiating PI3K-Akt-mTOR signaling cascade that leads to hyperphosphorylation of the key translational regulators, p70S6K and 4E-BP1. Consistently, ICOS costimulation promotes the formation of polysomes on IL-4 mRNA in a PI3K-signaling dependent manner. Here we provide evidence that IL-4 production is diminished during T-B co-culture when ICOS-PI3K signaling axis is disrupted in T cells. Thus, ICOS-PI3K signaling facilitates translation of IL-4 mRNA and this may augment IL-4 production during T-B interaction in germinal centers.

P3.06.05
Discovery of a novel immune checkpoint regulator of the B7 family that negatively modulates T cell activation

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Using Compugen's discovery platform, CGEN15001T was recently identified as a novel member of the B7/CD28 protein family. Ectopic membrane expression of CGEN15001T in HEK293 cells resulted in inhibition of T cell activation. To further investigate CGEN15001T function, a fusion protein designated CGEN-15001 was produced, consisting of the extracellular domain of CGEN-15001T fused to IgG Fc. CGEN-15001 inhibited activation of murine and human naïve or effector T cells, reduced Th1/Th17 responses while promoting Th2 responses and secretion of anti-inflammatory cytokines, and enhanced iTregs differentiation. In vivo, CGEN-15001 demonstrated therapeutic effects in murine models of multiple sclerosis (RR-EAE) and rheumatoid arthritis (CIA). In the CIA model, administration of CGEN-15001 to mice with existing disease resulted in reduction of clinical score, joint swelling and histological score. Increase in the collagen-specific IgG1/IgG2a ratio was observed in the serum of CGEN-15001 treated mice, in line with the Th1/Th17 to Th2 shift observed in vitro and in the RR-EAE model. Furthermore, short term treatment of RR-EAE from onset of disease remission resulted in long lasting remission and inhibition of epitope spreading, suggesting induction of immune tolerance.
CGEN15001T is expressed in various cancers, including hematological, carcinomas, sarcomas and melanoma, in both tumor cells and subtypes of tumor infiltrating immune cells.
The immunomodulatory activity of CGEN-15001T and its expression in tumors support its role as immune checkpoint. The therapeutic efficacy in murine models of rheumatoid arthritis and multiple sclerosis support the therapeutic potential of CGEN-15001 for treatment of autoimmune diseases, by restoring and maintaining immune balance.

P3.06.06
Nucleic acid-mediated T cell activation through a novel sensing mechanism induces Th2 responses

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Recent studies including ours have shown that Toll-like receptors (TLRs) are functionally expressed not only on innate immune cells, but also on T cells, and some TLR ligands exhibit costimulatory functions of T cells upon TCR stimulation. However, we found that costimulation by TLR3 ligand poly(I:C) or TLR9 ligand CpG-DNA was independent of TLRs, whereas TLR2 ligands-mediated costimulation was dependent on TLRs. Surprisingly, the costimulation function by DNA was independent of CpG motif. Furthermore, we found that genomic DNA complexed with antimicrobial peptides, but not genomic DNA alone were internalized into naïve T cells and induced costimulatory responses. We demonstrate that this nucleic acid-mediated costimulation does not require all known nucleic acid sensors such as RIG-I-like receptors (RLRs) and STING-dependent cytosolic DNA sensors, ASC inflammasome. This response was mediated by enhancing the activation of NFAT and NF-κB. We also found that nucleic acid-mediated costimulation resulted in promoting IL-4 expression and suppressing IFN-γ expression directly on T cells in the absence of the involvement of any innate cells. Collectively, these results suggest an unexpected role for nucleic acid-mediated costimulation that promotes Th2 responses through a unique mechanism different from those in innate immune system.

P3.06.07
Semaphorin 4A regulates activation of CD8+ T cells

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Semaphorins are soluble and membrane-bound proteins originally identified as axon guidance factors which are involved in the development of the neuronal system. However, accumulating evidence indicates that several semaphorins, so-called 'immune semaphorins' are crucially involved in various phased of immune responses, that is, from the initiation to the effector phases of immune responses. Among immune emaphorins, Semaphorin 4A (Sema4A) has been shown to be expressed in dendritic cells, regulating helper T-cells. Indeed, Sema4A-dificient mice display dis-regulation of helper T-cells, resulting in developed atopic dermatitis and airway hypersensitivity. However, the involvement of Sema4A in CD8+ T-cells has not been determined. Here, we found predominant expression of Sema4A in CD8+ T-cells. In addition, Sema4A-deficient CD8+ T cells were hypo-responsive to TCR stimulation by anti-CD3, in which production of cytokines, such as IFN-g, TNF-a, and IL-2 was significantly impaired. Moreover, the induction of the T-box transcription factors, Eomesodermin (Eomes) and T-bet, which are required for effector and memory CTL differentiation of CD8+ T-cells, was also decreased. Collectively, our findings indicate an important role of Sema4A in activation and differentiation of CD8+ T-cells.

P3.06.08
CD28 costimulates the CD4+ T cell tolerogenic response to exogenous antigen in vivo

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The survival of the host critically depends on the appropriate immune response to harmless exogenous antigens versus dangerous pathogens. The reaction of CD4+ T cells under inflammatory conditions has been analyzed in detail in the past. In contrast, the tolerogenic CD4+ T cell response is much less characterized and has often been regarded as an incomplete form of the inflammatory reaction.
Using an adoptive transfer system we compared the tolerogenic and inflammatory responses of antigen-reactive CD4+ T cells in vivo using phenotypic, functional, and gene expression analyses. The results show that the T cell program which is elicited by pure antigen contains numerous elements of the inflammatory reaction (e.g. IL-2), but is overall a tolerance-specific program including surface molecules (e.g. RANKL, CD160, 4 1BB) and chemokines (e.g. CCL1), which do not participate in the inflammatory reaction.
Secretion of IL-2 in response to pure antigen raised the question about an involvement of CD28 in the tolerogenic response. Although CD28 strongly modulated only a limited number of genes, it had a critical influence on IL-2 secretion, cell division, and expansion of CD4+ T cells in the tolerogenic reaction, mirroring its rather selective but decisive influence in the inflammatory T cell response. Thus, CD28 not only acts as a potent T cell costimulator under inflammatory conditions, but also has a major role in the tolerance reaction. Our data extend the current paradigm of CD28 costimulation beyond its known key role in the inflammatory T cell response and the homeostasis of regulatory T cells.

P3.06.09
TIM-3 does not act as a receptor for galectin-9

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T cell immunoglobulin and mucin protein 3 (TIM-3) was originally identified as a marker for murine T helper type 1 cells. TIM-3 was found to negatively regulate murine T cell responses. Galectin-9 was described as a binding partner that mediates T cell inhibitory effects of TIM-3. Moreover, it was reported that like PD-1 the classical exhaustion marker, TIM-3 is up-regulated in exhausted murine and human T cells and TIM-3 blockade was described to restore the function of these T cells.
Here, we show that the activation of human T cells is not affected by the presence of galectin-9 or antibodies to TIM-3. Extensive studies on the interaction of galectin-9 with human and murine TIM-3 did not yield evidence for specific binding between these molecules. Moreover, profound differences were observed when analysing the expression of TIM-3 and PD-1 on T cells of HIV-1 infected individuals: TIM-3 was expressed on fewer cells and also at much lower levels. Furthermore, whereas PD-1 was preferentially expressed on CD45RA-CD8 T cells, the majority of TIM-3-expressing CD8 T cells were CD45RA+. Importantly, we found that TIM-3 antibodies were ineffective in increasing anti-HIV-1 T cell responses in vitro, whereas PD-L antibodies potently reverted the dysfunctional state of exhausted CD8 T cells.
Our results are not in support of an interaction between TIM-3 and galectin-9 and yield no evidence for a functional role of TIM-3 in human T cell activation. Moreover, our data indicate that PD-1, but not TIM-3 is a promising target to ameliorate T cell exhaustion.

P3.06.10
Endothelial-lymphocyte interaction causes proliferation and transient Foxp3 enrichment of CD4+ T lymphocytes

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Peripheral tolerance is regulated in part by suppressor cells of the $Foxp3^+$ regulatory T cell (Tregs) phenotype, failure of which leads to immune disorders. The vascular endothelium forms the barrier between the blood and tissue but actively recruits and activates immune cells. Previously murine $CD4^+$ T lymphocytes were shown to be activated via interaction with endothelial cells (ECs) causing Treg induction. Using human ECs and $CD4^+$ T lymphocytes we now show endothelium dependent $CD4^+$ proliferation and transient $CD25^+$ $Foxp3^+$ expression.
$CD4^+$ T lymphocytes were co-cultured with naïve or TNF or IFNg activated human ECs, in the presence of phytohaemagglutinin (PHA) or anti-CD3/28 antibodies. After 72 or 120 hours, lymphocyte proliferation was assessed by CFSE dilution and Treg induction was determined using CD25, CD127 and Foxp3 expression using flow cytometry.
$CD4^+$ cells only proliferated in the presence of EC or when combined with EC - PHA or EC - CD3/28. This proliferation was not enhanced when the EC were activated with TNF or IFNg. At 72h, 80-90 % of the proliferated cells expressed $Foxp3^+$. By 120h this dropped to 20-30 % despite further proliferation. $Foxp3^+$ expression was not changed following activation of the EC with TNF or IFNg.
ECs act as accessory cells for $CD4^+$ proliferation, enriching the population of $Foxp3^+$ cells. The transient expression of $Foxp3^+$ however may show further regulation by EC on T cell phenotype. This potential induction of Tregs points to the EC-T cell interaction as a novel target for Treg control in inflammation.

P3.06.11
Setting an intrinsic brake on T cell division number: Roles for TCR affinity and IL-2 concentration

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Kinetic mechanisms mediate the selective expansion of high-affinity T-cells during an immune response. Such processes include changes in time to first division but not times through subsequent divisions, which are typically constant. The proportion of cells that survive through each division also alters with stimulation affinity and is thought to be a dominant form of affinity-based regulation. Here we investigate an additional mechanism where the number of divisions that a T-cell undergoes following stimulation is 'programmed' and, as a consequence, higher affinity cells progress further and come to dominate the response.
We first quantified the effect of TCR-Ag affinity on T-cell division progression and survival by tracking proliferation of wild-type or Bim-deficient OT-I T-cells stimulated with a range of different affinity OVA peptides. We found high-affinity T-cells underwent greater expansion even when survival was enforced by Bim deficiency. This difference in expansion between affinities was traced to differences in the maximum number of divisions the T-cells were able to undergo before reverting to the quiescent state. We examined other T-cell stimuli and identified IL-2 as a potent modulator of the T-cell division limit, contributing towards selective expansion. By progressively subculturing cells *in vitro*, and applying mathematical modeling we determined a typical dose response between IL-2 concentration and the average division number that ranged from ~2 to over 12 divisions.
Although we cannot exclude a role for survival in mediating selective expansion, these results show that the programmed division capacity of T-cells plays an important survival-independent role in regulating selective expansion.

P3.06.12
Role of metalloprotease ADAM17 in regulating ICOSL-mediated humoral immune response

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Immune cells regulate cell surface receptor expression during their maturation/activation. Although many of these receptors are regulated largely at the level of expression, protease mediated ectodomain shedding represents an alternative means of refashioning the surface of immune cells. Shedding is largely attributed to a family of ADAM metalloproteases, including ADAM17 also known as TACE (TNF-α converting enzyme). Although ADAM17 is well known to contribute to innate immune response by releasing diverse membrane proteins, such as cytokines (TNF-α), cytokine receptors (TNF-R1, TNF-R2, IL-6R) and adhesion molecules (L-selectin), much less is known whether/how this metalloprotease regulates adaptive immunity. To determine if ADAM17 contributes to regulating adaptive immune responses we took advantage of ADAM17 hypomorphic (ex/ex) mice, in which ADAM17 gene and protein expression is reduced by 90-95% as compared to WT littermates. Here we show that B cells express more activated ADAM17 compared to T cells and that ADAM17 deficiency results in spleen and lymph node enlargement, as well as increased levels of Ag-specific class-switched immunoglobulin production following immunization with ovalbumin. Moreover, we demonstrate that impaired down-regulation of surface levels of co-stimulatory molecule ICOSL in ADAM17 ex/ex mice might contribute to the development of exaggerated Ab responses. Therefore, our data suggest a functional link between ADAM17 and ICOSL in controlling adaptive immune responses.

P3.06.13
IL-35 is induced in human T cells upon co-stimulation via CD43 and PD-1

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Regulatory T cells (Treg) are not only crucial for the maintenance of immunological self-tolerance but are key mediators of infectious tolerance. IL-35, a member of IL-12 family is an inhibitory cytokine associated with Treg. Recently, we have shown that human rhinovirus (HRV) induces IL-35 producing Treg via up-regulation of B7-H1 (CD274) and sialoadhesin (CD169) on rhinovirus treated DCs (R-DC) as one of the immune evasion mechanisms. Thus, a combined set of co-stimulatory signals for T cells seems to be critical to induce IL-35. To further elucidate the underlying mechanism, the T cells isolated from human peripheral blood, were stimulated with a panel of plate-bound monoclonal antibodies (mAbs) against putative accessory T cell surface receptors. The IL-35 induction was analyzed by qPCR, intracellular staining and by sandwich ELISA. Among the various combination tested, co-engagement of CD3 and CD43 along with agonist anti-PD-1 mAb was identified to most potently induce IL-35, compared to the other prominent T cell activation pathways. However, upon stimulation via CD3+CD43+PD-1 the T cell signature cytokine profile (IL-2, IL-4, IL-10, IL-17, IL-22, IFNγ) remained unaltered compared to the other T cell activation pathways.
Though the primary effect of IL-35 is suppression of T cell proliferation, our data suggests that mechanism of suppression by such IL-35+Treg is rather dependent on the presence of antigen presenting cells (APC). Thus, our findings identify a novel pathway to induce immune-inhibitory T cells.

P3.06.14
High throughput generation of antibody secreting B-cell clones from peripheral blood of rabbits

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We have developed a robust high throughput B-cell cultivation method using peripheral rabbit B cells. This rapid procedure generates a diverse set of antibodies using blood of immunized rabbits. The entire workflow includes (i) the identification and isolation of single B cells from rabbit blood expressing IgG antibodies, (ii) an elaborate short term B-cell cultivation to produce sufficient monoclonal antigen specific IgG for comprehensive phenotype screens, (iii) the isolation of VH and VL coding regions via PCR from B-cell clones producing antigen specific and functional antibodies followed by the sequence determination, and (v) the recombinant expression and purification of IgG antibodies. Rabbits immunized with the human IL1RL1 antigen (also known as ST2, DER4, FIT-1, IL33R, ST2L, ST2V, T1) were used for the proof-of-concept study. The IL33-IL1RL1 ligand-receptor system plays an important role in autoinflammatory diseases such as asthma, ulcerative colitis or arthritis. The fully integrated and to a large degree automated platform yielded clonal and very diverse IL1RL1-specific antibodies. Due to the high IgG productivity of the cultivated B-cell clones the IL1RL1-inhibiting IgGs from individual animals could be identified already during primary screening, thus substantially lowering the workload for the subsequent B-cell PCR workflow. In summary, this powerful B-cell cultivation method has proven to be an efficient and robust tool for the rapid generation of antigen specific and functional monoclonal rabbit antibodies without sacrificing the immunized animal.

P3.06.15
Co-stimulatory effects of PD-1 in sublingual mucosa-mediated CD4$^+$ T cell responses

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Objective. PD-1 is a co-inhibitory receptor on T cells that binds two ligands, B7-H1 and B7-DC. The B7-H1:PD-1 pathway is involved in peripheral tolerance, especially at the local inflammatory responses. At the same time, co-stimulatory effects of either B7-H1 or B7-DC have been reported. The sublingual mucosa has been used as a route for sublingual immunotherapy and sublingual vaccination, which induce opposite immune responses. The mechanisms by which sublingual mucosa-mediated immune responses are controlled are unclear. In this study, we investigated the involvement of PD-1 in sublingual mucosa-mediated antigen-specific CD4$^+$ T cell responses.
Methods. Cell tracking dye-labeled DO11.10 T cells were transferred into RAG2KO or SCID mice, OVA was applied onto sublingual mucosa with or without a mucosal adjuvant, cholera toxin (CT), and then CD4$^+$ T cell responses were measured.
Results. Sublingual OVA-painting induced antigen-specific CD4$^+$ T cell proliferation of regional lymph nodes. CT did not clearly affect the ratios of dividing cells, but enhanced the ratios of IFN-γ-producing cells. PD-1 blockade using anti-PD-1 mAb or the use of PD-1-deficient DO11.10 T cells markedly inhibited the T cell proliferation and these were especially obvious in the presence of CT. PD-1 was comparably induced with the increasing of cell division. Induction of B7-H1 on sublingual mucosal dendritic cells (DCs) at the OVA-painted sites was markedly inhibited and MHC class II expression was reversely enhanced.
Conclusions. Our results suggest a novel co-stimulatory function of PD-1 in sublingual mucosa-mediated CD4$^+$ T cell responses.

P3.06.16
Combining molecular adjuvant or a sub-immunizing dose of inactivated Foot and Mouth Disease Virus (FMDV) with plasmids encoding P12A3C or VP1 protein enhances DNA vaccine potency

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In this study we evaluated the ability to induce protective immune responses using formulations including plasmids encoding the secreted form of VP1 protein (pVP1s) or the polyprotein P12A3C (pP1)of FMDV in combination or not with pCD40L and pIL15 or a sub-immunizing dose of inactivated FMDV (iFMDV).
BALB/c mice were inoculated id with pP1 or pVP1s vaccines in combination or not with a chemical adjuvant and one sub-immunizing dose of iFMDV. Negative controls were inoculated with empty plasmids, or adjuvant. No difference in antibody titers was found among the pP1 groups. On day 7 after the last immunization, vaccinated mice were challenged with $10^{4.5}$ TCID50 of FMDV. 75% and 50 % of animals were protected when received pP12A3C and pIL15 or pCD40L and the chemical adjuvant without a sub-immunizing dose of iFMDV), respectively. Animals vaccinated with pVP1s alone or with adjuvant with a single sub-immunizing dose of iFMDV enhanced dramatically the antibodies titers and were protected against the viral challenge, while negative controls were infected. Our results demonstrated that pP1vaccines were effective at priming and suggest that IL15 and CD40L can contribute to immune-enhancement and protection against FMDV challenge. Besides, an inoculation with pVP1 or pP1 and a sub-immunizing dose of iFMDV leads to complete protection and consequently may represent a useful strategy for improving the potency and efficacy of FMDV-DNA vaccines.

P3.06.17
The impact of CD27 signaling on Wnt-mediated T cell expansion

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The costimulatory molecule CD27 and its unique ligand CD70 are considerably involved in adaptive immunity. CD27 belongs to the tumor necrosis factor receptor (TNFR) family and is expressed on human and murine T, B and NK cells and hematopoietic stem cells. CD70 expression is restricted to activated lymphocytes and mature dendritic cells upon immune activation. Triggering of CD27 on T cells promotes T cell survival, expansion and differentiation. Whereas CD27-mediated T cell survival mechanisms have been studied for some time now, the way by which CD27 stimulation increases T cell proliferation is less well understood. We recently demonstrated that the CD70-CD27 interaction stimulates leukemia stem cell proliferation by increasing Wnt signaling via TNFR-associated factor 2 (TRAF2) and NCK-interacting protein kinase (TNIK).
We now hypothesized that CD27 may have an impact on T cell expansion via the Wnt pathway as well. To investigate a possible link between CD27 and Wnt signaling in T cells, we activated human CD8+ T cells in the presence or absence of inhibitory anti-CD27 monoclonal antibody. Proliferation, TNIK and b-catenin localization, Wnt-specific transcription factor activity and Wnt target gene mRNA levels were analyzed.
Blocking CD27 signaling attenuated nuclear localization of TNIK and b-catenin, resulting in reduced activation of the Wnt pathway. We propose that CD27 signaling promotes T cell proliferation by activating the Wnt pathway via TRAF2 and TNIK. Therefore, targeting the CD27-TRAF2-TNIK-b-catenin signaling axis may offer an attractive strategy to modulate CD8+ T cell responses in infections and autoimmunity.

P3.06.18
Constitutively active STAT3 acts as a tolerogenic factor in human CD4+ T-cells

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Purpose/Objective:
The signal transducer and activator of transcription 3 (STAT3) is a key integrator of signals provided by a multitude of cytokines and factors. Yet, the role of STAT3 in human CD4+ T-cells is not well established. Current research indicates Th17-inducing but also tolerogenic roles. We assessed the effects of STAT3 activation in human T-cells.
Materials and Methods:
A constitutively active mutant of STAT3 (STAT3C) was generated and cloned into an IRES-GFP harboring retroviral vector. Upon transduction into peripheral blood CD4+ T-cells phenotype, cytokine secretion, proliferation and suppressive potential in response to qualitatively different stimuli was analyzed.
Results:
STAT3C+ T-cells showed clear-cut transgene expression and phenotypically displayed an effector T-cell phenotype in regard to the characteristic regulatory T-cell markers CD25, CD39, CD127 and CTLA-4. Following anti-CD3 stimulation in concert with costimulatory signals via either CD80-CD28 or CD58-CD2, STAT3C+ T-cells were hyporesponsive and secreted a distinct cytokine profile. This included a significant reduction of IL-2, IL-13, IFN-γ and TNF-α secretion and a four-fold increase in IL-10 production. In co-cultures with CD4+ responder T-cells, STAT3C+ T-cells showed significant suppressive capacity following CD58 co-stimulation or moderate anti-CD28 co-stimulation, while supra-physiological levels of anti-CD28 costimulation abrogated this suppressive capacity. Suppression was contact-dependent but independent of IL-10 and TGF-β.
Conclusions:
Our data provide a clear-cut relation between activation of STAT3 and the acquisition of an anti-inflammatory cytokine profile and suppressive function. Consequently, the main function of STAT3 in

human CD4+ T-cells seems to be the regulation of tolerogenic processes.

P3.06.19
Adapters TRIM and LAX regulate transport and surface expression of CTLA-4 via Rab8 vesicles

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CTLA-4 potently inhibits T-cell activation, and yet primarily resides in intracellular compartments such as the *trans*-Golgi network (TGN). Little is known regarding the mechanism(s) by which the co-receptor is shuttled to the cell surface. We previously showed that the type I transmembrane adapter T-cell receptor interacting molecule (TRIM) facilitates the formation of CTLA-4 containing vesicles for transport to the cell surface. In this study, we demonstrate that the related transmembrane adapter LAX (linker for activation of X cells) binds and coordinates with TRIM and Rab8 the transport of CTLA-4 from the TGN to the cell surface. LAX co-localized with TRIM and CTLA-4 in Rab8 positive vesicles and bound to the small GTPase Rab8 via its N terminal region. Both adapters promoted the reorientation of the microtubule-organizing center (MTOC) to the site of TCR engagement for polarized CTLA-4 release, while disruption of LAX/Rab8 binding or down-regulation of Rab8 substantially reduced CTLA-4 surface expression. These findings suggest that the multimeric complex CTLA-4/TRIM/LAX, with LAX binding to Rab8, cooperates to mediate the transport of CTLA-4 to the cell surface resulting in inhibition of T-cell responses. In addition to uncovering a new coordinator of CTLA-4 surface expression on T-cells, our results suggest a novel function for type 1 transmembrane proteins in the intracellular transport of receptors to the cell surface.
This work was supported by the Wellcome Trust and BBSRC.

P3.06.20
Signaling through Flt3 on activated splenic B-cells drives plasma cell differentiation

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The tyrosine kinase receptor Flt3R plays an important role in early B-cell development. Mice lacking either the Flt3R or its ligand (FL) have reduced numbers of pre-B-cells in the bone marrow (BM) but normal mature B-cell numbers in the periphery and normal levels of serum Igs. Although Flt3R is down-regulated on pre-B-cells, this receptor reappears on activated B-cells in the spleen where its function is still unknown. In the present study we investigate how Flt3 signaling on activated B-cell affects terminal differentiation and antibody production.
Mice deficient for FL (FLKO) and their wild type (WT) counterparts were immunized against a T-cell dependent antigen (mBSA). FLKO mice were found to have an increased frequency of splenic Flt3+ B-cells, a population showing increased expression of MHCII, CD80, CD86 and CD40 when compared to Flt3- B-cells. Interestingly, the frequency of plasma cells was significantly increased in both spleen and BM of FLKO mice compared to WT, which was associated with an increased serum level of IgM and increased expression of the plasma cell associated genes IRF4, Blimp1 and XBP1. Although, serum levels IgG antibodies were not altered after immunization, FLKO mice failed to produce high-affinity IgG1 antibodies against mBSA.
We show that Flt3 expression on splenic B-cells define an activated B-cell population. Increased Flt3+ B-cells in FLKO mice are accompanied by a higher frequency of plasma cells and increased IgM production, but FLKO mice fail to produce high affinity IgG1 antibodies.
We propose that functional Flt3 signaling is essential for plasma cell differentiation.

P3.06.21
PD-1 blockage reverses immune dysfunction and hepatitis B viral persistence in a mouse animal model

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Persistent hepatitis B viral (HBV) infection results in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Recent studies in animal models of viral infection indicate that the interaction between the inhibitory receptor, programmed death (PD)-1, on lymphocytes and its ligand (PD-L1) play a critical role in T-cell exhaustion by inducing T-cell inactivation. High PD-1 expression levels by peripheral T-lymphocytes and the possibility of improving T-cell function by blocking PD-1-mediated signaling confirm the importance of this inhibitory pathway in inducing T-cell exhaustion. We studied T-cell exhaustion and the effects of PD-1 and PD-L1 blockade on intrahepatic infiltrating T-cells in our recently developed mouse model of HBV persistence. In this mouse animal model, we demonstrated that there were increased intrahepatic PD-1-expressing CD8+ T-cells and increased CD4+ FoxP3+ T cells in mice with HBV persistence, but PD-1 upregulation was resolved in mice which had cleared HBV. The Intrahepatic CD8+ T-cells expressed higher levels of PD-1 and lower levels of CD127 in mice with HBV persistence. Blockade of PD-1/PD-L1 interactions increased HBcAg-specific interferon (IFN)-γ production in intrahepatic T lymphocytes. Furthermore, blocking the interaction of PD-1 with PD-L1 by an anti-PD-1 monoclonal antibody (mAb) reversed the exhausted phenotype in intrahepatic T lymphocytes and viral persistence to clearance of HBV *in vivo*. Our results indicated that PD-1 blockage reverses immune dysfunction and viral persistence of HBV infection in a mouse animal model, suggesting that the anti-PD-1 mAb might be a good therapeutic candidate for chronic HBV infection.

P3.06.22
Convergent and divergent effects of costimulatory molecules in conventional and regulatory CD4+ T cells

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Costimulatory molecules of the CD28 family on T lymphocytes integrate cues from innate immune system sensors and modulate activation responses in conventional CD4(+) T cells (Tconv) and their FoxP3(+) regulatory counterparts (Treg). To better understand how costimulatory and coinhibitory signals might be integrated, we profiled the changes in gene expression elicited in the hours and days after engagement of Treg and Tconv by anti-CD3 and either anti-CD28, -CTLA4, -ICOS, -PD1, -BTLA, or -CD80. In Tconv, a shared "main response" was induced by CD28, ICOS, and, surprisingly, BTLA and CD80, with very limited CD28-specific (primarily Il2) or ICOS-specific elements (including Th1 and Th2 but not the follicular T signature). CTLA4 and PD1 had a very subtle impact in this system, similarly inhibiting the response to anti-CD3. Treg responded to the same costimulatory hierarchy and to the same extent as Tconv, but inducing different clusters of genes. In this reductionist system, costimulatory or coinhibitory engagement mainly elicits generic responses, suggesting that the variability of their effects in vivo result from temporal or anatomical differences in their engagement, rather than from inherently different wiring.

P3.06.23
The immunomodulatory potency of interleukin 2 applied as 'natural adjuvant' bound to virus-like nanoparticles is critically influenced by its membrane-anchor characteristics

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Decoration of virus-like nanoparticles (VNP) with functionally active, immunomodulatory molecules enhances particle immunogenicity and promotes selective immune recognition of the antigens they bear. In this study we investigated the influence of membrane characteristics of artificially membrane-bound cytokines. One (1Ig), two (2Ig) or four (4Ig) immunoglobulin(Ig)-like domains of CD16b were inserted between the model cytokine Interleukin 2 (IL-2) and the minimal GPI-anchor acceptor sequence of CD16b (GPI). We compared targeting of IL-2 fused to different membrane-anchors to lipid rafts of producer cells and VNP and tested the influence of the membrane-anchor on the biological activity and co-stimulatory potency in antigen-specific and non-specific assays. We identified a membrane-anchor - 2IgGPI - conferring an up to tenfold increase of targeting of IL-2 onto VNP, when compared to the minimal GPI-anchor acceptor sequence. When co-expressed on VNP with H-2D^{b} presenting the lymphocytic choriomeningitis virus glycoprotein peptide 33-41 (LCMV-GP$_{33-41}$), as antigen-specific signal 1, IL-2::2IgGPI was superior to IL-2::GPI to co-stimulate proliferation and accumulation of primary LCMV-GP-specific P14 TCR transgenic T-cells *in vitro.* Correspondingly, IL-2::2IgGPI induced increased numbers of terminally differentiated CD25^{high}CD127^{neg}KLRG1^{pos} effector cells with potent effector functions (IFNγ production and cytotoxic activity) when compared to IL-2::GPI. In contrast, co-stimulation with IL-2::GPI rather favored formation of CD25^{lo}CD127^{+}KLRG1^{-} memory precursor cells. Corresponding loss-of-function variants of IL-2 induced consistently less pronounced T-cell proliferation and differentiation. Artificially membrane-anchored cytokines decorating VNP used as convenient immunization platform might represent a novel interesting tool to shape immune responses.
Support: Christian Doppler Society, Austrian Science Fund-SFB 4609-B19 & Biomay AG.

P3.07 B cell development and plasma cell differentiation

P3.07.01
Differential expression of CD21 in different species

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All mature B cells in humans and mice are CD21 positive until B cells become plasma cells. This expression profile closely matches the role of CD21 in enhancing of BCR signaling, involution of CD21 in B cell responses and antigen presentation, and the promotion of B cell survival. However, studies in sheep show two populations of mature B cells that differ in expression of CD21. These CD21^{-} and CD21^{+} mature B cell populations were shown to have distinct recirculation characteristics, and phenotype or tissue distributions. Some reports in pigs also indicate that porcine mature B cells could express CD21 differentially. For these reasons we have analyzed expression profile of three mAbs IAH-CC51, BB6-11C9.6 and B-Ly4 that are routinely used to detect porcine CD21 on the surface of B lymphocytes. Cross-reactive studies show that CD21 is always present on the surface of all mature B cells but can be expressed in at least two differential forms. We used IAH CC51 mAb that recognize those forms together with anti-CD2 to define four subpopulations of B cells. Ontogenetic and in vitro culture studies, analysis of cell size, expression of CD11b and class-switched phenotype together with measurement of proliferation and cell death, revealed that these subsets represent functionally distinct populations of B cells. Phenotypic and functional features collectively suggest that distinct subsets of mature B cells can express differential forms of CD21, which can be significant for their function. This work was supported by Czech Science Foundation grant P502/10/0038.

P3.07.02
Immunologic Alterations in Long-Term Divers

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Diving could encounter the immune system of divers due to long term stressors and disasters. This study was designed to understand comprehensive effect of diving on the cellular and humoral components of immune system in a number of persons with long-term exposures. The cross-sectional study was developed on 25-male-expert divers. The immunologic parameters were compared in these individuals with 25 sex-age-matched healthy controls. Serum levels of IgA ($p<0.001$) and IgM ($p=0.003$) were significantly decreased in divers compared to the controls; while the levels of IgG and IgE ($p<0.001$) were significantly increased. Absolute count of B cells was significantly lower in the divers group, compared to the controls ($p<0.001$). Specific-antibody-responses of divers was also defective ($p<0.01$). An increased activity of classic pathway of complement components was detected in the divers ($p<0.001$). Absolute count of natural killer cells in the divers was also significantly higher than the controls ($p<0.001$). Humoral, cellular and complement components of the immune system could be involved during long-term diving.

P3.07.03
Class Switch Recombination Process in Common Variable Immunodeficiency

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Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by hypogammaglobulinemia and recurrent bacterial infections. Impaired antibody production in these patients is due to defect in differentiation of naïve B cell into plasma cells. Class switch recombination (CSR) which plays a critical role in the production of different Immunoglobulin classes may be defective in a group of CVID patients. The aim of this study was to investigate the CSR process of CVID patient's B lymphocytes, by evaluating the expression of IgE mRNA and protein in an IgE inductive medium. Peripheral blood mononuclear cells (PBMCs) from twenty nine CVID patients and 21 healthy controls were isolated and cultured in the presence of rhIL-4 and CD40L. IgE mRNA and IgE protein were measured by RT-PCR and ELISA techniques, respectively. Normal production of IgE mRNA was recorded in 23 out of 29 patients (79.31%) as well as all controls; while the remaining 6 patients (20.69%) were unable to express IgE mRNA indicating a defect in CSR. PBMCs of 16 out of 29 patients (55.2%) could not produce normal values of IgE comparing with controls after stimulation by IL-4 and CD40L. IgE production is impaired in most of the CVID patients. Defects in post CSR (IgE mRNA transcription) processes such as protein production and secretion can be the causative mechanism of CVID in patients with normal mRNA and impaired protein productions. Determination of these defects can help to clarify the various underlying mechanisms in etiology of CVID.

P3.07.04
Class Switch Recombination Process in Common Variable Immunodeficiency

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Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by hypogammaglobulinemia and recurrent bacterial infections. Impaired antibody production in these patients is due to defect in differentiation of naïve B cell into plasma cells. Class switch recombination (CSR) which plays a critical role in the production of different Immunoglobulin classes may be defective in a group of CVID patients. The aim of this study was to investigate the CSR process of CVID patient's B lymphocytes, by evaluating the expression of IgE mRNA and protein in an IgE inductive medium. Peripheral blood mononuclear cells (PBMCs) from twenty nine CVID patients and 21 healthy controls were isolated and cultured in the presence of rhIL-4 and CD40L. IgE mRNA and IgE protein were measured by RT-PCR and ELISA techniques, respectively. Normal production of IgE mRNA was recorded in 23 out of 29 patients (79.31%) as well as all controls; while the remaining 6 patients (20.69%) were unable to express IgE mRNA indicating a defect in CSR. PBMCs of 16 out of 29 patients (55.2%) could not produce normal values of IgE comparing with controls after stimulation by IL-4 and CD40L. IgE production is impaired in most of the CVID patients. Defects in post CSR (IgE mRNA transcription) processes such as protein production and secretion can be the causative mechanism of CVID in patients with normal mRNA and impaired protein productions. Determination of these defects can help to clarify the various underlying mechanisms in etiology of CVID.

P3.07.05
Allelically included B cells with two specificities are eliminated in the periphery

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The basis for monospecificity of B cells is allelic exclusion of immunoglobulin (Ig) heavy (H) and light (L) chain genes during early differentiation. Allelic exclusion at the IgH locus requires expression of the pre-B cell receptor containing the *mu* chain and an in-frame ("productive") VDJ rearrangement from one of the two IgH alleles inhibiting further IgH gene recombination.
We investigated to which extent mature human B cells carrying a frame-shift mutation in exon 1 of the *mu* gene (*mu*CH1) express productive VDJ-CH1 transcripts originating from the mutant allele. According to the feedback inhibition model of allelic exclusion, 12 % of these B cells may carry two productive VDJ rearrangements -one that has been expressed as VDJ-Ig*mu*CH1 but failed to assemble into a surface-expressed B cell receptor due to the mutation and one that has been expressed in a membrane bound *mu* chain and saved the B cell from elimination. It is possible that after antigen stimulation, class switch recombination might occur on both functionally rearranged alleles leading to generation of plasma cells and memory B cells with dual specificity thereby breaking allelic exclusion. We amplified, cloned and sequenced VDJ-CH1 transcripts from mature B cells from a heterozygous human carrying a frame-shift *mu*CH1mutation. We analyzed 950 unique clones of which 90% contained a productive VDJ rearrangement. Less than 1 % of the functionally rearranged transcripts originated from the mutant allele. These data suggest counter-selection of "double producers" when they expand in the peripheral immune system.

P3.07.06
High-throughput mutant mouse characterisation is a powerful tool to identify novel immune phenotypes

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As part of the Sanger Mouse Genetics Project (MGP) we have generated over 900 knockout mouse lines. Following expansion, dedicated groups of mice are phenotyped through an extensive and standardised battery of tests assessing development, physiology and metabolism, and yielding data on >280 parameters for every line with a biobank resource also available. The status of the immune system in naïve mice is tested by flow cytometric profiling of peripheral blood leukocytes (PBL). Complementing this, the response to infection is probed by challenging with two bacterial systems; a mucosal response to Citrobacter rodentium, and a systemic response to Salmonella Typhimurium. To date 564 alleles have been phenotyped in the naïve state, 495 of which have completed the infection challenges. Immune testing is an expanding area of the MGP with increased analysis in the naïve state to include the addition of spleen, lymph node and bone marrow flow cytometric profiling, cytotoxic T cell function, autoimmune diagnostics and epidermal immune cell composition have been added to the pipeline. New challenges will be added to probe a wider range of responses and will now include DSS, Influenza and Trichurius. This rich source of information is openly available on the Sanger Mouse Portal (http://www.sanger.ac.uk/mouseportal/).

P3.07.07
Functional analysis of synaptobrevin family members in secretion of antibodies by human plasma cells

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Plasma cells (PC) are B-lymphocytes terminally differentiated with the purpose of manufacturing and secreting immunoglubulins (Ig). VAMPs (Vesicle Associated Membrane Proteins) or v-SNAREs are a subfamily of SNARE (Soluble N-ethylmaleimide-sensitive factor Attachment protein REceptor). SNARE complex required for exocytosis are made up of three proteins, one from each SNARE subfamily (VAMPs, Syntaxins (STX) and SNAP25). The aim of this study is characterize the presence of several VAMPs proteins in human PC and examines their functional roles on Ig secretion process.
Expression of VAMP proteins PC was examined by Western-blot, immunofluorescence and immunoprecipitation assays (IP) were carried out in the human U266 cell line in order to identify which VAMPs can interact with other SNAREs that could participate in the SNARE complex for Ig secretion in antibody secreting cells. Experiments of loss of function were performed using specific iRNAs.
Western-blotting assays revealed that VAMP-2,-3,-4,-5,-7 and VAMP-8 but no VAMP-1 were expressed. The majority of v-SNAREs were localized in cytoplasmic vesicular structures with no overlapping staining patterns with any subcellular structures marked with LAMP1, EEA1, Giantin, Cadherin or Calnexin. IP assays demonstrated an interaction of VAMP7 with STX4 and SNAP23 and interactions between VAMP2 and VAMP3 with both STX3 and STX4. Functional results from iRNA assays shown a statistically reduction of antibody secretion with iRNA specific for VAMP2. We identified several possible SNARE complexes including SNAP23/STX3/VAMP2, SNAP23/STX3/VAMP3, SNAP23/STX4/VAMP2, SNAP-23/STX4/VAMP3 and SNAP23/STX4/VAMP7. However, previous and present iRNA functional assays only imply as SNARE complexes candidates to the ones containing VAMP2 and/or STX4.

P3.07.08
B cell responses toward bacterial pathogens in human tonsils

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The first contact between microorganisms and the human host takes place in the upper respiratory tract and oro-pharyngeal mucosa. Current data on B cell responses in nasopharyngeal-associated mucosa are limited because, more frequently, the immune memory response to a pathogen or a vaccine is analyzed in peripheral blood. Therefore, understanding the mechanisms underlying the effector and memory B cell responses at the site of host-pathogen interaction is of great interest in the vaccine field.
Aim of the present study was to evaluate effector and memory B cells from palatine tonsils of children and adult patients that underwent surgery.
We measured IgG+ and IgA+ plasmacells (PC) and memory B cells (MBC) specific for antigens known to be immunogenic from *S.aureus*, *S.pneumoniae* and *S. pyogenes*. Frequencies of PC and MBC were calculated as percentages of total IgG/IgA positive B cells in the tonsils. Microbiologic culture data from tonsil swabs were also available for each analyzed sample.
We demonstrate that the antigen recognition profile of MBC and PC is different in children versus adults for most of the tested antigens. In addition, we show a relationship between the pathogens isolated from the tonsils and the pattern of the antigen specific B cell response. We provide also evidence that antigens of the biofilm phase of *S.aureus* can be seen by the immune system, leading to the development of biofilm-specific MBC and PC. These data provide new insights in the profiling of the B cell response at the site of interaction between host and pathogen.

P3.07.09
Ectonuclotidase activity in class switch recombination of murine B cells

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Class switch recombination (CSR) is an irreversible somatic recombination mechanism by which B cells switch their surface immunoglobulin class expression from IgM and IgD to other isotype with distinct effector function. This diversification is essential for a protective adaptive humoral immune response. B cell receptor (BCR) activation together with exogenous signals, including tumor necrosis factor (TNF) family members, Toll-like receptors (TLRs) ligands or cytokines trigger CSR. Here we show that extracellular adenosine critically contributes to CSR in murine mature B cells. Murine naïve B cells release ATP upon coordinate B cell receptor (BCR) and Toll Like Receptors (TLRs) stimulation. The released ATP is hydrolysed to adenosine by plasma membrane ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1) CD39 and ecto-5'-nucleotidase (5'-NT) CD73. Remarkably germinal center and isotype switched B cells displayed higher expression of CD73. Moreover, CD39 and CD73 deficient B cells showed in vitro a significant impairment in their capacity to differentiate to class switch plasma cells upon BCR and TLR engagement, underlying the role of adenosine in the B cells isotype switching process. Ig CSR-deficiencies in human can be dependent from an intrinsic B cell defect, however most of them are still molecularly undefined and diagnosed as common variable immunodeficiency (CVID). Notably, CVID patients with impaired class switched antibody responses are selectively deficient in CD73 expression in B cells, suggesting that CD73 dependent adenosine generation contributes to the pathogenesis of this disease.

P3.07.10
Primary Antibody Deficient Patients and ENT Complication

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The main clinical presentation of patients with primary antibody deficiency (PAD) incorporates upper respiratory tract infections comprising otitis media, sinusitis and pneumonia. This study was designed to investigate clinical and paraclinical hearing loss associated complications in important types of PAD comprehensively. Among all diagnosed patients with PAD, a cross sectional study was conducted on 55 PAD patients with diagnosis of selective IgA deficiency (SIgAD), common variable immunodeficiency (CVID), X-linked or autosomal recessive agammaglobulinemia and hyper IgM syndromes (HIgM). All patients were undergone of physical examination, tuning fork tests, pure tone audiometry, speech audiometry and auditory brain stem response. Otologic complications were detected in 54.5% of PAD patients. Conductive hearing loss was the main otologic complication amongst PID patients (73.3%) followed by SNHL which was present in 8 cases. Otitis media with effusion (21.8%), chronic otitis media (16.3%), tympanic perforation (10.9%), tympanosclerosis (5.4%) and auditory neuropathy (3.6%) were other complications in PAD patients. CVID and XLA patients with prophylactic usage of antibiotic had lower rate of ENT complications (p=0.04) and Otitis media with effusion (p=0.027) respectively. Given the high prevalence of hearing loss in PAD patients, a systematic otological investigation is recommended as an integral part of the management and follow-up of these patients.

P3.07.11
IL-4 promotes B cell maturation in synergy with BAFF and confers resistance to cell death in negative selection checkpoints

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IL-4 plays an essential role in the activation of B cells. Constitutive expression of IL-4 results in mature B cell compartment hyperplasia and is associated with antibody mediated autoimmunity, suggesting failure of tolerance safeguard mechanisms. Here, we analyzed the effect of IL-4 on in vitro B cell maturation, from immature to transitional stages, and its influence on BCR-mediated negative selection. Starting either from purified IgMneg B cell precursors, or sorted bone marrow immature (B220lo IgMlo CD23neg) and transitional (B220int IgMhigh CD23neg) B cells from C57BL/6 mice, we compared the maturation effects of IL-4 and BAFF. We found that IL-4 stimulated the generation of CD23+ T2-like B cells from CD23neg transitional B cells, and this effect was comparable to BAFF. Remarkably, IL-4 showed unique protective effect against anti-IgM apoptotic signals on transitional B cell checkpoint, not observed with BAFF. Moreover, IL-4 and BAFF strongly synergized to promote B cell maturation, and again IL-4 rendered this process refractory to BCR-mediated cell death. IL-4 leads to the reduction of pro-apoptotic Bim protein levels, which are induced by BCR crosslinking. Diminished levels of intracellular Bim protein correlates with protection to BCR induced cell death. Evidences support the notion that down-modulation of Bim by IL-4 occurred in a post-transcriptional manner. Together, these results contribute to the understanding of IL4 role in B lymphocyte physiology, unveiling a previously undescribed activity of this cytokine on the maturation of B cells, which could have important implications on the breaking of B cell central tolerance in autoimmunity.

P3.07.12
The hierarchical process of differentiation of long-lived antibody-secreting cells is dependent on integrated signals derived from antigen and IL-17A

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Two B cell populations are responsible for humoral immune memory: memory B cells (Bmem) and the long-lived antibody-secreting cells (ASC). In this study, we proposed to confirm whether Bmem contribute to ASC differentiation. Using cellular suspensions of peritoneal cavity, spleen and bone-marrow from mice with chronic humoral response against *Thalassophryne nattereri* venom (48 d), we purified CD19-positive Bmem that were cultivated in an *in vitro* system in the presence of venom, or cytokines as IL-17A, IL-21, IL-23, and IL-33. We demonstrated the existence of a hierarchic process of differentiation in which Bmem differentiated in CD138-positive ASC with intracellular IgG and able to secretion. IL-17A or IL-21/IL-23/IL-33 improves the ability of venom to induce intracellular IgG of peritoneal derived-ASC. Only cognate stimulation with venom is sufficient to down-regulate the expression of CD45R/B220. BAFFR is up-regulated in splenic or bone marrow derived-ASC stimulated by venom, CpG or cytokines. Only splenic derived-ASC up-regulate Bcl-2 expression after CpG or the combination of IL-21/IL-23/IL-33. Finally, the activation of ASC for IgG1 secretion is triggered by venom proteins in peritoneal cavity and by the inflammatory milieu in medullar niche. These results show the importance of the integration of signals downstream of BCR and cytokine receptors in modulating ASC differentiation, focusing in the microenvironment niche of their generation. **Support by:** Fapesp and CNPq.

P3.07.13
Granzyme B-secreting B cells: suicidal or lethal?

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Serpin B9 (formerly PI-9) is a potent inhibitor of the human serine protease Granzyme B (GrB). GrB mediates target cell apoptosis when released by cytotoxic T lymphocytes or natural killer cells. These cells express high levels of intracellular Serpin B9 to protect themselves from misdirected GrB. Classically, PI-9 expression in accessory cells, such as B cells, was thought to protect them from accidental exposure to locally secreted GrB. By contrast, we recently reported that human B cells are able to secrete active GrB, and therefore Serpin B9 expression in B cells might serve a broader purpose than described to date. Here, we show that human B cells constitutively express Serpin B9, whereas GrB mRNA and protein are induced de novo by interleukin-21 (IL-21) and B cell receptor (BCR) cross-linking. Interestingly, the viability of cells remained high, suggesting the constitutive levels of Serpin B9 were able to protect B cells despite high expression of GrB. Using confocal microscopy, we show that GrB localises to the B-cell secretory pathway, through co-localisation with calreticulin, GM130 and EEA1; markers of the endoplasmic reticulum, Golgi and early endosomal compartments, respectively. Furthermore, IL-21/anti-BCR induces a unique transcriptional profile, different to naïve B cells or plasma cells, indicated by a distinct impact on the IRF4-Blimp1-Bcl6 axis. Our studies strongly suggest an important role for GrB secretion by B cells, pointing to a role in B cell homeostasis, either autoregulatory in the context of activation-induced cell death (AICD), or cytotoxic in our classical understanding of target cell death.

P3.07.14
Participation of arginine methyltransferase 1 in B cell development and differentiation

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Development and proliferation of B lineage cells are tightly controled by intracellular signaling network. Activity of these signaling components is modulated by post-translational protein modifications, including phosphorylation, acetylation, and methylation. Protein arginine methylation catalyzed by protein arginine methyltransferases (PRMTs) plays a crucial role in transcription and signal transduction. To examine the role of PRMT1, a major enzyme in mammalian cells, in B cells, we established B cell-specific PRMT1-deficient mice through Cre-lox approach. Development of B cells, but not T cells, was impaired in B cell-specific PRMT1-deficient mice. Concentrations of immunoglobulin including IgM and IgG1 in sera were also diminished in the PRMT1-deficient mice compared with control. NP-specific IgM and IgG3 responses were also decreased in the PRMT1-deficient mice following immunization with type II T-independent antigen NP-Ficoll in vivo. Moreover, generation of IgG3-positive cells induced by polyclonal B cell activator lipopolysaccharide (LPS) together with IL-4 and CD40-ligand (CD40-L) was reduced in the PRMT1-deficient B cells compared with WT cells. However, IgG3 concentration of the culture supernatant from the LPS-stimulated B cells was comparable between PRMT1-deficient and WT cells, suggesting that PRMT1 regulates IgG3 secretion from IgG3 plasma cells. Finally, arginine methylation of p19 and p21 were increased following stimulation with LPS/IL-4/CD40-L in WT B cells, whereas p19 methylation was missing in PRMT1-deficient B cells, suggesting that p19 is a possible target of PRMT1 responsible for B cell activation and differentiation. Together, arginine methylation catalyzed by PRMT1 plays a crucial role in B cell development and differentiation.

P3.07.15
Distinct processing of the pre-B cell receptor and the B cell receptor

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It is well established that pre-BCR signaling governs proliferation and differentiation of pre-B cells. It has been suggested that signaling by the µ heavy chains of the pre-BCR induces survival and differentiation of pre-B cells, while the surrogate light chains are essential for proliferation and clonal expansion. However, the mechanism by which pre-BCR µ chains initiate differentiation signals is not clear, as physiological ligands have not been identified. We have demonstrated that while oligosaccharide moieties of µ heavy chains in the BCR are of the complex type as expected, those of the pre-BCR on the surface of pre-B cells contain oligosaccharide moieties of the high-mannose type only. This is unique, because high-mannose glycans are generally restricted to the endoplasmic reticulum in mammalian cells. It is therefore hypothesized that mannose-specific lectin-like molecules may function as the non-antigen ligand that triggers the pre-BCR.
We further examined the processing of the unusually glycosylated µ heavy chains in pre-B cells. We demonstrated that the pre-BCR reaches the cell surface by a non-conventional brefeldin A-sensitive monensin-insensitive transport pathway. Although pre-BCR complexes consist of µ heavy chains with high-mannose oligosaccharide moieties, they are stably expressed in the plasma membrane and demonstrate turnover rates similar to those of the BCR. Thus, rapid internalization cannot account for their low surface expression. Rather, we demonstrated that the low pre-BCR abundance in the plasma membrane results, at least in part, from insufficient production of surrogate light chains, which appears to be a limiting factor in pre-BCR expression.

P3.07.16
A functional BCR in human IgA and IgM plasma cells

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Plasma cells are terminally differentiated cells of the B cell lineage that secrete antibodies at high rate. Serum IgG antibodies and consequently IgG PCs can persist for a lifetime, although displacement by newly generated PCs and susceptibility to FcgammaRIIB-mediated apoptosis increase their turnover. In contrast, the IgA PCs present in the lamina propria show a high turnover rate. These findings suggest different mechanisms of regulation of IgG and IgA PCs.
BCR expression is required for memory B cells survival, but is thought to be lost on mature plasma cells due to a secretory switch in the Ig mRNA. Here, we report that human IgA and IgM unlike IgG plasma cells express a membrane functional BCR associated with the Igα/Igβ heterodimer. Importantly, this is shown for in vitro derived as well as ex vivo plasma cells isolated from bone marrow and gut lamina propria. BCR crosslinking on IgA and IgM plasma cells led to Ca2+ mobilization, ERK1/2 and AKT phosphorylation and impacted survival of IgA plasma cells. These findings demonstrate a fundamental difference between human IgG, IgM or IgA plasma cells and suggest that the IgA plasma cell repertoire may be modulated by specific antigens with implications for the regulation of the mucosal immune system.

P3.07.17
The surrogate light chain selects Mu heavy chain based on its DH CDR-H3 sequence

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Formation of the Pre B cell receptor (Pre BCR) is a key step in B cell development. The Pre BCR is formed by the binding of the surrogate light chain proteins (SLC) to the Mu heavy chain (Mu HC). We sought to test the hypothesis that the sequence of the DH heavy chain complementarity determining region 3 (CDR-H3) differentially affects the interaction between SLC and Mu HC. We used Bromodeoxyuridine (BrdU) incorporation and analysis of apoptosis to assess B cell turnover, cell cycle progression and cell loss as functions of CDR-H3 content mainly at the Pre B cell stage. We also, measured the degree of formation of Pre BCR based on binding of SLC to Mu HC with different CDR-H3 contents. B lineages enriched for hydrophobic CDR-H3 had difficulty forming Pre BCR, increased cell loss and inefficient cell cycle progression at the transition from early to the late pre-B cell. On contrary, mice limited to use of a single, normal DH with tyrosine enriched CDR-H3 followed the wild-type pattern of B cell development. Our findings suggest that immunoglobulin heavy chains with hydrophobic CDR-H3s have poor binding to SLC, resulting in decreased cycling activity and increased apoptosis at the Pre B cell stage. These findings may explain how humans minimize the use of hydrophobic CDR-H3s in their developing B cells since humans lack Dμ protein formation. Moreover, explain decreased prevalence of neutralizing antibodies with CDR-H3s enriched for charged and hydrophobic amino acid contents in HIV, influenza and other infections.

P3.07.18
Therapeutic effects of anti-APRIL blocking antibody on murine IgA nephropathy

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Background:A proliferation inducing ligand(APRIL) is member of tumor necrosis factor and blocking selective antibody-mediated APRIL blockade delays disease development in lupus mouse model by preventing proteinuria, kidney lesions, and mortality. TACI/BCMA axis is critical for IgA production and the patients with IgAN show serum elevation of APRIL. However, APRIL antagonism alone has never been tested until now in IgAN. So we are purposed to reveal its effect on murine IgAN.
Methods:We treated female IgAN prone mice(grouped ddY mice), which was recently established by our group(J Am Soc Nephrol 23; 1364, 2012), with 100mg i.p. of anti-APRIL blocking monoclonal antibody(Apophe) or control mouse IgG(cIg) twice a week during 6weeks starting at 7weeks of age, and checked serum levels of IgA, albuminuria. Pathological changes and immunoflorescence findings of(IgA, IgG, IgM and C3) and also cellular effects in bone marrow, spleen and peripheral blood were also evaluated.
Results: Until 4weeks, Apophe treatment showed reduced albuminuria with decrease of serum levels of IgA. Serum levels of IgG in Apophe group after 4weeks was lower than those of cIg group. Glomerular IgA deposition, but not IgG and IgM, at 6weeks in Apophe group was significantly decreased than that in cIg(4.12±0.17 vs 4.48±0.16, p=0.001).Flow cytometric analyses revealed that Apophe treatment induced significant attenuation of Gr(-)/FcrRIV(+) monocytes in peripheral blood.
Conclusion:Anti-APRIL treatment ameliorates murine IgAN with decrease of serum levels of nephritogenic IgA and its glomerular depositions. Present findings suggest that APRIL pathway may be importantly involved in the pathogenesis of IgAN and thus a therapeutic target.

P3.07.19
SLAM-dependent and independent mechanism of IgE induction

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Accumulating evidence suggests that IgE production induced by epicutaneous sensitization with protein antigen are associated with the development of allergic diseases such as allergic asthma and food allergy as well as the pathogenesis of atopic dermatitis. However, the precise mechanism of IgE induction by epicutaneous antigen exposure remains to be elucidated.The costimulatory molecule SLAM (Signaling Lymphocytic Activation Molecule, also known as CD150) is a glycoprotein expressed on activated lymphocytes and antigen-presenting cells. It has been reported that SLAM signal regulates IL-4 production by Th2 cells and follicular helper T cells, suggesting the possible role of SLAM in Th2 response and IgE induction. In fact, previous report showed that SLAM-deficient mice were less susceptible to the development of hyper-IgE and allergen-induced airway hypersensitivity after intraperitoneal sensitization.Here we investigated the role of SLAM in IgE induction by epicutaneous sensitization. Unexpectedly, SLAM-deficient mice showed increased levels of antigen-specific IgE after epicutaneous immunization with ovalbumin (OVA) as wild-type mice, while the elevation of antigen-specific IgE after intraperitoneal OVA immunization was significantly attenuated in SLAM-deficient mice. These results indicate that, in contrast to intraperitoneal immunization, epicutaneous immunization with protein antigen

induces antigen-specific IgE production in a SLAM-independent manner. We further revealed the precise mechanisms of SLAM-dependent and -independent IgE induction, which may provide a fundamental finding for specifically suppressing IgE production by epicutaneous antigen exposure.

P3.07.20
Differences between IgG4+ and IgG1+ B cells provide insight into regulation of the IgG4 response

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IgG4 antibodies are a peculiar subclass of human immunoglobulins that display limited capacity to trigger effector mechanisms. Partly, this can be explained by the fact that IgG4 can exchange half-molecules, rendering bi-specific, monovalent antibodies. In allergy, IgG4 antibodies compete with IgE and decrease allergic symptoms. Because of these properties, IgG4 belongs to the humoral arm of immune tolerance. How this tolerogenic humoral immune response is regulated is unknown, as information on IgG4+ B cells is lacking. We isolated IgG4+ B cells and show that there are phenotypical differences between IgG4+ B cells and IgG1+ B cells. These differences may provide insights why the IgG4+ response is differentially regulated from the IgG1 immune response and whether there is crosstalk between the regulatory networks that control humoral and cellular immune tolerance. IgG4+ B cells express higher levels of the low affinity FcεR while expression of inhibitory FcγRIIb was not altered compared to IgG1 cells. Expression of complement receptors is reduced on IgG4+ B cells, possibly making them less sensitive to regulation via complement. Although IgG4+ B cells are part of the humoral tolerogenic repertoire, we show that IgG4+ B cells are not classical Bregs, since they are not CD24hiCD38hi and more importantly, do not produce IL-10. Taken together, these findings indicate IgG4+ B cells are differentially regulated than IgG1 B cells. The specific expression pattern of regulatory receptors on the IgG4+ B cells may provide insight in the pathways that control the tolerogenic humoral immune response.

P3.07.21
The role of the histone demethylase Jmjd3 in B-cell lymphopoiesis

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Jmjd3 (Kdm6B) is a Histone H3 lysine-27 demethylase involved in transcriptional regulation and lineage determination. Jmjd3 plays an important role in macrophage differentiation and activation 1-3. It is expressed also in other hematopoietic lineages including B-lymphocytes. Jmjd3 transcripts were detected throughout B cell development reaching the highest levels in B cell progenitors. The function of Jmjd3 in B cell development remains unknown. To study the role of Jmjd3 at defined stages of B cell lymphopoiesis, we generated Jmjd3 conditional knock-out mice (Jmjd3fl). Jmjd3fl mice were crossed to the Mb1-cre strain to inactivate the demethylase starting from pro-B cells. Preliminary flow cytometry analyses of cell suspensions from lymphoid organs of Jmjd3 conditional mutant mice revealed fewer bone marrow B cells and a reduction in the fraction of CD5+ B-1a peritoneal cavity B cells. Instead, differentiation of follicular and marginal zone B cells was largely unaffacted by Jmjd3 inactivation. These data indicate that Jmjd3 regulates early B-cell development and the establishment of a normal pool of CD5+ B-1 B cells. Experiments are underway to identify the molecular targets regulated by Jmjd3 in these B-cell subsets

P3.07.22
Protease-activated receptor (PAR)1 and PAR2 have opposing effects on B-lymphocyte function during different stages of maturation

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Protease-activated receptors (PARs) play an important role in immune responses, although their role in B-lymphocytes is unknown. In this study we demonstrate, for the first time, that PAR1 and PAR2 are expressed by human B-cell lines (Ramos, Raji and Epstein Barr Virus-transformed lymphoblastoid cell line 1 [EBV-LCL1] cells) representing different stages of B-lymphocyte maturation. Using PAR agonists (Ag), antagonists (At) and confirmed by murine wild-type (WT), PAR1 and PAR2 knock-out (KO) splenic B-lymphocytes, we reveal that PAR1 At, but not PAR2 At, potently inhibited proliferation in all stages of B-cell maturation, while markedly increasing cytokine secretion of interleukin (IL)-1b, IL-10 and tumour necrosis factor (TNF)-a. PAR1 At had differential effects on IL-6 secretion; increased IL-6 in Ramos and Raji cells, but complete inhibition in EBV-LCL1 cells. In Ramos and EBV-LCL1 cells PAR1 At increased and PAR2 At decreased immunoglobulin (Ig)M. IgG secretion was similarly regulated in EBV-LCL1 cells. Investigation into the PAR signalling pathways revealed that in Ramos cells, PAR2 signalling was via nuclear factor (NF)-kB in Ramos cells, while in Raji and EBV-LCL1 cells PAR1 and PAR2 signalling required extracellular signal-regulated kinase (ERK) activation. This was confirmed by IL-6 secretion following NF-kB and ERK inhibition. In conclusion, we report that PAR1 and PAR2 have largely opposing effects on B-lymphocyte function during different stages of maturation.

P3.07.23
Ataxia Telangiectasia Patients Presented by Class Switch Recombination Defect: Elevated Serum Levels of IgM

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Ataxia telangiectasia (AT) is a rare primary immunodeficiency disorder with various clinical manifestations. Increased serum levels of IgM and recurrent infections can be the presenting feature in a few cases of AT patients and may be initially misdiagnosed as hyper-IgM (HIgM) syndrome.
This study was designed to investigate class switch recombination (CSR) as a critical mechanism in B lymphocytes' maturation to produce different isotypes of antibody in response to antigen stimulation in AT cases with HIgM presentation. Quantitative IgE production after stimulation by IL-4 and CD40L was considered as an indicator for CSR function. We also compared their results with sex and age matched controls of AT patients with normal values of IgM.
We report four AT patients who presented episodes of recurrent infections during infancy and high serum levels of IgM. Laboratory evaluation in these cases revealed defective CSR while none of 3 controls had a defect in the CSR process.
The characterized defect in AT is a mutation in the ataxia telangiectasia mutated (ATM) gene. This gene may result in CSR defects due to impaired DNA break repair. A special association of AT and HIgM may indicate a new subgroup of AT patients according to their clinical phenotype and CSR condition.

P3.07.24
B cell development undergoes profound changes during pregnancy

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Mammalian pregnancy represents a challenge to the rules of immunology. The maternal immune system has to tolerate the semi-allogeneic fetus while protecting mother and fetus against infections. The participation of B cells in this important issue is poorly understood. Here, we aimed to analyze B cell development during early and mid-pregnancy in mice. Mature IgM^+IgD^+ (B220 gated) B cells were significantly augmented in the bone marrow of pregnant females (P) as compared to non-pregnant mice (NP). Similarly, IgM^+IgD^+ mature as well as $CD19^+CD138^+$ plasma and $CD19^+CD27^+$ memory B cells percentages were significantly higher in P mice as compared to NP mice. Alongside to a splenomegaly observed in P mice, B cell compartment was strongly affected by pregnancy in the spleen. P mice showed significantly lower percentages of $CD23^{hi}CD21^-$ (B220 gated) follicular B cells while significantly higher percentages of $CD23^-CD21^{lo}CD1d^{hi}$ (B220 gated) marginal zone and IgM^+IgD^+ mature B cells compared to NP controls. In peritoneal cavity, the percentages of $CD19^+CD23^+$(B2) and $CD19^+CD23^-CD5^-CD11b^+$ (B-1b) B cells were significantly higher while $CD19^+CD23^-CD5^+CD11b^+$ (B-1a) levels were strongly reduced in P mice as compared to NP mice. Mature B cells ($B220^+CD21^+$) were significantly elevated in paraaortic lymph nodes lymph that drain the uterus in P mice as compared to NP mice.
Our data demonstrate profound changes in B cell populations in both, bone marrow and periphery that suggests important regulations of B cell development during gestation.

P3.07.25
Influence of Wnt5a treatment on B-1 cells viability in vitro

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Hematopoietic Stem Cells (HSC) are pluripotent cells, which are self-renewing and able to give rise to all lineages of the blood. Recently, studies demonstrated the presence of signal transduction by Wnt pathway in lymphocyte development and HSC self-renewal. The expression of lymphoid and myeloid markers by B-1 cells, likewise in HSC, involves complex signaling pathways. Previous data from our group have showed that B-1 cells express components of Wnt/beta-catenin pathway, including Wnt ligants and receptors. In order to understand the implications of Wnt pathway in B-1 cells *in vitro*, the viability of B-1 cells co-cultivated with OP9 cells and treated with two distinct Wnt recombinants (Wnt3a or Wnt5a) were analyzed, after 72 hours. Our results suggest that B-1 cells treated with Wnt5a recombinant present a reduced number of viable cells. As is known, some cytokines are essential to maintain B-1 cells alive *in vitro,* such as IL-6 and IL-10. We evaluated the presence of some cytokines by CBA Th1/Th2/Th17 kit in our model. We have detected an increase in the IL-6 levels at non-treated and Wnt3a treated groups. Besides the Wnt5a treatment augments IL-10 levels, we observed a reduction of IL-6 levels. These data indicate that B-1 cells are responsive to Wnt5a, and implicates in changes of cytokine levels. The participation of a non-canonical Wnt/Ca^{2+} pathway in this modulation is being studied.

P3.07.26
Effects of Ouabain on B and T lymphocytes at mice

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Ouabain (OUA) is a steroid produced by the adrenal and found at mammalian plasma. Ouabain can regulate immune functions, for example, the inhibition of proliferation induced by mitogens. It has been suggested that OUA is released together with other glucocorticoids during stress situations. Here, we evaluated whether the release of Ouabain can regulate lymphocytes subpopulations. Balb/C or C57BL/6 mice were injected intraperitoneally with 0.56mg/Kg OUA for 3 days. B and T cells were analyzed by flow cytometry, 24 hours after the last injection. Our results show that in bone marrow and spleen there was a decrease in mature B cells. In the spleen was observed a reduction notably in follicular B cells and no effect in regulatory B or B1 cells was observed. The B1 peritoneal cells were not affected by Ouabain. In the spleen, OUA induced apoptosis of B cells *in vitro,* but not *in viv*o. Ouabain also partially inhibited the proliferation *in vitro* of splenic B cells in response to LPS. Furthermore, Ouabain raised the B lymphocytes migration to lymph nodes. At the thymus, OUA had no effect on T cell subpopulations, but the number of TCD4+Foxp3+ in the spleen was reduced significantly. In the case of T cells, OUA did not induce an increase on apoptosis or inhibition of proliferation in response to α-CD3. We did not observe an increase in the migration of T cells to the lymph nodes. Ouabain seems to use different mechanisms for the regulation of B and T cells subpopulations.

P3.07.27
Combination effect of LPS and MDP on antibody production by mouse B cells

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Toll-like receptors (TLRs) and Nod-like receptors (NLRs) are pattern recognition receptors (PRRs) that recognize specific conserved bacterial structures. Several studies have reported that B cells express both TLRs and NLRs, and the receptors signaling contribute to B cell activation. However, the roles of the receptors in antibody production by B cells are not fully understood. In this study, we investigated the effect of Nod2 agonist MDP in combination with TLR4 agonist LPS on B cell proliferation, viability, and antibody production *in vitro* culture of purified mouse spleen resting B cells. MDP cooperated with LPS to induce B cell proliferation and viability. Furthermore, MDP and LPS synergistically increased only both IgG2b production and germline (GL) γ2b transcripts (GLTγ2b) expression. In the experiment with Nod2- and TLR4-deficient mice, we found that the cooperative effect of MDP and LPS is dependent on the receptors, Nod2 and TLR4. On the other hand, LPS/MDP-induced B cell proliferation was mediated by JNK and RIP2/p38 pathway, but these pathways did not affect GLTγ2b expression. Taken together, our present study suggests that cross-talk between Nod2 and TLR4 signaling enhances B cell proliferation via RIP2/p38 and JNK pathways and specifically increases IgG2b production through increasing GLTγ2b expression. Thus, MDP in combination with LPS directly enhances B cell activation and selectively reinforces IgG2b production independent of T cells and B cell receptor (BCR) stimulation, and thereby MDP can be developed as an effective B cell adjuvant.

P3.07.28
Immunization of Common Variable Immunodeficiency (CVID) patients with Influenza induces increase in switched memory B cells and plasmablasts

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CVID is a primary antibody deficiency characterized by hypogammaglobulinemia, increased susceptibility to infections and impaired antibody response to specific antigens. Several immunological defects were described in this heterogeneous group of patients. Patients present defects in B lymphocytes maturation, resulting on alterations in differentiation, frequency and function of its subtypes. Immunization is an important measure to reduce infections however, in CVID patients response is controversial and experience, limited. Thus, our objectives were to evaluate the behavior of B cell subtypes and specific antibody production before and after

vaccination with influenza vaccine (H1N1 and H2N3) and the effect of vaccination on the number of infections. CVID patients were selected from PID outpatient clinic of Hospital das Clínicas, São Paulo. Blood samples were collected before and one month after vaccination. PBMCs were separated and stimulated with PWM, *Influenza* viral lysate and hemagglutinin peptide for 6 days. B cell subtypes (naïve, marginal zone, switched memory B cell and plasmablast) frequency and activation were evaluated by flow cytometry. Influenza specific antibodies were measured by ELISA and H1N1 specific antibodies by hemagglutination inhibition. Our results show reduction in the frequency of respiratory infections in the 12 month period after vaccination although no protective antibody levels were produced. Moreover, we observed a significant increase in the number of switched memory B cells and plasmablasts in CVID patients one month after immunization. These results reinforce the benefits of vaccination of CVID patients and bring some insights into the mechanisms by which this clinical improvement may occur.

P3.07.29
CD38 ligation promotes proliferation and ERK phosphorylation of bone marrow B-cell precursors in mice

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CD38 is a 45 KD transmembrane protein, expressed by immature and mature lymphocytes. Expression and function of CD38 throughout the B-cell differentiation pathway have been poorly studied in mice. Here we report CD38 expression from the earliest stages of B cell development. Pre-pro-B, pro-B, pre-B and immature B cells from murine bone marrow were all positive for CD38, but immature B cells showed the highest expression. Pro-B stage contains two distinct cell populations on the basis of CD38 expression that may correspond to developmental stages.
No differences were found between WT and CD38-/- mice in the B-cell lineage frequencies and absolute numbers within bone marrow, indicating that CD38 is dispensable during B-cell ontogeny, probably because its function may be being replaced by the homologous molecule CD157 which is also expressed from early states of maturation in the bone marrow. Interestingly, highly purified immature B cells proliferated upon CD38 ligation, and no apparent apoptosis was recorded under these conditions. Moreover, ERK was phosphorylated after CD38 crosslinking. In conclusion, the expression of CD38 is tightly regulated during the ontogeny of murine B-cells lineage and functional CD38 may play a role throughout the early development process of B cells as its engagement induces proliferation. This work was fully supported by Conacyt-México 153733.

P3.07.30
Patients with X-linked Agammaglobulinemia Survival Rate : Mortality and Morbidity

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Background: X-linked agammaglobulinemia(XLA) is a genetic disorder characterized by a defect in the generation of mature B cells, lack of antibodies production, and susceptibility to recurrent bacterial infections. Understanding of the risk factors responsible for morbidity and mortality in these patients can help in a better management of this disorder. However, there is a lack of specific studies in the literature regarding the morbidity and mortality of XLA patients. Objective: This study is designed to evaluate morbidities and mortality and survival rates in Iranian patients with XLA diagnosis during the past 20 years.Methods: We have registered the clinical data of the XLA patients and followed them up till 2010. At the time of diagnosis, a four-page questionnaire including complete medical information was filled for all patients. Follow-up information was gathered either by reviewing the patients' hospital records or regular visiting the patients. Results: Among 41 patients, 11 patients(26.8%) have died during follow up period. The most common first manifestation was pneumonia. All of the complications before the initiation of treatment such as pneumonia, otitis media and diarrhea, were reduced after immunoglobulin replacement except sinusitis and conjunctivitis. There were significant association between some immunologic and clinical characteristics such as lymphocyte subsets, consanguinity marriage and mortality.Conclusion:Despite recent advances in the treatment of XLA, these patients still suffer from severe complications. Associations between poor prognosis and clinical and some immunologic characteristics of the patients may help physicians to select poor prognoses patients at higher risk of mortality to develop prevention strategies for them.

P3.07.31
Retinoic acid acts as a selective human IgA switch factor

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Retinoic acid (RA) is known to have several activities which lead to potent mucosal IgA response. Nevertheless, its exact role in human IgA synthesis was not delineated yet. We asked if RA can specifically induce IgA isotype switching in human B cells. We found that RA increased IgA production and the expression of germ-line IgA1 and IgA2 transcripts (GLTα1 and GLTα2), and that this was paralleled by the increase of frequency of IgA-secreting B cell clones as assessed by limiting dilution assay. Under the same conditions, RA did not increase IgM and IgG production. Am80, an agonist of RA receptor (RAR) increased IgA production. In addition, the RA activity was abrogated by LE540, an antagonist of RAR, suggesting that RAR pathway is involved in RA-induced IgA production. Taken together, these results indicate that RA has activity as an IgA switch factor through RAR in human.

P3.07.32
Adaptor protein Swiprosin2/EFhd1 during early B cell development

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The pre-B cell receptor (pre-BCR) regulates proliferation and differentiation of pre-B cells by changing their transcriptome. One of the genes that become down-regulated by the pre-BCR is *efhd1*. Swiprosin2/EFhd1 and Swiprosin1/EFhd2 are two conserved proteins with 65% sequence homology, functional protein-interaction sites and two calcium-binding EF-hand domains. We have shown that in contrast to EFhd1, EFhd2 is expressed throughout all B cell stages and is involved in pro-apoptotic B cell receptor (BCR) signalling and in the germinal center reaction. EFhd1 has been implicated in carcinogenesis and cell proliferation. Its function in B lymphocytes, however, remains elusive.
Ectopic expression of EFhd1 in pro-B cells *in vitro* by retroviral transfection leads to slower decrease in surface c-kit expression, indicating slower differentiation into pre-B cells. Transgenic mice (*efhd1tg*) expressing EFhd1 constitutively under the VH promoter and Eμ enhancer reveal a trend to reduced B cells after the pre-B cell check point in the bone marrow (BM). In mixed BM chimeras, wild type B lymphocytes have a significant advantage over *efhd1tg* in the BM. Down regulation of EFhd1 by the pre-BCR therefore aids efficient subsequent B cell development. To analyse the role of EFhd1 during early B cell development, in addition to the *efhd1tg* model, a

conditional *efhd1*$^{-/-}$ mouse is being established. Future aims include phenotypic characterization of *efhd1*$^{-/-}$ mice, focusing on development, survival and homing of B lymphocytes. Functional redundancy of the homologous proteins EFhd1 and EFhd2 will be analysed by crossing *efhd2*$^{-/-}$ mice with *efhd1tg* mice and, later, *efhd1*$^{-/-}$.

P3.07.33
Serine/threonine phosphatase PP4 is required for pro-B cell development by promoting Ig VDJ recombination

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PP4 phosphatase regulates a number of crucial cellular physiologies. However, the role of PP4 in B cells has never been reported. Through the analysis of B cell-specific *pp4* knockout mice, we identify the essential role of PP4 in B-cell development. Deficiency of PP4 in B lineage cells leads to a strong reduction of pre-B cells in numbers, an absence of immature B cells and a complete loss of mature B cells. In PP4-deficient pro-B cells, Ig DJ_H recombination is impaired. In the meanwhile, Ig μ H chain expression is strongly reduced. Consistent with these findings, the residual PP4-deficienct pre-B cells show a cell cycle arrest at G1 phase. In addition, an increased apoptosis and an excessive DNA damage are detected in these mutant cells. We showed that the enforced expression of transgenic Ig in PP4-deficient mice drives B-cell development and overcomes IgM^+ B cells. Our study reveals a novel function of PP4 in pro-B cell development by promoting V_HDJ_H recombination.

P3.07.34
Y-Chromosome-linked B- and NK-cell deficiency in mice

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There are no primary immunodeficiency diseases linked to the Y chromosome, because the Y chromosome does not contain any vital genes. We have established a novel mouse strain in which all males lack B and NK cells and have Peyer's-patch defects. By 10 weeks of age, 100% of the males had evident immunodeficiencies. Mating these immunodeficient males with wild-type females on two different genetic backgrounds for several generations demonstrated that the immunodeficiency is linked to the Y chromosome and is inherited in a Mendelian fashion. Although multicolor fluorescence in situ hybridization (FISH) analysis showed that the Y chromosome in the mutant male mice was 1/3 shorter than that in wild-type males, exome sequencing did not identify any significant gene mutations. The precise molecular mechanisms are still unknown. Bone marrow chimeric analyses demonstrated that an intrinsic abnormality in bone marrow hematopoietic cells causes the B- and NK-cell defects. Interestingly, fetal liver cells transplanted from the mutant male mice reconstituted B and NK cells in lymphocyte-deficient *Il2rg-/-* recipient mice, while adult bone marrow transplants did not. Transducing the EBF gene, a master transcription factor for B cell development, into mutant hematopoietic progenitor cells (HPCs) rescued B-cell but not NK-cell development both *in vitro* and *in vivo*. These Y-chromosome-linked immunodeficient mice, which have preferential B- and NK-cell defects, may be a useful model of lymphocyte development.

P3.07.35
ACKR3 (CXCR7) expression on late germinal center B cells

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Atypical chemokine receptors (ACKRs) differ from typical receptors by their inability to trigger G-protein mediated signal transduction, such as chemotaxis, but instead sequester chemokine ligands for lysosomal degradation or transcystosis. The scavenger receptor ACKR3 (formerly CXCR7) binds with high affinity CXCL12 and somewhat lower affinity CXCL11. ACKR3 is expressed on non-hematopoietic cells and neoplasms, as well as on leukocytes. Here we investigated the function of ACKR3 at late stages of B cell maturation, when B cells differentiate into antibody-secreting plasmablasts before they become long-lived plasma cells. By using specific mAb we characterized two populations of $ACKR3^+$ cells in tonsillar mononuclear cells (TMCs), one being presumably memory B cells or early plasmablasts ($FSC^{low}CD19^+CD38^{mid}$) and the other being plasmablasts or early plasma cells ($FSC^{high}CD19^+CD38^+$). Further analysis of receptor expression on TMCs showed that ACKR3 is expressed on $CD19^+CD27^+$ memory B cells, on $CD19^+CD38^+CD138^-$ and intracellular immunoglobulin high ($icIg^{high}$) plasmablasts, but not on $CD19^+CD138^+icIg^{high}$ plasma cells. The transient expression pattern on B cells, suggests a potential contribution of ACKR3 in the process of final B cell maturation. By using an *in vitro* differentiation system, we found an marked inverse correlation of ACKR3 and CXCR5 cell surface levels, whereas expression of CXCR4 remained almost constant during maturation. Moreover transwell migration assays revealed that inhibition of ACKR3 markedly increases CXCR4-mediated chemotaxis towards CXCL12, especially at late stages of B cell maturation. The findings unveil an important role of ACKR3 in regulating the migration of plasmablasts at late stages of B cell maturation.

P3.07.36
Inhibition of germinal center formation and humoral immune responses by EB virus protein LMP1

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Germinal center (GC) is critical for the generation of high-affinity B cells. It has been reported that Epstein-Barr virus (EBV) preferentially infects GC B cells where EBV-encoded Latent membrane proteins (LMPs) such as LMP1 and LMP2a are expressed, suggesting that LMPs may modify B cell differentiation. However, it has not been well characterized how LMPs affect GC B cell differentiation. Here, we generated the knock-in mice expressing LMP1 in an activation-induced cytidine deaminase (AID) promoter-dependent manner ($LMP1^{Aid}$). Flow cytometric analysis showed the enlarged cell size and the increased expression of surface markers including CD95, CD69, CD80, MHC class II, and PD-L1 in $LMP1^+$ B cells when compared with $LMP1^-$ B cells. No typical GC was formed in spleens, lymph nodes and peyer's patches of immunized $LMP1^{Aid}$ mice although $CD38^{medium}GL7^+Bcl\text{-}6^-$ abnormal B cells and Tfh cells were generated. Antigen-specific antibody responses were severely impaired in immunized $LMP1^{Aid}$ mice. In addition, $LMP1^{Aid}$-derived B cells could suppress GC formation and antigen-specific antibodies production by wild type B cells in immunized bone-marrow chimera mice. When *in vitro* stimulated with IL-4, IL-5, and LPS, IgG1 and IgM production were markedly impaired in B cells from $LMP1^{Aid}$ mice, whereas IL-6 and IL-10 production were significantly enhanced. In conclusion, LMP1 expression in GC-committed B cells resulted in the impairment of typical GC formation and the inhibition of antibody production, suggesting that LMP1-expressing B cells might act as regulatory B cells to suppress humoral immune responses. We will discuss how EBV evades immune responses by using LMP1.

P3.07.37
B cell progenitors and precursors change their microenvironment in fetal liver during early development

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The microenvironments, in which B-lymphocytes develop in fetal liver, are largely still unknown. Among the non-hematopoietic cells we have identified and FACS-separated two subpopulations, $CD45^-TER119^-VCAM\text{-}1^+$ cells that are either $CD105^{high}LYVE\text{-}1^{high}$ or $CD105^{low}ALCAM^{high}$. Immunohistochemical analyses find three of four $c\text{-}Kit^+IL\text{-}7R\alpha^+B220^{low}CD19^-SLC^-$ B-progenitors in contact with vascular endothelial-type $LYVE\text{-}1^{high}$ cells on embryonic day 13.5. One day later $c\text{-}Kit^+IL\text{-}7R\alpha^+$ cells develop to $CD19^{-\ and\ +}$, SLC-expressing, DHJH-rearranged pre/pro and pro/preB-I cells. Less than 10% are still in contact with $LYVE\text{-}1^{high}$ cells, but half of them are now in contact with mesenchymally-derived $ALCAM^{high}$ liver cells. All of these $ALCAM^{high}$ cells, but not the $LYVE\text{-}1^{high}$ cells produce IL-7 and CXCL12, while both produce CXCL10. Progenitors and pro/preB-I cells are chemo-attracted *in vitro* towards CXCL10 and 12, suggesting that lymphoid progenitors with Ig gene loci in germline configuration enter the developing fetal liver at E13.5 from vascular endothelium, attracted by CXCL10, and then migrate within a day to an $ALCAM^{high}$ liver cell-microenvironment, differentiating to DHJH-rearranging, surrogate light chain-expressing pre/proB and pro/preB-I cells, attracted by CXCL10 and 12. Between E15.5 and E16.5 preB-I cells expand 10 fold in continued contact with $ALCAM^{high}$ cells, and begin VH- to DHJH-rearrangements in further differentiated $c\text{-}Kit^-IL\text{-}7R\alpha^-$ preBII cells.

P3.07.38
B cell-intrinsic role of DOCK2 in T cell-dependent humoral immunity

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The small GTPase Rac has been implicated in various cellular functions by acting downstream of multiple receptors including B cell receptor (BCR). However, its role in humoral immunity and the upstream regulator are poorly understood. DOCK2 is an atypical Rac activator predominantly expressed in hematopoietic cells. We found that DOCK2 is essential for BCR-mediated Rac activation and critically regulates immunological synapse formation. To examine B cell-intrinsic role of DOCK2 in humoral immunity, we used the knock-in mice expressing antigen-specific BCR in a class-switchable manner. When the knock-in mouse-derived B cells were transferred into wild-type mice with the cognate antigen, germinal center (GC) formation and class switch recombination occurred normally in the absence of DOCK2. However, while IgG+ wild-type B cells rapidly expanded and differentiated to syndecan-1+ plasma cells, these processes are severely impaired in DOCK2-deficient B cells, suggesting that DOCK2 regulates post GC B cell differentiation through immunological synapse formation. On the other hand, we also found that initial generation of extrafollicular plasma cells is impaired in the absence of DOCK2. DOCK2-deficient B cells exhibited a defect in EBI2-mediated Rac activation and failed to move properly to extrafollicular sites. These results thus indicate that DOCK2-Rac axis controls plasma cell differentiation, at least, by two distinct mechanisms during humoral immune responses.

P3.07.39
Impact of genetic deficiencies in the TLR signaling pathways on human IgM+IgD+CD27+ B cells

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We studied peripheral B-cell subsets in patients deficient for key factors of the TLR signaling pathways. All TLRs, except TLR3 that signals through the TRIF adaptor, require MyD88 and IRAK-4 to mediate their function. TLR4 and the TLR2 heterodimers (with TLR1, TLR6 and possibly TLR10) require in addition the adaptor TIRAP, whereas UNC-93B is needed for the proper localization of intracellular TLR3, TLR7, TLR8 and TLR9. We found that IgM+IgD+CD27+, but not switched B-cells, were strongly reduced in MyD88-, IRAK-4- and TIRAP-deficient patients. In contrast, the numbers of IgM+IgD+CD27+ B-cells were normal in the absence of TLR3, TRIF and UNC-93B, suggesting that UNC-93B-dependent TLRs, and notably TLR9, are dispensable for the presence of this subset in blood. Hence, we propose a role for TIRAP-dependent TLRs, (TLR1, 2, 6 and 10) in the development and/or maintenance of IgM+IgD+CD27+ B-cells (Weller et al. Blood. 2012; 120(25): 4992-5001). Interestingly, TLR10 whose expression is restricted to B and plasmacytoid dendritic cells was expressed at higher level in IgM+IgD+CD27+ compared to switched B-cells in healthy individuals. An exome sequencing strategy allowed us to identify patients with mutations in TLRs. We focused on 3 missense mutations in TLR10 predicted to possibly damage its structure and function. While 2 variants showed normal cell-surface expression when transfected into HEK293T cells, the third one remained intracytoplasmic. We will test on IgM+IgD+CD27+ B-cells in vivo the impact of this mutation as well as other mutations in TLR1,2 and 6 described by Ben-Ali et al. as deleterious (Hum Mutat. 2011;32(6):643-52).

P3.07.40
Follicular dendritic cells induce a new class of myeloid cells with unique B cell stimulating activities: specific dependence on IL-34/CSF-1R signaling pathway

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We have been analyzing roles for follicular dendritic cells (FDCs) in controlling germinal center (GC) reaction using a mouse FDC line, FL-Y, which was established in our laboratory, and has been shown to be useful as a model of native FDCs. When we cultured FL-Y cells with Lin-, c-kit+ spleen cells for 7-9 days, we observed expansion of a new class of CD11b+ myeloid cells (Gr-1-, CD11c-, F4/80+, CXCR4+, CCR2+, CX3CR1-), which was named FDMCs (FDC-induced myeloid cells). FDMCs had unique B cell-stimulating activities. Division of anti-CD40-stimulated B cells was markedly enhanced when they were co-cultured with FDMCs, and the FDMC-activated B cells acquired GC B cell-associated markers efficiently, suggesting a role for FDCMs in GC reaction. FL-Y cells produced CSF-1 and IL-34, both of which has been showed to induce macrophages development by signaling through a common receptor, CSF-1R. We examined the involvement of these cytokines in FDMC generation using neutralizing Abs. Interestingly, FDMC generation was clearly blocked by an anti-CSF-1R Ab, but neutralization of CSF-1 had no inhibitory effects. On the other hand, an anti-IL-34 Ab strongly suppressed FDMC generation. Similar IL-34-specific dependence was confirmed by the gene 'Knockdown' (KD) in FL-Y cells using shRNA, thus demonstrating that FDMC induction is specifically dependent on the IL-34/CSF-1R pathway. To our knowledge, this is the first report describing a CSF-1R-mediated signaling pathway that exclusively respond to IL-34. Further investigations are underway to elucidate molecular mechanisms underlying the specific action of IL-34.

P3.07.41

The Non-Canonical function of UNG in AID induced Antibody Gene Diversification

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Activation-induced cytidine deaminase (AID) is essential to antibody memory formation, which requires immunoglobulin (Ig) locus specific genomic alterations, such as class switch recombination (CSR) and somatic hyper mutation (SHM). It has been proposed that CSR and SHM are initiated by AID induced DNA cleavage that requires AID mediated dU formation in DNA and its removal by UNG. In contrast to AID deficiency, where both the processes were completely abolished, CSR and SHM were differentially affected in UNG deficiency, suggesting a novel function of UNG. Here we show that the *bona fide* uracil repair enzyme UNG acts non-canonically to regulate DNA end-joining and erroneous repair required for CSR and SHM, respectively. One of our intriguing observations is that UNG acts as a potent suppressor of AID induced SHM. In addition, a catalytic mutant of UNG was found to be as proficient as wild type UNG restoring the mutation load and base bias. It appears that UNG associated faithful base excision repair complex competes with the error prone polymerase complex like Rev1 involved in SHM. Further analysis of switch recombination junctions revealed that UNG promotes the alternative-nonhomologous end-joining (a-NHEJ) repair pathway. We provide evidence that the CSR defect attributed to the deregulated recruitment of critical DNA repair factors and improper S-S synapse formation. Therefore, UNG distinctly regulates CSR and SHM, which are dissociable and independent of its catalytic activity, and thus providing compelling evidence on its non-catalytic function in AID induced genomic instability.

P3.08 Th1 and Th2 cells

P3.08.01
Chemokine receptor (CKR) expression on memory CD4+ Th2 cells in mice during an allergen-mediated exacerbation of allergic asthma

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Chemokine-CKR interactions play an important role in allergic asthma. In asthmatics and experimental mice, CCR3, CCR4 and CCR8 are considered major players. However, data from mice lacking these receptors are inconclusive and the mechanisms underlying chemokine-CKR-mediated migration in allergic asthma remain unclear. We sought to characterize CKRs on CD3+CD4+CD62L-CD44+ memory Th2 cells in lungs of mice during allergen-induced disease exacerbation. We administered intranasal allergen to mice recovered from allergic asthma and compared CKR cell surface expression 3 and 7 days after allergen rechallenge on memory CD4+ T cells from the lungs and spleens of bicistronic IL-4 reporter-4get mice. We found no change in expression of any of the 13 CKRs on spleen IL-4+ or IL-4- CD4+ T memory cells. In contrast, allergen rechallenge increased the expression of CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CXCR3, CXCR4 and CXCR6 on IL-4+ and IL-4- memory CD4+ T cells in lung, but did not influence the expression of CCR3, CXCR2, CXCR5, and CXCR7. These data demonstrate differences in CKR expression on memory cells in spleen and lung and support the notion that there are organ-specific memory cell subpopulations. In addition, the increased expression of numerous CKRs on CD4+ T cells in lung during disease exacerbation suggests that either there are several memory cell subsets bearing distinct CKRs or that lung memory cells express high numbers of CKRs. Both possibilities would explain a lack of phenotype in CKR deficient animals and indicate that targeting CKRs to treat allergic asthma might be difficult.

P3.08.02
The role of CD244 in regulating *M. tuberculosis* antigen-specific $CD4^+$ T cell function

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CD244 (2B4) is a member of the signaling lymphocyte activation molecule (SLAM) family of immune cell receptors and it plays an important role in modulating NK cell and $CD8^+$ T cell immunity. The role of CD244/2B4 on human $CD4^+$ T cell function in active tuberculosis (TB) patients has not been reported so far. In this study, we investigated the expression and function of CD244/2B4 on $CD4^+$ T cells from active TB patients and latent infection individuals. Active TB patients had significantly elevated CD244/2B4 expression on *M. tuberculosis* antigen-specific $CD4^+$ T cells as compared with latent infection individuals, and the expression of CD244/2B4 was associated with disease persistence in active TB patients. Compared with CD244/2B4-dull and -middle $CD4^+$ T cells, CD244/2B4-bright $CD4^+$ T cell subset had significantly reduced expression of IFN-γ, suggesting that CD244/2B4 expression may modulates IFN-γ production in *M. tuberculosis* antigen-responsive $CD4^+$ T cells. Activation of CD244/2B4 signaling by cross-linking led to significantly decreased production of IFN-γ. Blockage of CD244/2B4 signaling pathway of T cells from patients with active TB resulted in significantly increased production of IFN-γ, compared with isotype antibody control. In conclusion, CD244/2B4 signaling pathway has an inhibitory role on *M. tuberculosis* antigen-specific $CD4^+$ T cell function.

P3.08.03
Dose-dependent differential effects of thrombin in allergic bronchial asthma

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Apart from its role in the coagulation system, thrombin plays an important role in the inflammatory response through its protease-activated receptor (PAR)-1, but its role in the immune response is unclear. This study evaluated the modulatory role in allergic bronchial asthma. Bronchial asthma was induced in mice by sensitization and challenge with ovalbumin. Mice with low but sustained coagulation activation had reduced allergic inflammation and allergic asthma was inhibited by low doses but worsened by high doses of thrombin. Allergic asthma was worsened by antithrombin, argatroban, hirudin and anti-thrombomodulin antibody. Mice with an increased concentration of an inhibitor of both thrombin and activated protein C had worsened disease. Heterozygous PAR-1 mice had less allergic inflammation but PAR-1 agonist worsened it. Allergic bronchial inflammation was worsened in mice that received adoptive transfer of PAR-1 agonist-treated Th2 cells compared to controls. Low concentrations of thrombin suppressed but high-dose of it enhanced maturation and secretion of cytokines in dendritic cells. The effects of thrombin on allergic asthma are dose-dependent with detrimental effects at high-dose being mediated by PAR-1 while low doses were protective via thrombomodulin. These data demonstrate that thrombin modulates the outcome in allergic bronchial asthma.

P3.08.04
Serine protease inhibitor characteristics of human T helper 2 cells

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Atopic diseases are characterized by IgE mediated immune responses towards allergens and high expression levels of T helper 2 (Th2) type cytokines. Many of the atopic allergens possess serine protease activity. A serine protease inhibitor SPINK5 gene is reported to play a suppressive role in atopic skin diseases. Interestingly, differentiating human Th2 cells have also shown elevated expression

levels of a serine protease inhibitor kunitz-type 2 (SPINT2). However, an in-depth understanding how protease inhibitors potentially modulate allergic reactions in atopic diseases is missing.
This study aims at characterizing the expression and secretion of SPINT2 from differentiating and fully polarized human Th2 cells and identifying the specific gene expression patterns of SPINT2 displaying Th2 cells.
We used naïve CD4+ T cells isolated from umbilical cord blood and activated them to induce differentiation of Th0, Th1 and Th2 cells. The kinetics of SPINT2 expression was detected at RNA and protein levels by qRT-PCR and flow cytometry. We examined the subset specific gene expression profiles with Affymetrix arrays from RNA extracted from Th2 cells sorted based on their SPINT2 surface expression.
Th2 cells expressed and secreted significantly more SPINT2 throughout the differentiation process compared to other Th subsets. With the genome wide gene expression analysis we found 97 differentially expressed genes in SPINT2 positive Th2 cells, including genes linked to NK cell associated cytotoxic pathways and chemokine genes related to asthma and migration of Th2 cells. The results are expected to gain new important information on serine protease inhibitor exploitation of Th2 cells.

P3.08.05
New column-free procedure for the enrichment of human Th1(CD4+CXCR3+) cells from peripheral blood

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Upon appropriate activation naïve T helper (Th) precursor cells proliferate and become polarized toward various cell subtypes distinguishable by their effector function and chemokine expression. Th1 cells regulate cellular immunity via the autocrine growth factor IL-2 plus IFN-γ and TNF-β, activating macrophages, cytotoxic T, NK and B cells in the effort to eliminate intracellular pathogens. Although no single specific marker is used to identify Th1 cells, they preferentially express the chemokine receptors CXCR3 and CCR5 and can be further characterized by intracellular staining for IFN-γ. We have developed a 2-step EasySep™ immunomagnetic column-free method for the enrichment of Th1(CD4+CXCR3+) cells from fresh peripheral blood nucleated cells. First, non-CD4 T cells are targeted for depletion using dextran-coated magnetic particles and a cocktail of antibody complexes. Labeled cells are separated using an EasySep™ magnet, and pre-enriched CD4 T cells are poured off. CXCR3+ cells are then positively selected from the pre-enriched CD4 T cell fraction. The procedure can be automated using RoboSep™. With an initial frequency of 10 ± 3% CD4+CXCR3+ cells, purities of 90 ± 4% (n=12) can be obtained. When stimulated, a high proportion of these cells produce IFN-γ (68 ± 12%) compared with total CD4 T cells and pre-enriched CD4 T cells. Less than 1% of the isolated cells produce cytokines associated with Th2 or Th17 cell subtypes (IL-4 or IL-17 respectively). Enrichment of unstimulated human Th1(CD4+CXCR3+) cells enables the investigation of chronic inflammation responses and mechanisms of regulation in human models of disease.

P3.08.06
Identification and characterization of the secreted form of TMEM98 that promotes Th1 cell differentiation both *in vitro* and *in vivo*

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Cytokines are secreted proteins that mediate the communication among immune cells. They play critical roles in the differentiation and effector function of $CD4^+$ T cells. More importantly, cytokines have made effective targets for drug development for a variety of diseases. Therefore, identifying novel potential cytokines will be of great value. We have established an immunogenetic platform to identify secreted proteins with modulatory effects on immune cells. In this study, we demonstrate that TMEM98 is a type II transmembrane protein that is secreted by a non-classical pathway. GEO profile analysis indicated that TMEM98 might be involved in anti-viral and anti-tumor immunity. We found that mouse $CD4^+$ T cells upregulated TMEM98 upon stimulation, especially on activated Th1 cells. When placed under Th1-skewing conditions, recombinant TMEM98 could increase both the percentage of IFN-γ^+ cells and IFN-γ secretion into culture medium. Moreover, T-bet expression, the key Th1 transcription factor, was also upregulated. These findings were confirmed *in vivo,* as recombinant TMEM98 administration significantly increased antigen-specific IFN-γ production and serum antigen-specific IgG2a in the mBSA-induced delayed-type hypersensitivity (DTH) model. Overall, these observations emphasize an essential role of TMEM98, a novel secreted protein, in promoting Th1 cell differentiation, thereby adding to the understanding of Th1 cell development.

P3.08.07
NFκB attenuates IL-5 production and upregulates T-box transcription factors in Th2-like T cells

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IL-5 plays important roles in eosinophil differentiation, expansion, and recruitment. The regulation of IL-5 seems critical for the treatment of eosinophil-mediated allergic reactions. However, the precise mechanisms for IL-5 regulation remain unknown. In this study, we investigated how IL-5 production is regulated. The transduction of GATA-3 into a murine T cell hybridoma as well as OT-II $CD4^+$ T cells resulted in acquiring the ability to produce IL-5 in response to an antigenic stimulus. This production was dependent on the cAMP-PKA pathway, but not on p38 activation. Neither transduction of MEK nor NFATc1 altered IL-5 production. On the other hand, transduction of NIK largely impaired IL-5 production. RelA and RelB similarly impaired IL-5 production. The transduction of GATA-3 decreased the expression of *Tbx21* and *Eomes*, but the additional transduction of RelA abrogated the decreased expression of GATA-3-induced *Tbx21* and *Eomes* in T cell hybridoma, and upregulated IFN-γ production from GATA-3-transduced OT-II $CD4^+$ T cells. These results suggested that strong enhancement of the NFκB pathway downregulates IL-5 production and upregulates T-box protein expression to shift an immune response from Th2 to inflammatory Th1.

P3.08.08
T-lymphocytes effectors/memory cells detection by intracellular cytokines staining in children of different age and adults

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The method of intracellular marker cytokines staining was applied in order to quantify normal levels of Th1, Tc1, Th2, Tc2, Th17, Tnc17 subpopulations in children of different age and in adults. The studied group consisted of 150 children and adolescents aged 7 months - 18 years and 45 adults of 25-45 years of age. It has been found that Th1 and Tc1-lymphocytes absolute and relative levels significantly lower in infants of 7-12 months of age than in adults. Simultaneously the Th2 lymphocytes count was similar in 7-12 months old infants and adults, whilst Tc2-lymphocytes absolute count was higher in 7-12 months old infants than in all other age groups. Th17, Tnc17 lymphocytes absolute counts were higher in 7-12 months old infants than in adults. Thus, Th2(Tc2)/Th17(Tnc17) immune response polarization was found in 7-12 months old infants which possibly helps Th2-mediated allergic disorders manifestation in atopy predisposed infants. Th1(Tc1) and Th2(Tc2) lymphocytes absolute counts were not different significantly in children older than 12 months old and in adults. Th2-lymphocytes levels decreasing and IL17A-positive cells levels increasing trends were identified in 15-18 years old adolescent group. This phenomenon can mediate the induction of autoimmune disorders predisposition at this age. Th1/Th2 counts proportion and Tc1/Tc2 counts proportion were 18.7 and 56.8, respectively in adults of 25-45 years of age. Correlations analysis

identified the strongest correlation between Th1/Tc1 subpopulations and memory cells. Relative increase in Th1 и Tc1 lymphocytes portion linked to T-memory cells pool formation reflects immune system maturation within the infants aging.

P3.08.09
Generation of mutants forms of mite allergen Der p 5 with reduce IgE binding

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Group 5 dust mite allergens Der p 5, Der f 5 and Blot 5 are part of a most important allergen group, and cause allergic disease. Based on the structure of Blot 5, linear and conformational epitopes of this allergen have been identified. Our aim is to characterized B-cell conformational epitopes of the Der p 5 allergen from *Dermatophagoides pteronyssinus* by using the web-server SEPPA and experimental validation of these results.
Two antigenic determinants: N57-K70 and R91-I100; the major surface-exposed charged residues in this region have been selected for site-directed mutagenesis. Single and multiple mutations in alanine have been introduced by PCR. Mutants have been produced in *Pichia pastoris,* purified and their stability and IgE binding capacity have been compared.
The data obtained show that the different mutants are stable at 37°C. Compared to rDer p 5, the mutants rDer p 5 E61A, rDer p 5 K65A, rDer p 5 K70A and rDer p 5 Q94AR95A display a very weak IgE reactivity, as measured by sandwich ELISA. These results will contribute to the determination of B cell epitopes to develop hypoallergenic variants of Der p 5 suitable for use in allergen-specific systemic immunotherapy or desensibilisation.

P3.08.10
The role of neuronal adhesion molecule AMIGO2 in T cell function and inflammatory diseases

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Introduction: The nervous and immune systems communicate with each other constantly and delicately to maintain own homeostasis. So far, many molecules have been identified to be expressed and utilized by both systems.
Objectives: To elucidate the roles that neuronal adhesion molecules play in the neuroimmune crosstalk, in order to provide a novel and global understanding of interaction between these two systems. AMIGO2, induced by HMGB1 in rat hippocampal neurons, is expressed mostly by endothelial, neuronal and immune cells and inhibits apoptosis and promotes neuronal survival, while little is known about its regulatory role on immune system, especially T cell function.
Methods: To study these, we generated a conventional AMIGO2 knockout mouse line. To investigate its role in T cell development, we immunophenotyped the splenocytes and thymocytes from KO mice by flow cytometry and found that T cells are slightly increased. T cell activation and proliferation assays show that KO T cells are significantly more activated and proliferative in response to CD3 ligation or ConA stimulation and survive better under mild stimulation in vitro. Interestingly, using acute EAE model, we found that KO mice have delayed onset of disease development. Currently, we are studying its role in regulating T cell differentiation and characterizing the molecular mechanisms underlying various immune regulatory functions, which may help us interpret the in vitro and in vivo results.
Conclusions: Absence of Amigo2 leads to enhanced T cell survival, activation, and proliferation in vitro, and increased amount of T cells and more resistance to EAE disease development.

P3.08.11
IL-27 regulates Th1 lymphocytes and induces Th17-dependent protection against experimental Trypanosoma cruzi infection

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IL-27 is a heterodimeric cytokine known to induce IL-10-producing Tr1 cells and to regulate Th1, Th2 and Th17 subsets, depending on the underlying disease. Because the infection caused by *Trypanosoma cruzi* normally induces heart inflammation mirrored by an outstanding migration of Th1 cells, we analyzed the regulatory role of IL-27 in this disease. After infecting WT, IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice, we verified an impressive cardiac inflammation and damage in the absence of Th17-related molecules (IL-17R$^{-/-}$, IL-23$^{-/-}$ and IL-6$^{-/-}$ mice), leading them to the premature death when compared to the control group. This cardiac inflammation was reflected by a Th1 deviation in the absence of Th17-related molecules, since that we found reduced IL-27 mRNA expression in the heart tissue in the absence of Th17-related molecules. The decreased IL-27 expression was associated with increased IL-2 levels in the heart tissue, which inhibited both Th17 and regulatory T cells. This milieu enriched by IL-2 promoted CXCL9 and CXCL10 chemokines in the heart tissue, thus inducing a deleterious Th1 migration to this organ, characterized by a local increased expression of IL-12, T-bet, iNOS, IFN-γ and TNF. To confirm the regulatory role of IL-27 in the Chagas Disease, we detected increased serum IL-27 levels in patients with free or mild cardiomyopathy when compared to patients with moderate or severe cardiomyopathy. Here, we showed that IL-27 was able to control a deleterious Th1 response in the heart tissue, thus preventing the striking myocarditis induced after *T. cruzi* infection.

P3.08.12
IL-4 receptor alpha signalling on dendritic cells is required for optimal Th2 responses in house dust mite induced allergic airway disease and schistosomiasis

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Th2 responses are responsible for the symptoms of allergic airway disease, but are required for protection against helminth infections such as schistosomiasis. While IL-4 is the main inducer of Th2 responses, the precise mechanisms leading to Th2 differentiation are unclear.The aim of this study was to determine the role of IL-4Ralpha signalling on DCs in the induction of Th2 type immune responses.CD11c^{cre}IL-4Ralpha]$^{-/lox}$ mice, lacking IL-4Ralpha expression on DCs and alveolar macrophages, were generated by gene targeting and site-specific recombination using the cre/*lox*P system under control of the CD11c locus, and tested in mouse models of house dustmite (HDM) -induced allergic airway disease and schistosomiasis. CD11c^{cre}IL-4Ralpha $^{-/lox}$ mice sensitized and challenged with HDM extract had reduced eosinophils in the bronchiolar lavage fluid and decreased airway hyperreactivity compared to littermate controls. This was associated with significantly reduced numbers of Th2 cytokine secreting CD4^{+} T cells and decreased levels of IL-33 in lung homogenates. In *Schistosoma mansoni* infection, both Th1 and Th2 responses were reduced in CD11c^{cre}IL-4Ralpha$^{-/lox}$ mice, which had noticeably smaller lymph nodes containing lower numbers of cells than littermate controls, indicating suboptimal activation of immune responses in the absence of IL-4Ralpha signaling on DCs. CD11c^{cre}IL-4Ralpha $^{-/lox}$ mice had increased morbidity and mortality, associated with smaller granulomas in the intestine and liver, raised TNF levels in the serum and a shift towards classical activation of macrophages.In conclusion, IL-4Ralpha signaling on dendritic cells promotes the development of Th2 responses against house dust mite allergens and the helminth *Schistosoma mansoni*.

P3.08.13
Differential mechanisms of contact hypersensitivity development to dinitrochlorobenzene (DNCB) in rats

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Contact hypersensitivity (CHS) is a T cell-mediated skin inflammatory reaction to haptens. As there are implications that genetic background might influence reaction, in this study CHS to dinitrochlorobenzene in Dark Agouti (DA) and Albino Oxford (AO) rats, which develop different type (Th1/Th2) of immune response to various stimuli, was investigated. More intense ear swelling response (ear thickness, cell infiltration, inflammatory mediator content in conditioned medium of ear skin explants) was observed in DA as compared to AO rats and was associated with higher proliferative activity (*ex vivo, in vitro* hapten- and IL-2-stimulated) and effector cytokine (IFN-γ, IL-17) production/mRNA expression in draining lymph nodes (DLN) during challenge phase. Concerning Th2 cytokines during challenge phase, unchanged IL-10 was noted in both strains, but increased IL-4 production only in AO rats. While relative number of $CD8^+$ cells in DLN was increased in both strains, number of $CD8^+CD11b^+$ was increased only in DA rats. Relative number of $CD4^+$ cells was decreased in DA (unchanged in AO rats), however number of $CD4^+CD25^+Foxp3^+$ was unchanged in both strains. During sensitization phase, DLN activity (*ex vivo* and IL-2-stimulated proliferation, IFN-γ and IL-17 production/mRNA expression) was increased in both strains, but lower in AO rats. IL-10 and IL-4 production were unchanged during this phase. Reduced lymphoproliferative activity and IFN-γ and IL-17 production, together with increased IL-4 production during challenge phase, could be underlying mechanisms of lower intensity of CHS expression in AO rats.
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P3.08.14
A novel strategy for integrating gene expression profiles derived from multiple publicly available datasets highlights novel hub genes and pathways in mouse models of allergic asthma

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There are 30 studies of gene expression profiling of lungs from mice with allergic asthma, which shed new light on the molecular mechanisms of disease, but the experimental designs between them differ. Nevertheless, relevant genes and pathways underlying disease pathogenesis may emerge from the integration of these datasets. From 12 publically available datasets, we selected 6 for meta-analysis on the basis of the microarray platform and *in vivo* experimental protocol. Our strategy was to combine a top down pathway-centered and a bottom up gene-centered analyses. The first approach consisted of selecting asthma-specific pathways from a combination of 'enriched biological terms' in each individual study. Secondly, we combined differentially expressed genes from individual datasets to create a gene list. From 22,690 genes and 131 samples, we obtained a core-network of more than 300 interconnected genes unraveling known (STAT1, JAK2) and unknown hubs (PU.1, CDK1). Additionally, we successfully selected known and unknown disease-related pathways, such as T-cell activation during an immune response, B-cell mediated immunity, phagocytosis and complement activation. The power of this strategy is the possibility to uncover key molecules underlying disease pathogenesis utilizing datasets derived from disparate experiments.

P3.08.15
Expression of cytokines and transcription factors in CD4+ T cell in myasthenia gravis

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When activated, CD4+ T cells differentiate and secrete cytokines to modulate other cells, influencing the pathogenesis of autoimmune diseases. Helper T cell subsets [Th1, Th2 and Th17, T follicular helper (Tfh)] as well as regulatory T cells (Treg) effect disease pathogenesis in many autoimmune diseases. In this study, Th cell subtypes are evaluated and compared with another autoimmune disease, namely rheumatoid arthritis (RA).
CD4+ T cell population from 63 generalized myasthenia gravis (MG) without thymoma (26 anti-AChR+, 18 anti-MuSK+ and 19 double-negative-MG), 12 RA patients and 20 healthy donors (CON) were included. TBET, GATA3, RORG, BCL6, CD40L and IFNG, IL10, IL17A, IL21 mRNA levels were determined semi-quantitatively by PCR with specific primer sets using Sybr Green. Quantification of target gene levels were performed by comparing 2 -αα CT values with GAPDH.
Expression of TBET, GATA3, RORG as well as CD40L was lower in MG than CON ($p= 0.018$, $p= 0.033$, $p= 0.026$ and $p<0.001$). The expression levels of IL10, IFNG, IL17A, IL21, BCL6 in CD4+ T cells were similar in the MG as well as in the subgroups with or without treatment. TBET and CD40L were also down regulated in CD4+ T cells of MG patients without treatment ($p= 0.01$). Similarly TBET, GATA3, RORG and CD40L expressions in RA were also decreased compared to CON (all $p< 0.001$).
Th1 downregulation and the lower levels of the B cell costimulation by CD4+T cells is implicated in the peripheral blood of MG patients.
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P3.08.16
MiRNA expression and function in CD4 T cells from asthmatic airways

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Atopic asthmatics are prone to developing T-helper type 2 (Th2) responses, and Th2 cells are central in the inflammatory response in the lung due to production of the pro-inflammatory cytokines interleukin-13 (IL-13), IL-5, and IL-4. Additionally, production of the cytokines IL-17A, IL-17F, and IL-22 by Th17 cells may be involved in airway smooth muscle remodeling and airway hyper-responsiveness in asthma. MicroRNAs (miRNAs) regulate gene expression programs by reducing the stability and translation of target mRNAs, and they are particularly important in cell identity decisions within the hematopoietic lineage. In order to understand the role of miRNAs in CD4 T cells in asthma, we determined the expression of a panel of miRNAs in CD4 T cells sorted from bronchoalveolar lavage fluid from asthmatic and healthy subjects, and found differential expression of a small number of miRNAs. Further functional analyses in both human and mouse CD4 T cells indicate that these miRNAs play a role in regulating the differentiation of naïve CD4 T cells into Th2 or Th17 effector cells, and may contribute to the pathogenic properties of T-helper cells in the airways of asthmatics.

P3.08.17
Differences in the polarization of the inflammatory response of patients with chronic obstructive pulmonary disease (COPD) secondary to smoking and to biomass smoke exposure

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Introduction. Smoking and exposure to biomass smoke cause the release of pro-inflammatory mediators, as well as the activation of T helper (Th) cells. This inflammatory response can lead to the development of COPD; however, there are different clinical features between patients with COPD secondary to smoking (sCOPD) and to exposure to biomass smoke (bsCOPD), and these may be due to the kind of predominant Th response.
Aim. Quantify the $TCD4^+$ cells subpopulations and the cytokines in peripheral blood of patients with sCOPD, bsCOPD and healthy controls (HC).
Methods. 41 patients were recruited, 24 sCOPD and 17 bsCOPD; we included 25 HC. Through flow cytometry the proportion of $TCD4^+$ cells subpopulations was defined. The serum cytokines were quantified through Luminex system. The differences between groups were evaluated with a Kruskal-Wallis test, and a post-hoc analysis was performed through a U of Mann Whitney test.
Results. A prevalence of Th17 cells was observed in the sCOPD group (10.3 ± 3.4%), when compared with bsCOPD (3.5 ± 0.9%) and HC (0.9 ± 0.4%) $p<0.001$. Th2 cells were predominant in the bsCOPD patients (4.4 ± 1.3 %), in comparison with sCOPD (2.5 ± 0.8 %), and HC (1.1 ± 0.4 %). IL-4 and IL-10 are in a higher concentration in bsCOPD versus sCOPD.
Conclusions. Our data show a polarization of Th17 cells in sCOPD, and of Th2 cells in bsCOPD. There are differences in the wsCOPD and the sCOPD cytokine profiles, which may explain some of the clinical features observed in the COPD phenotypes.

P3.08.18
Transgenic Expression of Survivin Compensates the OX40-Deficiency in Driving Th2 Development and Allergic Inflammation

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Two major types of signals are required for optimal T cell activation, with one derived from the T cell receptor (TCR) and the other from a number of costimulatory receptors. Emerging evidence has suggested that the protein survivin, a member of the inhibitor of apoptosis (IAP) family (also called baculoviral inhibitor of apoptosis repeat-containing 5 or BIRC5), may be an important intermediate contributing to the development of efficient T cell responses. However, the importance of survivin to T cell-driven inflammatory responses has not been demonstrated. Here, we show that survivin transgenic mice exhibit increased antigen-driven Th2 lung inflammation, and that constitutive expression of survivin reverses defective lung inflammation in the absence of OX40 costimulation. We found that while OX40-deficient mice are compromised in generating Th2 cells, airway eosinophilia, and IgE responses, survivin transgenic/OX40-deficient mice generate normal Th2 responses and exhibit strong lung inflammation. These results suggest that OX40 costimulation crucially engage survivin during antigen-mediated Th2 responses, and promote the notion that survivin could be a vital intracellular molecule of costimulation that regulates allergic responses or lung inflammation.

P3.08.19
Resistin, Tumor Necrosis Factor-α and Insulin Resistance in Normal Pregnancy

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Intoduction: Pregnancy is associated with glucose metabolism disorders and insulin resistance (IR). Insulin resistance may facilitate supply of appropriate nutrients particulary of glucose t! o fetus for fetal growth and metabolism. The mechanism responsible for insulin resistance has not been clearly stated. Objective: The purpose of this study was to evaluate the role of resistin and Tumor Necrosis Factor-α (TNF-α) in insulin resistance during pregnancy. Approach: Serum resistin and TNF-α concentrations were measured by ELISA in 86 healthy pregnant women (26, 23 and 37 of them in the 1st, 2nd and 3rd trimesters, respectively) and in 21 healthy non pregnant women in a cross sectional study. Results: Resistin concentration was significantly higher in the third trimester (9.5±3.3 ng mL1) as compared with non pregnant women (7±3.3 ng mL1). Serum TNF-α levels were also significantly increase in pregnant women (2.6±1.9 pg mL1) as compared with maternal healthy controls (0.8 ±0.7 pg mL1). There were significant correlations between gestational age and BMI ($r=0.28$, $p=0.01$), resistin ($r=0.36$, $p=0.002$) and TNF-α($r = -0.44$, $p<0.0001$). There was not a significant correlation between gestational age and Insulin Resistance (IR). We also did not found correlation between IR and resistin as well as between IR and TNF-α in pregnant women.Conclusion: TNF-α and resistin do not appear to contribute greatly to pregnancy-induced insulin resistance in healthy pregnancy.

P3.08.20
Identifying the T helper cell population involved in class-switching to IgE

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IgE antibodies are key players in allergic diseases and can be generated by direct switching of IgM/IgD cells, or by sequential switching of IgG1 cells to IgE. The characteristics of the IgG1 cells involved in the switching to IgE and the nature of IL-4 producing T cells involved in direct and sequential switching are not known. The goal of this work is to identify the T cell populations that help naïve and IgG1 cells to switch to IgE.
When comparing the memory/activated population of cells in the lymphoid organs of immunized TBmc 4get mice, we found at least 2 types of IL-4 producing cells identified based on PD1 expression, in agreement with published work. Real-time PCR analysis of the GFP^+PD1^+ cells in the lymphoid organs showed expression of IL-4, IL-21, CXCR5 and Bcl6, confirming its Tfh nature. Conversely, the GFP^+PD1^- population lacked Tfh markers, representing a Th2-like population. Using this system, we found that the Th2-like cells were the best helpers to induce naïve B cells to class-switch to IgE both in an in-vitro and an in vivo model.
We are also investigating the specific characteristics of IL-4 Tfh populations found in systemic and mucosal lymph nodes, to correlate with their differential ability to promote immunoglobulin class switching to IgE.
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P3.08.21
Retrogenic expression of human TCR in HLA-DR4 transgenic NOG mice

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Humanized mouse is a key technology for translating basic researches to clinical applications.
We have generated HLA-DR4 expressing NOG mice (DR4 NOG). In this meeting, we are going to report retrogenic expression of human

TCR genes from cow-milk allergy patients. We isolated 2 independent TCR genes from HLA-DR0405-restricted $CD4^+$ T cell clones (HA5.7 and YA4) specific for a bovine lactoglobulin (BLG) peptide. Using retroviral or lenti vectors, we introduced the TCR genes into HSC and transferred into DR4 I-$A^{-/-}$ NOG mice. The expression of the TCR was monitored by FACS using anti-TRBV antibody and bi-cistronic GFP. In 3 months after HSC-transplantation, $CD4^+$ T cells appeared in the peripheral blood and gradually expanded. When those mice were analyzed at 5 months post HSC-transfer, in the thymus, the skewing of GFP positive thymocytes to $CD4^+CD8^-$ SP cells were detected. In the periphery, both HA5.7- and YA4-derived $GFP^+TRBV^+CD4^+$ T cells were identified. Stimulation of the spleen cells with the BLG peptide in vitro induced significant proliferation of $GFP^+TRBV^+CD4^+$ T cells. These T cells also produced IFN-g in response to the peptide stimulation. When using HLA-matched HSC, anti-BLG IgG was also detected in the sera of the mice.
Collectively, our results suggest that human TCR genes can be expressed in humanized mice in a functional manner. This system may help recapitulating human food allergy. We are now generating GM-CSF/IL-3 transgenic DR4 I-$A^{-/-}$ NOG mice, in which human myeloid cells including mast cells can develop.

P3.08.22
The effect of toll-like receptor 4 on the development of acquired immunity against Salmonella infection

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Interaction of TLR4 with the lipopolysaccharide of *Salmonella enterica* serovar Typhimurium (*ST*m) rapidly activates innate immunity to control microbial growth *in vivo*. Effective clearance of the bacteria requires engagement of acquired, antigen-specific immunity. It is unclear whether TLR4 is essential for the development of classical T_H1 immunity against *ST*m. To address this question, *Tlr4*$^{-/-}$ and congenic wild-type mice were challenged with attenuated *ST*m. *Tlr4*$^{-/-}$ mice had significantly higher microbial burden confirming the crucial role TLR4 plays in restricting bacterial growth early in infection. Bacterial clearance, however, which is primarily driven by CD4-dependent T_H1 immunity, was unimpaired in *Tlr4*$^{-/-}$ mice. To investigate this further, we first characterised the ability of *Tlr4*$^{-/-}$ and wild-type bone marrow-derived dendritic cells (BMDCs) to polarise wild-type naïve $CD4^+$ T cells in response to *ST*m *in vitro*. We found that *Tlr4*$^{-/-}$ BMDCs were impaired in polarising wild-type naïve T cells as opposed to their wild-type counterparts. We then assessed the quality of immunological memory by stimulating $CD4^+$ T cells obtained from the spleens of immunised mice with *ST*m cell extract. IFN- and Il-2 production by *Tlr4*$^{-/-}$ $CD4^+$ T cells was substantially reduced compared to that produced by wild-type cells. *Tlr4*$^{-/-}$ mice immunised with live *ST*m were as protected as wild-type when exposed to what would be a lethal infection in unvaccinated animals. Our *in vitro* work clearly suggests that TLR4 is indispensable for the development of robust T_H1 responses against *ST*m. *In vivo*, however, compensatory mechanisms exist that drive pathogen clearance and generation of protective immunity.

P3.08.23
Formation of the utero-(pre) placental blood flow in pregnant women with threatened abortion in early pregnancy due to impaired immune-hormonal homeostasis

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Results. In pregnant women with threatened abortion in the first trimester, due to the low level of progesterone, compared with rates of women with physiological pregnancy, was shown increased production of cytokines Th I and indicators of vascular resistance (resistance index (RI) and pulse index (PI)) in the spiral arteries which were shown by increasing concentration of α-TNF (14,65 pg/ml(14.03-16,2) and 7.2 pg/ml(6.3-7,5), respectively, p< 0,01), γ-IFN (9,34pg/ml (8.16-12,1) and 3.95 pg/ml(3.55-4.35), respectively, p<0,01) and IL-2 (9.74 pg/ml(7.3-12,77) and 5.1 pg/ml(4,3-5,9), respectively, p<0,01) with a parallel decrease in the concentration of IL-4 (0.68 pg/ml(0.58-0,84) and 1.49 pg/ml(1.38-1,64), respectively, p<0,01) and IL-10 (4.96 pg/ml(4.2-5.94) and 9.2 pg/ml(8.59-9,6), respectively, p<0,01), as well as increased RI (0,67(0,65-0.69) and 0.55(0,49-0,62), respectively, p<0,01) and PI (1,35(1,32-1,38) and 0.84(0,78-0,91), respectively, p<0,01) in the spiral arteries. At the same time, was revealed correlation between γ-IFN and RI (ρ=0,43; p<0,01) and PI (ρ=0,58; p<0,01) of spiral arteries, between α-TNF and RI (ρ=0,39; p<0,01) and PI (ρ=0,52; p<0,01) of spiral arteries and between the level of IL-4 and RI (ρ=-0,42; p<0,01) and PI (ρ =-0,59; p<0,01) of spiral arteries.
Conclusions. In pregnant women with threatened abortion in the first trimester, due to the low level of progesterone, a systemic shift was revealed in the balance of regulatory cytokines towards Th1 and their correlation to the vascular resistance in the spiral arteries, which attests to a disturbance in the formation of the utero-(pre-) placental blood flow and can be primary predictor of placental insufficiency.

P3.08.24
Myofibroblast Apoptosis, Removal from the Lungs and Attenuation of Fibrosis by FasL+ Immune Cells

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Immune surveillance can be exerted upon self cells, usually after being transformed, senescent or infected; however, we previously found, in the bleomycin (BLM) experimental-model of idiopathic-pulmonary-fibrosis, that fibrotic-lung myofibroblasts resist Fas-induced apoptosis and escape immune surveillance. Yet, mechanisms for physiological scar removal from sites of injury remain obscure. Here we show that upon resolution of fibrosis, myofibroblasts are susceptible to CD4+T cell-induced apoptosis and to T cell-dependent immune surveillance in allogeneic-BALB/c, but not in immune-deficient congeneic-SCID mice. Impaired immune surveillance, as detected, in SCID mice, or in humans under immunosuppressive therapy, results in the development or worsening of lung fibrosis, thus showing that immune surveillance is important for limiting aberrant fibroblast accumulation and development of fibrosis in vivo. FasL-negative mouse primary-T cells, and primary-T cells or human Jurkat T cell-line, in which we interfered with FasL/Fas interaction, failed to induce myofibroblast apoptosis. Accordingly, following IT-BLM, when compared to FasL-deficient mice with wild-type (wt) immune cells (wt/gld), FasL-deficient (gld/gld) mice, accumulate myofibroblasts in their lungs and develop fibrosis. Our study indicates that, functional FasL+ immune cells and CD4+ T cells, may control abnormal in vivo myofibroblast accumulation by inducing myofibroblast cell death and that immune surveillance represents an important extrinsic component of myofibroblasts apoptosis and a fibrosis barrier. Factors interfering with immune cell-myofibroblast interaction via Fas/FasL may abrogate immune surveillance. Annulling these factors may pave a new direction to control human lung fibrosis.

P3.08.25
Impact of immunosuppressive drugs on killing capacity and cytokine production of BKV-specific T cells

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Immunosuppression (IS) of kidney allograft recipients is currently the indispensable method to avoid graft rejection. Nevertheless, suppression of the cellular immunity is an important risk factor for the reactivation of Polyomavirus BK (BKV) and the development of BKV-associated nephropathy (BKVAN). However, the impact of diverse immunosuppressive drugs on the multifunctionality and cytolytic capacity of BKV-specific T-cells remained unclear.
BKV-specific T-cell lines were generated from BKV-seropositive healthy donors and cultured under treatment of Prednisone,

Mycophenolate Mofetil (MMF), Rapamycin, Cyclosporin A (CsA) and Tacrolimus, respectively. Subsequently, phenotype, proliferation, cytokine production and killing capacity of the obtained BKV-specific T-cells were assessed by flow cytometry.
As expected, IS had a negative effect on T-cell proliferation, headed by MMF. The downregulation of IFNg production by calcineurin inhibitors (as previously described) and by prednisone was observed, whereas Tacrolimus revealed the highest suppressive potential. Furthermore, calcineurin inhibitors and Prednisone, but not Rapamycin and MMF, deteriorate the multifunctionality of BKV-specific T-cells. Surprisingly, even the CsA, Tacrolimus and Prednisone treated T-cells revealed their virus-specific cytolytic abilities.In case of BKV-reactivation the reduction or break of CsA, Tacrolimus and/ or Prednisone treatment might be required to restore the beneficial multifunctionality of naturally occurring BKV-specific T-cells. However, in a future setting of a possible BKV-specific adoptive T-cell transfer, such a change of the IS drug regimen seems to be non-essential, as the virus specific cytolytic capacity itself is less affected. These findings need to be considered in context of an adoptive T-cell-therapy as well as for vaccine development to ensure therapeutic feasibility.

P3.08.26
Bromodomain-Containing-Protein 4 (BRD4) Regulates RNA Polymerase II Serine 2 Phosphorylation in Human CD4+ T Cells

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Transcriptional elongation by RNA polymerase II (Pol II) is regulated by positive transcription elongation factor b (P-TEFb) in association with Bromodomain-containing protein 4 (BRD4). We used genome-wide chromatin immunoprecipitation sequencing in primary human CD4+ T cells to reveal that BRD4 co-localizes with Ser2-phosphorylated Pol II (Pol II Ser2) at both enhancers and promoters of active genes. Disruption of bromodomain:histone acetylation interactions by JQ1, a small-molecule bromodomain inhibitor, resulted in decreased BRD4 binding, reduced Pol II Ser2, and reduced expression of lineage-specific genes in primary human CD4+ T cells. A large number of JQ1-disrupted BRD4 binding regions exhibited di-acetylated H4 (lysine-5 and -8) and H3K27 acetylation (H3K27ac), which correlated with the presence of histone acetyltransferases and deacetylases. Genes associated with BRD4/H3K27ac co-occupancy exhibited significantly higher activity than those associated with H3K27ac or BRD4 binding alone. Comparison of BRD4 binding in T cells and in human embryonic stem cells revealed that enhancer BRD4 binding sites were predominantly lineage-specific. Our findings suggest that BRD4-driven Pol II phosphorylation at serine 2 plays an important role in regulating lineage-specific gene transcription in human CD4+ T cells.

P3.08.27
Effect of extremely low frequency electromagnetic field (ELF-EMF) on some cytokines production of rats

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A link between exposure to extremely low frequency electromagnetic field (ELF-EMF) and some cancers has been suggested. This can be due to ELF-EMF effects on the immune system, where $CD4^+$ T cells play an important role. Naïve $CD4^+$ T cells differentiate into effector T_H1, T_H2 and T_H17 subsets according to the presence of different cytokines. T_H1 cells are critically important in protecting us against tumors. Therefore, the present study was designed to test the hypothesis that ELF-EMF can modulate cytokines like IFN-γ, IL-4, IL-6 and IL-12 that regulate the $T_H1/T_H2/T_H17$ balance. 40 adult male Wistar rats were randomly assigned to ELF-EMF-exposed and sham-exposed control groups. The ELF-EMF-exposed group was exposed to a flux density of 100 μT, frequency 50 HZ, 2h/day/7 days a week for 3 months. The sham-exposed control group was placed in identical chambers without ELF-EMF. Results show that serum IL-12 levels are decreased from 418±47pg/ml in sham-exposed controls to 300±23pg/ml ($p<0.05$) in ELF-EMF-exposed rats. PHA-activated IL-6 production in spleen cultures (1356±92 pg/ml) and total blood cultures (418±40 pg/ml) of ELF-EMF-exposed rats was higher ($p<0.001$) compared with controls (905±74 pg/ml), (182±26 pg/ml), respectively. However, the serum levels of IFN-γ, IL-4 and IL-6 and levels of IFN-γ, IL-4 and IL-12 in whole spleen cultures and total blood cultures of the two groups were not significantly different. In conclusion, ELF-EMF may change the $T_H1/T_H2/T_H17$ balance towards down-regulation of T_H1 (decrease of IL-12) type response and upregulation of T_H17 (increase of IL-6) type response.

P3.10 Th17 cells

P3.10.01
New light of the il23/th17 in pemphigus foliaceus

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The pemphigus foliaceus (PF) is considered as a Th2 disease, with a partial shift to Th1 during the remission phase. This classical Th1/Th2 paradigm previously defining PF could be challenged with the recent discovery of Th17 T cells. The IL-23/IL-17 axis plays an important role in many autoimmune skin diseases. The aim of our research was to investigate the expression of IL-23, IL-17 and TNF-α on PF patients and healthy individuals skin biopsies.
Biopsies obtained from 17 PF patients and 17 healthy controls were assessed by immunohistochemical analysis using specific polyclonal antibodies (anti-IL17, anti-IL23 and anti-TNF α). Immunohistochemical staining for IL-17 positive T cells showed the presence of these cells only in PF specimens, but these cells do not appear to be abundant in PF skin (5% of lymphocyte in PF skin). Interestingly, a significant correlation between IL17 expression and antibody titers was observed ($r=0.878$; $p=0.021$). Keratinocytes and dendritic cells in lesional PF skin expressed higher levels of IL-23 compared with normal skin (0.794±0.356 and 24.7% in patients versus 0.588±0.264 and 14.1% in healthy controls, respectively). Specific staining for TNF-α in cryostat sections confirmed the higher expression of this cytokine by monocytes and fibroblastes in PF versus controls skin ($p=0.023$ and $p<0.0001$, respectively). Furthermore, the TNF-α, IL17 and IL23 expression appeared to be significantly correlated in PF skin compared with normal skin, as well.
Taken together, these results suggest that the IL-23/Th-17 axis may play a central role in the pathogenesis of the PF.

P3.10.02
Differential mucosal expression of Th17 related genes between inflamed and non-inflamed colon of patients with Crohn's disease

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A causal role is attributed to inflammation in several diseases through cellular mediators and cytokines. These molecules are involved in inflammatory bowel diseases including Crohn's disease (CD) and have both tissue-protective and inflammatory effects in the gut.
The main objective of this study is to evaluate the implication of Th17 cells in colonic inflammation in CD. Th17 are considered

developmentally distinct from Th1 and Th2 cells and characterized as preferential producers of interleukin 17A. Th17 lymphocytes are significant and sometimes even decisive in the establishment of a protective immune response during bacterial and fungal infections The expression level of the Th17 related cytokines: IL22, IL17A and the IL23 receptor where compared between inflamed and non inflamed colonic tissues from the same patients.
A total 20 macro-dissected intestinal tissue samples from CD patients were obtained during colonoscopy. The mRNA expression level of each gene was determined by RT-PCR. Evaluation of mRNA expression levels in colonic and control samples from 20 patients revealed that IL17A, IL22 and IL23R were expressed at higher levels in inflamed biopsies.
The increased expression of these genes was predominantly observed in inflammatory samples from CD patients and associated with more intense inflammation. Our findings suggest the implication of Th17 cells in the pathogenesis of CD. This study demonstrates that tissue inflammation in CD patients is clearly associated with increased expression levels of Th17 related cytokines.

P3.10.03
The role of β2 adrenergic receptor signaling during the reciprocal differentiation of Th17 and Foxp3+ Treg cells

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The nervous system can interact with the immune system via the sympathetic nervous system. $CD4^+$ T cells express adrenergic receptors, mainly the β_2 adrenergic receptor (β_2AR). β_2AR signaling in $CD4^+$ T cells seems to impair Th1 differentiation. The aim of this study was to investigate the impact of β_2AR signaling in the reciprocal differentiation of Th17 and $Foxp3^+$ Treg cells. Sorted naïve $CD4^+$T cells ($CD4^+CD62L^+Foxp3^-$) from $Foxp3^{gfp}$ KI mice were cultured with plate bound anti-CD3, IL-6, TGF-β, IL-23, IL-1β, TNF-α, anti-IL-4 and anti-IFN-γ for 3 or 5 days. A specific β_2AR antagonist was administered in the culture. The expression of IL-17A was determined by intracellular cytokine staining and ELISA. We observed that in control cultures about 12-16% of the naïve $CD4^+$ T cells differentiated into IL-17-producing cells. The treatment with the specific β_2AR antagonist did not change the percentage of IL-17-producing $CD4^+$ T cells as compared with control. However, the expression of IL-17 was significantly reduced in cells after β_2AR antagonist treatment. Confirming this result, we also found lower IL-17 levels in the supernatants from cultures treated with the β_2AR antagonist as compared to those observed in control cultures. Following β_2AR blockade, the lower IL-17 production by anti-CD3 activated $CD4^+$ T cells cultured under Th17-skewing conditions was associated with higher frequency of $CD4^+$ T cells expressing Foxp3 (3 day). Our data suggest that the endogenous production of catecholamines can impact the differentiation of Treg cells and the production of IL-17 by CD4+ T cells via β_2 adrenergic receptor signaling.

P3.10.04
Regulation of cytokine gene expression in human T helper cells: role of IL-2 and pSTAT5 in the activation-dependent down-regulation of IL-17 production

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TH17 cells represent a distinct lineage of CD4+ T helper cells characterized by the production of IL-17A, IL-17F, and IL-22 that are involved in autoimmunity as well as in host defence .In humans,TH17 differentiation is induced by IL-6, IL-1β, IL-23 and TGF-β and requires the activation of transcription factors such as RORγt and STAT3.
Here we show that, following activation, TH17 memory cells transiently down-regulate IL-17 production while, in some cases, up-regulating IL-10 production in a reciprocal manner. Regarding the activation-dependent reciprocal switch from IL-17 to IL-10 production, we demonstrate that IL-17 producing cells can be recovered from activated CD4+ CCR6+ memory T cells which produce IL-10 and that were isolated from peripheral blood or synovial fluid of patients suffering from Juvenile Idiopathic Arthritis (JIA). We further demonstrate that the down-regulation of IL-17 in TH17 cells is accompanied by the down-regulation of RORγt. Based on published on our findings we propose a mechanism of activation-dependent down-regulation of IL-17 that involves the competition of IL-2-induced pSTAT5 with pSTAT3 for binding to the IL-17 locus, thereby reducing IL-17 transcription. Interestingly we further found that also TH2 cells down-regulate their signature cytokine IL-4 upon activation, while the expression of the master transcriptional regulator GATA3 is maintained and IL-2 does not seem to play a role in the process of down-regulation. Inhibition of IL-17 production in activated TH17 cells represents a new regulatory function of IL-2 besides its role in inhibiting TH17 differentiation, and in the development, survival and function of regulatory T cells.

P3.10.05
Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4.

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BACKGROUND: IL-17A has been suggested to play a pathogenic role in bronchial asthma and other allergic disorders. OBJECTIVE: Study of the relationship between human IL-17A-producing CD4+ T cells (TH17) and IL-4-producing CD4+ Tcells (TH2). METHODS: T-cell clones generated from the CCR6+CD161+ fraction of human circulating CD4+ T cells, which contains virtually all TH17 cells, as well as circulating CD4+ T cells from both healthy subjects and patients with asthma, were assessed by flow cytometry for their cytokine production profile. RESULTS: A small proportion of CCR6+CD161+CD4+ T-cell clones showed the ability to produce both IL-17A and IL-4 (TH17/TH2). TH17/TH2 clones also produced IL-5, IL-8, IL-9, IL-13, IL-21, and IL-22 and displayed the ability to induce the in vitro secretion of IgE. A very few
TH17/TH2 cells were found among circulating CD4+ T cells from normal subjects, but their proportions were significantly increased in the circulation of patients with chronic asthma. TH17/TH2 cells could not be derived from naive umbilical cord blood CD4+ T cells under any experimental condition. However, when circulating memory CCR6+CD161+CD4+ T cells were cloned under appropriate polarizing conditions, TH17/TH2 clones originated in the presence of IL-4, suggesting that an IL-4-rich microenvironment may induce the shifting of memory TH17 cells into TH17/TH2 cells. CONCLUSION: Because of its peculiar functional properties and the increased numbers in the circulation of patients with bronchial asthma, this previously unknown population of TH17/TH2 cells may play some role in the pathogenesis of this disease.

P3.10.06
Generation of effector T cells from costimulation from immune complexes and complement

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CD4+ T helper (TH) cells are the major driver of host immunity. Costimulation from CD28 is essential for the generation of the CD4+ T effector population. Lack of CD28 costimulatiory signal results in an anergic population. We earlier reported changes associated with T cell activation from signals derived from immune complexes (ICs) and C5b-9, a byproduct of complement activation. Now we report that the signal from the ICs and C5b-9 successfully substitutes for the CD28 requirement in generation of IL-17A+ and IL-22+ populations. Further activated CD4+ T cells engage ICs via FcγRIIIA and produce high levels of IFN-γ. T-bet was not required for this IFN-γ production. IFN-γ contributes to tissue damage and regulates the function of TH17 and Treg cells by inducing T-bet. Elevated levels of ICs are present in infections, cancers, and their defective clearance lead to the development of autoimmunity. In such scenarios, IFN-γ produced from IC stimulation could influence the expression of key regulators of peripheral tolerance such as PD1 and ICOS. Thus, we demonstrate the first non-membrane associated costimulator and new IFN-γ producing population that is FcγRIIIA+. These results are vital for

understanding the disease pathology and for the development of safe biological therapies. In many patients biologics upon infusion forms ICs.

P3.10.07
Role of Notch receptor signaling in CD4+ T helper 1 and T helper 17 cell differentiation

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CD4+ T helper cells (TH) are involved in host defense to pathogens and in the maintenance of immune homeostasis. Upon antigen-specific recognition, naive CD4+ T cells differentiate into several functionally distinct subsets of CD4+ TH cells characterized by the secretion of a specific pattern of effector cytokines. A relationship between TH1 and TH17 cells has been reported, illustrated by counter-regulation and also by the plasticity of TH17 cells that can differentiate into TH1 cells. However, the mechanisms involved in this control are not defined. Among the different factors contributing to the differentiation of CD4+ TH cells, Notch receptor signaling has been reported to influence either the differentiation and/or the function of CD4+ TH cells. To investigate the contribution of Notch signaling in the control of TH1 and TH17 cell functions, mice carrying a T cell-specific deletion of both Notch1 and Notch2 (N1N2ΔCD4Cre) were infected with the protozoan parasite Leishmania major. Despite their resistant C57BL/6 genetic background, N1N2ΔCD4Cre mice were unable to control lesion development and parasite growth, due to impaired secretion of IFN-γ by TH1 cells. Moreover, upon restimulation with L. major, the absence of Notch receptors also prevented the secretion of TH17 cytokines by dLN cells. These results demonstrate a new crucial role for Notch signaling in the differentiation of functional TH1 and TH17 cells during the course of this infection. Furthermore, they suggest that the modulation of Notch signaling in immune responses involving both TH1 and TH17 immunity could impact on disease development.

P3.10.08
Defective Th17 development in human neonatal T cells involves reduced RORC2 mRNA content

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The neonatal immune system shows an impaired response upon activation compared to adults. This phenomenon may involve $FOXP3^+$ regulatory T cells (Treg) and Th17 cells.
In order to investigate this, we compared $CD4^+CD25^-CD45RO^-$ naive T cells from human cord blood (CB) with cells from adult peripheral blood (APB). Cells were activated *in vitro* by anti-CD3 in the presence of viable antigen presenting cells (APC). The induction of regulatory T cells and Th17 cells was analysed by flow cytometry. Cytokine production was measured by multiplex immunoassays and RORC and IL-17 mRNA content by PCR.
Activation of CB cells resulted in a significantly higher percentage of functional Treg than in APB. No Th17 cells were observed in CB, whereas in APB cultures IL-17, IL-21 and IL-22 were produced in significant quantities. As inflammatory cytokines produced by APC have a major role in directing T cells towards a Th17 phenotype, we tested IL-1β and IL-6 concentration in cell culture supernatants. High amounts of these cytokines were found in APB, but not in CB cultures. Addition of recombinant cytokines IL-1β, IL-6 or IL-23 could not imprint a Th17 phenotype in CB cells, but merely reduced the amount of induced Treg. Measurement of RORC2 mRNA - coding for RORγT protein, the Th17 defining transcription factor - showed a significantly lower content in CB compared to APB after activation.
These data show that factors upstream of RORC transcription can prevent Th17 development and can have implications for understanding Th17 and Treg development and neonatal immune maturation.

P3.10.09
Phagocytosis of different source of infected apoptotic cells: does a trigger to Th17 or Th1 differentiation?

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Phagocytosis of infected apoptotic cells (ACs) with *Escherichia coli* by dendritic cells (DCs) promotes the production of anti-inflammatory cytokines such as TGF-β but also pro-inflammatory cytokines as IL-6 and IL-23 and these cytokines microenvironments induce the Th17 differentiation. The *aim* of our study is to understand whether distinct sources of PAMPs could trigger the same immunostimulatory effect. Therefore, we co-cultured DC with apoptotic cells infected with Gram-negative and Gram-positive bacteria to evaluate the cytokine profiles produced in response to efferocytosis by dendritic cells. As a source of infected apoptotic cells, C57BL/6J mice were intraperitoneally injected with 1 mL thioglicollate plus live *E. coli* or live *Streptococcus pneumoniae,* and 13 h later the cells were collected from peritoneal cavity lavage. Then, these infected apoptotic neutrophils were irradiated with UV (350mJ) to induce apoptosis. BMDC were co-cultured at 1:3 ratio with both infected apoptotic neutrophils during 18h and supernatant was collected for the *measurement* of IL-1α, IL-1β, IL-6 and TGF-β by ELISA. Our results showed that distinct cargo of infected apoptotic cells induced a differential cytokine profile, such as IL-1α (AC+*E.coli*, 255±51 and AC+*S.pneumoniae*, 986.7±59.8 pg/ml) and IL-1β (AC+*E.coli,* 803±17 and AC+*S.pneumoniae,* 1439 ±170.4 pg/ml), TGF-β (AC+*E.coli,* 705±31 and AC+*S.pneumoniae,* 509±81) and IL-6 (AC+*E.coli,* 26740±1109 and AC+*S.pneumoniae*, 2625±363.5 pg/ml). Our results show that DCs are able to discriminate differentially the cargo from infected apoptotic cells and the next step is to further understand how it impacts the adaptive response.

P3.10.10
Cytokine regulation of immunopathology in *Echinococcus multilocularis*

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Alveolar echinococcosis (AE) is a rare but potentially fatal disease. It is caused by infection with the larval (metacestode) stages of Echinococcus multilocularis (Em). A Th1 / Th2 imbalances is the most important immunologyical change in the course of a parasitic infection. Th17 cells have been identified as a new subset of T helper cells and several cytokines are involved in regulating their activation and differentiation. Th17 cells and IL-17 play an important role in the parasitic diseases. During the course of E.m infection course, our results showed a trend of constantly increasing levels of IL-17 was observed. IL-17 levels were elevated significantly ($P < 0.05$, respectively) at 6 and 10 month post-infection. TGF-β1 concentration was also elevated significantly at 6 and 10 months. Levels of IL-6 reached a maximum at 2 days post-infection , then dropped at 1 month, followed by a steady rise until 2 months. Levels of IFN-γ increased after Em infection, reaching a peak at 3 months, and decreased thereafter. IL-4 levels remained low until 2 months, and increased after 3 months. TGF-β was highly expressed in the late infection, and showed a similar phase variation to IL-17, which indicates that TGF-β1 may have a important role in promoting Th17 cell differentiation. With the growth of hydatid cysts, this process, and not a serious inflammation reaction infiltration formed the fibre layer and separated the hydatid cyst from the host tissue. This protective mechanism can effectively evade the host immune response.

P3.10.11
Pharmacological modulation of epigenetic regulation of Treg and Th17 differentiation in vitro

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Depending upon signals from innate immune cells induced by pathogenic agents, naïve $CD4^+$ T cells differentiate into several effector fates including T helper 1 (Th1), Th2, and Th17. Also, instead of becoming effector cells, $CD4^+$ T cells can become regulatory cells that help in suppressing immune responses. These effector and regulatory $CD4^+$ T cell lineages protect the body from infection but, in some cases, contribute to autoimmunity and inappropriate inflammation. Although transcription factors are critical in regulating cellular differentiation, epigenetic factors also play important roles in T cell differentiation. Posttranslational modifications of histones, such as acetylation and methylation, are implicated in regulating gene expression by controlling chromatin structure and DNA accessibility.
We aimed to pharmacologically modulate the differentiation of Th17 and Treg cells *in vitro*, by altering the acetylation and methylation status of histone H3.
We show that the methyltransferase inhibitor Methylthioadenosin (MTA) decreases the differentiation of naïve $CD4^+$ T cells towards Th17 cells *in vitro* in nearly 50%, while increasing global H3K27me3 levels, without affecting the differentiation towards Tregs.
In the other hand, the histone deacetylase inhibitor Trichostatin A (TSA) increases the differentiation of naïve $CD4^+$ T cells towards Tregs nearly 10%, with a concomitant increase in global H3ac levels. Moreover, Tregs generated in the presence of TSA showed increased suppressive function, probably by mechanisms involving CD39 and CD73.
Our work suggests that epigenetic drugs could be used to modulate the differentiation and function of Th17 and Tregs. [Financed by Fondecyt 1100448 (MR),1100557 (MRB), 1120170 (AL)]

P3.10.12
Independent molecular control of IL-17 and IL-22 in the human Th17 response

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IL-17 and IL-22 are cytokines considered distinctive of Th17 cells and associately produced by such cells. To approach the problem of the relationship in the expression of IL-17 and of IL-22 we analyzed the production of these cytokines, as well as of IFN-γ, in memory Th cells specific for Candida albicans, commonly regarded as a classic exemple of Th17 cells, or for M. tuberculosis, a pathogen which requires production of IFN-γ for resolution of infection. Moreover, naïve $CD4^+$ T cells were cultured in the presence of Th17-promoting mixture of IL-1β, IL-6 and IL-23 or supernatant from DCs stimulated with zymosan (SNDCzym), or perturbating these microenvironments using various combinations of IL-1β, IL-6 and IL-23, or with SNDCzym in which various combinations of IL-1β, IL-6, IL-12 and IL-23 have been neutralized using specific antibodies or IL-1RA.
Ex vivo, M. tuberculosis-specific memory Th cells express IFN-γ, while IL-17 and IL-22 cells are virtually absent; candida-specific memory Th cells expressed consistent percentages IL-17, IL-22 or IFN-γ, with a significant correlation between expression of IL-17 and IL-22 in different donors, suggesting a dependent expression of IL-17 and of IL-22 in these memory cells.
However, in naive Th cells, perturbation of the Th17-promoting microenvironments identified (a) an independent production of IL-17 and of IL-22, depending on the combination of the cytokines IL-6, IL-23 and IL-1, which played pivotal and sometimes opposing roles in the regulation of IL-17, IL-22 and IFN-γ expression, (b) correlation profiles between Th effector cytokines and Th – associated genes, including transcriptional regulators.

P3.10.13
Transfection of human naïve $CD4^+$ T-cells with mir-326 and study of their differentiation to Th-17 cell

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Th17 cells are known to play a critical role in adaptive immune responses to several important extracellular pathogens. T helper 17 (Th17) cells have important roles in autoimmune diseases, inflammatory immune responses and cancers.Therefore, it is essential to understand the mechanisms that regulate Th17 differentiation. The development of T cells is also controlled by complex protein signalling networks that are subject to regulation by miRNAs. Here, we report invitro differentiation of naïve CD4+ Tcells to Th17 cells in the presence of miR-326. Naïve $CD4^+$ T cells were isolated from normal blood samples and cultured in X-VIVO 20 serum-free medium. Purified cells were treated with combinations of polarizing cytokines (TGF-β, IL-1β,IL-23 and IL-21) and miR-326 followed by analysis of the expression of characteristic genes and their relevant cytokines by real-time quantitative RT-PCR and flowcytometry method, respectively. Our studied showed that cells transfected with miR-326 could produce more Il-17 compared to cells differentiated in Th17-polarizing conditions, as determined by protein and mRNA levels detected by flow cytometry and Real Time PCR. IFN-γ was also slightly increased in T cells transfected with miR-326 compared to cells stimulated by cytokines. Our data show a critical role for miR-326 in Th17 differentiation and cytokines related to this subset.

P3.10.14
Activation of TGF-β-induced non-Smad signaling pathways during Th17 differentiation

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T helper (TH)17 cells, a proinflammatory subset of effector T cells, are involved in the pathogenesis of autoimmune diseases such as multiple sclerosis. Although the cytokine TGF-β has been shown to positively regulate the development of murine interleukin (IL)-17 producing CD4+ T cells, the canonical Smad-dependent intracellular signaling pathway seems to be dispensable for TH17 differentiation. By using a protein antibody array we characterized the phosphorylation status of signaling proteins downstream of the TGF-β receptor during differentiation of naïve murine CD4+CD62L+ T cells to the TH17 phenotype. To identify Smad-independent signaling pathways we compared the protein phosphorylation between wild type T cells and T cells with overexpression of the Smad pathway inhibitor Smad7. We found that proteins of the PI3K/AKT/mTOR pathway are activated at specific phosphorylation sites during TH17 differentiation, independently of Smad signaling. Pharmacological inhibition of these pathways caused decreased IL-17 production by differentiated TH17 cells in vitro. Our data show that Smad-independent intracellular signaling pathways contribute to TGF-β-induced TH17 differentiation, and expand our understanding of inflammatory pathways relevant for multiple sclerosis.

P3.10.15
The aryl hydrocarbon receptor: differential contribution to Th17 and Tc17 development

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The aryl hydrocarbon receptor (AhR) has been shown to play an important role in the polarization of T helper (Th) cell subsets; specifically it is required for optimal Th17 cell activation. Th17 cells

provide immunity against extracellular pathogens and are implicated in autoimmune and allergic diseases. An analogous subset of T cytotoxic (Tc) cells, designated Tc17, has also been identified but their dependence upon the AhR is relatively unknown. In the current investigations the role of the AhR in cytokine production by Th17 and Tc17 cells has been compared. Tc (CD8+) and Th (CD4+) cells were isolated by negative selection from the peripheral lymph nodes of naive mice and polarized with plate-bound anti-CD3 and anti-CD28 antibodies and appropriate cytokine cocktails (Th1/Tc1: interleukin [IL]-12; Th17/Tc17: IL-6, transforming growth factor-β and IL-1β). Cell differentiation was assessed as a function of mRNA and protein expression for interferon-γ and key IL-17 cytokines. Both Tc17 and Th17 cells displayed a type 17 profile demonstrated by relatively low interferon-γ and high IL-17 and IL-22 production. Using a selective AhR antagonist (CH-223191), Th17 and Tc17 cell activity was reduced significantly. In contrast, addition of the natural AhR agonist 6-formylindolo[3,2-b]carbazole (FICZ) markedly enhanced cytokine expression for Th17 cells only. These data demonstrate that the optimal development of Th17 cells is much more dependent upon AhR activation than is the development of Tc17 cells, thus endogenous AhR ligands play a much greater role in driving Th17 cell responses in vitro.

P3.10.16
T cell-dependent IgA induction by plastic Th17 cells

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Th17 cells are an essential T helper subset for the mucosal host defense against extracellular pathogens. Effector functions of Th17 have been studied in terms of neutrophil migration and the induction of anti-microbial peptides in epithelial cells. However, a Th17-mediated humoral immune response remains largely unknown. We investigated whether Th17 cells are responsible for antigen-specific immunoglobulin (Ig) A response at mucosal sites. IgA secreted in the lumen is important for the neutralization of toxins and response to pathogens, but also critically involved in shaping the diversity of the commensal microbiota. Intestinal Peyer's patches are essential lymphoid organs for the generation of T cell-dependent IgA production for gut homeostasis. Using IL-17 fate reporter mice we show that endogenous Th17 cells in lymphoid organs of naïve mice home preferentially to the intestine and are maintained independently of IL-23. In Peyer's patches such Th17 cells acquire a T follicular helper (Tfh) phenotype with high levels of Bcl-6, IL-21, CXCR5 and PD-1 following the downregulation of IL-17 and Rorgt and induce the development of IgA-producing germinal center B cells. Bone marrow (BM) chimera mice deficient in Th17 cells (Rorc-/- BM to Tcra-/-) fail to generate antigen specific IgA responses following a single challenge with cholera toxin, providing evidence that Th17 cells are the crucial subset required for high affinity T cell-dependent IgA production.

P3.10.17
A novel assay format for ELISA measurement of antigen-specific human IL-21 production by peripheral mononuclear cells

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Interleukin (IL)-21 regulates the activation and development of NK, B and T cells. Dysfunctional regulation of IL-21 has been implicated in several conditions, in particular autoimmune diseases. *In vitro* studies of IL-21 are thus of high relevance and functional methods for the quantitation of IL-21-levels as well as IL-21-producing cells are vital. The aim herein was to develop ELISA and ELISpot assays for *in vitro* analysis of IL-21-levels and IL-21-producing cells, respectively.
Monoclonal antibodies (mAb) to hIL-21 were made. Several mAbs identified, neutralized the biological effect of IL-21 as measured by inhibition of IL-21-induced IgG production in human PBMC.
By ELISpot, high numbers of IL-21-producing cells were found after stimulation (Phytohaemagglutinin; PHA) of human PBMC, weaker but positive responses were also observed after antigen-specific stimulation with *Candida albicans* extract or Tetanus toxoid. In contrast, demonstration of IL-21-levels in cell culture supernatants by ELISA was only feasible after PHA stimulation. Hypothesizing is that the lack of detection of antigen-induced IL-21 in cell supernatants was due to cellular receptor consumption of IL-21 during cell culture. PBMC were stimulated with antigen in the presence of the neutralizing anti-IL-21-detection mAb. Following this procedure, it was possible to measure IL-21 by ELISA also after antigenic stimulation.
Addition of a neutralizing antibody to the cell culture enables detection of antigen specific human IL-21-produced by human PBMC and may generally facilitate the analysis of IL-21 in *in vitro* cultures. This strategy may be applicable also to the ELISA analysis of other cytokines/factors that are difficult to detect.

P3.10.18
Ecto-nucleoside triphosphate diphosphohydrolase 7 controls Th17 cell responses through regulation of luminal ATP in the small intestine

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Extracellular ATP is released from live cells in controlled conditions, as well as dying cells in inflammatory conditions, and, thereby, regulates T cell responses, including Th17 cell induction. The level of extracellular ATP is closely regulated by ATP hydrolyzing enzymes, such as ecto-nucleoside triphosphate diphosphohydrolases (ENTPDases). ENTPDase1/CD39, which is expressed in immune cells, was shown to regulate immune responses by downregulating the ATP level. In this study, we analyzed the immunomodulatory function of ENTPDase7, which is preferentially expressed in epithelial cells in the small intestine. The targeted deletion of Entpd7 encoding ENTPDase7 in mice resulted in increased ATP levels in the small intestinal lumen. The number of Th17 cells was selectively increased in the small intestinal lamina propria in Entpd7(-/-) mice. Th17 cells were decreased by oral administration of antibiotics or the ATP antagonist in Entpd7(-/-) mice, indicating that commensal microbiota-dependent ATP release mediates the enhanced Th17 cell development in the small intestinal lamina propria of Entpd7(-/-) mice. In accordance with the increased number of small intestinal Th17 cells, Entpd7(-/-) mice were resistant to oral infection with Citrobacter rodentium. Entpd7(-/-) mice suffered from severe experimental autoimmune encephalomyelitis, which was associated with increased numbers of CD4(+) T cells producing both IL-17 and IFN-γ. Taken together, these findings demonstrate that ENTPDase7 controls the luminal ATP level and, thereby, regulates Th17 cell development in the small intestine.

P3.10.19
Human Th17 cells: a unique cell type in health and disease

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Human Th17 cells, an effector memory $CD4^+$ T helper cell subset, are important for releasing pro-inflammatory cytokines in bacterial immunity. In disease, Th17 cells have been detected in the active sites of inflammation such as in the synovial fluid of arthritic joints.
To study the rare population of Th17 cells we have developed a method for the isolation, expansion and long term culture of human Th17 cells (cells that exclusively produce IL-17A upon activation), to compare its unique characteristics to Th1 cells (produces IFN, and not IL-17A) and Th17 polyfunctional cells (cells capable of co-producing high levels of IL-17A and IFN). Th17 cells and Th17 polyfunctional cells selected for by the surface markers CCR6 and CCR4, while the Th1 cells lack both. The cells are cultured without polarising cytokines and are stably committed to being characteristically a Th17, Th1 or Th17 polyfunctional cell in the long term *in vitro* culture, even upon additional restimulations with antigen presenting cells to further expand cell number. Typically up to $1x10^7$ cells is expanded from the initial seeding of 2 cells per well.

Comparative analysis of gene expression between the three cell subsets has allowed for the identification of markers unique upregulated in Th17 cells from healthy peripheral blood, and also comparative differences with cells sourced from an active site of inflammation.
This characterisation of Th17 cells will enable a better understanding of the potential pathogenic nature of this rare cell type, enabling specific targeting in both basic research and clinical therapeutics for disease.

P3.10.20
Therapeutic effect of RORγt-DNA-binding domain in TH17-mediated Autoimmune diseases

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Interleukin (IL)-17 secreting T helper cell, T_H17 plays a crucial role in causing autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) which is an animal model of multiple sclerosis (MS). Although previous studies reported the molecular and cellular mechanisms by which EAE is induced, a therapeutic drug that cures the disease by modulating T_H17 differentiation and its function has not been developed yet. Transforming growth factor (TGF)-beta1/IL-6 and IL-21 induced T_H17 differentiation required expression of a transcription factor, retinoic acid receptor-related orphan receptor gamma t (RORγt). Here, we demonstrate that a cell permeable form of DNA-binding domain (DBD) of RORγt (tRORγt-TMD) competitively inhibits the functions of endogenous RORγt while its ligand-binding domain (RORγt-LBD) does not achieve such effects. In the presence of HH-RγD, $CD4^+CD25^-CD62L^{high}$ naïve T cells couldn't differentiate into T_H17 but it was shown that such treatment did not entail any inhibitory effects against T_H1 or T_H2 differentiation, which suggests T_H17-specific functionality of HH-RγD. EAE treated with HH-RγD significantly reduced clinical severity/incidence of EAE and showed a dramatic reduction in the number of inflammatory cell infiltration into the central nervous system (CNS). In summary, we demonstrate that a HH-RγD can selectively control T_H17 differentiation *in vivo* and *in vitro*, and can be a novel therapeutic reagent to treat many inflammatory diseases associated with T_H17.

P3.10.21
Distinctive features of classic and nonclassic (Th17 derived) human Th1 cells

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T helper17 (Th17) lymphocytes represent a third arm of the CD4(+) T-cell effector responses, in addition to Th1 and Th2 cells. Th17 cells have been found to exhibit high plasticity because they rapidly shift into the Th1 phenotype in inflammatory sites. In humans, Th1 cells derived from Th17 cells express CD161, whereas classic Th1 cells do not; these Th17-derived Th1 cells have been termed nonclassic Th1 cells. In this study, we examined similarities and differences between classic and nonclassic human Th1 cells by assessing a panel of T-cell clones, as well as CD161(+) or CD161(-) CD4(+) T cells derived ex vivo from the circulation of healthy subjects or the synovial fluid of patients with juvenile idiopathic arthritis. The results show that nonclassic Th1 cells can be identified based on CD161 expression, as well as the consistent expression of retinoic acid orphan receptor C, IL-17 receptor E, CCR6, and IL-4-induced gene 1, which are all virtually absent in classic Th1 cells. The possibility to distinguish these two-cell subsets by using such a panel of markers may allow the opportunity to better establish the respective pathogenic roles of classic and nonclassic (Th17 derived) Th1 cells in different chronic inflammatory disorders.

P3.10.22
Discovery of potent and selective retinoid related orphan receptor gamma (ROR-Gamma) inverse agonists for the treatment of Th17 mediated diseases

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Background: Th17 cells play a key pro-inflammatory role in a variety of autoimmune diseases. The nuclear hormone receptor RORγ controls the differentiation of Th17 cells and expression of IL-17. Methods: Novel RORγ inverse agonists were designed using structure and knowledge based methods. Compounds were screened in a RORγ radio-ligand binding assay using ^3H 25-Hydroxycholesterol, as well as in a cell based reporter assay to demonstrate inverse agonism. Selected compounds were screened against RORα to evaluate selectivity. Crystal structure of RORγ in complex with known inverse agonists as well as novel compounds, were solved. Th17 differentiation assay was developed using primary mouse CD4+ve T-cells to determine functional effect of the compounds. Pharmacokinetic profile in mice was determined for selected compounds. Results: Novel hits from multiple structural classes have been identified, with IC_{50} in the range of 5 - 500 nM in binding assay. Compounds from the lead series demonstrated good activity (< 1 μM) in reporter assay. Co-crystal structure of novel compounds clearly showed the mode of binding. Several compounds demonstrated > 10 fold selectivity against RORα in a reporter assay. Compounds from multiple series have shown significant inhibition of IL-17 release from differentiated Th17 cells. Lead compounds have shown good pharmacokinetic properties in mice. Conclusions: We have identified novel and structurally diverse small molecule inverse agonists of RORγ. Selectivity against RORα and cell based activity has been demonstrated for compounds from multiple series. Relevant efficacy models have been established and profiling of lead compounds in these models in on-going.

P3.10.23
Unravelling the transcriptional circuit regulating IL-10 production in human Th17 cells

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IL-17 producing $CD4^+$ cells (Th17) are a subset of effector T helper cells known to play an important role in host defence against fungi and extracellular bacteria, but also involved in tissue inflammation and autoimmune diseases, such as multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis. The function of Th17 cells depends critically on the range of cytokines produced and on the balance between pro- and anti-inflammatory cytokines. Autoreactive Th17 cells producing IFN-γ and GM-CSF are pathogenic in a mouse model of EAE, while Th17 cells producing IL-10 are not. We have recently shown that *C. albicans*-specific human Th17 cells produce IL-17 and IFN-γ, while *S. aureus*-specific human Th17 cells produce IL-17 and, after restimulation, IL-10. While the ontology of the two different Th17 subsets has been clarified, it still remains elusive what is the transcriptional circuit that regulates the expression of the immunoregolatory molecule IL-10. Using a combination of transcriptional profiling and epigenetic approach, we identified the transcription factor c-MAF as a candidate for the regulation of IL-10 production in human Th17 cells, thus potentially representing a discriminant factor between pathogenic and non pathogenic Th17 cells.

P3.10.24
Differential regulation of T cell responses and disease severity by mycoplasma and its superantigen in autoimmune diabetic mice

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Infection has been extensively documented as a serious problem causing high mortality and morbidity in diabetic populations. Due to the complex nature of immunological mechanisms, little is known regarding how an infection affects the outcomes of diabetes. Our laboratory has previously established a mouse model of septic shock by infecting mice with the Mycoplasma arthriditis. M. arthritidis produces a potent superantigen (SAg) MAM, which has been shown to be at least partially responsible for the lethal toxicity induced in certain susceptible mouse strains. In the present study, we have investigated how diabetic immune cells respond to the M. arthritidis and MAM SAg. By using two well-defined animal models of diabetes, STZ and NOD mice, we found that STZ mice infected with M. arthritidis developed severe lethal toxicity and the immune cells predominantly produced pro-inflammatory cytokines TNFα, IL-6, IL-1β, chemokines CCR2/4 and Tbx21, a Th1-related transcription factor; while MAM-activated T cells from NOD mice display a mixture of Th1 and Th17 profiles which are associated with the elevated levels of ICOS molecule and a unique transcription factor LSF (tfcp2). Since ERK, a signaling protein of MAPK family, is reported to be responsible for the phosphorylation of LSF in T cell activation, we propose a novel ERK-LSF-ICOS signaling cascade is required for the Th1/Th17 development in NOD mice in response to MAM. Our findings further suggest that the inhibition of these molecules might be potentially therapeutic in the alleviation of mortality and morbidity in diabetic individuals with severe infections.

P3.10.25
Th17 and IL17 expression in Autoimmune Polyendocrine Syndrome Type 1 and Addison disease: what's the difference?

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Introduction: Autoimmune Polyendocrine Syndrome Type 1 (APS-1) is an autosomal recessive disorder caused by mutations in AIRE; patients are often affected by chronic mucocutaneous candidiasis (CMC). There are increasing evidences that T cell subpopulation Th17 is involved in protection against *Candida* species infections. Addison disease (AD) is the most common cause of autoimmune primary adrenal cortex insufficiency.
Aim: Evaluate Th17 cells in APS-1 patients with CMC and in AD.
Materials and methods: We studied 8 APS-1 patients with CMC and 14 patients affected by AD (not APS-1). As controls we considered 20 healthy volunteers. Flow cytometry analysis using monoclonal antibodies against CD3, CD4, CD8, CD5, CD19, CD56, CD16, CD57 was performed; Th17 population was studied using monoclonal antibodies against IL23R and IL17. We determined the RORγt expression, a transcriptional factor involved in Th17 differentiation, with real time PCR .
Results: In APS-1 patients we found an increased level of IL23R and increased expression of RORγt; IL17 was normal. In patients with AD we observed an increase of IL23R and IL17; RORγt was normally expressed. In healthy group IL23R, IL17 and RORγt were normal.
Discussion: Our findings reveal an interesting difference between APS-1 suffering from CMC and the AD patients: while Th17 count was increased in both groups, IL17 was increased only in patients with AD; furthermore in APS-1 patients there was a higher expression of RORγt. This suggests a function impairment of IL17 in ASP-1, probably due to autoantibodies against IL17 recently described in these patients.

P3.10.26
The kinase PKCα selectively upregulates interleukin-17A during Th17 cell immune responses

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Transforming growth-factor β (TGFβ) has been implicated in T helper 17 (Th17) cell biology and in triggering the expression of interleukin-17A (IL-17A), which is a key Th17 cell cytokine. Deregulated Transforming growth-factor β receptor (TGFβR) signaling has been implicated in Th17-cell-mediated autoimmune pathogenesis. Nevertheless, the full molecular mechanisms involved in the activation of the TGFβR pathway in driving IL-17A expression remain unknown. Here, we identified protein kinase C α (PKCα) as a signaling intermediate specific to the Th17 cell subset in the activation of TGFβRI. We have shown that PKCα physically interacts and functionally cooperates with TGFβRI to promote robust SMAD2-3 activation. Furthermore, PKCα-deficient (Prkca(-/-)) cells demonstrated a defect in SMAD-dependent IL-2 suppression, as well as decreased STAT3 DNA binding within the Il17a promoter. Consistently, Prkca(-/-) cells failed to mount appropriate IL-17A, but not IL-17F, responses in vitro and were resistant to induction of Th17-cell-dependent experimental autoimmune encephalomyelitis in vivo.

P3.10.27
IL-17 is predominantly expressed by neutrophils, but Th17 cells are associated with survival in cervical cancer

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Cervical cancer is the fourth leading cause of death by cancer in women worldwide. The role of IL-17 in cancer, produced both by Th17 and innate immune cells, has remained unresolved. We studied the phenotype of IL-17 expressing cells in 26 cervical, head and neck, ovarian, endometrial, prostate, breast, lung and colon carcinoma specimens using immunohistochemical double and immunofluorescent triple stainings. In cervical cancer, the majority of IL-17 positive cells were present in the tumor stroma, 66% neutrophils (CD15 positive), 23% mast cells (tryptase positive), 8% innate lymphoid cells (CD127 positive) and 3% Th17 cells (CD3 positive). 82% of the neutrophil population expressed IL-17, compared to 1% of the $CD3^+$ T cells. Neutrophils were the large majority (21-69%) of the IL-17 positive cell population in the six other cancer types, compared to a minority (maximum of 6%) of Th17 cells. Also in these other cancer types, the majority of neutrophils expressed IL-17 (43-92%), compared to a maximum of 1% of T cells. The effect of the IL-17 expressing cells on clinico-pathological parameters was studied in 160 cervical cancer specimens. Although there was no effect of the number of IL-17 positive cells, neutrophils or mast cells on survival (based on the median number of cells), Th17 cells were positively correlated with improved survival (p=0.02). A high number of IL-17 positive cells and neutrophils were correlated to less vaso-invasion (p=0.003). In conclusion, although IL-17 was predominantly expressed by neutrophils, a high number of Th17 cells correlated with improved survival.

P3.10.28
Characterisation of a population of human CCR6 TH1 cells expressing RORC

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CD4+ T helper (TH) subsets can be defined by the cytokines they produce and their transcription factor expression. The paradigm of Tbet and IFNγ denoting TH1 cells and RORC and IL-17 signifying a TH17 cell is well established. Initially these subsets were thought to have a stable phenotype however there are small subsets of cells that co-express cytokines normally attributed to separate lineages (i.e. IFNγ+IL-17+). TH1/TH17 cells can be induced in vitro through IL-12

stimulation of TH17 cells. There is evidence that the TH1/TH17 cells can switch off IL-17 and become a TH1 cell.
We have shown that human peripheral blood CD4+ TH1/TH17 cells express lower levels of IFNγ per cell than single IFNγ secretors (MFI Median=143.7 compared with median=72.4 [p=0.034]), though a similar level of IL-17 than single IL-17 secretors (MFI Median=59.23 compared to median=73.61 [p=0.32]). TH1/TH17 cells co-express Tbet and RORC both at protein and mRNA level and all express the chemokine receptor CCR6. Non classical TH1 cells have been classified in the literature as CD161+ IFNγ+. We are characterising a population of non-classical TH1 cells that express CCR6. We have established CCR6+ non-classical TH1 cells co-express RORC and Tbet and have other signatures of TH17 cells such as IL-23R expression but secret neither IL-17a nor IL-17f. We are investigating the effect of IL-23 on non-classical TH1 cells. We also wish to discover if there are transcriptional reasons why no IL-17 is detectable if RORC is expressed.

P3.10.29
Rarity of human T helper 17 cells is due to retinoic acid orphan receptor-dependent mechanisms that limit their expansion

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The reason why CD4(+) T helper 17 (Th17) cells, despite their well-known pathogenic role in chronic inflammatory disorders, are very rare in the inflammatory sites remains unclear. We demonstrate that human Th17 cells exhibit low ability to proliferate and to produce the T cell growth factor interleukin-2 (IL-2), in response to combined CD3 and CD28 stimulation. This was due to the upregulated expression of IL-4-induced gene 1 (IL4I1) mRNA, a secreted L-phenylalanine oxidase, which associated with a decrease in CD3ζ chain expression and consequent abnormalities in the molecular pathway that allows IL-2 production and cell proliferation. High IL4I1 mRNA expression was detectable in Th17 cell precursors and was strictly dependent on Th17 cell master gene, the retinoid acid related orphan receptor (RORC). Th17 cells also exhibited RORC-dependent CD28 hyperexpression and the ability to produce IL-17A after CD28 stimulation without CD3 triggering. Our findings suggest that the rarity of human Th17 cells in inflamed tissues results from RORC-dependent mechanisms limiting their expansion.

P3.10.30
Disequilibrium of Tregs and TH17 cells in the pathogenesis of autoimmune skin disorder: Pemphigus vulgaris

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Background: Pemphigus vulgaris(PV) is an auto-immune blistering disease forming auto-antibodies against desmoglein 3 (Dsg3). Treg and TH17 cells play important role in autoimmune diseases. Reason of paucity of Treg cells in PV lesions and Involvement of homing receptor and its ligand needs to be explored. We aim to assess the frequency of Treg and TH17 cells, circulatory and quantitative expression of their cytokines, transcription factors, chemokine receptor-ligands in PV.
Methodology: 25 active PV patients (confirmed by clinical, histological and anti-Dsg3 levels), 15 controls (non-inflammatory lypoma patients) were included. Serum TGF-β, IL-17, CCL20 and CCL22 levels were analysed by ELISA. Molecular expression of Foxp3, CCR4, CCL22 and RORγt, CCR6, CCL20 was performed by Real time PCR. Frequency of Treg and TH17 cells was assessed by flow cytometry.
Results: Frequency of TH17 cells was significantly higher than Treg cells ($p<0.02$, $p<0.008$) in circulation of PV patients vs controls. For Tregs, levels of TGF-β were significantly elevated whereas CCL22 lowered in patients. Foxp3 expression was higher in tissues whereas relative expression of CCR4 was significantly lower in both blood and tissue ($p<0.05$) of patients. For TH17, significantly elevated level of IL-17 was found in patients. Relative expression of RORγt, CCR6 and CCL20 was significantly high in both blood and tissue of patients ($p<0.001$) as compared to controls.
Conclusion: Augmented expression of RORγt, CCR6, CCL20, Foxp3, CCL22 and lower expression of CCR4 in tissue signifies chemokine receptor-ligand imbalance of Treg cells that might affect the suppressive ability of iTregs over TH17 in the pathogenesis of PV.

P3.10.31
Characterization of the immune mechanisms involved in periodontal disease in obese rats.

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Periodontal disease (PD) is a chronic inflammatory disease that affects the tissues that support the teeth and is the most prevalent bone disorder in humans. The etiological agent is the dental plaque however the host immune response determinates the susceptibility and progression of PD. Obese individuals have a higher susceptibility to periodontal disease and this is more severe, therefore this study aimed to evaluate the immune mechanisms involved in the development and progression of induced periodontal disease in obese rats. The obesity experimentally mimicked by high fat diet (HFD) is characterized by inducing impaired glucose tolerance and hyperinsulinemia. These animals showed a higher protein and gene expression level of pro-inflammatory cytokines, such as IL-17, IL-6 and IFN-γ indicating a polarization toward Th1 and Th17 immune profile. These pro-inflammatory cytokines profile stimulate the induction of matrix metalloproteinases (MMP) expression and inhibition of their regulators as TIMPs, and overexpression of RANKL (receptor activator of nuclear factor kappa B ligand) and inhibition of osteoprotegerin (OPG), which imbalances is responsible for the degradation of the extracellular matrix and bone resorption in PD. Our results allowed to correlate the HFD induced metabolic changes resulted in exacerbation of inflammatory response in gingival tissue with PD.

P3.10.32
The histamine H_4 receptor mediates inflammation in models of arthritis via inhibition of Th17

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The histamine H_4 receptor (H_4R) has been shown to drive inflammatory responses in models of asthma, colitis and dermatitis and in these models it appears to impact both innate and adaptive immune responses. Many of these models involve Th2 mechanisms and the effect of the H_4R was postulated to be driven by inhibition of these T cell responses. The effect on T cells prompted the question as to whether the H_4R could modulate responses of other T cell subtypes and, therefore, have a role in autoimmune disease. In this study both H_4R-deficient mice and a specific H_4R antagonist were utilized to investigate the involvement of the H_4R in mouse arthritis models. Both H_4R-deficient mice and mice treated with the H_4R antagonist exhibited reduced arthritis disease severity in both collagen antibody-induced arthritis (AIA) and collagen-induced arthritis (CIA) models. This was evident from the reduction in disease score and in joint histology. In the CIA model treatment with the H_4R antagonist reduced the number of Th17+ cells in the lymph node and the total production of IL-17. Th17 cell development in vivo was reduced in H_4R-deficient mice or in mice treated with an H_4R antagonist. Finally, in both mouse and human blood treatment with an H_4R antagonist reduced the production of IL-17 when cells were stimulated in vitro. These results implicate the H_4R in disease progression in arthritis and in the production of IL-17 from Th17 cells; thus supporting future clinical exploration of H_4R antagonists for the treatment of rheumatoid arthritis.

P3.10.33
IL-2 interferes with CD4+ T cell homing by reducing the expression of the α4 integrin

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To provide optimal host defense against pathogens naive CD4+ T cells differentiate into different T helper cell subsets after they have encountered their cognate antigen. This process is accompanied by a massive IL-2 production, which in turn modulates the expression of cytokine receptors to help specify and maintain differentiated states. More specifically, IL-2 induces Th1 polarization by enhancing expression of T-bet and IL-12Rβ2 subunit. Similarly to Th1, IL-2 stimulates Th2 differentiation by inducing IL-4Rα and keeping the Il4 gene locus accessible. Moreover, IL-2 was shown to be crucial for the reciprocal balance between Th17 cells and FOXP3+ regulatory T cells (Tregs). Whereas in the absence of IL-2 the numbers Treg cells decline, the numbers of Th17 cells increase. As shown previously, IL-2 seems to be not only crucial for the T cell fate but also for their homing capacities as IL-2 suppresses CCR6, which is important for Th17 cells gut-homing (Wang et al., Mucosal Immunol., 2009).We could observe a similar mechanism for the integrin alpha 4 chain (ITGA4) of the specific gut-homing molecule α4β7 integrin complex. Addition of IL-2 to Th17 cells led to a dose-dependent decrease of the itga4 expression. Inhibition of IL-2 induced STAT5 resulted in an increase of itgα4 both on RNA and protein levels. Understanding the molecular mechanisms underlying itga4 modulation by IL-2 and subsequent function in gut migration would contribute to the knowledge of T cell driven inflammatory diseases of the gut like Crohn's disease and could help to improve clinical therapies.

P3.10.34
Infected apoptotic adherent or non-adherent cells instruct DCs to trigger Th17 differentiation

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The phagocytosis of infected apoptotic cells (AC) by dendritic cells (DC) promotes the production of anti-inflammatory cytokines such as TGF-β but also pro-inflammatory cytokines as IL-6 and IL-23, resulting in the differentiation of Th17 cells. We have asked ourselves whether the quality of infected cell could differentially impact the DC instruction toward Th17 using infected adherent or non-adherent cells. C57BL/6J mice were intraperitoneally injected with 1 mL thioglicollate plus 106 live E. coli and 13 h later the cells were collected from peritoneal cavity lavage. Apoptotic cells were generated by UV irradiation (350mJ) and confirmed by Annexin-V/PI detection. BMDC were co-cultured at 1:3 ratio with adherent or non-adherent infected apoptotic cells for 18 h and supernatant was collected for differentiation assay and detection of Th-17 related cytokines, IL-6 and TGF-β, by ELISA. For the phagocytosis assay the infected apoptotic cells were labeled with CFSE. Our results showed that DC were able to phagocyte both adherent and non-adherent cells in a similar way and instruct Th17 differentiation as well. Interestingly, non-adherent cells were capable to induce a 3-fold higher production of IL-6 together with a small amount of TGF- β, while adherent cells were less capable to produce IL-6 and better at promoting TGF-β production. Our result shows the ability of DCs to be instructed from diverse infected cells and further promotes differentiation of Th17 cells. The result suggests that apoptosis of infected cells are a standard instruction for DC to trigger Th17 response.

P3.10.35
Protein Kinase C θ regulates Th1/Th17 plasticity

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Mature CD4+ cells can polarize into functionally distinct T helper subsets (Th1/Th2/Th17/iTreg). This differentiation is often regulated by opposing molecular pathways, but still some plasticity between the subsets exists. This phenomenon is especially remarkable in case of Th1 versus Th17 lineage development, although underlying mechanisms are not fully understood. Protein Kinase C θ (PKCθ) is a well established player in proximal T cell receptor signaling, but less is known about its contribution to differentiation and function of particular Th subsets.
We investigated the role of PKCθ in Th17 differentiating cells. According to our observations, in vitro differentiated PKCθ-defficient CD4+ cells express normal levels of Th17 marker genes (IL-17, RORγt), but at the same time produce more factors typical for the Th1 subset (IFNγ, T-bet). Increased expression of IFNγ and T-bet in PKCθ-deficient cells was specific for Th17-promoting conditions and this phenomenon could not be attributed to TGF-β resistance. In concert with increased IFNγ production, also phosphorylation of STAT1 was enhanced and sustained in PKCθ-defficient cells. This phenotypical plasticity switch was confirmed also in vivo, in the mouse model disease EAE (Experimental Autoimmune Encephalomyelitis).
Our observation suggests that PKCθ is involved in modulation of molecular pathways underlying Th1 and Th17 lineages differentiation and their plasticity. Defining mechanisms of such contribution will not only clarify the flexibility of T cell polarization, but may also shed light on the pathogenesis of autoimmunity and cancer.

P3.10.36
Myeloid-derived suppressive cells promote Th17 cell differentiation and IL-17 production

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Myeloid-derived suppressive cells (MDSCs) have been implicated in the pathogenesis of several diseases, including cancer and autoimmune disorders. We found that CD11b+Gr-1+ MDSCs from tumor- or experimental autoimmune encephalomyelitis (EAE)-bearing mice promote the differentiation of naïve CD4+ T cell precursors into Th17 cells in a highly efficient manner. The presence of MDSCs also results in elevation of IL-17A production and upregulation of the orphan nuclear receptor RORA and RORC in T cells. IL-1beta-derived from MDSCs is identified as a major mediator of the enhanced Th17 differentiation, which involves the IL-1 receptor on CD4+ T cells. Depletion of MDSCs leads to significantly reduced Th17 response and decreased clinical scores of EAE as well as myelin injury. Our data reveal a new feature of MDSC action in the shaping of inflammatory Th17 cell responses and provide additional insights into the pleiotropic functions of this myeloid cell population. These new findings support the concept of targeting MDSCs for potentially effective intervention of Th17 cell/IL-17-mediated immunopathology in cancer and autoimmune diseases (e.g., multiple sclerosis).

P3.10.37
Runx1-deficiency in CD4+ T Cells Causes Fatal Autoimmune Inflammatory Lung Disease due to Spontaneous Hyperactivation of Cells

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The Runx1 transcription factor is abundantly expressed in naïve T cells but rapidly down-regulated in activated T cells, suggesting that it plays an important role in a naïve stage. In the present study, we showed that mice harboring Runx1-deleted CD4+ T cells developed a fatal autoimmune lung disease. CD4+ T cells from these mice were spontaneously activated and preferentially homed to the lung. At early age, lung peribronchiovascular regions of the mice were infiltrated with CD4+ lymphocytes, while at later stage, interstitial regions were massively occupied with immune cells and alveolar spaces were filled with granular exudates that resembled pulmonary alveolar proteinosis in humans. Mice suffered from respiratory failure as well as systemic inflammatory responses which caused early dead at around 7-8 months old. Runx1-deleted CD4+ T cells in the mice expressed various cytokines including IL-17 and IL-21 which promoted neutrophils and monocytes activation and triggered formation of IgG+ plasma B cells, respectively. Among these, the deregulation of IL-21

transcription was likely to be associated with Runx-binding sites located in an IL-21 intron. Our data indicate that Runx1 plays an essential role in repressing the transcription of cytokine genes in naïve CD4+ T cells and thereby maintains cell quiescence.

P3.10.38
The effects of traditional Chinese medicine type on Th17 cells in chronic hepatitis C patients received antivirval therapy

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Objective: To observe the change of Th17 cells and IL-17 expression in patients with chronic hepatitis C virus infection before and after treatment with peginterferon alfa-2α plus ribavirin with or without traditional Chinese medicine type “prescription of harmonizing and tonifying liver and spleen”.
Methods: The study consisted of 80 subjects, including 24 patients treated with peginterferon alfa-2α plus ribavirin, 26 patients treated with peginterferon alfa-2α plus ribavirin and traditional Chinese medicine type, and 30 healthy controls. The frequency of circulating Th17 cells were analysed by flow cytometry. The peripheral serum IL-17 levels and HCV RNA were measured by ELISA and Real-Time PCR.
Redults: The frequency of Th17 cells was increased in patients with chronic hepatitis C compared with healthy controls. The elevated prevalence of Th17 cells is positivedly associated with ALT and HCV RNA. In addition, after 12 weeks’ treatment, Thl7 cells were significantly decreased in patients treated with peginterferon alfa-2α plus ribavirin and traditional Chinese medicine type than patients treated with peginterferon alfa-2α plus ribavirin.
Conclusion: Thl7 cells are correlated with the severity of liver inflammation and decreased after treatment with peginterferon alfa-2α plus ribavirin and traditional Chinese medicine type. Traditional Chinese medicine type can improve immune function in CHC patients treated with peginterferon alfa-2α plus ribavirin.

P3.10.39
Distinct Th17 inductions contribute to the gender bias in CVB3-induced myocarditis

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Viral myocarditis is often caused by coxsackievirus B3 (CVB3) infection and occurs more frequently in male. So far the mechanisms for this sex difference are not fully elucidated. Th17 cells are required for the development of CVB3-induced myocarditis, but their impact on the gender bias in viral myocarditis is still unknown. Here, the frequencies of splenic Th17 cells in CVB3-infected male and female mice were compared, and the impact of sex hormones on Th17 cell differentiation was also evaluated. We found that in infected male mice, Th17 cell frequency was remarkably increased and significantly higher than that in female mice. When neutralizing IL-17 by monoclonal antibody, the male prevalence of myocarditis was obviously abolished, further confirming the effect of Th17 cells on gender bias in viral myocarditis. It was also found that estradiol significantly inhibited the Th17 differentiation post CVB3 infection both in vitro and in vivo. However, testosterone showed no such effects. In this study, we reported that Th17 cells were predominantly induced in CVB3-infected males than females as the inhibitory effect of estrogen on Th17 differentiation, and played an important role in the sex differences in the sensitivity to CVB3-induced myocarditis. This study may help us understand the role of Th17 cells in viral myocarditis and facilitate the development of corresponding therapeutic strategies.

P3.10.40
The predominant Th17 phenotype in gastric cancers may be associated with HLX expression decrease blocking Th1 cell specific T-box transcription factor

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Th0 cells differentiate into Th1 or Th2 depending on multiple transcription factors acting on specific time points to regulate gene expression. Th17 cells, a subset of IL-17 producing T cells distinct from Th1 or Th2 cells has been described as key players in inflammation and autoimmune diseases as well as cancer development. In this study, we investigated the expression level of IL-17, IFN-γ, RORγt, *HLX* and *T-bet* and Th17-related cytokines in gastric cancer tissues and assessed the possible mechanism and their clinicopathological parameters. Serum, peripheral blood mononuclear cells (PBMC), tumor and adjacent normal tissues were obtained from 60 patients with gastric cancer. IL-17, IL-21, IL-23, IFN-γ, RORγt, *HLX* and *T-bet* mRNA expression levels were quantified by real-time RT-qPCR; Serum IL-17, IL-21, IL-23, and IFN-γ were detected by ELISA; Th17 infiltration was examined by immunofluorescence. Expression of IL-17, IL-21, IL-23 and RORγt mRNA was found to be significantly up-regulated in PBMC compared with healthy people; whereas, expression of IFN-γ, *HLX* and *T-bet* was obviously down-regulated. Serum IL-17, IL-21, IL-23 were increased, IFN-γ was decreased. And Th17 cells infiltrated the cancer tissue. All the data indicated that Th17 was a predominant phenotype in gastric cancers; the data also indicated that the Th1 response was inhibited. From the above data we speculated that the predominant Th17 response in gastric cancers may be associated with HLX expression decrease blocking Th1 cell specific T-box transcription factor

P3.10.41
A probiotic strain of Lactobacillus L casei stimulates gene expression in PBMC in obese people

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Objective: We tried to determine whether dietary supplementation with yogurt, enriched by some strains of probiotics with or without weight reduction diet, could be effective on gene expression of PBMCs from obese subjects.
Design: This randomized doubled-blind controlled clinical trial was performed recruiting 75 healthy persons with BMI=25-40. Participates consumed 200 g/day yogurt containing Lactobacillus acidophilus La5, Bifidobacterium BB12 and lactobacillus casei DN001 108 CFU/gr. Subjects were randomly assigned to three groups: Those who consumed conventional yogurt under low calorie diet (LCD) (n=25), subjects who got probiotic yogurt with LCD (n=25) and those who consumed probiotic yogurt without any LCD (n=25) for 8 wk. Fasting blood samples were taken at baseline and after a 8 wk intervention to measure changes of gene expression for FOXP3, T-bet, GATA3, TNF-α, IFN-γ ,TGF-β and ROR-γt in PBMCs.
Results: Expression of ROR-γt was reduced (p=0.007) while FOXP3 expression enhanced ($p<0.001$) significantly in all three groups after intervention. Changes in expression of TNFα, TGFβ, and GATA3 were not significant between groups after intervention (p=0.39, p=0.43 and p=0.16, respectively). Expression of T-bet was significantly decreased in probiotics groups ($p<0.001$). Expression of IFNγ was decreased significantly in all three groups after intervention.
Conclusions: Weight reduction diet and probiotic yogurt had synergistic effects on gene expression in subgroups of T lymphocytes in overweight and obese persons, and the combination of a weight reduction regimen and consumption of probiotic yogurt appears to be more effective at improving immune vigor and reducing inflammatory mediators than consumption of probiotics alone.

P3.10.42
Antagonistic regulation of human Th17 and GM-CSF-producing T helper cells

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The pathogenicity of Th17 cells in mouse models of autoimmunity has recently been associated with their production of granulocyte-macrophage-colony-stimulating factor (GM-CSF). Here, we have analyzed the expression of GM-CSF by human T helper cells. The induction of GM-CSF expression by human T helper cells is constrained by the IL-23/STAT3/ROR-γt/Th17 cell axis but promoted by the Th1 axis. While STAT3 blocks GM-CSF induction, STAT5 promotes it. Ex vivo, most GM-CSF+ T helper cells co-express IFN-γ and T-bet. A distinct subset of GM-CSF+ T cells does not express Th1, Th2 and Th17 signature cytokines or master transcription factors, and thus constitutes an independent T helper cell lineage, the Th-GMCSF subset. Our findings suggest a role for GM-CSF+ T cells in the inflamed brain of multiple sclerosis patients as well as in the healthy skin.

P3.11 Gamma-delta T cells

P3.11.01
CD2 expression determines two lineages of γδ T cells in swine

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Swine together with ruminants and birds belongs to the group of γδ-high species in which γδ T cells are regularly present in the blood and secondary lymphoid organs and may account for >70% of all T cells. This is in sharp contrast with γδ-low species such as rodents and humans in which γδ T cells are rare in circulation and are preferentially limited to epithelia. In this respect, γδ-low species almost lack circulating $CD2^{-}$ γδ T cells that are numerous in γδ-high species. Analysis of porcine γδ T cells shows that $CD2^{+}$ and $CD2^{-}$ γδ T cells differ in many aspects. These two subsets have differential distribution in the blood and secondary lymphoid organs, differential expression of TCR, differential capacity to modulate TCR expression, differential susceptibility for proliferation, differential capacity to modulate CD8 expression and differential expression of other auxiliary molecules and TCRγ chains. CD2— γδ T cells are also absent in immature thymocytes. However, repertoire of TCRδ is polyclonal in both subsets indicating there is the same extent of diversification and equal capability of immune responses. The tissue distribution of $CD2^{+}$ and $CD2^{-}$ γδ T cells is also independent of bacterial colonization and it is already established in the thymus. Results collectively indicate that $CD2^{+}$ and $CD2^{-}$ γδ T cell subsets represent two independent lineages and that $CD2^{-}$ γδ T cells is a specific lineage that is missing in the blood of humans and mice. This work was supported by Czech Science Foundation grant P502/12/0110.

P3.11.02
IL-17-producing gamma-delta T cells are crucial for the development of autoimmune arthritis in IL-1 receptor antagonist-deficient mice

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A high proportion of IL-17 producing-gamma-delta T (γδ17) cells was detected in joints of Il1rn-/- mice, a model of rheumatoid arthritis, whose development depends on IL-17 and T cells. However, their pathogenic roles are not well understood. To clarify the roles of γδ T cells and CD4+ T cells in the development of arthritis, γδ T cells or CD4+ T cells were depleted in Il1rn-/- mice using antibodies. The development of disease was suppressed in both cases, suggesting both γδ T cells and CD4+ T cells were involved in the pathogenesis. Adaptive transfer experiment showed that the combination of CD4+ T cells and γδ17 cells were required for the development of arthritis in scid/scid mice, while γδ17 cells alone could induce in the Il1rn-/- background because Il1rn-/-nu/nu mice still developed arthritis. Furthermore, we generated mice with γδ17 cells, but without Th17 cells, by adoptively transferring Il17-/-Il1rn-/--CD4+ T cells into nu/nu mice in which γδ17 cells are present. We found that these mice still developed arthritis and that only γδ T cells produced IL-17, indicating that extrathymic γδ17 cells are also important for the development of arthritis in the absence of Th17 cells. These observations suggest that joint-specific CD4+ T cells or IL-1 production directs tissue specificity and γδ17 cells play effector functions in the development of arthritis.

P3.11.03
Changes in Granzyme B expression in gamma delta T-cell in (HTLV-1)-associated myelopathy/tropical spastic paraparesis patients

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Human T-cell lymphotropic virus type 1 (HTLV-1) is an etiologic agent of adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HAM/TSP is a chronic inflammatory disease characterized by the loss of motor movement in response to spinal marrow cells destruction by T lymphocytes. HTLV-1 preferentially infects T CD4+ lymphocytes. The efficient immune response seems to be important to control viral replication and associates disease development. γδ T cells are increasingly recognized as having important functional roles in a range of diseases, such as infection, autoimmunity and cancer. Thus far, the relationship between HTLV-1 infection and γδ T lymphocytes during HAM/TSP disease is poorly understood. In this study, we compared the phenotype of these cells of health donors and HTLV-1 carriers. The total γδ T cell population was not significantly altered in HTLV-1 infected donors. Similar results were observed in T γδ cells subtypes, analyzing Vδ2, Vγ9 and CD27 expression. In HAM/TSP patients, the percentage of granzyme B expression in γδ T cells was significantly lower than in asymptomatic patients and non-infected donors. These results suggest that the reduction of granzyme B expression impairs γδ cytotoxic activity related to proviral load control and HAM/TSP progression.

P3.11.04
Involvement of γδ-T cells in the pathogenesis of autoimmune skin disorder: Pemphigus vulgaris

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BACKGROUND: Pemphigus vulgaris (PV) is an autoimmune, intraepithelial, blistering disease affecting the skin and mucous membranes, mediated by circulating autoantibodies. In most of the autoimmune skin disorders auto reactive T cells are critical for the induction and regulation of antibody production. γδ-T cells are primarily located in epithelial surfaces and participates in many autoimmune diseases. In this maiden attempt we elucidated the involvement of γδ-T cells in pathogenesis of PV by phenotypic characterization and their cytokine polarization in PV patients.
METHODOLOGY: 30 cases of active PV confirmed by histopathology (H & E) & anti-Desmoglein 3 levels and 30 healthy individuals as

controls were included. Serum levels of IFN-γ as TH1 marker and IL-4 as TH2 marker were estimated by high sensitivity ELISA test. Phenotypic determination of γδ-T cells and their intracellular cytokine polarization was quantified by Flow cytometry (FACs).
RESULT: Circulatory level of IL-4 was found to be significantly higher ($p<0.001$) whereas IFN-γ was low ($p<0.01$) in patients than controls. FACs result showed higher percentage of γδ-T cells expressing IL-4 (6.8%) than IFN-γ (2.7%) in PV patients as compared to controls The FACs results were in concordance with our circulatory cytokine levels.
CONCLUSION: FACs result explained increased population of IL-4 expressing γδ-T cells in PV patients and found to be TH2 polarized. The involvement and induction of γδ T cells in Pemphigus Vulgaris immune dysregulation reinforces the need of detailed analysis by observing cytotoxicity of γδ T cells. This approach might be beneficial in designing targeted therapy in future.

P3.11.05
Exploiting NKG2D and lymphoid stress surveillance response in seronegative spondyloarthritis

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A number of biomarkers are currently being investigated in patients with seronegative spondyloarthitis (SpA) since they may be used to predict exacerbation and monitor the clinical response to an intervention.
Natural killer group 2, member D (NKG2D) is an activating receptor present on the surface of natural killer (NK), CD8 T cells, γδ T cells and under certain conditions CD4 T cells. NKG2D receptor binds to a family of ligands that are distant relatives of major histocompatibility complex class I molecules.
Environmental stress such as genotoxic/oxidative stress, heat shock and viral infections stimulate NKG2D ligands expression. Recent studies have demonstrated that NKG2D ligands are induced on proliferating rheumatoid arthritis (RA) synoviocytes and may thus contribute to progression of rheumatic disease.
Here we investigate the expression of NKG2D ligands in synovial tissue from SpA patients and we compare it with samples from RA patients and healthy controls. We monitor NKG2D regulation also in in-vitro cultured SpA and RA synoviocytes in response to physiological stimuli such as oxidative stress, heat-shock and mechanic stretch.
Downregulation of NKG2D receptor on peripheral blood mononuclear cells (PBMC) represents a physiological regulatory mechanism of NK and gd T cells in response to activation and it is mediated by the production of pro-inflammatory proteins.
Hence the production of key cytokines by circulating NKG2D+ cells may indicate the impaired response of the immune system to stress in rheumatic disease.
We believe that understanding the contribution of NKG2D pathway in rheumatic pathologies may provide rational for emerging targeted therapies.

P3.11.06
T Cell Dysregulation in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis

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Gamma delta (γδ) T lymphocytes comprise about 1-10% of peripheral lymphocytes. Although, functionally distinct from the more diverse αβ T cells, γδ T cells have important roles in pathogenesis and inflammation. γδ T cells secrete cytokines and chemokines essential for regulating adaptive immunity. In diseases such as Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) decreased activity of cytotoxic T cells and elevations in FOXP3 secreting T cells have been reported. Dysregulation in T cell function may be an important component of the CFS/ME immune profile. Hence, the objective of this study was to determine the role of γδ T cells in CFS/ME patients. 20 CFS/ME patients (age=51.8±1.74 years) and 30 non-fatigued controls (age= 53.19±1.35years) were recruited for the study. Inclusion into the CFS/ME group was based on the Centre for Disease Prevention and Control (CDC 1994) criteria definition for CFS/ME. 10mL of whole blood was collected from all participants. The whole blood samples were stained with a cocktail of monoclonal antibodies containing CD4, CD62L, CD27, CD45RA, CD3, CD11a, CD94, V delta-1 and V delta-2. Following which samples were analysed on the flow cytometer. The statistical analysis used to determine differences in the data was ANOVA with significant set at P=0.05. Compared to non-fatigue controls, modifications in cell number were observed in the CFS/ME patients. The results demonstrate that CFS/ME may be characterised by discrepancies in other subsets of T cells.

P3.11.07
***In vivo* IFN-alpha/ribavirin treatment modulates Vγ9Vδ2 T-cell function during chronic HCV infection**

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In chronic HCV infection, treatment failure and defective host immune response highly demand improved therapy strategies. Vγ9Vδ2 T-cells represent a good target for HCV immunotherapy, since phosphoantigen (PhAg)-activated Vγ9Vδ2 T-lymphocytes are able to inhibit subgenomic HCV replication by IFN-γ release. A profound impairment of IFN-γ production by Vγ9Vδ2 T-cells during chronic HCV infection was previously shown. Interestingly, *in vitro* IFN-α partially restored Vγ9Vδ2 T-cells responsiveness to PhAg, by stabilizing IFN-γ-mRNA.
In order to verify how *in vivo* IFN-α/RBV treatment could affect Vγ9Vδ2 T-cells phenotype and responsiveness to PhAg in HCV-infected patients, 10 subjects underwent a longitudinal study before and after treatment. IFN-α/RBV therapy did not significantly modify Vγ9Vδ2 T-cell numbers and differentiation profile. Interestingly, Vγ9Vδ2 T-cell responsiveness remained unmodified until 3 weeks of therapy, but dropped after one month, suggesting that repeated *in vivo* IFN-α administration in the absence of TCR-mediated signals results in Vγ9Vδ2 T-cell anergy.
The present work defines the window of possible application of combined strategies targeting Vγ9Vδ2 T-cells during chronic HCV infection; specifically, the first 3 weeks from the beginning of treatment may represent the optimal time to target Vγ9Vδ2 T-cells *in vivo*, since their function in terms of IFN-γ production is preserved.

P3.11.08
γδ T cells are required for the development of autoimmune anti myeloperoxidase glomerulonephritis

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The relationship between γδ and αβ T cells in the generation of autoimmunity is not well defined. This study explores the role of γδ T cells in the pathogenesis of autoimmune anti-myeloperoxidase (MPO) anti-neutrophil cytoplasmic antibody (ANCA) associated glomerulonephritis (GN) by comparing the development of autoimmunity to MPO and resulting GN among WT and TCRδ-/- mice. Autoimmunity was induced by MPO immunization in Freunds Adjuvant (CFA) and GN triggered using a subnephritogenic dose of antiglomerular basement membrane antibody. Deficiency in γδ T cells resulted in significant attenuation of functional (proteinuria; 3.2±0.3 vs 2.3±0.2mg/24hr, $p<0.05$) and histological (abnormal glomeruli; 34.4±2.9 vs 20.0±3.1%) renal injury and glomerular leukocytic infiltration (macrophage; 5.3±0.7 vs 1.3±0.7cells/glomerular cross section [c/gcs], neutrophils; 1.4±0.2 vs 0.3±0.06c/gcs and CD4 T cell; 1.4±0.3 vs 0.3±0.06c/gcs), $p<0.05$. Attenuated renal injury observed in TCRδ-/- mice correlated with decrease in systemic anti-MPO autoimmunity with decreased MPO specific CD4 T cell responses

assessed by; dermal MPO induced DTH (swelling 0.24±0.04 vs 0.02±0.01Δmm) and decreased frequency of IFNγ producing CD4 T cells (Elispot 82.2±19.0 vs 20.1±7.1cells), all $p<0.05$. However humoral immunity was unaffected (similar ANCA titres between groups; 0.3±0.03 vs 0.3±0.05OD_{450nm}, p=0.7). Analysis of dendritic cells (DC) in lymph nodes draining immunisation sites showed significantly reduced DCs ($6.3x10^4$±$7.4x10^3$ vs $4.2x10$±$4.1x10^3$ cells/draining LN) with increased percentage of DC apoptosis (1.6±0.3 vs 3.4±0.6%), $p<0.05$. Thus γδ T cells affect pathogenic anti MPO autoimmunity by optimising the development of CD4 T effector adaptive autoimmune responses.

P3.11.09
Differentiation of human peripheral blood Vδ1 T cells expressing Natural Cytotoxicity Receptors: implications for immunotherapies and the understanding of γδ T cell function

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Natural Cytotoxicity Receptors (NCRs) were originally identified as specific natural killer cell activating receptors that, upon binding to their endogenous ligands, trigger the killing of tumor cell targets. Here we describe a population of Vδ1+ T cells from human peripheral blood that can differentiate in vitro to express the NCRs NKp30, NKp44 and NKp46. We show that the expression of NKp30 endows Vδ1+ T cells with the ability to lyse, in vitro, both hematologic tumor cell lines as well as chronic lymphocytic leukemia cells isolated from patients. Moreover, we found that these cells are also able to produce CC chemokines upon the triggering of NKp30, which suppresses HIV-1 viral replication in CD4+ T cells in vitro. This evidence not only further discloses the key role of NK cell receptors in certain γδ T cell functions, but also suggests that Vδ1+ T cells specifically harbor a powerful therapeutic potential through the induction of NCRs, whose function can be manipulated for the treatment of various diseases.

P3.11.10
Mycobacterium tuberculosis antigen specific changes of CDR3 length distribution of Vδ2 chain of human γδ T lymphocytes

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The human γδ T cells can be activated by both non-peptide and peptide antigens from Mycobacterium tuberculosis (Mtb). Recent studies show that phosphate antigen activated γδ T cells fail to, but Mtb activated γδ T cells potentially inhibit intracellular Mtb growth. Mtb heat resistant antigen (Mtb-HAg), a peptide antigen, has been shown to preferentially stimulate Vγ9Vδ2 T cells. However, the characteristic of diversity of TCR Vδ2 region of Mtb-HAg stimulated Vγ9Vδ2 T cells has not been investigated. In this study, by using size spectratyping of length of CDR3 fragment of Vδ2 gene, the length of the most dominant peak of CDR3 fragments of Vδ2 gene in Mtb-HAg activated γδ T cells was significantly larger than that of γδ T cells from fresh PBMC. In addition, the frequency of the most dominant peak of Vδ2-CDR3 fragments of γδ T cells in patients with pulmonary TB (0.28±0.06) was significantly higher than that in health donor subjects (0.20±0.03) ($p<0.05$). The RT-PCR products of dominant peaks of Vδ2-CDR3 fragments of PBMC from four TB patients were cloned and sequenced. All cloned Vδ2 CDR3 regions contained conserved "CACD" and "KLIFGKG" at the N- and the C-terminus, respectively, but there were distinct different inner sequences of most dominant peak of the Vδ2-CDR3 regions among these TB patients. In conclusion, Mtb antigen or infection biased distribution of Vδ2-CDR3 fragment of Vγ9Vδ2+ T cells indicates that Mtb infection induces the oligoclonal expansion of human γδ T cells.

P3.11.11
Respiratory syncytial virus infection influences FasL-mediated apoptosis of γδ T cells in a murine model of allergen sensitization

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We have reported previously that adoptive transfer of γδ T cells increased the number of inflammatory cells as well as the percentage of eosinophils in the lungs of allergic mice, suggesting that γδ T cells may play a proinflammatory role in OVA-induced allergic responses. RSV infection before sensitization to OVA decreased the number of Th2-type γδ T cells. However, the underlying mechanisms remain unknown. In this study, by using BALB/c mice that were inoculated intranasally with live RSV before or after sensitization to OVA, we found that adoptive transfer of γδ T cells from OVA-sensitized and challenged mice enhanced not only the number of inflammatory cells but also the production of Th2 cytokines in the lungs and allergy-related antibodies in the serum, further confirming that γδ T cells act as pro-inflammatory cells or a promoter for the development of allergic asthma. RSV infection before sensitization to OVA decreased the number of total γδ T cells as well as activated γδ T cells by augmenting apoptotic death of the γδ T cells. Furthermore, prior RSV infection increased the absolute number of FasL-expressing γδ T cells in the lungs of allergic mice. Blocking FasL with monoclonal antibody diminished the percentage of apoptotic death of the pulmonary γδ T cells. These results suggest that RSV infection protects against the subsequent development of OVA-induced allergic responses by enhancing apoptotic death of pulmonary γδ T cells via FasL-dependent pathway.

P3.11.12
Sensing cellular stress by γδ TCR

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Human non-Vδ2 γδ T cells constitute a first line of defence in epithelium and organs by recognizing modified cells as virus infected or transformed cells. This reactivity suggests recognition of stress-associated self-antigens by non-Vδ2 TCRs. In order to identify these uncharacterized antigens, we set up a strategy comprising mice immunization with tumor cells targeted by non-Vδ2 TCRs and selection of mAbs able to specifically abrogate the reactivity of γδ TCR transductants against these targets.
In this study, we focused on a Vγ8Vδ3 TCR, which conferred to transductant a restricted reactivity against U373MG glioblastoma cell line. A blocking mAb (FMS-01) was generated against U373MG that specifically abrogated recognition by both Vγ8Vδ3 transductant and T cell clone, suggesting that FMS-01 mAb targets the antigenic ligand of Vγ8Vδ3 TCR. Surprisingly, we showed that modification of U373MG culture conditions as high level cell confluence, thermic choc, hypoxic stress or CMV infection, increased the reactivity of Vγ8Vδ3 transductant and T cell clone. These stress conditions were able to induce Vγ8Vδ3 transductant activation against other glioblastoma cell lines (U343MG and U251MG). This recognition of stressed cells was TCR dependent and blocked by FMS-01 mAb. Moreover, stress increased expression of FMS-01 targeted antigen that directly correlated with Vγ8Vδ3 transductant activation. Identification of FMS-01 targeted antigen is in process.
In conclusion, our results show that stress conditions can induce the expression of γδ TCR antigens. Because these antigens are critical for γδ T cell recognition, their identification could lead to new immunotherapeutical treatments in cancer such as glioma.

P3.11.13
Human Vγ9Vδ2 T cells can selectively promote TH1 or TH2 responses via interactions with dendritic cells or B cells in vitro

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The Vγ9Vδ2 subset of human γδ T cells can induce maturation of dendritic cells (DC) into antigen-presenting cells (APC) and B cells into antibody-secreting plasma cells. Since B cells are capable of presenting antigens to T cells, we investigated if Vγ9Vδ2 T cells can influence antigen presentation by these cells. Vγ9Vδ2 T cells, B cells and monocytes were isolated from human blood. Vγ9Vδ2 T cells were expanded by phosphoantigen stimulation and monocytes were induced to differentiate into immature DC. Vγ9Vδ2 T cells were co-cultured with equal numbers of DC or B cells. Expression of APC markers, production of cytokines and antibodies, and stimulation of T cells by the DC or B cells was then measured using flow cytometry and ELISA. Vγ9Vδ2 T cells induced expression of CD86 and HLA-DR and the secretion of IL-6, IL-12, TNF-α and IFN-γ by DC. They augmented the ability of DC to stimulate proliferation and IFN-γ production by antigen-specific and alloreactive T cells. In contrast, Vγ9Vδ2 T cells induced CD86 expression, but downregulated HLA-DR expression, by B cells and they promoted the release of IL-4 and IgG, IgA and IgM by B cells. B cells matured with Vγ9Vδ2 T cells stimulated proliferation but not cytokine secretion by conventional T cells. These data suggest that Vγ9Vδ2 T cells can induce maturation of DC and B cells into APCs, but while they prime DC to stimulate TH1 responses, they prime B cells to stimulate TH2 responses.

P3.11.14
Understanding the crosstalk between γδ T cells and osteoclasts in patients with breast cancer

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Bone metastasis is a common cause of morbidity in breast cancer patients. Aminobisphosphonates are widely used in treatment of bone metastasis and skeletal disorders. Aminobisphosphonates increase endogenous pool of isopentenyl pyrophosphate (IPP) of mevalonate pathway in tumor cells, which acts as potent antigen for γδ T cells. γδ T cells are unique subsets of T-lymphocytes that are activated by aminobisphosphonates and phosphoantigens (bromohydrin pyrophosphate, IPP). The present study aims at understanding the crosstalk between γδ T cells and osteoclasts in patients with breast cancer metastasis. Multicolor flow cytometry showed higher expression of RANKL on γδ T cells of breast cancer patients. Stimulation with aminobisphosphonate (zoledronate) increased RANKL expression on γδ T cells. Osteoclasts generated from $CD14^+$ monocytes of breast cancer patients and healthy individuals in the presence of zoledronate activated γδ T cells showed reduced osteoclastogenesis as analysed by 23c6 mAb (CD51/61) and DAPI staining. Osteoclasts generated in the presence of cell free supernatants of activated γδ T cells showed reduced resorption area (pits) on Osteoclast Activity Assay Substrate. Cytokine analysis of activated γδ T cells showed presence of IFN-γ, antiosteoclastogenic cytokine. Zoledronate activated γδ T cells have high propensity to migrate to bone and inhibit osteoclastogenesis. Aminobisphosphonate activated γδ T cell based immunotherapy would be a promising approach to treat bone metastasis in breast cancer.

P3.11.15
Toll-like receptor 2 ligand enhances effector function but not antigen presentation properties of gamma-delta T cells

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Background: Activated gamma-delta T cells possess potent cytotoxicity against broad range of cancer cells. They are also involved in the activation of dendritic cells through their expression of cytokines. Gamma-delta T cells also acquire antigen presenting cell properties upon activation. These properties make gamma-delta T cells an attractive platform for cancer immunotherapy. Toll-like receptors (TLR) 2 and 3 are expressed by gamma-delta T cells. We aim to explore if TLR2 and TLR3 ligands could enhance the functions of activated gamma-delta T cells and thus enhancing their usefulness for immunotherapy.
Methods: Peripheral blood gamma-delta T cells of healthy donors were activated by zoledronate (Zometa, Novartis, USA). Activated cells were expanded by culturing in the presence of IL-2 (100ng/ml), IL-15 (10ng/ml), and either TLR2 ligand Pam3CSK4 (100ng/ml) or TLR3 ligand Poly(I:C) (100ng/ml) for up to 14 days. The cells were assayed for antigen presenting cell markers (such as CD86, HLA-DP,DQ,DR and CCR7) and cytokine expression (IFN-gamma and TNF-alpha) by flow cytometry.
Results: Upon activation, gamma-delta T cells rapidly upregulated granzyme, as well as CD86 and HLA-DP,DQ,DR expression that peaked at days 7-10. Neither the addition of Pam3CSK4 nor Poly(I:C) resulted in any enhancement in these expression. However, Pam3CSK4 stimulated enhancement in CCR7 and IFN-gamma expression.
Conclusion: TLR2 and TLR3 ligands (Pam3CSK4 and Poly(I:C) respectively) did not enhance the expression of antigen presenting cell markers on gamma-delta T cells. However, Pam3CSK4 was found to upregulate IFN-gamma and CCR7 expression, implying its potential in enhancing cytotoxicity and lymph node-homing properties of gamma-delta T cells.

P3.11.16
Phenotypic characterisation of Human Vγ9Vδ2 T-cells

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Human Vγ9Vδ2 T-cells are commonly divided into subtypes using CD27 and CD45RA surface expression, reflecting their cytokine production, proliferative and plastic potential; [27^+RA^+] Naïve-T_N, [27^+RA^-] Central Memory-T_{CM}, [27^-RA^-] Effector Memory; T_{EM}, and a [27^-RA^+] T_{EM} subset expressing CD45RA-T_{EMRA}. However, the use of these markers fails to adequately describe Vγ9Vδ2 T-cell biology.
Objective: To characterise the different Vγ9Vδ2 T-cell phenotypes using different cell surface markers.
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient separation from the peripheral blood of 20 healthy subjects and stained for cell surface receptors: CD3, Vδ2, CD27, CD45RA, CD28, CD11a, CD16 and CD56. Flow cytometry was used to determine extracellular marker expression on Vγ9Vδ2 T-cells.
Results: Our results highlight the heterogeneous nature of Vγ9Vδ2 T-cell subsets defined using CD27 and CD45RA. Notably, T_N cells include both a $CD11a^{hi}$ and $CD11a^{lo}$ subset, while T_{CM} cells can be sub-divided, often equally, into $CD28^+$ and $CD28^-$ populations. We have also identified a mutually exclusive relationship between the expression of CD28 and CD16 on T_{CM}, T_{EM} and T_{EMRA} cells. As CD28 and CD16 have important functional roles in T-cell activation, we are presently investigating the functional abilities of the populations described.
Conclusion: We have identified considerable surface heterogeneity in T_N, T_{CM}, T_{EM} and T_{EMRA} Vγ9Vδ2 T-cell subsets suggesting a comparable degree of functional heterogeneity. A better understanding of Vγ9Vδ2 T-cell phenotypes should provide improved insight for the study of these cells in associated diseases such as; Crohn's disease, Behçet's disease and Bisphosphonate-related osteonecrosis of the jaw (BRONJ).

P3.11.17
Human peripheral Vδ1+ γδ T cells can develop into αβ T cells

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The T cell compartment can be divided in two large classes: αβ and γδ T cells. It is believed that they develop in the thymus from a common progenitor and that the choice between αβ and γδ T cell fate is the first lineage decision made by progenitors after they commit to the T-cell lineage. However, here we show that peripheral Vδ1+ γδ T cells in an inflammatory environment can transdifferentiate into αβ T cells. Upon their extrathymic route of differentiation, that resembles well-characterized molecular program of thymic αβ lineage development, Vδ1+ T cells upregulate CD4+ coreceptor, develop Vδ1+ CD4+CD8+ double positive cells, show heterodimeric CD8αβ, transcribe RAG, preTα and express a particular Vβchain on their surface. Simultaneously inflammation confers controlled initiation of rearrangement in the TCRα locus. Transdifferentiation of Vδ1+ T cells at the clonal and bulk-culture level into functional CD4+ or CD8+ αβ T cells via a Vδ1+/αβ+TCR double positive stage suggests that, upon inflammatory stimuli, Vδ1+ T-cell conversion participates in the induction of adaptive immune responses. Identifying an innate T cell as an αβ T-cell progenitor and showing the developmental steps linking the progenitor to adaptive, thymus-independant αβ T-cell responses as inflammatory conditions is of utmost relevance and will deeply impact evaluation of immune responses in infection, malignancy and autoimmune processes.

P3.11.18
The regulatory role of TCR Vδ1$^+$ NKT cells in systemic sclerosis patients with interstitial pneumonia

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Purpose. Interstitial pneumonia (IP) is a serious complication in systemic sclerosis (SSc). However, the exact mechanism of IP remains elusive. The purpose of this study is to clarify the role of TCR Vδ1$^+$ NKT cells in SSc patients with IP.
Methods. The proportion of TCR Vδ1$^+$ NKT cells in PBMCs from healthy controls (HC, n=22) and SSc patients (n=35) was analyzed by flow cytometry. In SSc patients with IP, the correlation between proportion of TCR Vδ1$^+$ NKT cells in PBMCs and serum KL-6 levels was analyzed. Cytokine (IFN-γ, TNF-α, IL-4, IL-17) and chemokine (CCL2, CCL3, CCL4, CCL5) secretion assay using TCR Vδ1$^+$ NKT cells from HC and SSc patients was performed.
The effect of culture supernatant of TCR Vδ1$^+$ NKT cells on fibroblast proliferation was evaluated.
Results. The proportion of TCR Vδ1$^+$ NKT cells was significantly higher in IP-negatives (mean±SEM, 1.03±0.32%) than IP-positive patients (0.28±0.07%, $p<0.05$) and HC (0.23±0.09%, $p<0.05$). In IP-positive SSc patients, the proportion of TCR Vδ1$^+$ NKT cells correlated negatively with serum KL-6 values (r=-0.464, $p<0.05$).
Upregulation of CCL3 and downregulation of IFN-γ production were noted in TCR Vδ1$^+$ NKT cells of IP-positive SSc patients upon TCR stimulation compared with HC. Fibroblast proliferation was promoted with medium supplemented with culture supernatant derived from IP-positive SSc patients, whereas that from HC was not.
Conclusions. TCR Vδ1$^+$ NKT cells might play a regulatory role in the pathogenesis of IP in SSc patients.

P3.11.19
γδ T cells react against the murine cytomegalovirus

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The cytomegalovirus (CMV) is a large beta herpesvirus and various layers of the innate and adaptive immune system provide control in CMV-infected individuals. In this study we focused on the protection mediated by γδ T cells against this virus.
Using the murine model we studied protective capacities of γδ T cells after infection with the murine cytomegalovirus (MCMV). We furthermore started to characterize γδ T cells reacting and expanding after CMV infection.
Adoptive transfer of γδ T cells purified from spleens and lymph nodes of infected donor mice protected immunocompromised hosts (Rag-/- mice) from the lethal course of disease and virus titers were markedly decreased 14 days after infection in several organs.
To examine, whether there is a role for γδ T cells during MCMV infection in immune competent mice as well, we infected wildtype C57BL/6 and TCRδ-/- mice and compared viral titers on days 3 and 5 after infection. TCRδ-/- mice showed significant higher viral titers in several organs. This indicates that γδ T cells also play a role in immune competent mice during the early stage of infection.
We further characterized the γδ T cells reacting and expanding after CMV infection regarding their expression of certain surface molecules using flow cytometry. The percentage of CD44 expressing γδ T cells increased after infection, what points to an activated state of these cells. In addition we observed a long-lasting increase in NKG2D expressing cells after infection, which is concomitant with down modulation of CD27.

P3.11.20
TCR requirements for γδ T cell development

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Gamma delta (γδ) T cells play important roles in immune responses against pathogens and tumours which seem to strongly correlate with the secretion of cytokines such as IFNγ and IL-17A. Recent evidence suggests that γδ cells are predominantly pre-committed to certain effector fates during thymic development; T cell receptor (TCR)-agonists favouring the development of IFNγ-producing γδ cells (that are variously identified by CD27, CD122 and NK1.1), whereas the absence of ligand interactions was suggested to generate IL-17A-producing γδ cells (identified as CD27(-), but expressing CCR6). The aim of this study was to determine the role of the TCR and TCR signalling in the development of γδ T cell subsets and in the adoption of effector fates in the thymus.
Our results demonstrate that at least 4 distinct subsets of γδ T cells can be identified in both the periphery and the thymus of C57BL/6 mice. These subsets display distinct cytokine-secreting and proliferative potential as well as variable TCR usage. Our data in vitro demonstrate a γδ developmental sequence where CD27(-) IL-17A-secreting γδ T cells are generated from CD27(+) precursors. In addition, we find that TCR signalling induced by cross-linking with an activating antibody or in a ligand-independent manner does not favour the development of CD27(-) IL-17A-secreting γδ T cells. Whilst our results suggest that effector fate of γδ subsets is not likely to correlate with ligand-dependent versus ligand-independent signalling it remains to address whether it correlates with quantitative and/or qualitative differences in TCR signalling between the different subsets.

P3.11.21

CD30L/CD30 signaling plays a critical role in maintenance and activation of IL-17A-producing γδ T cells in mucosa-associated tissues in mice

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CD30 ligand (CD30L, CD153), a member of the TNF superfamily, and its receptor CD30 are important for differentiation and activation of $CD4^+$ Th17 cells. In the present report, we demonstrate that the IL-17A-producing γδ T cells normally developed in the fetal thymus, whereas $Vγ1^-Vγ4^-$ γδ T cells expressed Vγ6/Vδ1 gene transcript selectively decreased in mucosa-associated tissues in naïve CD30KO or CD30LKO mice. Moreover, CD30 and CD30L were expressed preferentially by $Vγ1^-Vγ4^-$ γδ T cells in naïve mice. The bacteria clearance was attenuated by the impaired response of the IL-17A-producing γδ T cells and decreased infiltration of neutrophils in CD30KO or CD30LKO mice*. In vivo* administration of agonistic anti-CD30 mAb restored the ability of protection against *L. monocytogenes* by enhancing $Vγ1^-Vγ4^-$ γδ T cells producing IL-17A not only in WT but also CD30LKO mice. Taken together, it appears that CD30L/CD30 signaling plays an important role in the maintenance and activation of IL-17A-producing γδ T cells presumably bearing Vγ6 in the mucosa-associated tissues of mice.

P3.11.22
Developing a protocol for large scale generation of antigen-pulsed gamma-delta T cells for cancer immunotherapy

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Background: Gamma-delta T cells are members of the innate immune system that display potent cytotoxicity against broad range of cancer cells. Activated gamma-delta T cells also acquire antigen presenting cell functions. We therefore aim to explore and develop a protocol for adopting antigen-pulsed gamma-delta T cells as dual function cytotoxic cell-plus-vaccine platform for cancer immunotherapy.
Methods: Gamma-delta T cells in the peripheral blood mononuclear cells (PBMC) of healthy donors were activated by zoledronate and expanded by culturing in the presence of IL-2 (100ng/ml) and IL-15 (10ng/ml), followed by magnetic isolation and pulsed with Epstein-Barr virus latent membrane protein 2 (LMP2)-derived peptide library. Cytotoxicity of the cells was assessed by DELFIA BATDA fluorometric assay against human cancer cell line targets. Antigen presenting cell markers (CD86, HLA-DP,DQ,DR and CCR7) and intracellular cytokine expression (granzyme B, IFN-gamma and TNF-alpha) were assayed by flow cytometry.
Results: Upon activation, gamma-delta T cells rapidly upregulated granzyme B and IFN-gamma expression, as well as CD86 and HLA-DP,DQ,DR expression that peaked at days 7-10. At day 14, between 30-60 fold expansion of peripheral blood gamma-delta T cells was achieved. Cytotoxicity assays showed that these cells retain potent non-HLA-restricted killing of human cancer cell lines. Co-culturing of LMP2 peptide-pulsed gamma-delta T cells with autologous PBMCs for 14 days resulted in up to seven-fold expansion of LMP2 epitope-specific CD8 T cells.
Conclusion: Gamma-delta T cells generated by this protocol achieved good expansion and possessed potent cytotoxicity and antigen presentation functions. The protocol will be validated on NPC patient PBMCs.

P3.11.23
The role of phosphoantigen-expanded human γδ-T cells in the control of Epstein-Barr virus infection

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Background. Epstein-Barr virus (EBV) is a ubiquitous infectious agent which infecting more than 90% of the world's population. And it is well known to be linked to several specific human malignancies. Also, in immunocompromised patients, EBV causes lymphoid proliferation that results in the appearance of lymphomas. The limitations of current therapies for treating EBV-associated disorders have led to the development of alternative strategies to enhance host immunity against EBV. The antiviral activities of γδ-T cells against other varied viruses have been demonstrated. However, little is known about their antiviral activities against EBV infection in human.
Methods. Human Vγ9Vδ2-T cells were expanded by phosphoantigen aminobisphosphonate pamidronate (PAM), and then co-cultured with EBV-transformed human autogolous B cells (EBV-LCLs). The antiviral mechanisms of Vγ9Vδ2-T cells against EBV in vitro were determined.
Results. PAM selectively activated and expanded human Vγ9Vδ2-T cells, and up-regulated the expression of TRAIL, NKG2D and perforin in Vγ9Vδ2-T cells. The PAM-expanded human Vγ9Vδ2-T cells can efficiently recognize and kill the autologous EBV-LCLs and significantly inhibited viral replication in vitro. The cytotoxicity of Vγ9Vδ2-T cells against EBV-LCLs was dependent on NKG2D, TRAIL activation and was mediated by Fas-Fas ligand and perforin-granzyme B pathways. The killing of EBV-LCLs by Vγ9Vδ2-T cells was also dependent on cell-cell contact and required the activation of EBV-transformed cells.
Conclusion. PAM-activated Vγ9Vδ2-T cells can effectively kill autologous EBV-infected B cells in vitro, suggesting a novel approach by using phosphoantigens to activate human Vγ9Vδ2-T cells against EBV infection.

P3.11.24
Assessment of immune dysfunction in IgA-nephropathy patients

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Although IgA nephropathy (IgAN), which features IgA deposition in kidney mesangium, is considered a benign condition, a high proportion of patients progress to renal failure.
Methods: The immune profile was assessed in 36 patients with biopsy-proven primary IgAN and 30 healthy donors (HD). Flow cytometry measurement of lymphocytes phenotype, T cells proliferation in response to phytohemagglutinin (PHA), γδ T cells response to isopentenyl pyrophosphate (IPP) and ELISA for serum IgA determination were used. The stimulation index (SIγδ) was determined as a ratio between the amount of T cells in medium and IPP-stimulated T cells.
Results: IgAN patients demonstrated significantly decreased number of circulating CD19+ B cells predetermined by reduced CD19+5- B2 lymphocytes. In addition, CD19+ B-cells in IgAN patients had elevated membrane-bound IgA1 expression (20,1(9,8÷40,8)% vs 9,9(5,9÷14,1)% in HD, $p<0,05$).
Serum IgA was significantly higher in IgAN than in HD, with 37% of patients having IgA level upper the normal 95th percentile.
T cells with αβ T-cell receptor (TCR) were in the normal range and there wasn`t difference in T cells response to 2,5mg/ml PHA between patients and HD. However decrease of circulating CD3+ T lymphocytes expressed Vδ2 TCR was established (1,9(1,2÷2,9)% in IgAN vs 3,2(1,6÷5,8)% in HD, $p<0,05$) and their significant expansion in response to 5mM IPP was observed. SIγδ for patients was 11,1(7,4÷70,0) in comparison to 5,4(3,1÷8,4) for donors.
Conclusion: Immune profile of IgAN patients, including both B cells arm of immune system and γδ T lymphocytes function, contribute to filling gaps in our knowledge about IgAN development.

P3.12 Follicular helper T cells

P3.12.01
MyD88 activated Monocyte-derived CD11b+ dendritic cells enhance Follicular Helper T cell differentiation

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Adjuvant combinations could result in synergistic enhancement of the immune response. In this context, how Toll-like Receptor (TLR) agonists contribute to antigen-specific T cell-dependent B cell responses was assessed in vivo. We found that when vaccine adjuvant was combined to MyD88-dependent but not to Trif-dependent TLR agonists, differentiation of antigen-specific T Follicular helper cells (Tfh), which regulate high-affinity B cells, was increased without changing the overall magnitude of the antigen-specific T cell response. This phenomenon correlated with an enhancement of germinal center reaction, antigen-specific plasma cells and circulating antibodies. Increase of Tfh differentiation was driven in vivo by enhanced production of IL-6 by antigen-presenting monocyte-derived CD11b+ dendritic cells. In contrast, nor B cells or plasmacytoid dendritic cells that also secreted IL-6 in response to TLR agonist were involved as shown in vivo in their absence. Thus, some TLR agonists imprint the specialized program of Tfh function needed to promote high-affinity B cell protection in vivo specifically through T cell differentiation by enhancing IL-6-production by monocyte-derived CD11b+ dendritic cells.

P3.12.02
Regulation and B-cell help mediated by distinct subsets of IL-10-producing T-cells

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IL-10 is a B-cell growth factor produced by CD4+ helper T-cells, but also mediates suppression of T-cell responses by regulatory T-cells. We have recently identified two subsets of IL-10 producing CD4+ CCR6+ memory T cells and IL-7R- effector T cells with suppressive capacities in human blood (Haringer et al., JEM 2009, Rivino et al. JEM 2010).
Here, we identified a phenotype that distinguishes IL-10-producing regulatory T-cells and IL-10-producing helper cells in humans and mice. We directly compared memory and effector T cells for the regulation of IL-10 production, and analysed their phenotypes and B-help functions in peripheral blood and in secondary lymphoid organs.
IL-10-producing regulatory T-cells co-expressed CCR5 and PD-1, but were negative for CCR6 and IL-7R. Purified CCR5+CCR6-IL-7R-CD4+ T-cells did not display B helper functions because they failed to up-regulate CD40L. Moreover, they actively suppressed T-cell proliferation and T-cell dependent IgG production. Conversely, IL-10-producing CCR6+IL-7R+CD4+ T-cells helped B-cell antibody production in an IL-10-dependent manner. CCR6+CD4+ T-cells secreted IL-10 in B-cell follicles but were distinct from conventional follicular helper T-cells, which were CCR6- and helped independently of IL-10.
Experiments in mice confirmed findings with human cell populations. CCR6+IL10+cells provided antigen specific B cell help, while IL10-producing CCR5+PD1+ cells exerted suppressive functions.
In conclusion, two distinct IL-10-producing T cell subsets are present in human and mice blood and lymphoid organs, which can be distinguished by chemokine receptor expression. We propose that these two subsets have opposing functions in humoral immune responses.

P3.12.03
Role of Notch signal in the generation of follicular helper T cells (TFH) and memory T cell

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Follicular helper T cells (TFH) are specialized T cell subset localizing in germinal centers (GCs), and their secreting cytokines, IL-4 and IL-21 facilitate B cell help in the antibody responses. Conserved noncoding sequence 2 (CNS2) have been characterized as an intrinsic enhancer element specific for the IL-4 expression in TFH cells. However, it still remains unclear how CNS2 is specifically activated on TFH cells, and whether TFH cells can be memory cells. Here we demonstrated that deficiency of Rbpj, a downstream transcription factor of Notch signaling completely abrogated the CNS2 mediated IL-4 expression in TFH cells, indicating that Notch signaling determine an identity of TFH cells. Antigen priming promoted the plasticity of CNS2 active TFH cells to differentiate into TH2 and TH17 cells. Furthermore, CNS2 active TFH cells reduced the expression of CXCR5 and re-localized in T cell area as IgG1 and IgE regulating memory cells that rapidly form and re-entered into the GCs after antigen priming. In contrast, the transfer of the TFH cells derived from Rbpj deficient mice diminished memory formation. These findings indicate that Notch signaling is intrinsic to control the IL-4 expression in TFH cells and to maintain the memory T cells.

P3.12.04
Role of BCL6 and PD-1 in CD4 memory T cell development

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Follicular T helper (TFH) cells have been shown to play an indispensable role within the germinal center (GC). To study the effects of TFH cell loss, our lab has developed a mouse line wherein BCL6, the master transcription factor behind TFH cell differentiation, is conditionally deleted in specific cell subsets ($BCL6^{fl/fl}$). When mated to Cre-CD4 mice, loss of BCL6 specifically in CD4 T cells resulted in a complete absence of GCs. Data from control mice confirmed the correlation between BCL6 and PD-1 expression, with the highest levels of both found in TFH cells. Further analysis showed the few $CD4^{+}$ $CXCR5^{+}$ $ICOS^{+}$ $PD\text{-}1^{hi}$ cells found in $BCL6^{fl/fl}Cre^{CD4}$ mice have higher rates of apoptosis than those in control mice. Because previous studies have shown that Bcl6 regulates T cell memory, we investigated whether there were any effects on CD4 T cell memory development and found $BCL6^{fl/fl}Cre^{CD4}$ mice have an altered memory cell phenotype, with higher levels of central memory cells and fewer effector memory cells than control mice. Furthermore, memory cells in these mice show significantly lower rates of apoptosis as well as surface expression of PD-1. Taken together, this data suggests a complex regulatory network involving BCL6 and PD-1 expression, which effects not only TFH cell differentiation, but the development of CD4 T cells into effector versus central memory cells as well.

P3.12.05
B cell antigen presentation induces Tfh-differentiation from Th2 cells in vitro

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During T-dependent immune responses, after antigen presentation from B cells to CD4 T cells, B cells proliferate extensively to form germinal center (GC), and CD4 T cells differentiate into distinct effector helper T cell subsets, such as Th1, Th2, and follicular helper

T (Tfh) cells. To clarify the mechanisms of the differentiation of B cells and CD4 T cells, we analyzed B cells and T cells co-cultured in the culture system we have recently established, in which B cells are cultured on feeder cells expressing CD40 ligand and BAFF. By using this culture system, we show that, when cultured with naïve B cells in the presence of antigens under the Th2 conditions (i.e. with IL-2 and IL-4), in-vitro-derived Th2 cells differentiate into cells with Tfh-like phenotype (CXCR5+ PD-1+ ICOS+ and IL-21 producing). Surprisingly, these Th2-derived Tfh-like cells retained the function of producing IL-4 and expressed GATA3 mRNA. On the other hand, B cells cultured with Th2 cells, in the presence of antigens, differentiate into cells with pre-memory like phenotype (B220+ CD38+ FAS+ GL7+) and plasma cells (Blimp1+ CD138+). Thus, we demonstrate efficient in vitro differentiation of Th2 cells into Tfh-like cells that also retain the nature of Th2 cells, which are reminiscent to a recently proposed Tfh subset. We also show that antigen-presenting B cells are necessary for the differentiation of Tfh-like cells.

P3.12.06
ICOS is critical for maintenance of the T follicular helper phenotype

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The development of T follicular helper (TFH) cells can be divided into different phases: the early expression of the master transcription factor Bcl-6 in the T cell zone, the upregulation of CXCR5 and migration towards the B cell zone, and late phases where fully matured TFH cells interact with germinal center (GC) B cells. The costimulatory molecules CD28 and ICOS both have been shown to be important for TFH cells.
Using an *in vivo* T/B cooperation system with antigen-specific T and B cells, we dissected the requirement for CD28 and ICOS in different phases of TFH development. Whereas CD28 was already critical for very early steps, ICOS knock-out T cells normally upregulated Bcl-6 and migrated towards the B cell zone. However, these functions reversed in late phases. Blocking of CD28 with CTLA-4-Ig did not have any effect on already differentiated TFH cells. In contrast, blockade of the ICOS pathway resulted in a complete loss of the TFH phenotype within 48 hours. The reversion of the TFH phenotype started within hours by downregulation of molecules important for the localization of TFH cells, leading to redistribution to the T cell zone. This further resulted in breakdown of the GC response and loss of GC B cells within the next 4 days. We could show that continuous ICOS costimulation is required for a unique transcriptional program which stabilizes the TFH phenotype. These findings are important in the context of treatment for autoimmune diseases where TFH cells play a critical role for pathology.

P3.12.07
A positive feedback loop between splenic long-lived plasma cells and follicular helper T cells

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Long-lived plasma cells (LL-PCs) are crucial for persisting chronic inflammatory disorders, but how they impinge on the pathogenesis remains unclear. We found that Foxp3+ regulatory T cell-depleted autoimmune mice, referred to as K/BxNsf, exhibited an abnormal accumulation of autoantigen-specific LL-PCs in their spleens but not in the bone marrow. K/BxNsf LL-PCs were less susceptible to the cytotoxic action of cyclophosphamide and escaped from immune complex-mediated apoptosis by downregulating FcγRIIb. They expressed lower levels of costimulatory molecules and the intracellular machinery for antigen presentation than conventional short-lived plasma cells (SL-PCs), which was associated with their reduced capacity of CD4+ T cell priming. Most importantly, antigen-pulsed LL-PCs drove CD4+ T cells to induce follicular helper T cell-specific factors such as BCL-6 and IL-21, while SL-PCs failed to do. Thus, our results suggest that, unlike SL-PCs, LL-PCs abnormally accumulated in the spleen of autoimmune mice form a positive feedback loop with follicular helper T cells, thereby favoring the persistence of humoral autoimmunity. [Supported by a grant from KRF 20110015826 and 201200000000945].

P3.12.08
Roles of follicular helper T (TFH) cells in antibody based protective immunity in influenza virus vaccination

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The elevation of antibody's affinity for virus neutralization is achieved by class-switch and somatic mutation of B cells in germinal center (GC). GC formation is highly dependent on unique helper T cell subset, follicular helper T (TFH) cells. The transcriptional repressor Bcl6 is a master regulator of TFH cells and GC formation, and its deletion results in the loss of TFH cells. To address the role of TFH cells in the influenza A virus (IAV) infection, we evaluated the protective responses for IAV in the T cell and B cell specific Bcl6 deficient mice (Bcl6f/f CD4-cre and Bcl6f/f mb1-cre). During the IAV infection and vaccination process, Bcl6 deficiency severely diminished the IAV specific IgG1 production in the consequence of loss of TFH cell and GC formation. However, the Bcl6 deficient mice exhibited normal IgG2b and IgG2c productions, which is a dominant antibody specific for virus HA antigen. Furthermore, in normal IAV vaccinated mice, the IgG2c producing B cells resided in both inner and outer GC area, while the IgG1 producing B cells were preferentially resided in inner area of GC. Serum from the vaccinated Bcl6 deficient mice had a protective immunity as efficient as normal mice, suggesting that IgG1 and IgG2b&c responses may be controlled independently by distinct helper T cell subset, and the IgG2b&c antibody responses and the protective immunity against IAV are independent on the TFH cells and GC formation.

P3.12.09
Costimulation through GITR increases follicular helper T cell formation and leads to control of a chronic viral infection.

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The glucocorticoid-induced TNF receptor family-related protein (GITR) is an important costimulatory receptor on T cells. We have previously shown that enhanced costimulation through GITR increases the formation of both effector and regulatory $CD4^+$ T cells. Here we explored whether it could also affect humoral immunity and T cell help to B cells. Although development of mature B cells was not affected in GITRL transgenic (tg) mice, we found that the number of follicular helper T cells ($CXCR5^+$ $PD1^+$ $CD4^+$ T cells, Tfh) was significantly increased, including the absolute number of Tfh-B cell conjugates, as revealed by ImageStream analysis. Tfh from GITRL tg mice had normal expression levels of ICOS, SLAM and CD44 and slightly lower levels of CD62L and CCR7 compared to wild-type (WT) littermates. Interestingly, Tfh from GITRL tg mice produced more IFN-γ and IL-10, which was accompanied by a biased antibody repertoire (decreased IgG3 and increased IgA, IgG2a and IgG2b). Since Tfh have been implicated in the late control of viral replication, we infected WT and GITRL tg mice with LCMV Clone 13. Surprisingly, at day 30 after infection, we could not detect viral genome in spleen and liver from GITRL tg mice, while WT mice were still infected. Also, PD-1 expression was strongly decreased on virus-specific $CD8^+$ T cells, which correlated with faster viral clearance. All in all, these results indicate that GITR-mediated costimulation enhances the control of chronic viral infections, by boosting and modulating Tfh cell responses.

P3.12.10

The implications of skin epidermal and dermal antigen-presenting cells in T follicular helper cell polarization

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T follicular helper cells (TFH), a lineage of CD4+ T cells, play a pivotal role in isotypic switch of immunoglobulin (Ig) after interaction with professional antigen-presenting cells. The cutaneous vaccination route has been shown to be more effective at inducing both cellular and mucosal immune responses. Mouse pre-clinical studies have demonstrated a good level of efficacy of intradermally-injected poly-lactic-acid nanoparticles (PLA-NPs), coated with the HIV-derived protein p24, in initiating a favorable immune response. The skin contains numerous dendritic cells (e.g. epidermal Langerhans cells (LCs) and dermal CD207+ dendritic cells (CD207+ dDCs)), specialized in the capture and presentation of antigens. As TFH are highly implicated in IgA class switching, we thus questioned the role of LCs and dermal DC in the induction of TFH for shaping IgA production.
Our results in mice model highlight two different transport pathway of ID-injected p24-NPs to the aDLN: i) rapid and passive migration to the subcapsular sinus and ii) transport by skin DCs and inflammatory cells. While free p24-NPs are taken up by LN-resident DCs, cell-bearing NPs are located in T and B cell zones. We noted the high efficacy of ID-injected p24-NPs to induce TFH polarization and the expansion of IgA-secreting B cells in aDLNs. Interestingly, deficiency in LCs affects both responses. In contrast free draining NPs were not able to induce neither TFH nor IgA responses.
Our work highlights the importance of skin DCs in eliciting a strong antibody response and contributes to our knowledge of the complex cellular mechanisms orchestrating intradermal vaccination.

P3.12.11
Regulation of follicular helper T cells in Peyer's patches by endoluminal ATP

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Follicular helper T (T_{FH}) cells have emerged as the specialized supplier of B cell help during T cell dependent humoral response. T_{FH} home B cell follicles trough the expression of CXCR5 and express high level of several costimulatory molecules including ICOS, PD1 and CD40L. Precursor of T_{FH} cells in Peyer's patches (PPs) are regulatory T cells (T_{regs}) that lose FoxP3 expression. The development of T_{FH} necessitates the B-T cells interaction and the expression of the transcription factor B cell lymphoma 6 (Bcl6). The purinergic P2X7 receptor is a non-selective cationic channel activated by extracellular ATP. Prolonged P2X7 stimulation or high concentration of ATP provokes opening of a pore permeable to molecules up to 900 Da and cell death. *P2rx7* is a T_{regs} signature gene and is upregulated upon T_{regs} conversion to T_{FH} cells in PPs, rendering this T cell subset particularly sensitive to apoptosis induction by extracellular ATP.
p2rx7 deficiency led to significant increase in T_{FH} cells in PPs with increased ICOS that in turn boosted CXCR5 expression. PPs from *p2rx7*$^{-/-}$ mice showed significant expansion of GC B cells in PPs and increase in fecal IgA. Increased T_{FH} differentiation in PPs is a T cell intrinsic property of *p2rx7*$^{-/-}$ T cells since *p2rx7*$^{-/-}$ T_{regs} reconstituted PPs in *cd3*$^{-/-}$ reconstituted mice more robustly than wild-type cells. Oral administration of ATP led to a strong decrease in T_{FH} cells thereby showing that endoluminal ATP can control through P2X7 T_{FH} cells abundance and adaptive mucosal immunity in the gut.

P3.12.12
TLR9 signaling acts on multiple elements of the germinal center to enhance antibody responses

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Recent studies have demonstrated important roles of nucleic acid sensing toll-like receptors (TLRs) in promoting protective antibody responses against several viruses. To dissect how recognition of viral nucleic acids by TLRs enhances germinal center (GC) responses, mice selectively deleted for MyD88 in B cells or dendritic cells (DCs) were immunized with antigen bound to a TLR9 ligand. TLR9 signaling in DCs boosted GC magnitude by expanding follicular helper T cells (TFH) and as a consequence, antigen-specific GC B cells, whereas in B cells it improved GC quality through affinity maturation, class switch to IgG2a, and enhanced B cell memory. Qualitative changes resulting from B cell-intrinsic and -extrinsic effects, as well as quantitative effects emerging from DCs, were associated with changes in the relative numbers of TFH and FoxP3+ follicular regulatory CD4+T cells (TFR) and in modulation of their expression of the costimulatory receptors ICOS and PD-1. These data indicate a widespread role for TLR/MyD88 signaling in DCs and B cells in the enhancement of antibody responses through coordinated regulation of multiple elements of the GC response.

P3.12.13
Antibody production and T forlicular helper response in mice immunized with modified HIV-1 envelope gp120

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5%-25% of HIV-1 infected individuals produce broad neutralizing antibodies (bNAb). A HIV-1 vaccine (RV144) has shown a protection partially associated with epitopes within envelope V2 regions. It is well accepted that both NAbs and non-NAbs are important for protection against HIV infection. However, the mechanisms for antibody production in HIV-1 vaccination are still less known. In the present study, to develop HIV-1 envelope immunogens and explore T follicular helper function in Ab production, we modified the envelope derived from a HIV-1 primary R5 strain, immunized mice using DNA prime-protein boost strategies. Antibody production, T cell development as well as B cell differentiation by the vaccination have been examined (This work was supported by a Grant of Natural Science Foundation of China 81271824).

P3.13 Regulatory T cells: basic aspects

P3.13.01
Increased $CD8^+$ regulatory T cells in breast cancer

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Cancer progression and metastasis has been correlated with the presence of immunosuppressive factors. Regulatory T cells (Tregs) are partially responsible for attenuating antitumor immune responses in patients with cancer. Tregs are a heterogeneous population of cells composed of subsets with different phenotypes and functions. Most of these cells constitutively express Forkhead box protein P3 (Foxp3), a transcription factor critical for Tregs cell development and function. $CD4^+$ Tregs have been extensively studied in many different types of cancer, however less is known about $CD8^+$ Tregs. In the present study, we evaluated the frequency of $CD8^+$ Tregs in 20 patients with breast cancer. Blood samples, lymph nodes and tumor tissues were evaluated for the proportion of $CD8^+$ $CD25^+$, $CD8^+$ $Foxp3^+$, and $CD8^+$ $CD25^+$ $Foxp3^+$ T cells as a percentage of the total $CD8^+$ cells, by flow cytometric analysis with triple-color staining.
The results showed the mean (+SE) ratio of CD4/CD8 in blood, lymph node and breast tissue were 1.73 (+0.7), 3.73 (+0.45) and 0.51

(+0.09), respectively. The percentage of $CD8^+$ $CD25^+$ T cells (28.3+7.2), $CD8^+$ $Foxp3^+$ (30+7.4), $CD8^+$ $CD25^+$ $Foxp3^+$ (25.7+7.1) cells in tumor tissue were significantly (P<0.01) higher than those of PBMCs. Moreover, the percentage of $CD8^+$ $Foxp3^+$ in tumor was significantly higher than that of lymph node (P<0.01).
An enrichment of CD4 T cells in lymph node and CD8 T cells in tumor tissue is evident. In addition, it seems that $CD8^+$ Tregs are accumulated at tumor sites compared with peripheral tissues and may participate in forming a suppressing environment.

Regulatory T cells during tumor development in the APCmin/+ mouse model of colon cancer

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Regulatory T cells (Treg) are important to prevent autoimmunity, microbial driven inflammation and allergy. In colorectal cancer, Treg infiltration is associated with a better patient outcome, in contrast to most other tumours. To evaluate the function of Treg in colorectal cancer, we have used $APC^{min/+}$mice that spontaneously develop tumours in the intestine due to a mutation in the *APC* tumor suppressor gene.
Co-culture experiments with $CD4^+CD25^+$Treg and $CD4^+CD25^-$ responder T-cells was performed to examine the function of Treg. Splenic Treg from $APC^{min/+}$mice suppressed the proliferation of conventional wild-type T cells in a dose-dependent manner to the same extent as wild-type Treg. This suppression was independent of age and tumor burden. Cytokines was examined in the supernatants of suppression assay and revealed an increased production of IL-17 and a decreased production of IFN-γ compared to responder cells cultured alone. The frequencies of Treg, $CD4^+$ and $CD8^+$ were determined by flow cytometry and immunoflorescence and revealed an accumulation of Treg and a decrease of conventional T-cells in the tumors. To further evaluate if Treg actively inhibit lymphocyte migration into tumors, $APC^{min/+}$mice were crossed with DEREG mice, in which Treg can be selectively depleted through injections with diphteria toxin. Treg depletion resulted in an increased infiltration of conventional T cells into intestinal tumors.
In conclusion, our results show that $APC^{min/+}$mice have functionally active Treg and that Treg depletion leads to an increased infiltration of T cells into the tumors, suggesting that Treg have important effects on local T cell recruitment to tumors.

P3.13.03
Asymptomatic *Leishmania major*-infected individuals exhibit lower frequency of regulatory T cells compared to the healed individuals

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Recent studies have suggested critical roles for regulatory T cells (T_{reg}) in leishmaniases. Here using human subjects, we investigated the role of these cells in individuals healed from *Leishmania major* infection (symptomatic), compared to healthy subjects with positive leishmanin skin test (asymptomatic). Volunteers from an endemic area of *L. major* in Iran (Damghan) were screened with leishmanin skin test. Peripheral blood samples from both symptomatic (16) and asymptomatic (18) groups along with two control groups from healthy residents of either the endemic (15) or a non-endemic area (10) were collected. The percentage of T_{reg} ($CD4^+CD25^+Foxp3^+$) was determined by monoclonal antibodies using flow cytometry. Moreover, the responder T ($CD4^+CD25^-$) and T_{reg}-enriched ($CD4^+CD25^+$) cells were isolated (n=4) by magnetic cell sorting and the suppressive capacities of the latter were interpreted by in vitro proliferation assays. The results revealed that the asymptomatic group had significantly lower percentage of T_{reg} (0.43 ± 0.07%), compared to the symptomatic cases (0.83 ± 0.17 %; p<0.05). The co-culture of the isolated responder ($CD4^+CD25^-$) and T_{reg}-enriched ($CD4^+CD25^+$) cells in 1:1 ratio caused significantly lower percentage of proliferation in the healed and the asymptomatic groups (57 % ± 0.06 and 61% ± 0.14, respectively; p<0.05), compared to the controls. These data suggest that despite the similar suppressive activity of T_{reg} in both groups, the lower frequency of the T_{reg} in the asymptomatic persons may prolong the Th1 responses, leading to persistence of a very limited number of the parasite in the inoculation sites and prevention of lesion formation.

P3.13.04
Analysis of the Treg cell proteome by differential quantitative mass spectrometry

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Regulatory T cells (Treg cells) are involved in the regulation of immune responses to pathogens and tumors as well as in the regulation of the immune homeostasis. While gene expression profiles of Treg cells have been generated to identify targets, only limited data are available on the protein level. We now performed differential quantitative mass spectrometry of the Treg cell proteome.
Murine Treg cells and conventional CD4+ T cells (Tconv cells) were FACS purified. The isolated proteins from both populations were differentially labeled with stable isotopes. Differential quantitative mass spectrometry was performed to measure relative protein abundance. The experiment was replicated 4 times and identified targets were confirmed by flow cytometry as far as antibodies were available.
We were able to identify a total of 5225 proteins, while 3756 were present in all 4 replicates, among those 164 were significantly differentially expressed in Treg cells compared to Tconv cells. The generated mass spectrometry data correlated with the gene expression data for many candidates. Classic regulatory T cells markers like Foxp3, GARP, IL2rβ, GITR and Nrp1 were highly expressed on Treg cells compared to Tconv cells on both RNA and the protein level. In addition, the proteome data set was enriched for differences that affected the Treg population as a whole unlike the mRNA data. The comparison of RNA and protein data allowed us to identify interesting targets that were differentially regulated between the RNA and protein. We are currently working to characterize identified targets in Treg cell development and function.

P3.13.05
Association between SNP rs 2234711 and tuberculosis

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Introduction: The FOXP3 gene encodes a transcription factor thought to be essential for the development and function of regulatory T cells(Treg), which are play a key role in balancing immune responses to maintain peripheral tolerance against antigens .The Studies have found an association between common polymorphisms in FOXP3 and some of infection diseases. However, data about FOXP3 gene in TB is limited. The aim of present study was to analyze the association between FOXP3 gene polymorphism and susceptibility to TB in the North of IRAN.
Methods: In a case-control study, 183 TB patients and 183 controls were recruited according to age and gender. We investigated one FOXP3 promoter single nucleotide polymorphism (SNP), -3279 A>C (rs3761548) by using PCR sequence-specific primer (PCR-SSP) technique.
Results: We found no evidence for increased risk associated with the FOXP3 and rs3761548 (-3279 AC) genotype (P > 0.05).
Conclusion: In this study, we showed that there were no differences between TB patients compared to controls. It is concluded that understanding the relationship between TB and Foxp3 gene needs additional experiments in different populations.

P3.13.06
Prolactin Effect in Cd4+Cd25hicd127low/- Regulatory T Cell Function in Human

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Among its many functions, prolactin participates in the immune response favoring the activation, differentiation and proliferation of T cells. However, the mechanisms through which prolactin participates in the regulation of regulatory T cells (T_{reg}) are still unknown. Our goal was to determine whether prolactin plays a role in T regulatory cell function.
We determined the expression of prolactin and its receptor in regulatory T cells and effector T cells (T_{eff}) from 15 healthy individuals. Furthermore, we evaluated the functional activity of regulatory T cells by examining proliferation and cytokine secretion in cells activated with anti-CD3/CD28 in the presence or absence of prolactin. We report that regulatory T cells constitutively express prolactin receptor, whereas T effectors cells require stimulation with anti-CD3/CD28 to induce prolactin receptor expression. On the other hand, expression of prolactin is constitutive in both populations. In cell cultures, we found that the addition of prolactin inhibited the suppressor effect mediated by regulatory T cells, reducing suppression in the T_{reg}:T_{eff} culture from 37.4 to 13 % when compared to prolactin is added to co-cultures of T_{reg}:T_{eff} ($p < 0.05$). While, cultures favored a Th1 cytokine profile, with increased production of TNF and IFN. We report for the first time that prolactin receptor expression is constitutive in regulatory T cells, unlike T effectors cells, which require stimulation to induce prolactin receptor expression. Prolactin inhibits regulatory T cell function, apparently through the induced secretion of Th1 cytokines.

P3.13.07
Regulatory T-cells of mouse display distinctive surface glycosylation patterns and enhanced responsiveness to lectin-induced proliferation

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Surface protein glycosylation status profoundly influences T-cell functional characteristics. We aimed to compare surface glycosylation profiles of regulatory (T-reg) and conventional (T-conv) $CD4^+$ mouse T-cells that may underlie specialised functions. $CD4^+$ T-cells were isolated from C57BL/6-FoxP3-EGFP mice in which GFP expression is exclusive to T-reg. Surface glycosylation and proliferation was then evaluated by multi-colour flow cytometric analysis using a panel of 17 biotinylated lectins (carbohydrate binding proteins), labelling with the cell proliferation dye eFluor670® and gating on T-regs (GFP^+) and T-conv (GFP^-) at rest and following activation.
Freshly-isolated splenic T-regs demonstrated significantly higher surface binding of 5/17 lectins (GSL-I, DSL, PHA-E, PHA-L and SNA-I) compared to T-conv. These differences were maintained following 5-day culture without an activation stimulus or following weak activation stimulus (0.1 - 1.0 ug/ml Concanavalin A). However, in strongly stimulated cultures (anti-CD3/anti-CD28 antibodies), the lectin-binding profiles of T-conv changed to resemble those of T-regs.
In the resting state, T-reg from subcutaneous lymph nodes showed similar lectin binding profiles to splenic T-reg. However, T-reg from thymus and bone marrow had additional variances in lectin binding levels compared to T-conv from the same sites. In 5-day cultures of eFluor670®-labeled $CD4^+$ splenic cells stimulated with a low concentration of Concavalin A preferential proliferation of T-reg occurred and the proportion of GFP^+ cells increased from an average of 17% at baseline to 37% of the total cells.
Differential surface glycosylation profiles of T-reg and T-conv may reflect an inherently higher *in vivo* activation status of T-reg which varies across anatomical location.

P3.13.08
Immunological consequences of HIV co-infection with M. Leprae

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Brazil is one of the countries considered highly endemic for leprosy infection. Brazil also has the largest number of HIV infections in South America. Co-infection of *M. Tuberculosis* with HIV is known to impact the immunopathology of both pathogens. However, little is known about the impact of *M. leprae* on HIV-induced disease and vice versa. In this study, we assessed the immunological impact of HIV/*M. Leprae* co-infection in subjects in the state of Pará, Brazil. The study subjects were divided into four groups: healthy controls, HIV seropositive patients, patients with leprosy alone, and leprosy and HIV-1coinfected patients. We quantified T cell activation, regulatory T cells (T_{regs}) and assessed Th-17 cells. We found higher immune activation and negatively correlation between T_{regs} and activation markers in co-infected patients. An increase of T_{reg} cells in the periphery, and a loss of the Th17 to T_{reg} ratio in HIV. In conclusion, we believe these findings can be related to disease progression for HIV and Leprosy infection.

P3.13.09
PDCD5 negatively regulates autoimmunity by upregulating $FOXP3^+$ regulatory T cells and suppressing Th17 and Th1 responses

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Maintenance of FOXP3 protein expression is crucial for differentiation and maturation of regulatory T (Treg) cells, which play important roles in immune homeostasis and immune tolerance. Emerging evidence indicates that Histone acetyltransferase (HAT) Tip60 can enhance FOXP3 stability by upregulating its acetylation in regulatory T cells. We have previously demonstrated that programmed cell death 5 (PDCD5) interacts with Tip60, enhances its stability and HAT activity. In the present study, We demonstrate that PDCD5 interacts with FOXP3, increases acetylation of FOXP3 in synergy with Tip60 and enhances the repressive function of FOXP3. In PDCD5 transgenic (PDCD5tg) mice, overexpression of PDCD5 enhanced the level of FOXP3 protein and percentage of $CD4^+CD25^+FOXP3^+$ cells. Naïve $CD4^+$ T cells from PDCD5tg mice were more sensitive to TGF-β-induced Treg polarization and expansion. These induced Tregs retained normal suppressive function *in vitro*. We further investigated the role of PDCD5 in experimentally-induced autoimmune encephalomyelitis (EAE). Severity of EAE in PDCD5tg mice was significantly reduced relative to that of wild-type mice. The beneficial effect of PDCD5 likely resulted from increases of Treg cell frequency, accompanied by a switch from the predominant pathogenic Th17/Th1 response to the protective Th2-type immune activity. Activation-induced cell death enhanced by PDCD5 was also linked to this process. This is the first report revealing that PDCD5 activity in T cells suppresses autoimmunity by modulating Tregs. This work was supported by a grant from the National Key Basic Research Program of China (973, 2011CB910103) and the National Natural Science Foundation of China (30871263).

P3.13.10
The endocannabinoid 2-Arachidonoylglycerol potentiates Foxp3 expression and Regulatory T cell functions through its metabolism

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The CD4+CD25+ regulatory T cells (Tregs) are a unique population of T cells that maintain peripheral immune tolerance and inhibit autoreactive T cells. At least two subpopulations of Tregs exist, named naturally occurring Tregs (nTregs) and induced Tregs (iTregs). Several in vitro and in vivo models demonstrating the induction of iTregs from CD4+CD25- naive T cells by prolonged or repeated antigenic stimulation or by IL-10 or TGF-β support the possibility that Tregs can be generated in the periphery. Although the transcription factor Foxp3 is considered one of the specific markers of Tregs, the immunosuppressive mechanisms exerted by Tregs are not fully known. Given the recent evidences demonstrating the important role of the endocannabinoid system (ECS) in the modulation of several immune responses, we investigated for the first time whether endocannabinoids may interfere with Tregs induction and function. Herein, not only we show that nTregs express several members of the ECS (cannabinoid receptors and endocannabinoid-metabolizing enzymes), but also that the endocannabinoid 2-arachidonoylglycerol (2-AG) up-regulates Foxp3 and potentiates the regulatory phenotype of iTregs, in a mechanism which is independent by cannabinoid receptors. This 2-AG-induced effect in driving a “super-Treg” phenotype is blocked by selective COX-2 inhibition, suggesting that 2-AG could participate in the physiopathological regulation of iTregs induction and maintenance through one of the lipid intermediates derived from COX-2-induced 2-AG metabolism.

P3.13.11
Altered expression of miR-146a and miR-155 in Th2 and IgG expressing B cells in the autoimmune disease pemphigus foliaceus

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MicroRNAs are a class of small non-coding RNAs ranging from 18 to 25 nucleotides that regulate gene expression by complementary binding to mRNA sites. MicroRNAs have been increasingly implicated in normal immune function. Pemphigus foliaceus (PF) is a blistering autoimmune skin disease characterized by a Th2-dependet production of pathogenic IgG autoantibodies. We quantified by qRT-PCR six candidate microRNAs (miR-145, miR-146a, miR-148a, miR-155, miR-338-5p, and miR-1321) in three lymphocyte subpopulations ($CD4^+CD294^+$ Th2-like, $CD4^+C25^+$ T, and IgG expressing B cells [IgG^+ B cells]) from six healthy controls, six patients with inactive disease (iPF) and seven patients with active lesions (aPF). In Th2-like cells, miR-146a was 4-fold upregulated in patients with inactive disease compared to patients with active lesions (P=0.014), suggesting that the expression of this microRNA might be negatively related to disease activity. In IgG^+ B cells, miR-155 was 2-fold upregulated in patients with inactive disease compared to healthy controls (P=0.009) and exhibited a tendency for upregulation in these patients when compared to those with active lesions (P=0.082), allowing us to hypothesize that this microRNA might have a relevant role in PF remission. Moreover, miR-1321 revealed a tendency of upregulation in Th2-like cells of PF patients (iPF+aPF) compared to healthy controls (P=0.079). Expression of miR-1321 in these cells negatively correlated to that of its predicted target BLYS (BAFF), a B cell stimulator known to be important for PF pathogenesis (iPF, r_S=-1.00, P<0.05; aPF, r_S=-0.49, ns). In conclusion, miR-146a, miR-155 and miR-1321 arise as potentially new mediators of therapeutic intervention for PF.

P3.13.12
DX5+ CD4+ T cells modulate CD4+ T-cell response via inhibition of IL-12 production by DCs

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DX5+CD4+ T cells have been shown to dampen collagen-induced arthritis and delayedtype hypersensitivity reactions in mice. These cells are also potent modulators of T-helper cell responses through direct effects on CD4+ T cells in an IL-4 dependent manner. To further characterize this T-cell population, we studied their effect on DCs and the potential consequences on T-cell activation. Here, we show that mouse DX5+CD4+ T cells modulate DCs by robustly inhibiting IL-12 production. This modulation is IL-10 dependent and does not require cell contact. Furthermore, DX5+CD4+ T cells modulate the surface phenotype of LPS-matured DCs. DCs modulated by DX5+CD4+ T-cell supernatant express high levels of the co-inhibitor molecules PDL-1 and PDL-2. OVA-specific CD4+ T cells primed with DCs exposed to DX5+CD4+ T-cell supernatant produce less IFN-γ than CD4+ T cells primed by DCs exposed to either medium or DX5−CD4+ T-cell supernatant. The addition of IL-12 to the co-culture with DX5+ DCs restores IFN-γ production. When IL-10 present in the DX5+CD4+ T-cell supernatant is blocked, DCs re-establish their ability to produce IL-12 and to efficiently prime CD4+ T cells. These data show that DX5+CD4+ T cells can indirectly affect the outcome of the T-cell response by inducing DCs that have poor Th-1 stimulatory function.

P3.13.13
T regulatory cells in cancer

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Recent evidences show that anti-tumour immunity and cancer immunotherapy are negatively impacted by effects of T regulatory cells (Tregs). We and others showed that Tregs are expanded in peripheral blood and tumour microenvironment of cancer patients, which correlates with poor prognosis and reduced survival. Our recent work has further established that Treg infiltration of tumours is correlated with a lack of some patients' responsiveness to therapy. Additionally, we found that adoptive transfer of Treg-depleted autologous T cells in renal cell carcinoma (RCC) patients following conditioning chemotherapy provided a transient reduction of circulating Treg levels which was associated with improved antitumor immune response to the tumour-associated antigen 5T4.
There are two main populations of $CD4^+$ Tregs with different origins: thymus-derived natural Tregs (nTregs) and peripherally induced Tregs (iTregs). Conversion of conventional T cells into iTregs is proposed as a potential mechanism for Treg expansion in cancer. Discovery of markers that distinguish iTregs from the natural ones is of great importance and currently under intensive investigations; this might allow targeting iTregs in specific protocols. We found that the majority of peripheral and tumour-infiltrating $FoxP3^+$ Tregs expresses Helios, an Ikaros family transcription factor, and $FoxP3^+Helios^+$ Tregs possess more suppressive phenotype and characteristic, as compared to $FoxP3^+Helios^-$ Tregs. Despite the recent debate of the suitability of Helios as a marker of nTregs, Helios expression could be a potential target in cancer.
These observations suggest that Tregs are implicated in the immunopathology of cancer and their specific targeting may improve the efficacy of immunotherapeutic modalities.

P3.13.14
Regulatory T cells in acute lymphoblastic leukemia

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T regulatory cells have an important and specific role in malignant diseases such as acute lymphoblastic leukemia (ALL); these cells participate in a negative regulation of immunologic response, limiting the capacity to eliminate carcinogenic clones. Objective. The purpose of this study is to assess the percentage and function of T regulatory cells because it is suggested that function or expression increased in these cells in patients with ALL could be inhibiting an adequate anti-tumor response. Materials an Methods. We evaluated of percentage and function of T regulatory cells CD4+CD25+FOXP3+, CD4+CD39+FOXP3+ and CD4+CD25+CTLA-4+ in peripheral blood pediatric patient with acute lymphoblastic leukemia and healthy volunteers by Flow cytometry. Results. We observed significantly diminished CD4+CD25highCTLA-4+ cells levels and augmented FOXP3+ and CD39+ expression in CD4+ T cells in patients in LLA patients compared to healthy controls. Only three samples have been analyzed for the cell function of T regulatory cells, the remaining samples will be eventually evaluated Discussion. These results suggest LLA patient's exhibit an important activation of the immune response, possibly against carcinogenic clones and the absence of CD4+CD25highCTLA-4+ T regulatory cells could be contribute to the pathology observed in LLA patients.

P3.13.15
T Lymphocyte-mediated suppression of murine granulopoiesis by oral allergen pretreatment of sensitized/challenged mice

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Oral ovalbumin pre-exposure (oral OVA), also termed "oral tolerance induction", effectively prevents the subsequent allergic pulmonary inflammation in sensitized/challenged (S/C) mice, reducing lung eosinophil (Eos) infiltration. In OVA-S/C mice, bone-marrow (BM) eosinophilopoiesis is suppressed by the antihelminthic and antiallergic agent diethylcarbamazine, a 5-lipoxygenase inhibitor, through a proapoptotic mechanism requiring both inducible Nitric Oxide Synthase (iNOS) and CD95L, the ligand for death receptor CD95/Fas. The role of BM regulation by iNOS/CD95L in the effects of oral OVA, remains to be established. We examined oral OVA effects on granulopoiesis in S/C mice. BP-2, BALB/c and C57BL/6 wild-type (WT) mice, and BALB/c mutants lacking CD95L (*gld*), were orally given OVA in water, and subsequently S/C with OVA. Granulopoiesis was evaluated in liquid and semisolid BM cultures. Spleen T cells from oral OVA/S/C donors, or from Water/S/C controls, were transferred to syngeneic recipients, which were subsequently S/C with OVA before evaluation of BM eosinopoiesis. Oral OVA/S/C BP-2 mice had significantly decreased, relative to Water/S/C controls: a) Eos numbers in BM; b) IL-5-stimulated eosinophilopoiesis and GM-CSF-stimulated neutrophilopoiesis; c) GM-CSF-stimulated Eos colony formation; d) OVA-specific circulating IgE levels. Oral OVA was effective in BM CD95L-deficient mice. The effects of oral OVA on BM Eos numbers were duplicated by transfer of splenic T cells from oral OVA/S/C donors to syngeneic recipients, which were subsequently S/C, demonstrating a novel regulatory effect of oral OVA-induced T cells on granulopoiesis. **support:** CNPq-FAPERJ-CAPES

P3.13.16
Augmented levels of CD4+CD25high Treg cells in peripheral blood of patients with acute and chronic brucellosis

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Brucellosis remains one of the most common zoonotic diseases worldwide. In humans, Brucellosis can be a serious, debilitating and sometimes chronic disease. Different mechanisms can be postulated as to the basis for the induction of the chronic status of infectious diseases that T regulatory cells are one of the most important related mechanisms. For the first time, the current study was designed to determine whether percentage of CD4+CD25+ Treg cells in peripheral blood is changed in human Brucellosis samples (Acute form:n=16, Chronic form: n=10) in comparison to control group(n=15). In brief, Heparinized venous blood was obtained from both patients and healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque density gradient centrifugation. For surface staining, cells were incubated with the respective mAbs for CD4, CD25 and finally $CD4+CD25^{high}$Treg cells are evaluated by FACS. The results have revealed a new finding in relation to Treg cells and human Brucellosis. Our study indicates that the number of $CD4^{+}CD25^{high}$Treg increases in the peripheral blood of acute and chronic forms of Brucellosis samples compared with healthy groups and this increase in chronic group is further. $CD4^{+}CD25^{+}$ regulatory T cells (Treg) play a central role in the prevention of autoimmunity and in the control of immune responses to transplants, allergens, tumors, and infectious microbes by down-regulating the function of effector $CD4^{+}$ or $CD8^{+}$ T cells. In conclusion, a correlation seems to exist between increased $CD4+CD25^{high}$Treg cells and disease progression from healthy state to acute and chronic brucellosis.

P3.13.17
Association of circulating regulatory T cell number with the incidence and prognosis of diffuse large B cell lymphoma

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Regulatory T (Treg) cells are essential for maintaining immune tolerance. High Treg frequencies have been reported in peripheral blood and tissue samples of patients with solid tumors while their role in lymhomas, including diffuse large B cell lymphoma (DLBCL) has not been clearly established. In this study we analyzed the circulating Treg numbers in 27 patients with newly diagnosed DLBCL and 17 healthy individuals. Tregs were detected by flow cytometry based on CD4+CD25highFoxP3+ phenotype. In addition, the expression of CD45RA, HLA-DR, CD62L, CD39, and CTLA4 was analyzed. The number of circulating Treg cells was lower in DLBCL patients than in healthy controls: median 23 (range, 4-107)/μL versus 41 (19-104)/μL ($p=0.04$). In particular, the number of Tregs expressing CD45RA (naïve Tregs), HLA-DR (marker of activation) and CD62L (L-selectin) was decreased in the DLBCL group. Lower (below median) number of circulating Tregs was associated with reduced chance of achieving complete remission (29% versus 69%, $p=0.05$) and reduced probability of even-free survival (24% versus 84% at one year, $p=0.0004$), independently on the International Prognostic Index. We conclude that low number of circulating Tregs may be associated with susceptibility to DLBCL incidence and poor prognosis. Our observations require confirmation in larger patient population.

P3.13.18

Maintenance of memory regulatory T cells in peripheral tissues

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The skin has unique immunological characteristics and is especially plagued by T cell mediated inflammatory disorders. To study CD4+ T cell responses to epidermal self-antigen, which are largely undefined, we established a mouse model that features tetracycline-inducible expression of chicken ovalbumin (Ova) in the epidermis under the control of the keratin K5 promoter. Expression of antigen in the skin elicits a T cell (DO11.10) dependent inflammatory dermatitis, which is associated with IFN-γ and IL-17 production by DO11 T cells. This disease develops despite the presence of high starting numbers of natural DO11 Foxp3-expressing regulatory T cells (Tregs), and, in fact, these Tregs proliferate in response to skin-Ag recognition. Treg are activated by peripheral self-antigen to increase their suppressive function, and a fraction of these cells survive as memory regulatory T cells (mTregs). mTregs persist in nonlymphoid tissue after cessation of Ag expression and have enhanced capacity to suppress tissue-specific autoimmunity. These mTregs express specific effector memory T cell markers and localize preferentially to hair follicles in skin. Memory Tregs express high levels of both IL-2Rα and IL-7Rα. Using a genetic-deletion approach, we show that IL-2 is required to generate mTregs from naive CD4+ T cell precursors in vivo. However, IL-2 is not required to maintain these cells in the skin and skin-draining lymph nodes. Conversely, IL-7 is essential for maintaining mTregs in skin in the steady state. These results elucidate the fundamental biology of mTregs and show that IL-7 plays an important role in their survival in skin.

P3.13.19
Beta2 adrenergic receptor signaling in $CD4^+$ $Foxp3^+$ regulatory T cells enhances their suppressive function in vitro

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Beta2 adrenergic receptor (B2AR) signaling is known to impair Th1-cell differentiation and function in a cAMP-dependent way. $Foxp3^+CD4^+$ regulatory T (Treg) cells play a key role in the regulation of immune responses and are essential for maintenance of self-tolerance. Nevertheless, very little is known about adrenergic receptor expression in Treg cells or the influence of noradrenaline on their function. We showed by qPCR, immunofluorescence and western blot that sorted $Foxp3^+$ Treg cells express B2AR. B2AR activation by specific agonist in Treg cells leads to increased intracellular cAMP levels and to PKA-dependent
CREB phosphorylation. For suppression assays, sorted naïve T cells and Treg cells were cultured (proportion 1:0,25) with irradiated splenocytes and soluble anti-CD3. Before setting up the cultures, Treg cells were treated or not with B2AR agonist. After 3 days, naïve T cell proliferation was analyzed by flow cytometry. We found that B2AR signaling enhances the suppressive activity of Treg cells since naïve T cell proliferation was about 40% lower than the one observed in control cultures. B2AR-mediated increase in Treg-cell suppressive function was associated with decreased IL-2 mRNA levels in responder $CD4^+$ T cells and improved Treg-cell-induced conversion of $CD4^+$ $Foxp3^-$ cells into $Foxp3^+$ iTreg cells. Moreover, B2AR signaling increased CTLA-4 expression in Treg cells in a PKA-dependent way. Finally, we found that PKA inhibition totally prevented the B2AR-mediated increase in Treg-cell suppressive function. Our data suggest that sympathetic fibers are able to regulate Treg-cell suppressive activity in a positive manner through B2AR signaling.
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P3.13.20
Role of cutatneous regulatory T cells in regulation of immune homeostasis and contact hypersensitivity reaction in the skin

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There is accumulating evidence that $CD4^+Foxp3^+$ regulatory T (Treg) cells actively suppress pathological and physiological immune responses, thereby contributing to the maintenance of immunological self-tolerance and immune homeostasis and to suppressive control of a broad spectrum of immune responses. We and others have previously shown that there are a substantial number of Treg cells in the skin even in a steady state. However, it remains unclear whether Treg cells in non-immunized skin contribute to the maintenance of immune homeostasis and the suppression of antigen-specific immune responses, e.g., contact hypersensitivity (CHS). To address this issue, we investigated whether the selective elimination of cutaneous Treg cells would lead to the dysregulation of immune homeostasis in the skin and exacerbate CHS reaction. Intradermal injection of an immunotoxin targeting human CD2 (hCD2) into ear skin of $Foxp3^{hCD2}$ mice, in which Treg cells specifically express hCD2 on their cell surface, markedly reduced Treg cells only in the treated skin, but neither in other organs nor in the blood. Selective depletion of Treg cells in the skin led to the spontaneous development of inflammation in the treated skin. In addition, CHS reaction elicited at the Treg cell-depleted ear was exacerbated and prolonged, accompanied by increase of immune cell invasion and overproduction of Th1- and Th2-related cytokines. These results indicate that Treg cells existing in the skin are essential for the proper regulation of immune homeostasis and antigen-specific immune response in the skin.

P3.13.21
Genome-wide DNA methylation profiling identifies hypomethylated genes with the Forkhead-binding motif in human regulatory T cells

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Regulatory T cells (Tregs) prevent the emergence of autoimmune disease. Prototypic natural Treg (nTreg) are programmed by the transcription factor Forkhead-box P3 (FOXP3) and can be identified by demethylation at the FOXP3 locus. To further explore the methylation landscape of nTregs we performed genome-wide methylation profiling of human resting nTregs (rTreg) and conventional naïve CD4+ T cells (Naïve). We detected 2,315 differentially methylated CpGs between these two cell types, many of which clustered into 127 regions of differential methylation (RDM). T cell activation induced changes in 466 individual CpGs and 18 RDMs in Naïve but did not alter DNA methylation in rTreg. Gene set testing of these 127 RDMs revealed enrichment for common Treg signature genes, genes that bind FOXP3 and genes upregulated by FOXP3, predominantly in hypomethylated RDMs. A putative Forkhead-binding motif was overrepresented in promoter-associated RDMs, suggesting that methylation regulates gene expression by influencing FOXP3 binding. To exemplify this we showed that TIGIT, coding for an immune suppressive receptor, was hypomethylated and upregulated in Treg, but its expression was reduced in individuals at risk for autoimmune type 1 diabetes. These findings provide new insight into epigenetic regulation of human nTreg and the potential to exploit differential methylation as an immune biomarker in human diseases.

P3.13.22
Identification of PLAU as a critical gene for the suppressive function of memory-like CD4+ regulatory T cells

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The plasminogen activator urokinase (*PLAU*) is known for its role in fibrinolysis and late-onset Alzheimer diseases. However, we here demonstrate that *PLAU* is also important for the suppressor function of both human and murine CD4+ regulatory T cells (Tregs). The prediction of PLAU as an important gene is made through a systems biological network strategy directly from an undirected Treg-specific correlation network which we reconstruct from a high-time-resolution transcriptome during the very early activation process of human Tregs/$CD4^+$ T-effector cells (Teffs). The validation results show that the membrane-bound PLAU regulates the expression of several known Treg key genes, e.g., FOXP3 and EOS. Moreover, our analysis unveils that *PLAU* is particularly important for memory-like $CD44^{high}CD62L^{low}$ but not $CD44^{low}CD62L^{high}$ Tregs. Detailed mechanistic investigation shows that PLAU mediates Treg suppressor function via STAT5 and ERK signaling pathways but not via PLAU-PLAUR interaction between Treg and Teffs. Further analysis also shows that the development of the thymic $Cd4^+Cd25^+Foxp3^+$ Tregs was significantly enhanced in $Plau^{-/-}$mice, which might compensate the impaired suppressive function of memory-like Tregs and consequently not generate spontaneous autoimmune disorders. Our study highlights not only the power of the proposed network strategy but also a critical role for *PLAU* in Treg suppressor function.

P3.13.23
CD2-mediated regulation of peripheral CD4+CD25+ regulatory T cell apoptosis accompanied by downregulation of Bim

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Extensive studies on CD4+CD25+ regulatory T (Treg) cells suggest they are important in regulating immune responses. However, mechanisms of peripheral Treg cell homeostasis are unknown. We found that stromal cells isolated from secondary lymphoid organs such as spleen and lymph nodes could support the survival of Treg cells. This was dependent on CD2 engagement and a direct interaction between Treg cells and stromal cells. In the presence of stromal cells, Bim, a pro-apoptotic factor, was partially decreased in Treg cells. This effect could be inhibited by anti-CD2 blocking antibodies, indicating that stimulation through CD2 on Treg cells regulates Bim expression, which may be relevant to Treg cell apoptosis. Therefore, Treg cell interactions with stromal cells through CD2 may be essential for Treg cell survival. Surprisingly, the expression of CD2 ligands on stromal cells was not detected. Thus, it is not clear how CD2 on Treg cells contributes to a direct interaction with the stromal cells and participates in survival support for Treg cells. Taken together, CD2 stimuli were mandatory for Treg cell survival with reduced Bim expression, but CD2 may not function as a direct receptor for molecules on stromal cells.

P3.13.24
Diurnal changes in trafficking receptor expression on thymus-deriving natural regulatory T cells

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Natural $CD4^+CD25^+Foxp3^+$ regulatory T (nTreg) cells play a crucial role in the maintenance of self-tolerance and immune homeostasis. nTregs develop in the thymus and migrate to the periphery as a mature population of T lymphocytes. nTregs can suppress activation, proliferation and effector functions of various immune cells. Currently, the mechanisms regulating nTreg development, migration to peripheral lymphoid organs and suppressive function are still explored. As we have shown in our previous study various aspects of nTreg biology are controlled by circadian rhythms. Here we demonstrate that the expression of trafficking receptors show diurnal rhythmicity suggesting the involvement of hormonal regulation in nTreg migration. The aim of the study was to analyse the expression of CD62L and CCR9 on thymus-deriving nTregs of C57BL/6 mice in time intervals correlated with the circadian rhythm of endogenous glucocorticoids synthesis. CD62L direct nTregs to secondary lymphoid tissue and CCR9 to non-lymphoid tissue. We also analysed the expression of these markers after treatment of mice with the synthetic glucocorticoid, dexamethasone. C57BL/6 mice were injected i.p. with Dex at the dose of 1,25mg/kg/day. The analysis of nTreg markers expression was synchronized with the circadian rhythm of glucocorticoids synthesis. The results of the first part our studies in brief indicate that after administration of dexamethasone 1/ the differences in trafficking receptor expression were gender-dependent 2/ trafficking receptors expression on nTregs reveal diurnal rhythmicity, which was in accordance to circadian rhythm of glucocorticoids synthesis.
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P3.13.25
Dexamethasone dose-dependent effect on nTreg generation in vitro in thymocytes co-cultures with JAWSII cells

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Endogenous glucocorticoids have a broad impact on immune cells development and function because of their immunosuppressive activity, ability to induce apoptosis and modulate the maturation of antigen-presenting cells. In our research we have examined the effect of dexamethasone, a synthetic glucocorticoid, on thymocyte development *in vitro*. We have created an *in vitro* model promoting CD4+CD25+Foxp3+ thymocyte development using anti-CD3 antibodies and JAWSII immature dendritic cells as the sources of activatory signals. In experiments we have used thymocytes derived from C.Cg-*Foxp3*^{tm2Tch}/J mice. The aim of the study was to establish conditions for maintaining the appropriate level of JAWSII cells activation basing on the expression of their costimulatory molecules and MHCII. Moreover, using this model we have examined the effect of dexamethasone (in range of concentrations 10^{-12} - 10^{-6} M) onto: JAWSII cell line viability and costimulatory markers expression, viability of thymocytes co-cultured with JAWSII cells, distribution of major thymocytes subsets and CD4+CD25+Foxp3+ cells generation *in vitro*. Our research indicate that dexamethasone used at low concentrations, which do not induce apoptosis of thymocytes and maintain a weak expression of MHCII and costimulatory molecules on JAWSII cells, may play a role of a selection factor for nTreg generation in vitro.
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P3.13.26
Donor-specific regulatory t cells generated on donor b cells are superior to CD4+CD25high cells in controlling alloimmune responses in humanized mice

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Regulatory T cells have been proposed as important players in controlling donor reactive immune responses after transplantation. Several clinical trials had evaluated their safety in the treatment of GVHD after hematopoietic stem cell transplantation. As opposed to this lymphopenic setting a variety of animal studies had shown, that the impact of polyclonal Tregs in organ transplantation is small. Rather, the antigen-specificity of Tregs plays a major role, since

transplant tolerance is - as shown in animals models - related to donor-specific regulatory T cells. Direct comparison of polyclonal with alloreactive Tregs revealed the solely effectiveness of antigen-specific Tregs in respect to the inhibition of graft rejections.
Yet, due to the lack of a specific alloreactive Treg marker currently polyspecific CD4+CD25high Tregs are being tested in clinical trials in autoimmunity and transplantation. For the generation of donor-specific Tregs donor material is usually limited. Here we developed a method to generate large quantities of activated donor B cells by stimulation of donor PBMCs with 3T3 fibroblast expressing CD40L. These activated donor B cells were potent stimulators of CD4+CD25 high Tregs, which could be efficiently expanded. These donor reactive Tregs inhibited an allo-MLR in a donor-specific way and were far more potent in inhibiting an alloimmune response in humanized mice compared to the current gold standard of polyspecific CD4+CD25high Tregs. The generation of donor-specific Tregs could all be performed under GMP conditions. Donor-reactive Tregs could be a valuable tool to control immune responses after transplantation where polyspecific Tregs have failed so far.

P3.13.27
TIP60 Positively Regulates ThPOK-Mediated Repression of Eomesodermin in Human CD4+ T Cells

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The abundant expression of IFNγ in ThPOK-deficient CD4+ T cells requires the activation of Eomesodermin (Eomes); however, the underlying mechanism of this phenomenon remains unclear. Here we report that ThPOK directly binds to the promoter region of the Eomes gene to repress its expression in CD4+ T cells. We identified the histone acetyltransferase TIP60 as a corepressor of ThPOK-target genes, where ectopically expressed TIP60 increased ThPOK protein stability by promoting its acetylation at its K360 residue to then augment the transcriptional repression of Eomes. Moreover, knockdown of endogenous TIP60 abolished the stabilization of ThPOK in CD4+ T cells, which led to the transcriptional activation of Eomes and increased production of IFNγ. Our results reveal a novel pathway by which TIP60 and ThPOK synergistically suppresses Eomes function and IFNγ production, which could contribute to the regulation of inflammation.

P3.13.28
The effects of 5-azacytidine on the enhancement of CD4+CD25highFOXP3+ T cells from the cultures of human CD4+CD25- T cells

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FOXP3 is a master transcription factor for regulatory T (Treg) cells. Stable FOXP3 expression is required for the maintenance of Treg cell function. Epigenetic regulation of FOXP3 expression has been proposed to be important for the development and suppressive function of Treg cells. However, the induction of human Treg cells through epigenetic manipulation has not been demonstrated. We found that human $CD4^+CD25^-$ T cells partially differentiated into $CD4^+CD25^{high}FOXP3^+$ cells upon suboptimal stimulation. The addition of 5-azacytidine (AzaC), a DNA methyltransferase inhibitor, alone enhanced this $CD4^+CD25^{high}FOXP3^+$ T cell population. The production of interleukin (IL)-2 was elicited as activating $CD4^+CD25^-$ T cells treated with AzaC. However, similar level of transforming growth factor (TGF)-β and reduced IL-10 was detected. By examining FOXP3 expression in AzaC-treated cells with neutralizing anti-IL-2 antibody, the increased IL-2 was important for enhancing FOXP3 expression. Furthermore, AzaC-treated $CD4^+CD25^{high}$ T cells expressed higher FOXP3, CTLA-4 and GITR at the comparable level to natural Treg cells. Unlike TGF-β-induced $FOXP3^+$ T cells that were indicated to lack regulatory function, AzaC-treated $CD4^+CD25^{high}$ T cells potently suppressed the proliferation of allogeneic $CD4^+$ T cells. The suppression was cell contact-dependent. Together, the results have significant implication for using epigenetic strategy to enhance functional Treg cells for clinical application.

P3.13.29
Ctirical roles of the transcriptional regulator IκB-ζ in regulatory T cells

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The transcriptional regulator IκB-ζ is a nuclear IκB protein harboring ankyrin-repeats that interact with NF-κB. It is robustly induced in macrophages upon stimulation with lipopolysaccharide and plays an essential role in expression of the secondary response genes represented by interleukin (IL)-6. Previous studies have shown that IκB-ζ is also induced in T cells in response to transforming growth factor (TGF)-β + IL-6 and cooperates with the transcriptional factor RORγt to express IL-17. Accordingly, IκB-ζ-deficient mice are resistant to experimental autoimmune encephalomyelitis. Here we show that IκB-ζ plays critical roles in the immune homeostasis through regulatory T cells (Tregs). T cell-specific IκB-ζ-deficient mice exhibited reduced numbers of Tregs and, along with aging, developed splenomegary and lymphocytic infiltration into various tissues including the lung and saliva along. It was found that stimulation of T cells with TGF-β alone up-regulated IκB-ζ expression and that co-expression of the NF-κB subunit p65 and IκB-ζ synergistically activated the Foxp3 promoter. In fact, IκB-ζ-deficient T cells exhibited impaired generation of $Foxp3^+$ Tregs in response to TGF-β *in vitro*. In addition, adoptive transfer of IκB-ζ-deficient Tregs, but not the wild-type Tregs, failed to prevent colitis induced by $CD4^+CD25^-CD45RB^{hi}$ T cells. These results indicate crucial roles of IκB-ζ in generation and effector functions of Tregs.
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P3.13.30
Differential sialylation dictates the fate of regulatory T cells

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Galectin-1 (Gal-1), an endogenous lectin found at sites of inflammation and tumor growth, plays key roles in immune tolerance and homeostasis. This lectin specifically interacts with lactosamine-enriched N-glycans and core-2-O-glycans on cell surface glycoproteins. Here we investigated the impact of Gal1-glycan interactions in the physiology of regulatory T (Treg) cells. Using a panel of plant lectins, we analyzed the glycosylation profile of naturally-occurring Treg cells (nTregs) isolated from the spleen of C57BL/6 mice. Glycophenotypic analysis revealed higher frequency of asialo-core-1-O-glycans and complex N-glycans with terminal a2-3-linked sialic acid (SA) on LacNAc residues on nTregs. This effect was accompanied by the presence of large amounts of a2-6-linked sialic acid (SAa2-6), a glyco-epitope that is restrictive for Gal1 binding in both nTregs and inducible Tregs (iTregs), versus to Tact cells. Similar results were obtained using lectin binding assays and mass spectrometric analysis. Notably, iTregs exhibited lower capacity to bind Gal1 compared to Tact cells ($p<0,01$), and increased expression of the a-2,6-sialyltransferase 1 (ST6Gal1), an enzyme responsible of incorporating SAa2-6 residues. When naive CD4+T cells were differentiated into iTregs, binding of Gal1 decreased and SAα-2,6 increased in a time-dependent manner. In an in vivo model, effector T cells from mice immunized with ovalbumin (OVA) exhibited a higher capacity to bind Gal1 as compared to iTregs ($p<0,01$). Moreover, iTregs were considerably more resistant to Gal1-induced cell death than Th17 and Tact cells. Our results uncover a glycosylation-dependent mechanism which selectively dictates the fate of Treg cells.

P3.13.31

Thymic precursors of T regulatory cells are resistant to caspase/granzyme B mediated degradation of Linker for activation of T cells.

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Partial proteolysis of T cell receptor (TCR) signalosome plays a role in modulating the strength and duration of signaling. Using a newly developed method for assessment of LAT proteolytic degradation, we provided evidence that thymic T regulatory cell precursors (tT-regs) are resistant to LAT degradation upon TCR cross linking. We found that this resistance is partially due to the defect in Caspase 8 activation upon TCR engagement. In line with this finding we also observed that other extrinsic pro apoptotic signals delivered to thymocytes (i.e. Fas ligation or dexamethasone treatment) are unable to activate Caspase 8 selectively in tT-regs. In order to gain a better insight into mechanisms responsible for this phenomenon we evaluated the expression of several molecules that may counteract Caspase 8 activation or its external membrane targeting in tT-regs. Our data may shed light on mechanisms responsible for tT-reg resistance to the strong TCR mediated signals otherwise leading to negative selection of conventional, FoxP3 negative, thymocytes.

P3.13.32
Effect of gestational age and mode of delivery on CD4+ T cells and regulatory T cells (Treg) in neonatal cord blood

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Purpose: Regulatory T cells (Treg), a subset of CD4+ T cells expressing FoxP3 and CD25, comprise 1-3% of CD4+ T cells in adult peripheral blood and have been shown to suppress immune responses. The aim is to determine the effect of gestational age and mode of birth on Tregs in cord blood of human newborns.
Methods: Cord blood of 200 newborns was collected between June 2012 and January 2013 and mononuclear cells (CBMCs) were analyzed using four-color flow cytometry. Tregs (CD3+, CD4+, CD25high, FoxP3high) were identified, and FoxP3 mean fluorescence intensity (MFI) measured. Suppressive index (SI) was calculated as MFI of FoxP3/Treg for individual Treg suppressive capacity.
Results: CD4+ percentage of CBMCs was 19±9% at 23-32 weeks gestational age (n=14), 12±10% between 33-36 weeks (n=35) and 12±9% at term (n=72) (p=0.03). Tregs remained at 11±5% until completion of gestation, then diminished to 7±5% (p<0.001). SI increased over 10-fold during gestation (55±10635 at 23-32 weeks, 111±11863 at 33-36 weeks, 1679±12963 at term, p=0.7). Mode of delivery did not affect Treg levels (vaginal delivery (n=30): 6±4% vs. caesarean section (n=42): 8±6%; p=0.13).
Conclusions: CD4+ T cells in cord blood decrease significantly at mid-third trimester. Tregs are significantly lower in term newborns but had higher suppressive activity. Gestational age and mode of birth effects on Treg suppressive activity may have implications for pre-term infants who cannot suppress an antigen-specific response to newly acquired gut flora or intestinal epithelium, potentially leading to symptoms of necrotizing enterocolitis.

P3.13.33
Adenosine production through CD39 and CD73 mediates development of sepsis-induced immunossupression via A2a receptor

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Introduction and Aim: Sepsis is regularly accompanied by the development of a pronounced immunosuppression, which is characterized by expansion of regulatory T cells (Treg cells), reduced proliferation and function of effector T cells and increased susceptibility to secondary infection. Adenosine is a purine nucleoside that has been implicated in the modulation the immune system. Here, we investigate the role of ectonucleotidases (CD39 and CD73) and adenosine in the establishment of sepsis-induced immunosuppression. **Methods and Results:** C57BL/6 mice were subjected to severe sepsis induced by cecal ligation and puncture and treated with antibiotic (ertapenem), resulting in 50% of survival. At 15th day after CLP, sepsis-surviving mice showed an enhanced number of Treg cells in spleen and impaired T cell proliferative response, which was associated with high susceptibility to secondary infection induced by *L. pneumophila*. Notably, sepsis-surviving mice treated with CD39 inhibitor (ARL671516) and CD73 inhibitor [adenosine 5΄-(α,β-methylene)diphosphate] had reduced number of Treg cells, improved of T cells proliferative response, low bacterial load in lungs and spleen and improved survival to secondary infection compared to control mice. Moreover, sepsis-surviving mice treated with A2aR antagonist [8-(3-chloro-styryl)caffeine] also showed reduced number of Treg cells and improved of T cells proliferative response, which was associated with reduced levels of bacteria in lungs and spleen and an improved survival after secondary infection. **Conclusion:** Our studies demonstrate that extracellular production of adenosine by ectonucleotidases orchestrates the establishment of sepsis-induced immunosuppression. **Financial support:** FAPESP.

P3.13.34
CD4+ regulatory T lymphocytes in preeclamptic women and in their newborns: preliminary results

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Objective: Our aim was to study the proportion of regulatory T cells in preeclamptic women and in their newborns.
Methods: The CD4+CD25+high T-reg cells, and hormone profile were analyzed in 23 women with preeclampsia (PE) and in 14 newborns. Also, fourteen healthy women (HC) and 5 newborns were included as controls.
Results: The T-regs cells proportion was 2.93% in PE and vs. 5.54% in HC (p = 0.031). The CD4+CD25+high cells were in PE 3.71% vs. HC 7.41% in HC, (p= 0.020); Total IgG level was 847 in PE vs. 955 in HC (p= 0.117). In the umbilical cord blood samples we found 4.56% in T regs cells in PE vs.8.54% in HC (p = 0.201). While gamma-delta T-cells were 3.61 in PE vs. 20.87% in HC, (p= 0.063). Interestingly, CD4+CD69+ cells were 10.03% in PE and 28.22% in HC (p=0.190).
Conclusions: We found decreased proportion of T-regs cells in preeclamptic women as previously have been reported. Also we observed a tendency of decreasing levels of IgG in women with PE, in relation to the hypothesis of the increased of immunologic response mediated by T-cells. Regarding, hormone profiles we found no statistical significance. In the newborns we found a tendency of decreased T-regs cells, gamma-delta T-cells, and a low expression of CD69CD4 cells, an important early activation marker expressed by lymphocytes. Nevertheless, the number of patients included in this study is still small, but we can suggest that PE has a repercussion in the immunological cell profile of their newborns.

P3.13.35
The influence of regulatory T cells on the response to the measles and DTP vaccines in Gambian infants

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Despite the key role of Regulatory T cells (Tregs) in regulating immune responses to infections, very few studies have reported on their role in controlling the immunogenicity of vaccines in infants. It is still not clear if pre-existing Tregs interfere with the generation of

vaccine-induced immunity, and whether they are induced following vaccination in infants.
Using a dataset from a randomized trial of 9 month-old infants vaccinated with either measles vaccine (MV) alone, MV + DTwP (diptheria, tetanus, whole cell pertussis combined vaccine) or DTwP alone; Treg frequencies were examined overtime. The influence of the baseline Tregs on the measles antibody response, response to DTP antigens; and the production of both inhibitory and pro-inflammatory cytokines following overnight stimulation with vaccine specific/non-specific antigens were evaluated.
We observed a weak negative correlation between circulating Tregs and measles antibody levels ($r = -0.2077$; $p=0.0482$); but not with response to the DTP antigens. Following overnight stimulation with the TT antigen, there was a weak yet significant inverse correlation between the Treg frequencies and production of IL-4, IL10 and TNFα ($p<0.001$). We also showed that there was a significant decline in Treg frequencies in all donors by 19 months of age ($p<0.001$).
These data suggest that Tregs may have a role to play in controlling the immunogenicity of vaccines in infants. The influence of pre-existing Tregs on the MV response may differ from that of DTP. These findings provide useful background data for further studies looking to provide strategies to improve vaccine immunogenicity in infants.

P3.13.36
Homeostatic competition of regulatory T cells in the peripheral compartment of syngeneic and semi-allogeneic hosts

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The $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) population is kept in stable frequencies in the secondary lymphoid organs for the majority of lifetime, despite permanent thymic emigration or in face of thymic involution. A continuous competition is expected, thus, among recently thymus-emigrated and resident Treg cells (either natural or post-thymically-induced). In the present work, we analyzed the kinetics of Treg and non-Treg cell renewal, using a protocol of successive syngeneic or semi-allogeneic T cell transfers into athymic hosts as a model to understand the physiological competition between newly thymus emigrated T cells and resident T cells in the periphery. Our results showed a preferential incorporation of Treg cells into the previously colonized environment, in comparison to conventional $CD4^+$ T cells (non-Treg). In the semi-allogeneic condition, the Treg turnover kinetics was even more expressive, suggesting that a different density of MHC-peptides complexes in the periphery may affect the level of Treg cell colonization. In the syngeneic hosts, although the first-transferred population did not attain numbers seen in euthymic hosts, the entry of subsequently transferred Treg cells was apparently impaired. These findings are in accordance with a permanent evolution of Treg cell repertoire throughout life and suggest that Treg renewal is linked to the diversity of TCR repertoire. To know the rules determining Treg cell survival versus replacement in the periphery is crucial for the understanding of their repertoire evolution and for the envisaged use of Treg cell transfer as a therapy to restore tolerance to self-antigens in autoimmune diseases.

P3.13.37
Regulatory T cells are preferentially preserved over non-Tregs within neonatal-derived T cells present in adult mice

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Regulatory T cells (Treg), essential to control self/nonself responses, were also associated with neonatal acquired tolerance. Peculiar features of the neonatal lymphoid compartment might influence the substitution kinetics of the thymic-derived Treg population, affecting the maintenance of self-tolerance acquired in the neonatal period and the preservation of Treg repertoire diversity. To evaluate Treg and non-Treg cell peripheral homeostasis at different ages, athymic mice (Thy1.2+) were grafted with colonized thymic lobes from either neonatal or adult congenic donors (Thy1.1+) and the frequencies of donor- and host-derived T cells were followed by FACS. Neonatal-derived T cells were widely replaced by host-derived lymphocytes after the second month, while a substantial proportion (>30%) of donor T cells remained in the adult thymus-grafted hosts. Donor- and host-derived T cells contained similar proportions of Tregs in mice grafted with adult thymus. In contrast, the residual fraction of Thy1.1 neonatal-derived T cells contained a two-fold enrichment in Tregs when compared to the Thy1.2 host-derived T cells, and persisted for at least ten months. We obtained similar data using a co-transfer model of neonatal and adult T cell suspensions into athymic mice. Our results suggest that when facing adult competitors, an event expected during ontogeny, resident neonate-derived T lymphocytes are mostly replaced, but a Treg-enriched cohort is long-term preserved among the reminiscent cells. A higher affinity for peripheral self-peptides, in comparison to non-Treg cells, may promote this preferential long-term survival of neonatal Treg cells, which can be important for maintenance of self-tolerance memory.

P3.13.38
Identification of an endogenous subpopulation of regulatory CD8+ T cells specific for a tissue-restricted tumor associated antigen in tumor-bearing TRAMP mice

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The immune system exerts a dual host-protective and tumor-promoting action in cancer patients and, in this scenario, prostate cancer (PC) has been largely studied for defining new immunotherapeutic strategies that also target immunosuppressive mechanisms. Regulatory CD8+ T cells have been reported in PC patients but their real role in cancer progression still remains elusive. Here we provide evidences that endogenous low avidity CD8+ regulatory T cells from the spleen of tumor-bearing transgenic adenocarcinoma of the mouse prostate (TRAMP) mice are responsible for the establishment of specific tolerance against Tag, which in this model behaves like a tissue-restricted tumor associated antigen. In vitro, splenic CD8+ T cells from tumor-bearing TRAMP and not from age-matched wild-type males or healthy TRAMP mice inhibited priming of T cells by altering the antigen presenting capacity of matured dendritic cells. When adoptive transferred into wild-type recipients, only splenic CD8+ T cells from tumor-bearing TRAMP mice significantly impaired the Tag-specific CTL response induced by vaccination with dendritic cells pulsed with Tag. Moreover, this effect appeared to be antigen-specific, since these cells did not weaken the immune response induced by vaccination against the exogenous antigen ovalbumin. The CD8-mediated suppression directly correlated with Tag overexpression and tumor progression in TRAMP mice. All together, our data confirm and extend previous findings showing that indeed, an endogenous population of regulatory CD8+ T cells exists in tumor-bearing TRAMP mice that may contribute to tumor-associated tolerance in this model. A further characterization of this population will likely identify novel therapeutic targets for PC.

P3.13.39
CD39 is highly involved in mediating the suppressive activity of tumor-infiltrating CD8+ T regulatory lymphocytes

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CD39 is an ectoenzyme, present on different immune cell subsets, which mediates immunosuppressive functions catalyzing ATP degradation. It is not known whether CD39 is expressed and implicated in the activity of CD8+ regulatory T lymphocytes (Treg). In this study, CD39 expression and function was analyzed in both CD8+ and $CD4+CD25^{hi}$ Treg from the peripheral blood of healthy donors as well as from tumor specimens. CD39 was found expressed by both

CD8+ (from the majority of healthy donors and tumor patients) and CD4+CD25^{hi} Treg, and CD39 expression correlated with suppression activity mediated by CD8+ Treg. Importantly, CD39 counteraction remarkably inhibited the suppression activity of CD8+ Treg (both from peripheral blood and tumor microenvironment) suggesting that CD39-mediated inhibition constitutes a prevalent hallmark of their function. Collectively, these findings, unveiling a new mechanism of action for CD8+ Treg, provide new knowledge on intratumoral molecular pathways related to tumor immune escape, which could be exploited in the future for designing new biological tools for anticancer immune intervention.

P3.13.40
The hunt for a natural regulatory T cell ligand

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Natural T regulatory cells (nTregs) develop in the thymus along with conventional T cells (Tcons) in a similar fashion. While the exact cues for nTreg cell differentiation in the thymus is still a topic of rigorous study, it is widely accepted that TCR signal strength during positive selection is an important determinant. Various data provide evidence that nTreg TCR signal strength against self-peptide MHC falls someplace in between the level at which Tcon TCRs just pass positive selection and the level at which cells are deleted due to negative selection. This is reflective of the fact that mature Tregs are reactive to self antigens while mature Tcons are ideally reactive to foreign antigens in the context of MHC. A useful tool to help understand nTreg differentiation and function would be to find a natural antigen for nTregs; however, finding a natural antigen has proven elusive. Here we propose a technique to search for a natural Treg antigen by making nTreg TCRs “more” autoreactive followed by screening of a peptide library.

P3.13.41
CD8+ regulatory T cells recognize donor MHC class II-derived immunomodulatory epitopes in an allotransplantation model

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We recently reported that in a rat MHC mismatched heart allograft model, treatment with CD40Ig leads to indefinite allograft survival mediated by CD8+CD45RClow Tregs. Although essential, the exact role of TCR/MHC/peptide interaction in Treg activity is still unknown. We therefore studied their recognition properties by characterizing the allogeneic peptide(s) recognized.
Allogeneic peptides were derived from polymorphic regions of donor MHC molecules (RT1.A, RT1.B, RT1.D). 82 overlapping peptides of 16 aa were tested in a coculture of Tregs with syngeneic pDCs (ratio 1:4) and facs analysis was performed at day 6. Two peptides led to a strong activation of Tregs, as shown by the upregulation of CD25 (24.4% to 28.9% of expression). These antigen-specific Tregs displayed a modified phenotype (MHC-II, CD28, CD71 upregulated). For suppression assay, peptide-activated Tregs were sorted at day 6 and cocultured with CFSE+CD4+ effector T cells and allogeneic pDCs. Thus, allospecific Tregs were shown to maintain their suppressive activity in vitro after stimulation. For in vivo study, 0.5 mg/day of peptide was delivered continuously by a mini osmotic pump implanted intraperitoneally in recipients. Interestingly, we showed that administration of the dominant allopeptide, from day -7 to day 21 after transplantation, resulted in prolongation of allograft acceptance with an indefinite survival in 25% of the recipients. These rats showed a higher number of Tregs in spleen compared to untreated ones, an inhibition of alloantibody responses and no sign of chronic rejection.
This study demonstrated that antigen specific CD8+CD45RClow Tregs recognize 2 allogeneic epitopes with a therapeutic potential.

P3.13.42
Interferon-alpha plays complex roles in human regulatory T cell deprogramming into Th1-like cells

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Regulatory T cells (Treg) are classically viewed as immune suppressive cells tipping the balance between host defense from pathogens and prevention of excessive immunity. Recent data indicate that Treg can dynamically adapt to different contexts and even acquire the competence for unexpected inflammatory functions in peculiar cytokine contexts. The purpose of this study was to characterize the functional changes induced in Treg upon exposure to IFN-alpha, a cytokine with well-known antiviral properties. In vitro, in neutral culture conditions, IFN-alpha slightly increased Tbet and IFN-gamma expression by Treg; in a Th1-prone (IL2 plus IL12) condition, the addition of IFN-alpha further enhanced IL-12-driven IFN-gamma production, suggesting a role for IFN-alpha in supporting Treg deprogramming into Th1-like cells. IFN-alpha also exerted an anti-proliferative effect against both conventional T cells (Tconv) and Treg, without affecting the Treg-intrinsic suppressive function in Tconv-Treg cocultures. To evaluate the effects of Treg exposure to IFN-alpha in vivo, Treg were analysed by flow cytometry in patients with chronic HCV infection undergoing peginterferon/ribavirin therapy, before and 2 days after the starting of therapy. In the majority of patients, we could observe a decreased Treg frequency and an enhanced IFN-gamma production by both Tconv and Treg after treatment. IFN-gamma secretion by the two cell subsets showed a linear correlation, suggesting that Tconv-derived IFN-gamma may play a role in Treg diversion into Th1-like cells. Our data indicate that, under Th1 conditions, IFN-alpha may amplify Th1-type inflammation by inhibiting Treg expansion and fostering Treg deprogramming into Th1-like cells.

P3.13.43
Inducible IL-10 secreting CD49b+Treg cells as cell based-therapy for rheumatoid arthritis

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Adoptive transfer of Tregs is a promising approach to restore tolerance in autoimmune disease. However several subsets of Tregs need to be precisely defined to clearly establish which Tregs will be able to dampen efficiently the immune response in the various settings. We previously showed the potential of IL10-secreting induced CD49b+ Tregs to protect and prevent an experimental model of arthritis. Nevertheless the optimal dose, the phenotype and the in vivo-suppressive mechanism of these Tregs remain unknown. Here, we investigated and compared the therapeutic potential of CD25+FoxP3+ and induced CD49b+ FoxP3- Tregs in experimental models of arthritis or inflammation. Tregs were generated in naïve mice following repetitive injections of immature DCs. Tregs purification was based on the negative selection of CD4 T cells isolated from spleen and liver of vaccinated mice. Several doses of CD49b+ Tregs were intravenously injected at day 28 in established arthritis. We observed that the injection of 105 CD49b+ or CD25+ cells reverse clinical symptoms of arthritis while interestingly, a lack of efficacy of higher doses was observed. In vitro suppressive experiments confirmed the similar efficiency of both populations and phenotype analyses of CD49b Tregs showed expression profile of several Tregs specific markers (LAP+, LAG+, CTLA-4high). Moreover, in an OVA-specific model of inflammation, we demonstrated the higher impact of the CD49b Tregs on the proliferation of effector cells. Altogether, our results confirm the therapeutic potential of IL-10 secreting T cells in experimental model of arthritis in curative settings and unravel their mechanism of suppression.

P3.13.44
Systems analysis of innate activation reveals IL-1 family members as key modulators of T cells susceptibility to regulation and novel targets for therapeutic intervention

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Rational regulation of T cells responses is the ideal goal of tolerance promoting therapies. However, our understanding of the factors and mechanisms that modulate the susceptibility of T cells to regulation is limited. We and others have demonstrated that regulatory T cells (Treg)-mediated suppression of T cells is compromised in an inflamed environment. We aimed to identify inflammatory mediators that lessen T cell regulation. We implemented a bio-informatic analysis to evaluate the secretion of 23 cytokines by maturing murine dendritic cells. This analysis suggested that IL-1α and IL-1β could parallel IL-6 in reducing the efficacy of Treg. Using a CFSE-based, in vitro mouse T cell activation system, we confirmed that both IL-1α and β exert a profound negative effect on Treg-mediated suppression. In a mouse model of inflammatory bowel disease based on adoptive transfer of T cells +/- Treg, Ab-mediated blockade of IL-6 signaling delayed disease development through a process dependent on the presence of Treg. When Myd88-KO T cells (unable to respond to IL-1 family members) were used in this model, we observed a significant delay of weight loss, corroborating the involvement of this family of cytokines in modulating immune-regulation. Finally, we investigated the production of inflammatory cytokines following orthotopic vascularized composite (hind-limb) allotransplantation. IL-6, IL-1α, IL-1β, and particularly IL-18 (an IL-1 family member) accumulated at significant levels and for prolonged time in transplanted tissues. These observations provide evidence that these cytokines play an important role in promoting rejection, by antagonizing immune-regulation, and are necessary targets for therapeutic interventions.

P3.13.45
CD39 expression on Treg cells is genetically determined and can be further modulated upon activation

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CD39 defines a subset of regulatory T cells (Treg) with high suppressive capacity, and has been often associated with an activated status of regulatory T cells. The fraction of CD39-expressing cells within the Treg compartment varies widely (5% to 80%) in the human population, increasing slowly with age. However, the expression of CD39 on Tregs over time in one individual is very stable, and clonality analysis shows an unbiased repertoire. Moreover, in vitro activation of primary CD39-negative Treg cells using a Treg-tracking system resulted in only minor upregulation of CD39 in less than one third of the cells, making it unlikely that activation alone is responsible for the very high percentages of CD39+ Tregs found in some donors. We show here that CD39+ Treg are present in early compartments such as thymus and cord blood. CD39+ Treg in healthy adults express higher levels of CD25, FOXP3, memory markers and chemokine receptors involved in migration to inflammatory sites compared to their CD39-negative counterparts. However, in cord blood and young infants they exist as naïve cells, suggesting that -at least in part- they egress the thymus already as CD39 positive Tregs. Of note, thymic CD39+ Treg already show superior suppressive activity compared to CD39- Tregs. Finally, we show that donors with different levels of CD39 on Tregs can be segregated according to a SNP in the CD39/ENTPD1 gene, clearly indicating that the expression of CD39 on Tregs is genetically determined.

P3.13.46
ART2 blockade during cell preparation preserves the vitality and function of Tregs and iNKT cells

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Release of NAD+ during preparation of murine T cells causes ADP-ribosylation of cell surface proteins, which is catalyzed by the ecto-enzyme ADP-ribosyltransferase-2 (ART2). ART2 mediated ADP-ribosylation of the P2X7 ion channel induces gating of P2X7. This in turn mediates a cascade of downstream events including ecto-domain shedding of CD27/CD62L, externalization of phosphatidylserine, and ultimately cell death. ART2 shows catalytic activity even at 4 °C when cells are prepared on ice. Gating of P2X7, in contrast, occurs efficiently only when the cells are brought to 37 °C. Thus, the phenotypic and functional changes induced by ADP-ribosylation of P2X7 only become visible when cells are returned to 37 °C, e.g. in vitro for functional assays or in vivo for adoptive transfer studies. P2X7 is predominantly expressed by Foxp3+ regulatory T cells (Tregs) and invariant natural killer T cells (iNKTs). These cells are highly sensitive to activation of P2X7 via ART2. Using a Nanobody (clone s+16a) that blocks the enzymatic activity of ART2 during cell preparation we were able to markedly increase the vitality of Tregs and iNKTs in vitro. Further, we were able to greatly enhance the sensitivity of in vitro T suppression assays and to expand liver iNKTs in vitro. Finally, we observed that blockade of ART2 during cell preparation was also beneficial for the adoptive transfer of Tregs and indispensible for the successfull adoptive transfer of liver iNKTs. Therefore, our findings underscore the potential of Nanobody s+16a as valuable tool to improve and facilitate research on Tregs and iNKT cells.

P3.13.47
miR-21 transfection can differentiate human naïve T cells to regulatory phenotype

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Regulatory T cells are important players of the immune system that downregulate the immune responses. T regs are considered the main mediators of peripheral tolerance. Their mechanisms of action and clinical applications are subjects of considerable debate currently. These cells are involved in preventing autoimmunity and defective development and function of these cells can result in autoimmune disease.
Increasing evidence supports the role of miR-21 in the regulation of Foxp3 expression in Treg cells. In this research we tried to clarify whether miR-21 transfection to naïve CD4+ T cells is useful in the generation of iTregs in vitro. We separated naïve T cells using negative isolation magnetic kit and cultured them in the presence of anti-CD3, anti-CD28 and Treg polarizing cytokines or miR-21 transfection. We investigated in vitro differentiation of miR-21-transfected naïve CD4+ T cells to iTregs and then compared these iTregs to cytokine-differentiated iTregs and negative controls. We showed that expression of Foxp3, TGF-b, and IL-10 are increased in miR-21 transfected naïve T cells in comparison to cytokine-differentiated iTregs and negative controls.
Conclusion: Our data demonstrate that mir-21 is effective in the in vitro differentiation of naïve T cells to iTregs.

P3.13.48
Nr4a nuclear orphan receptors are essential for thymic regulatory T cell development and immune homeostasis

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Regulatory T cells (Treg) develop from progenitor thymocytes following engagement of the T cell receptors (TCR) with high affinity ligands, but the underlying molecular mechanisms are still unclear. Here, we demonstrate that the Nr4a orphan nuclear receptors, which are immediate-early genes up-regulated by TCR stimulation in

thymocytes, had an essential roles in Treg development. Mice that lack all Nr4a factors could not produce Treg cells and died within three weeks after birth due to systemic multi organ autoimmunity. Nr4a receptors directly activated the Foxp3 promoter, and forced activation of Nr4a receptors bypassed low-strength TCR signaling to drive the Treg developmental program. Our results our observations not only identified Nr4a receptors as critical components of Treg development, but also suggest that they act as the coordinators of the proper fate decision of CD4+ T cells in the thymus, by translating the strength of TCR signaling to transcriptional programs, thus working as crucial contributors to immune homeostasis.

P3.13.49
CD8+CD122+CD49d- regulatory T cells maintain immune homeostasis by Fas/FasL pathway-mediated killing of activated T cells

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One of the best-characterized $CD8^+$ regulatory T cells are $CD8^+CD122^+$regulatory T cells that were proved to play important roles in maintaining the immune homeostasis. However, $CD8^+CD122^+$ cells also contain a considerable percentage of memory T cells. Therefore, some marker(s) to distinguish regulatory T cells from memory T cells are needed. We found that CD49d, the α-chain of integrin, can separate $CD8^+CD122^+$ cells into two populations ($CD49d^-$ and $CD49d^+$). In this study, we established 2 assay systems, i.e. in vitro co-culture of $CD8^+CD122^-$ T cells and candidate cells of Treg, and in vivo co-transfer of $CD8^+CD122^-$ T cells and candidate cells of Treg. In the in vitro assay, the number of $CD8^+CD122^-$ indicator cells dramatically decreased after 48-72 h of co-culture if the candidate cells had the regulatory activity. In the in vivo assay, $RAG\text{-}2^{-/-}$ mice transferred with $CD8^+CD122^-$ T cells alone died within 14 weeks after the T cell transfer whereas mice transferred with a mixture of $CD8^+CD122^-$ T cells and $CD8^+$ Treg survived longer than 14 weeks. When the regulatory function of $CD8^+CD122^+CD49d^-$ cells and $CD8^+CD122^+CD49d^+$ cells was tested, $CD49d^-$ cells showed regulatory function both in vitro and in vivo experiments but $CD49d^+$ cells did not. Furthermore, $CD8^+CD122^-$ cells taken from *lpr* mice were not well regulated by wild type mice-derived $CD8^+CD122^+CD49d^-$ cells and $CD8^+CD122^+CD49d^-$ cells taken from *gld* mice did not regulate wild type mice-derived $CD8^+CD122^-$ cells. These results indicate that the regulation between $CD8^+CD122^+CD49d^-$ cells and $CD8^+CD122^-$ cells is mediated by Fas-FasL dependent cytotoxicty.

P3.13.50
Regulatory T cells in peripheral blood and tissue of active generalized vitiligo

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Vitiligo is an organ specific autoimmune depigmentation disease. Enhanced T cell mediated immunity and deficiency in regulatory T cells (Tregs) function has been implicated in the pathogenesis of generalized vitiligo (GV).
Tregs were characterized as CD3+ CD4+ CD25+ FOXP3+ in peripheral blood by flow cytometry, and the mRNA transcript levels of FOXP3, TGF-β and IL-10 were determined in peripheral blood and lesional, perilesional and non-lesional tissue by quantitative real time RT-PCR and immunolocalized by confocal microscopy.
Significant decrease in the frequency of Tregs was observed in active GV compared to healthy controls ($P < 0.05$). In peripheral blood, no siginificant difference was observed for mRNA transcript levels of FOXP3 and IL-10 ($P > 0.05$) but mRNA levels of TGF-β ($P < 0.05$) were significantly decreased in patients with active GV and healthy controls. Significant differential expression levels were observed for FOXP3 and TGF-β in lesional, peri-lesional non-lesional tissue of active GV.
Decrease in the percentage of Tregs in peripheral blood of active vitiligo patients suggests that there is deficiency in Treg frequency. Reduced mRNA levels of FOXP3 and TGF-β in lesional tissue suggests that there may be defect in their suppressive function or homing to skin leading to breakdown of peripheral tolerance and T cell mediated selective killing of melanocytes.

P3.13.51
Characterization of the role of hydroxyprostaglandin dehydrogenase in regulatory T cells

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Regulatory T cells (T_{reg} cells) are indispensable for self-tolerance and immune homeostasis. The forkhead transcription factor FOXP3 is essential for T_{reg}-cell development and function. We could identify hydroxyprostaglandin dehydrogenase (HPGD) as significantly higher expressed in human T_{reg} cells compared to conventional $CD4^+CD25^-$ T cells. HPGD fulfills an important function in the metabolism of eicosanoids and prostaglandins and is one of the major PGE_2-metabolizing enzymes. Notably, HPGD expression was specific for naturally occurring T_{reg} cells as HPGD was not upregulated during $CD4^+$ T-cell differentiation and even induced T_{reg} cells. Furthermore, exclusively in T_{reg} cells, HPGD expression could be specifically modulated by Interleukin-2. Minuscule amounts of IL-2 were sufficient to strongly upregulate HPGD expression. Blockade of JAK3/STAT5 signaling inhibited HPGD upregulation in T_{reg} cells. Moreover, the dependency of HPGD expression on IL-2-dependent signaling could be confirmed using reporter assays. Interestingly, FOXP3-ChIP experiments showed binding of FOXP3 to the HPGD promoter and loss of function experiments as well as reporter assays revealed that FOXP3 modulates rather than suppresses expression of HPGD. Furthermore, the dependency of HPGD expression on the extracellular microenvironment also indicates a tissue-specific expression and potential function of HPGD within T_{reg} cells. Taken together, the present study demonstrates that HPGD represents a novel gene, which is specific for human T_{reg} cells. Although the relevance of HPGD for T_{reg}-cell function remains to be elucidated, the present study indicates a tissue-specific role of HPGD in T_{reg} cells and provides a basis for further research to determine the role of HPGD for T_{reg}-cell function.

P3.13.52
Regulatory T cell plasticity in HCV-related liver inflammation and tumor

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Several evidences suggest that regulatory T cells (Treg) are functionally heterogeneous and plastic. For instance, in inflammatory milieus, Treg can produce pro-inflammatory cytokines, while a suppressive program characterizes tumor-associated Treg. Coinhibitory/costimulatory receptors expressed by Treg in particular conditions, such as PD-1 and OX40, may influence Treg sensitivity to cytokines and Treg program.
The aim of our study is to characterize the features of Treg in peripheral blood, hepatocellular carcinoma (HCC) and the surrounding tumor-free liver tissue, obtained from patients with chronic HCV infection.
Few Treg can be detected in tumor-free not cirrhotic liver tissue, expressing PD-1 and secreting IFN-γ ex vivo. Instead HCC and tumor-free cirrhotic liver tissues are infiltrated by abundant Treg that poorly secrete IFN-γ, despite a high expression of T-bet (Th1-related

factor). Interestingly, these Treg upregulate OX40, which is not constitutive in human Treg cells. OX40 expression and IFN-γ secretion are mutually exclusive, suggesting that OX40 correlates with protection from Treg deprogramming into Th1-direction. OX40L, the ligand for OX40, can be detected on a variety of immune cells at the HCC site.
These observations have been recapitulated in experiments in vitro. Indeed, Treg of healthy donors upregulate OX40 when stimulated with IL-2 and/or TNF-α in vitro. These OX40+ Treg are protected from the acquisition of IFN-γ production under Th1 conditions.
In conclusion, Treg can reprogram their functions toward suppression or inflammation; moreover, HCV-related cirrhosis may orchestrate a immunosuppressive signals fostering Treg expansion and favoring tumorigenesis.

P3.13.53
Application of novel Brilliant Violet™ fluorophores for multicolor flow cytometric analysis: phenotyping of regulatory T cells and conventional T cell subsets by surface and intracellular staining

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Multicolor flow cytometry (MCFC) is one primary technology for identification of lymphocyte subsets such as regulatory T cells (Tregs). Many MCFC users remain restricted by limited fluorophore availability, and the increasingly common violet laser (405nm) is still under-exploited by most users.
Here, we introduce usage of the highly sensitive polymeric molecule family excited by the violet laser that BioLegend first introduced with the following members: Brilliant Violet (BV) 421™, BV510™, BV570™, BV605™, BV650™, BV711™, and BV785™. All BV fluorophores are excited at 405nm and differ in their emission spectra, making them ideal candidates for MCFC. All new BV molecules yielded antibody conjugates with significantly increased brightnesses and signal-to-noise-ratios as compared to spectrally equivalent fluorophores.
BV-conjugated antibodies were used to analyze Tregs. In a 15-color surface marker panel, we demonstrated that all seven BV molecules can be multiplexed to analyze up to 7 different markers (CD25, CD3, CD19, CD161, CD27, HLA-DR, and CD127) off the violet laser. Despite using additional fluorophores excited by other laser lines, whose emissions contribute further to spectral overlap, the compensation range remained acceptable, and target populations were clearly resolvable. BV-conjugated antibodies were also successful in a 15-color panel for detection of intracellular cytokines (IFNγ, IL-2, IL-4, IL-10, IL-17A, IL-22), Foxp3 and surface antigens due to their excellent stability following fixation and permeabilization.
Thus, the novel family of Brilliant Violet™ fluorescent molecules provides superior tools for the violet laser, further enhancing the capabilities of MCFC for studying the phenotypic and functional heterogeneity of the immune system.

P3.13.54
PPAR-alpha deficiency modulates Treg cell functions and contributes to inhibit tumor growth in mice

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Background: There is a paucity of studies presenting the association of cancer progression with $CD4^+CD25^+$ regulatory T cell (Treg) prevalence. Increase inflammation in tumor bed can inhibit tumor growth, and peroxisome proliferator-activated receptor-alpha (PPARα), known as one regulator of inflammation, may therefore modulate tumor growth. Objectives: Here, we investigate the modulation by PPARα deficient-Treg cells of melanoma tumor growth in mice. Methods: CD4+ or CD8+ T cells isolated from spleen of wild type and PPARα-null mice were co-cultured with Treg cells of both types of mice. Cell migration was analyzed and cytolitic molecule release by CD8+ T cells was measured. Furthermore, B16 melanoma cells were sub-cutanously implanted in wild type (WT), PPARα-null and lymphopenic RAG2-/- mice in order to examine their tumorigenicities. Results: PPARα deficiency decreased the capacity of Treg cell to suppress CD4+ and CD8+ T cell proliferation. PPARα deficiency also diminished the ability of Treg cells to migrate toward chemokines. Moreover, melanoma tumor growth rate was much higher in WT mice than in PPARα-null animals. Dynamic analysis showed that Treg cells suppressed degranulation of perforin-release marker by CD8+ T cells and PPARα deficiency increases this cytolytic molecule release which participates to tumor rejection. Interestingly, adoptive transfer of splenic T cells into lymphopenic RAG2-/- mice inhibited tumor progression in these animals. Conclusion: Our findings suggest that PPARα deficiency increases the suppressive capacity of Treg cells and enhances the efficacy of CD4+ helper and cytotoxic CD8+ T cells and, thus, may provide a target for inhibiting tumor growth.

P3.14 B cells in autoimmunity and regulatory B cells

P3.14.01
Immunoglobulins, Zn and NO in newly diagnosed children with type 1 diabetes

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Background: Type 1 diabetes (T1D) is characterized by autoimmune aggression against pancreatic beta cells. Abnormalities in immunoglobulins and reactive oxygen and nitrogen species were reported especially in adult patients. Additionally, the human beta-cell-specific zinc (Zn) transporter Slc30A8 (ZnT8) has been recently defined as a major target of humoral autoimmunity in human T1D.
Objectives: To determine the levels of immunoglobulins, nitric oxide (NO), and Zn in newly diagnosed children with T1D.
Materials and methods: A retrospective matched-case control study was conducted at the Department of Pediatrics of Mother & Child Medical Center of Tlemcen (Northwest of Algeria).
Results: The levels of immunoglobulins, NO, Zn and albumin/globulin ratio were altered in type 1 diabetic patients as compared to healthy subjects; while, those of alpha-1, alpha-2, beta and gamma globulins were similar in both groups.
Conclusions: The alterations in Zn uptake and the excess of NO production and immunoglobulins could play key roles in the immuno-inflammatory response involved in the autoimmune process of T1D.

P3.14.02
Fibrinogen-like protein 2 (FGL2)/fibroleukin induces long-term allograft survival through regulatory B cells

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Fibrinogen-like protein 2 (FGL2)/fibroleukin has been previously described as being produced by CD4+ Tregs and to prolong graft survival. We recently showed in a rat model of heart transplantation tolerance dependent on regulatory CD8+ T cells that these cells showed overexpression of FGL2. In the present manuscript we show that in vivo overexpression of FGL2 through gene transfer without any further treatment resulted in inhibition of graft rejection. Adoptive cell transfer of splenocytes from FGL2-treated rats with long-term survival (> 100 days) inhibited acute and chronic rejection in a donor-specific and infectious tolerance manner since adoptive transfer up to a fourth consecutive recipient resulted in transplantation tolerance. Adoptive cell transfer also efficiently inhibited anti-donor antibody production. Analysis of all possible cell populations among splenocytes revealed that B lymphocytes were sufficient and enough for this adoptive cell tolerance. These B cells were also capable of inhibiting the

proliferation of CD4+ T cells in response to allogenic stimuli. This is the first description of regulatory B cells generated by FGL2 and capable of inducing infectious tolerance.

P3.14.03
FcγRIIb modulates IL-10 secretion by regulatory B cells: Implications for systemic sclerosis

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An IL-10-secreting subset of B cells, called regulatory B cells or Bregs, which participates in tolerance preservation in humans and mice, has been recently described. In humans, most Bregs are contained within the population of CD19+ CD24high CD38high transitional B cells. FcγRIIb is a receptor that inhibits the activation of B cells when recognizing the Fc fraction of IgG-containing immune complexes that are simultaneously bound to the BCR. Systemic sclerosis (SSc) is an autoimmune disease characterized by an extensive fibrosis of skin and organs. Activated B cells have a major role on SSc pathogenesis through the secretion of pro-fibrotic cytokines and disease-associated autoantibodies, however the molecular basis accounting for this B cell hyperactivity remains poorly understood. Our aim is to study the effect of FcγRIIB activation on BCR-mediated IL-10 secretion by B cells, and to analyze the frequency of transitional B lymphocytes and the expression of FcγRIIB in these cells among SSc patients. We demonstrate that stimulation of B cells with the F(ab)'2 fraction of an anti-BCR antibody induces an increased IL-10 production compared to the complete immunoglobulin. We also observed an increased population of transitional B cells in SSc patients, with a higher FcγRIIB expression, related to healthy controls. These results suggest that FcγRIIB expressed on Bregs inhibits BCR-mediated IL-10 secretion, and that SSc patients could have a misregulation in IL-10 production due to an increased FcγRIIB expression on transitional B cells.
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P3.14.04
Effects of fingolimod therapy on B cell biology in multiple sclerosis

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Fingolimod is the first oral immunotherapy for multiple sclerosis (MS). It is a sphingosine-1-phosphate receptor modulator that sequesters lymphocytes inside the lymph nodes. In this study, we aimed to investigate the effect of fingolimod on B cell subtypes and antigen presentation capacity in MS.
Peripheral blood mononuclear cells were isolated from the peripheral blood of 43 MS patients, collected before the first administration of fingolimod, after 1 month and then every 3 months for 18 months. Flow cytometry was performed to analyze ex vivo B cell subtypes (naive B cells, memory B cells, double negative cells, plasmablasts) and expression of costimulatory (CD80, CD86) and antigen presentation markers (HLA DR/DP/DQ).
After 1 month, fingolimod caused a decrease in the total number of lymphocytes and the percentage of B cells. The amount of cells remained low during follow-up. No changes were observed regarding B cell subtype distribution or antigen presentation capacity. After 3 months of treatment, an increase in the percentage of naive B cells (40% to 56%) was accompanied by a decrease in the percentage of memory B cells (20% to 10%). Furthermore, the percentage of HLA-DR/DP/DQ expressing B cells was significantly decreased, while a trend was observed towards a higher percentage of CD80 and CD86 expressing B cells.
To conclude, fingolimod causes a redistribution of B cell subtypes and a change in antigen presentation function of B cells in MS. The effects of fingolimod on B cell biology will be further studied both ex vivo and in vitro.

P3.14.05
Altered lymphoid organogenesis yet normal development of T-cell mediated autoimmunity in mice with skewed B-cell differentiation

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B cell receptor (BCR) signaling determines B cell differentiation, maturation and activation, and may potentially alter T cell-mediated immune responses. The Epstein-Barr virus latent membrane protein 2A (LMP2A) supports the activation and survival of B cells by mimicking a constitutively activated BCR, and its expression levels modulate peripheral differentiation of B cells. In this study we used two transgenic strains of BCR-deficient mice expressing LMP2A in mature B cells, where either follicular and marginal zone differentiation (D_HLMP2A mice) or B-1 cell development (V_HLMP2A mice) were supported, and evaluated the effects of skewed B lymphocyte differentiation on lymphoid organogenesis and T cell responses in vivo. Compared to naïve wild type animals, naïve transgenic strains displayed alterations in the composition of lymphoid organs. Immunization with the self antigen $PLP_{185-206}$ was also characterized by changes in the dynamics of distinct innate and adaptive immune cell subsets in transgenic mice. However, ex vivo T cell proliferation to $PLP_{185-206}$ peptide measured in immunized D_HLMP2A and V_HLMP2A mice was similar to that detected in immunized control mice. Finally, clinical expression of experimental autoimmune encephalitis in both LMP2A strains was identical to that of wild type mice. In conclusion, mice with skewed B cell differentiation driven by LMP2A expression in BCR-negative B cells display normal development of a T-cell mediated disease model of autoimmunity, suggesting that compensatory mechanisms support the generation of T cell responses. This study was supported by the Italian Ministry for Health (Ricerca Corrente and Progetto Giovani Ricercatori 2009-1607206)

P3.14.06
Accumulation of circulating autoreactive naïve B cells reveal defects of early B cell tolerance checkpoints in patients with Sjögren's syndrome

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Sjögren's syndrome (SS) is an autoimmune disease characterised by high affinity circulating autoantibodies and peripheral B cell disturbances with predominance of naïve and reduction of memory B cells. The stage at which errors in B cell tolerance checkpoints accumulate in SS is unknown. Here we determined the frequency of self- and poly-reactive circulating naïve and memory B cells of SS patients.
Single B cells were sorted by FACS from peripheral blood of SS patients and healthy donors (HD). RNA was used to amplify Ig VH and VL genes and PCR products were cloned and expressed as recombinant monoclonal antibodies displaying identical specificity of the original B cells. Recombinant antibodies were tested towards different antigens to determine the frequency of autoreactivity and polyreactivity.
We analysed 353 VH and 293 VL sequences and obtained 114 recombinant antibodies from naïve (n=66) and memory (n=48) B cells of 4 SS patients and compared their autoreactive profile to 45 clones from 2 HD. Analysis of the VH and VL gene usage showed no significant differences between SS and HD. Conversely, we observed accumulation of circulating autoreactive naïve B cells in SS as

demonstrated by increased reactivity towards Hep2 cells (43.1% SS vs 25% HD) and ENA (19.6% SS vs none), Ro/SSA and/or La/SSB.
Here using an efficient strategy to express recombinant antibodies from single B cells we demonstrated an elevated frequency of autoreactive naïve B cells in the circulation of SS patients supporting the existence of early defects in B cell tolerance checkpoints in SS.

P3.14.07
Characterization of regulatory B cells in rodent model, able to transfer allograft tolerance via the TGFbeta pathway

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Numerous reports have highlighted the central role of T_{reg} in tolerance, but few studies have investigated the B cell aspect. We described in a model of cardiac allograft tolerance in rat, induced by a short-term treatment, the accumulation in the blood and grafts of inhibited mature B cells ($sIgM^{int}$ IgD^{neg} IgG^{neg} $CD27^{het}$ $CD5^{neg}$ $CD24^{neg}$) blocked at the switch recombination process and over-expressing BANK-1, an inhibitor of the CD40-mediated B cell activation.
Interestingly, we demonstrated the presence of mature B_{reg} in the tolerant spleen, able to transfer a TGFβ-dependant donor-specific tolerance to a new recipient. In addition, *in vitro*, these B_{reg} suppress TNFα secretion in a MLR. Moreover, following transfer, these B_{reg} migrate or generate new B_{reg} and T_{reg} that accumulate locally in the graft but not in the spleen. Furthermore, following *in vitro* anti-CD40 stimulation, these B_{reg} are refractory to modulate Prdm1 and Bcl6 expression and express high level of granzyme B and BANK-1, suggesting partially anergic B cells with a killer phenotype. We observed in a similar mouse model of skin-graft tolerance the accumulation, in the draining lymph node, of activated $GL7^+$ $CD95^+$ B cells with $FoxP3^+$ T_{reg}, without an expansion of T_{eff}, suggesting a crosstalk between T_{reg} and B_{reg}.
These data demonstrate the presence of mature/memory B_{reg} able to transfer donor-specific allograft tolerance, with a phenotype similarly reported in patients operationally tolerant to kidney transplant. Therefore, our rodent models offer the opportunity to decipher the mechanisms of action of Breg in transplantation and to study their therapeutical potential.

P3.14.08
Protection from autoimmune nephritis in mice transgenic for BAFF, but lacking CD19

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B cell activating factor belonging to the TNF family (BAFF or BLys) is required for B cell survival and maturation. However, mice transgenic for BAFF (BAFFTg) develop spontaneous autoimmunity, resembling Systemic Lupus Erythematosus (SLE), in a T cell-independent manner. The autoantibodies which trigger disease in BAFFTg mice, are produced by marginal zone (MZ) and B1 cells, but not follicular B cells. Experiments using BAFFTg splenectomized mice suggested that it was not MZ or B1a B cells, but rather B1b B cells that were important in the development of autoimmune nephritis. We have developed a mouse cross between BAFFTg and CD19 knockout (ko) mice, in which MZ, B1a and B1b B cells are reduced. We observed that BAFFTgxCD19ko mice had reduced titres of autoantibodies compared to BAFFTg mice, and these mice resembled CD19ko animals with respect to autoantibody development. The BAFFTgxCD19ko animals were also protected from the splenomegaly observed in the BAFFTg animals. In aged mice BAFFTg display complement deposition in the glomeruli within the kidney, whilst BAFFTgxCD19ko were protected from this (as were the CD19ko mice). In addition, the BAFFTg glomeruli were larger, and more segmented than the BAFFTgxCD19ko, both signs of impaired function. Together these results point towards the pathogenic role of B1b B cells in autoimmune nephritis. This work also raises the prospect that targeting CD19 in SLE patients may prove efficacious.

P3.14.09
BAFF polymorphisms in Tunisian systemic lupus erythematosus patients

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Introduction: To investigate the association between polymorphisms in the 5' regulatory region of the B-lymphocyte activating factor (BAFF) gene, disease susceptibility and serum BAFF (s-BAFF) levels in Tunisian systemic lupus erythematosus (SLE) patients.
Methods: A case-control study including 124 patients with SLE and 152 healthy volunteers. The molecular study was done by PCR-RFLP although the s-BAFF was done by ELISA (Quantikinine ®, R & D Systems).
Results: The frequency distribution of genotypic and allelic polymorphisms -2701 T/A and -871 C/T BAFF was similar for patients and controls, while, the variant allele C of the polymorphism -2841 T/C of BAFF gene seems to be associated to susceptibility to SLE in Tunisian patients. The analytical study of polymorphism -2841 T/C of BAFF gene, based on clinical and biological manifestations of SLE, shows that the homozygous genotype T/T was significantly less prevalent in SLE patients expressing anti-dsDNA compared to those who do not express this type of self-Ac (p=0,021). Also, the homozygous genotype T/T (-2701) were significantly more prevalent in SLE patients with cutaneous vasculitis (p= 0,036). s-BAFF levels were elevated in SLE Tunisians patients more than in healthy subjects and it was also significantly higher in SLE patients positive for anti-dsDNA (s-BAFF levels = 1948,28 pg/ml) compared to those who do not express this type of self-Ac (s-BAFF levels = 1281,51 pg/ml) (p=0.007).
Conclusion: -2841 T/C polymorphism of BAFF gene could be a susceptibility factor in Tunisian SLE population and seems to influence the regulation of the production of anti-dsDNA.

P3.14.10
Direct PD-1-mediated suppression of autoreactive B cells by regulatory T cells

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Autoantibodies mediate various and heterogeneous forms of autoimmune diseases. On the one hand they can induce general autoimmunity like e.g. in systemic lupus erythematosus (SLE). On the other hand disease manifestations can affect just a single organ like e.g. the kidney, in certain forms of glomerulonephritis. T_{regs} are known to contribute to the maintenance of peripheral tolerance by suppressing the T helper cells and therefore T_{regs} can also control B cells, albeit indirectly [1,2]. This does not rule out the possibility of a direct (Treg - B cell) suppression, supported by several *in vitro* studies [3,4], but strict *in vivo* evidence is lacking. In previous studies we showed that B cells are suppressed in an antigen-specific manner *in vivo* [5,6], but the molecular mechanism remained unknown. Here, we report that most of such B cell suppression was mediated through the inhibitory cell surface molecule programmed death-1 (PD-1). Suppression required PD-1 expression on autoreactive B cells and expression of one of the two PD-1 ligands on T_{regs}. PD-1 ligation inhibited activation of autoreactive B cells, suppressed their proliferation and induced their apoptosis. Intermediate $PD\text{-}1^+$ cells, such as T helper cells, were dispensable for suppression. These findings demonstrate *in vivo* that T_{regs} use PD-1 ligands to directly suppress autoreactive B cells and identify a previously undescribed peripheral B cell tolerance mechanism against tissue autoantigens.

P3.14.11

Zap70+ b cells and plasmablasts as markers of disease activity and remission in systemic lupus erythematosus renal involvement

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The study aim was to analyze differences in B-cells subsets in patients with renal systemic lupus erythematosus (SLE) based on disease activity and to evaluate possible biomarkers of active nephritis and remission.
60 SLE patients with renal involvement (49 females; mean age 36.9±11.0 years; 37 with active and 23 with inactive nephritis) were analyzed for distribution of circulating peripheral blood B-cell subpopulations by staining for surface markers CD45, CD19, CD38, IgD, CD27 (CD27/IgD classification (1)) and intracellular marker ZAP-70 (2) by flow cytometry. All patients had a WHO class III or IV nephritis. Fourteen active patients were reassessed for PB B-cell subpopulations at renal remission achievement.
The 37 patients with active nephritis showed higher percentages of CD19/ZAP70+cells compared to 23 with renal remission (13.1±10.5%vs5.4±4.5%, respectively; p=0.002), and of plasmablasts (CD19+CD27/CD38+ cells: 10.6±7.4%vs6.3±5.8%, respectively; p=0.03).
In the 60 SLE renal patients, the percentage of CD19+/ZAP-70+cells correlated with disease activity index (SLEDAI) (r=0.44, p=0.002), inversely with complement fractions (C3: r=-0.44, p=0.001, C4: -0.45, p=0.001), the number of lymphocytes (r=-0.55, p<0.001) and of the CD19+B-cells (r=-0.61, p<0.001). Moreover, ZAP70+B-cells directly correlated with the memory cells subsets (CD27+IgD-: p=0.002, CD27-IgD-: p=0.01) and with plasmablasts (p=0.004).
The 14 patients evaluated at remission achievement showed a reduction of ZAP70+ B-cells percentage with respect to nephritis onset (p=0.002), and of plasmablasts (p=0.05).
The pool of CD19+/ZAP70+cells is associated with SLE activity parameters and B-cell memory compartment in lupus nephritis patients. Expansion of ZAP70+B-cells and plasmablasts characterize active renal disease and their reduction the remission state.

P3.14.12
The precursor BCR suppresses T cell-dependent autoimmune responses

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A hallmark of autoimmune diseases is the production of isotype-switched autoantibodies derived from T cell-independent (TI) and/or -dependent (TD) responses. Typically TI responses take place extrafollicularly and TD responses in germinal centers (GC). GCs are structures found in peripheral lymphoid organs requiring T follicular helper (TFH) cell differentiation and cognate B:T cell interactions. The precursor B cell receptor (pre-BCR), assembled from antibody heavy chains together with surrogate light chain, is involved in positive selection of the VH repertoire. It is also involved in negative selection of the antibody repertoire, an unexpected finding as this receptor is expressed on precursor-B cells. Its absence leads to the production of isotype-switched autoantibodies. The autoreactive B cells in pre-BCR-deficient mice lack markers of known B cell subsets (CD21-23-), whereas little else is known about these. Because pre-BCR-deficiency represents a B-cell intrinsic defect we hypothesized that the isotype-switched autoantibodies would arise from TI reactions. Here, we show that in pre-BCR-deficient mice the autoreactive CD21-23- population, unexpectedly, consists of GC B cells, and that GCs containing TFH and follicular dendritic cells develop spontaneously. GCs appear normal supporting B cell survival and expansion, and Ig isotype-switching and somatic hypermutation. Inhibition of B:T cell interactions completely abolishes these structures leading to reduced serum IgG2b/c autoantibody levels. Our results suggest that inadequate pre-BCR expression not only cell intrinsically subverts the central and peripheral selection of the BCR repertoire, but also influences the peripheral TCR repertoire with major implications for the development of autoimmunity.

P3.14.13
Functional properties of resident B cells in arterial walls of human atherosclerotic patients

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Ig repertoires were analyzed by RT-PCR on tissue extracts from carotid endarterectomy samples of 13 atherosclerotic patients. Cytokine, surface marker and transcription factor expression was studied on adventitial lymphocytes isolated by laser capture microdissection (LCM).
In adventitia, B lymphocytes constituted diffuse infiltrates or formed small cell clusters. BCR sequence analysis revealed that individual samples contained each a limited number of B cell clones. Functional α1, α2, γ1 and γ2 mRNAs comprised the majority of H chain mRNAs. IgA could be the only expressed H chain isotype whereas IgM was frequently absent. The λ/κ ratio of L chain usage was inverted. Ig V regions were hyper mutated and used recurrent V(D)J associations with some noticeable sequence convergences toward identical CDRs. AID was expressed in several arterial walls, in keeping with the observation of a local H chain class switch. Using CDR3 sequences as clonotypic markers, we found that plaque and adventitia repertoires were different and that B cells were trafficking between adventitia and draining lymph nodes. Three adventitial B cell antibodies reconstructed by genetic engineering proved to be autoantibodies recognizing discrete arterial wall proteins.
Most resident B lymphocytes were CD20 negative plasmablasts. However, they lacked markers of terminal differentiation to plasma cell (CD138 and Blimp-1). LCM isolated adventitial B lymphocytes expressed IL-6, GM-CSF and TNF-α whereas IL-2, IL-4, IL-10, M-CSF and IFN-γ were not detected.
These features witness for a local and antigen-driven maturation selecting activated oligoclonal adventitial B lymphocytes able to act on inflammation and disease progression.

P3.14.14
Epigenetic manipulation of protective and pathogenic antibody responses with histone deacetylase inhibitors

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Histone deacetylase inhibitors (HDACi) are new anti-cancer agents capable of remodeling chromatin and inducing widespread expression changes in gene expression. Although approved for the treatment of certain hematological malignancies, we understand little about their effect on the protective immune system. As HDACi have been demonstrated to be particularly potent against malignancies originating from the B lymphocyte lineage, we examined the ability of this class of compounds to modify naïve, pathogenic and memory B cell responses. We found that HDACi were capable of inducing a broad range of biological effects on normal lymphocytes including apoptosis, proliferation and differentiation. Importantly, treatment of autoimmune mice with HDACi significantly reduced B cells and pathogenic plasma cell numbers whilst other immune components were relatively unaffected. As predicted from these results, wildtype immunized mice treated with HDACi had a significantly impaired primary antibody response and were not capable of forming functional germinal centers. However, treatment with HDACi had no significant effect on circulating memory cells leaving secondary antibody responses intact. These studies suggest that HDACi may have a potential for the treatment of patients with B cell driven autoimmune conditions. Additionally, they suggest that although treatment with HDACi induces short-term immunosuppressive effects, there is no

long-term detrimental effect on the host immunological memory response.

P3.14.15
Systemic immunoglobulin G to human dentin fractions decreases during orthodontic treatment

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Limited data exists regarding the systemic immune response and orthodontic treatment. The objective of this study was to evaluate serum IgG levels to human dentin extract (HDE) and its fractions during orthodontic treatment. The study included 34 patients before orthodontic treatment (T0), at 6-7 months of treatment (T1) and at 12-13 months of treatment (T2). Serum IgG levels were determined by ELISA, using human dentine extract and its fractions as antigens. Periapical radiographs of maxillary incisors were analyzed by digital subtraction radiography. Dentine extract samples were submitted to chromatography and analyzed by SDS -PAGE. The two main chromatography fractions (I and II) analysis by SDS-PAGE resulted in components with MM > 200 kDa (I) and less than 50 kDa (II). The IgG levels to HDE were similar in all period analyzed ($p>0.05$), but decreased levels of IgG in T1 or T2 than T0 to fraction I ($p<0.05$) and decreased IgG levels in T2 than T1 and T1 than T0 ($p<0.05$) to fraction II were detected. In conclusion, orthodontic treatment modulated the IgG levels to dentin components with MM > 200 kDa and less than 50 kDa, regardless of root resorption degree.
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P3.14.16
Inhibition of formation of IgA1-containing immune complexes in IgA1 nephropathy

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IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide characterized by glomerular deposition of galactose (Gal)-deficient IgA1 (Gd-IgA1)-containing immune complexes (ICs). These deposits that activate mesangial cells are likely derived from the circulating ICs consisting of Gd-IgA1 bound by antiglycan antibodies. Here, we tested a new strategy for potential disease-specific therapy of IgAN. The approach was based on disrupting ICs formation to lessen their deposition and pathological impact. We tested various fragments derived from IgA1 antibody as competitors of IC formation. The competitors were prepared as recombinant proteins expressed in E. coli subsequently glycosylated in vitro using recombinant glycosyltransferases. The system used sera of IgAN patients containing anti-glycan IgG that were supplemented with Gd-IgA1 with or without a competing inhibitor. The inhibitors included non-glycosylated or Gal-deficient heavy chain and CH1-CH2 fragment of heavy chain of IgA1. Our results showed that glycosylated CH1-CH2 fragment of Gd-IgA1 blocked formation of new ICs in a glycan-specific manner. Further analyses indicated, that glycosylated CH1-CH2 could contribute also to partial disruption of ICs presented in the IgAN patient's serum. In contrast, nonglycosylated fragments CH1-CH2 did not interfere with ICs formation or stability. Thus, utilization of such competitors may open new avenues for future disease-specific therapeutic approaches for IgAN.
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P3.14.17
Can B cells induce the generation of regulatory T cells?

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Regulatory CD4+CD25+Foxp3+ T cells (Tregs) develop in thymus as natural regulatory T cells or are generated in lymphoid organs as induced Tregs. Generation of Tregs involves antigen-presenting cells (APC), mostly immature dendritic cells. It was demonstrated that Foxp3+ Tregs were found in T-B area of peripheral lymphoid organs pointing on the role of Tregs in inhibition of adaptive immunity or on a role of B cells in Treg generation. B cells can also play a role of APC in adaptive immunity to protein antigens. B cells, similarly to dendritic cells can be activated through Toll-like receptors, which dependently of signal strength modulate the expression of costimulatory molecules resulting in inhibition of activation of conventional T helper cells or Tregs induction. The aim of the study was to analyze the kinetics of the expression of B cell molecules crucial for antigen presentation process (CD80, CD86, CD40, MHCII) to T cells in conditions of B cell activation through Ig receptors, TLR4 and TLR7. C.Cg-*Foxp3*^{tm2Tch}/J mice were used in experiments. Distribution of CD4+CD25+Foxp3+ T cells was analyzed in co-cultured activated B cells and peripheral CD4+ T cells. Similar examination was performed with co-cultures with thymocytes as a source of natural Tregs. The results of our study demonstrated the constant expression of MHCII, while expression of costimulatory molecules changed depending on stimuli dose and incubation time resulting in changes of Foxp3+ T cells distribution.
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P3.14.18
IL-7 modulates B cells survival and activation by inducing BAFF and CD70 expression in T cells

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Interleukin-7 (IL-7) promotes the maintenance and activation of peripheral T cells, whereas it does not act directly on mature B cells due to the lack of IL-7Ra expression on these. We report here that, in spite of the insensitivity of B cells to IL-7, high concentration of IL-7 can lead to increased B cell survival and antibody production in the presence of T cells, without the use of any further B cell stimulatory signal. IL- 7 promoted B cell activation through inducing CD70 expression on resting T cells, particularly on CD4þ memory cells. The interaction of CD70 molecules with the B cell costimulatory receptor CD27 led to B cell proliferation, the accumulation of CD38 þ CD20- plasmablasts and antibody production. In addition, IL-7 treatment induced BAFF secretion from resting peripheral T cells thereby promoting B cell survival. IL-7 levels can increase in lymphopenic conditions, in autoimmune diseases or in patients receiving T cell regenerative IL-7 therapy. Based on our findings high IL-7 levels can lead to increased B cell activation by inducing the B cell regulatory proteins CD70 and BAFF in resting T cells. Such activity might be beneficial in short term immune-stimulatory IL-7 therapies; permanently increased IL-7 levels, on the other hand, can contribute to impaired B cell tolerance.

P3.14.19
Bendamustine increases interleukin-10 secretion from B cells

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Background and Purpose: Bendamustine is an alkylating agent approved for B cell malignancies such as lymphoma and chronic lymphocytic leukemia. However, the mechanisms of its action to B cells have not been fully clarified. The objective of this study is to investigate the effects of bendamustine on B cell function and to explore a possible clinical application of bendamustine to systemic autoimmune diseases.
Materials and Methods: A human B cell line, Ramos, was cultured in the presence of various concentrations of bendamustine for four days. The proliferation of the cells was analyzed by XTT assay and the secretions of IgM and cytokines by the cells were measured by ELISA.
Results and Discussion: The cellular proliferation of Ramos cells by 10% fetal bovine serum was significantly inhibited in the presence of 25-100 μM of bendamustine in a dose-dependent manner. Concordantly, IgM secretion from Ramos cells was significantly inhibited with 25-100 μM of bendamustine (by approximately 40%-70%). Interestingly, the secretion of IL-10 by Ramos cells was dramatically (at least >10-fold) increased in the presence of 25-100 μM of bendamustine. Although bendamustine has been known to inhibit the dimerization of STAT3, two STAT3 inhibitors (S3I-201 and Stattic) did not increase IL-10 from Ramos cells, suggesting the involvement of other mechanisms than STAT3. Our results suggest that bendamustine may provide a novel therapeutic tool for autoimmune diseases through anti-inflammatory effects of IL-10, in addition to the suppression of B cell growth and activation. The underlying molecular mechanism of our findings should be further elucidated.

P3.14.20
B-1 lymphocytes modify global gene expression by murine melanoma cells

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It is known that immune system components can contribute to tumor progression and inflammatory cells can influence almost all aspects of cancer progression, including the ability to form metastasis. Recent studies have attributed an important role to B-1 cells, a subset of B lymphocytes, in melanoma progression. In vitro interaction between B16 melanoma cells and B-1 lymphocytes lead to an increased in metastatic potential of B16 lineage but the molecular effects induced by B-1 cells in B16 have not yet been elucidated. In this study we used a microarray approach to assess gene expression profile in B16 melanoma cells after contacting B-1 lymphocytes. The microarray analysis identified upregulation in genes involved with metastatic progression, such as ctss, ccl5, cxcl2 and stat3. RT-qPCR confirmed increase in mRNA expression in B16B1 samples. Considering that previous studies showed the role of ERK1/2 MAPK cascade in melanoma cells after contacting with B-1, RT-qPCR was performed with RNA from melanoma cells before and after contacting B-1 cells and treated or not with inhibitors of ERK phosphorylation. The results showed that stat3, ctss and cxcl2 increased expression in B16B1 but their expression decreased when ERK / MAPK inhibitor was used. Stat3 was verified and validated at the protein level by Western blot analysis. Results showed that STAT3 expression was significantly increased in B16B1, suggesting that this pathway can also contribute to increase metastatic phenotype in our model. These results showed that B-1 cells induce important changes in the global gene expression of B16 melanoma cells.

P3.14.21
A novel flow-cytometry-based method to evaluate autoreactivity enables the study of B cell tolerance checkpoints in mice

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During maturation, B cells rearrange their V(D)J segments to produce functional immunoglobulins.
Along the developmental pathway, there are checkpoints to censor autoreactive B cells that may arise. In autoimmune diseases, these checkpoints are not effective and autoreactive B cells enter the mature B cell pool. Here we present a new approach to study censoring checkpoints.
Nuclear antigens were extracted from Hep-2 cells, labeled and used to stain splenocytes. The fraction of nuclear antigen-reactive (ANA+) cells is higher in the immature compartments and decreases with the maturation stage. The innate-like compartments of MZ and B1 B cells contain a significant fraction of ANA+ B cells.
To prove that the ANA+ B cells bear autoreactive antibodies, ANA+ and ANA- B cells were sorted and stimulated in vitro to secrete antibodies. Only antibodies from ANA+ B cells react with dsDNA and exhibit a rim nuclear pattern on Hep-2 slides.
The frequency of ANA+ B cells was compared between non-autoimmune (BALB/c, C57B/6) and lupus-prone (NZBxNZW/F1, MRL/lpr) mice. B cells from lupus-prone mice contain a higher percentage of ANA+ B cells than healthy mice, highlighting the altered censoring checkpoints present in lupus.
Our method allows for the studying censoring checkpoints in a fast and inexpensive manner. We propose this approach in order to expand the knowledge acquired from transgenic models, and overcome the limitation of studying tolerance one B cell receptor at a time.

P3.14.22
Strain-specific positive and negative regulatory roles for TLR9 in a mouse model of SLE

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Antinuclear antibodies (ANA), particularly against double stranded DNA (dsDNA), are a hallmark of Systemic Lupus Erythematosus (SLE). A point mutation dysregulating CD45 phosphatase activity (CD45E613R) results in a SLE-like phenotype on a 129-C57BL/6 (B6) genetic background. However, CD45E613R mice backcrossed to a B6 genetic background fail to develop ANA while CD45E613R mice on a BALB/c background develop anti-dsDNA IgG antibodies at 100% penetrance. Despite similar hyperresponsive ITAM signaling in multiple immune cell lineages, neither strain develops glomerulonephritis. A genetic modifier screen between B6 and BALB/c CD45E613R mice identified the pattern recognition receptor TLR9 as a potential mediator of anti-dsDNA antibodies. Interestingly, regardless of the presence or absence of the CD45E613R mutation, BALB/c B cells are hyporesponsive to CpG stimulation relative to B6 B cells. Here, we use the CD45E613R.BALB/c model, which lacks the interference and feedback of end organ disease, to elucidate which cellular immune compartments must be dysregulated for autoantibody production. We find that CD45E613R B cells are necessary and sufficient for anti-dsDNA IgG antibodies, while αβ T cells are dispensable. Mixed bone marrow chimeras demonstrate an intrinsic requirement for TLR9 in polyclonal B cells for anti-dsDNA IgG antibody production, indicating a positive regulatory role for TLR9 in BALB/c. Surprisingly, genetic ablation of TLR9 in the autoimmune-resistant B6 background permits ANA production, indicating TLR9 can also play a negative regulatory role. Taken together, our data support the hypothesis that the net strength of signal during B cell development dictates the autoreactivity of the B cell repertoire.

P3.14.23
Mast cells contribute to the expansion and differentiation of IL-10-producing B cells

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B cells are classically considered as positive regulators of the immune response, however the regulatory suppressive function of distinct B-cell populations has been recently described. Several studies have demonstrated that B cells acquire the ability to suppress immune responses in an IL-10-dependent manner, but the cell types involved in the induction of regulatory B cells are currently unknown. The evidence of a direct B-mast cell (MC) interaction together with data demonstrating the role of MCs as important regulators of immune responses, led us to investigate whether MCs could favor the development of IL-10-producing B cells.
We observed that MCs are able to increase the percentage of IL-10$^+$ B cells, regardless of their activation status, while they do not influence IL-10 secretion. MCs-induced IL-10$^+$ B cells are predominantly found within the CD1d$^+$CD5$^-$ B-cell subset; moreover they are CD23$^+$ and express high levels of CD19 and CD21, suggesting that they could derive from T2-MZP B cells. Among the different mechanisms that could be responsible for the expansion of IL-10-producing B cells by MCs we show the importance of both the CD40-CD40 ligand interaction and of soluble factors released by MCs even in the absence of activation. Furthermore, the *in vivo* absence of MCs reduces the percentage of CD19$^+$IL-10$^+$ cells in mouse bone marrow, lymph nodes and peritoneum. Altogether our data show that MCs can influence the differentiation of IL-10-producing B cells and this can be seen as a very relevant observation in the context of immunologically mediated inflammatory reactions.

P3.14.24
Abnormal distribution of B-cells populations associated with impaired regulatory functions in the Chronic Humoral Rejection

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In kidney transplantation, the composition of the B cell compartment is increasingly being identified as an important determinant for graft outcome. Whereas naïve and transitional B cells have been associated with long term allograft survival and operational tolerance, memory B cells have been linked to graft rejection and graft loss. Chronic antibody mediated rejection (cABMR) now represents a major complication in transplantation and is a challenge in current therapeutics. In this study, we show that patients with cABMR display a unique B cell phenotype with a reduction in the ratio of "activated B cells"/"memory B cells" associated with an impaired immunosuppressive activity. We further show that the regulatory functions of B cells depend on their maturation status. We established that "activated B cells" were able to induce the expansion of regulatory T cells and to mediate durable suppressive function on autologous T cell proliferation through a TGFb/IDO axis, that was deficient in B cells from cABMR patients. Phenotypic and functional analysis of the B cell compartment could be indicated for appropriate follow-up after transplantation and drive therapy in the establishment of transplant tolerance processes.

P3.14.25
The autoantibodies to GM-CSF found in pulmonary alveolar proteinosis are generated through somatic mutations

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Pulmonary alveolar proteinosis (PAP) is a rare and severe autoimmune disease caused by the production of autoantibodies that neutralize GM-CSF causing the loss of alveolar macrophages and the accumulation of lipoproteinaceous material within the alveoli. By immortalizing memory B cells from several PAP patients we isolated 17 monoclonal antibodies that bind to human GM-CSF with high affinity. By cross-competition experiments using surface plasmon resonance we established a map of the antigenic sites of GM-CSF and showed that 3 antibodies can bind simultaneously to a single GM-CSF molecule. Interestingly, the recombinant germlined version of the antibodies did not bind to GM-CSF, indicating that somatic mutations are essential for binding. Finally, using a site-specific serological assay we identified rare healthy blood donors with low levels of non-neutralizing GM-CSF antibodies. These findings support a stochastic model where autoantibodies are generated by accidental somatic mutations that occur in the course of the response to irrelevant antigens.

P3.14.26
CD38 and splenic B lymphocytes involvement in autoimmune disease using a murine model for systemic lupus erythematosus

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Mouse models contribute to our understanding of human diseases. Several strains of systemic lupus erythematosus (SLE)-prone mice, including New Zealand Black (NZB), F1 hybrids of NZB x New Zealand White (NZW) (B/W F1), MRL/Mp-lpr/lpr (MRL/lpr) and BXSB mice, are important in the research of this autoimmune disease. Furthermore, several genes and signaling molecules have shown to have a role in SLE pathologies such as: Lyn, CD22, SHP-1 or the sle1, sle2 and sle3 congenic models. CD38 is a transmembrane receptor able to induce activation, proliferation, and survival of human and mouse lymphocytes; this molecule is expressed along the B cell ontogeny, in addition, CD38 is an ectoenzyme involved cell adhesion and is used as a disease marker for leukemia and myeloma, also is a dependable negative prognostic marker for chronic lymphocytic leukemia (CLL). Some reports suggest a function for CD38 in autoimmunity of human and mice, but there are not reports that clarify the role of this molecule in SLE. To study the implication of CD38 in the SLE pathogenesis we compared proteinuria, serum ANA and anti-dsDNA antibody production, body-weight, nephritis and mortality in the CD38-deficient (CD38-/-) compared to the wild type (WT) mice. We expect that the pathologic manifestations of nephritis will appear significantly earlier in the CD38-/- mouse as well as changes in the survival rate.

P3.14.27
Generation of autoreactive and poly-reactive antibody producing cells during gamma-herpesvirus infection

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Autoantibody production is sometimes associated with persistent infections such as HIV, hepatitis C virus and Epstein-Barr virus (EBV) in humans. However, little is known about the underlying mechanisms how peripheral B cell tolerance is compromised during such viral infections. Murine γ-herpesvirus 68 (MHV68), a closely related virus

to EBV, induces serum IgG autoantibodies, which are vigorously produced at two or three weeks after infection, and gradually reduced following weeks, while high titer of serum anti-MHV68 antibodies are maintained for months. By employing this infection model, we investigated how virus- and autoreactive B cells are generated and selected during the germinal center (GC) reaction. To this end, we cloned and expressed monoclonal antibodies from splenic IgG+ GC B cells, which MHV68 preferentially targets in the latent infection. Reactivity profiles of cloned antibodies revealed that in addition to MHV68-reactive clones, a significant proportion (~20%) of GC B cells exhibited dsDNA autoreactivity. Intriguingly, one third of virus-reactive clones were polyreactive to dsDNA, insulin and/or cardiolipin. However, autoreactive prevalence in MHV68-infected cells was not found. Furthermore, higher frequencies of autoreactive and polyreactive clones were found in the plasma cell fraction than in GC B cells, indicating that these clones were positively selected to be plasma cells. PCR-based reversion of somatic hypermutations (SHMs) to germline sequences diminished autoreactivities of several polyreactive clones. Taken together, MHV68-induced auto- and polyreactive antibodies are, at least in part, attributed to SHMs given in the GC reaction.

P3.14.28
B cell-derived IL-10 suppresses inflammatory disease in Lyn-deficient mice

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Lyn kinase deficient mice represent a well-established genetic model of autoimmune/autoinflammatory disease that resembles systemic lupus erythematosus (SLE). We report that interleukin-10 (IL-10) plays a crucial immunosuppressive role in this model, modulating the inflammatory component of the disease caused by myeloid and T cell activation. Double mutant *lyn*$^{-/-}$*IL-10*$^{-/-}$ mice manifested severe splenomegaly and lymphadenopathy, dramatically increased pro-inflammatory cytokine production and severe tissue inflammation. Single mutant *lyn*$^{-/-}$mice showed expansion of IL-10-producing B cells. Interestingly, WT B cells adoptively transferred in *lyn*$^{-/-}$ mice showed increased differentiation into IL-10-producing B cells that assumed a similar phenotype to endogenous *lyn*$^{-/-}$ IL-10-producing B cells, suggesting that the inflammatory environment present in *lyn*$^{-/-}$mice induces IL-10-producing B cell differentiation. B cells, but not T or myeloid cells, were the critical source of IL-10 able to reduce inflammation and autoimmunity in double mutant *lyn*$^{-/-}$*IL-10*$^{-/-}$ mice. IL-10 secretion by B cells was also crucial to sustain transcription factor Forkhead Box P3 (Foxp3) expression in Tregs during disease development. These data reveal a dominant immunosuppressive function of B cell-derived IL-10 in the Lyn-deficient model of autoimmunity, extending our current understanding of the role of IL-10 and IL-10-producing B cells in SLE.

P3.14.29
B-cell delivery of tolerogenic fusion proteins via anti-CD20 targeting

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We previously demonstrated that retroviral delivery of IgG fusion proteins in B cells ex vivo could block or reverse immune responses in rodent models of multiple sclerosis, type 1 diabetes and hemophilia. Our model was based on the tolerogenic antigen-presenting capacity (APC) of B cells and the exquisite tolerogenicity of IgG carriers demonstrated decades ago, and shown to be effective in preventing and/or treating autoimmune disease models. We recently rendered this gene therapy model B-cell specific in vivo by engineering a viral vector that was pseudotyped with a single chain anti-CD20 (scCD20). In order to create a non-viral¬ delivery system taking advantage of the tolerogenicity of IgG fusion proteins directed into B cells, we have prepared an scCD20 fusion with target peptides on a human IgG4 scaffold. We term this fusion a B-cell-specific antigen IgG fusion tolerogen (BAIT). Thus, in principle, CD20+ B cells could be directly "infected" in vivo with this BAIT to deliver target epitopes in tolerogenic B cells. In this report, the scCD20 murine anti-huCD20 BAIT was constructed with myelin oligodendrocyte glycoprotein (MOG) immunodominant peptide 35-55 (or a control OVA peptide) at the C-terminus. Herein, we show that these BAITs could specifically "infect" normal human B cells and Raji B lymphoma. Expression of an immunodominant peptide was determined by presentation to T-cell receptor transgenic T cells, and the effect on EAE will be presented using huCD20 transgenic B cells as tolerogenic APCs. The BAIT approach should be a novel therapeutic for MS. (Supported by NIH grant AI035622)

P3.14.30
CD19 expression on B cells controls type 1 diabetes (T1D)

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Type 1 diabetes (T1D) is an autoimmune disease that results from mononuclear infiltration of pancreatic islets and targeted destruction of the insulin producing beta cells. The inflammatory infiltrate consists of many cell types including B lymphocytes capable of processing and presenting antigen to the T cells that kill beta cells. A crucial role for B lymphocytes in T1D pathogenesis has been established since non-obese diabetic (NOD) mice lacking B cells are protected from developing T1D. We show that CD19 expression on B cells, important for the internalization of membrane-bound antigens, is elevated in NOD mice, and results in increased signaling. Furthermore, while NOD B cells deficient in CD19 can adequately present peptide antigen and promote the expansion of T cells with specificity for the soluble beta cell protein pro-insulin, they possess significantly diminished capacity to expand T cells with specificity for the membrane-bound autoantigen, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP). We propose that elevated CD19 on NOD B cells leads to increased uptake of membrane-bound antigen, mediating the expansion of autoreactive T cells specific for membrane-bound autoantigens which are critical for invasive insulitis, beta cell destruction and T1D.

P3.14.31
Limited somatic hypermutation in IgA of transglutaminase 2 specific as well as gluten specific plasma cells of celiac disease intestinal lesions

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Celiac disease (CD) is an immune-mediated disorder with a strong HLA association to HLA-DQ2 and HLA-DQ8 that is caused by intolerance to cereal gluten proteins. A hallmark of the disease is IgA and IgG antibodies to the autoantigen transglutaminase 2 (TG2) as well as the exogenous antigen gluten. The generation of both anti-TG2 and anti-gluten antibodies are dependent on intake of gluten and associated with HLA-DQ2 and HLA-DQ8. A model where TG2-specific B cells receive help from gluten-specific T cells by taking up complexes of gluten and TG2 could explain the gluten and HLA dependent formation of anti-TG2 antibodies.
The consequence of this model is that TG2-specific and gluten-specific B cells may receive help from the same gluten-specific T cells, despite their different B cell receptor specificities. We have previously shown that intestinal TG2-specific IgA+ plasma cells show limited degree of somatic hypermutation, restricted VH gene usage and are highly abundant in the disease lesions. Here we compare intestinal gluten-specific and TG2-specific IgA+ plasma cells. Gluten-specific plasma cells have restricted VH and VL gene usage. As for the TG2-specific plasma cells, the somatic hypermutation is limited as

compared to gut IgA plasma cells with other specificities. The low degree of somatic mutation in gluten is surprising and may suggest that gluten-specific T cells influence the degree of mutations in antibodies produced by the exposure to gluten in celiac disease.

P3.14.32
Hydrodynamic DNA vaccination for induction of mouse monoclonal antibodies specifically reacting with N-acetylgalactosaminide α2,6-sialyltransferase II (ST6GalNAc II)

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IgA nephropathy (IgAN) is associated with galactose (Gal) deficiency of some IgA1 hinge-region (HR) O-glycans. Gal-deficient IgA1 plays a key role in IgAN, as such molecules are recognized by anti-glycan antibodies, form immune complexes that deposit in the glomerular mesangium and induce glomerular injury. O-glycosylation of IgA1 is initiated in Golgi apparatus of IgA1-producing cells by attachment of N-acetylgalactosamine (GalNAc) to some Ser/Thr residues in the HR, followed by addition of Gal to the GalNAc. Sialic acid (SA) could be attached to Gal by an α2,3-sialyltransferase and/or to GalNAc by α2,6-sialyltransferase II (ST6GalNAc II). Premature SA attachment to GalNAc would prevent addition of Gal, thus resulting in Gal deficiency.
The goal of our study is to determine whether overexpression of ST6GalNAc II, described in IgA1-producing cells from IgAN patients, is associated with abnormal localization of the enzyme, i.e., a shift from trans-Golgi toward cis-Golgi apparatus, and thus contributes to production of Gal-deficient IgA1. We developed ST6GalNAc II-specific monoclonal antibody for fluorescence-microscopy analysis of ST6GalNAc II intracellular localization. cDNA of human ST6GalNAc II was cloned into DNA vaccination plasmid and hydrodynamically administered to mice. Recombinant ST6GalNAc II protein was used for screening of ST6GalNAc II-specific antibodies from sera of DNA-immunized mice and monoclonal antibodies secreted by hybridoma cells. Analyses by fluorescence microscopy revealed that IgA1-producing cells from IgAN patients have increased fluorescence signal for ST6GalNAc II in trans-Golgi and a partial shift toward the cis-Golgi compared to IgA1-producing cells from healthy controls.
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P3.14.33
B cell extrinsic MyD88 and FcR common gamma chain control contraction of the autoreactive extrafollicular B cell response

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Both MyD88 and Fc Receptor (FcR) common gamma chain signaling drive proinflammatory responses. In B cells, MyD88 is required for anti-DNA and anti-RNA autoantibody responses in vivo. Mechanisms within innate immune cell types during this response are not well understood. Transgenic mice have been useful in elucidating how autoreactive Rheumatoid Factor (RF) B cells are activated. In lupus-prone mice, RF B cells undergo an isotype-switched extrafollicular (EF) plasmablast response. A similar response ensues when these cells are exposed in vivo to anti-chromatin antibodies, which presumably form immune complexes (ICs) with chromatin shed from dying cells. ICs could also stimulate myeloid cells via FcR and TLRs, which in turn could influence the EF response. To investigate this, we transferred RF B cells and anti-chromatin antibodies into mice lacking FcR common gamma chain, MyD88 or both. Initially, expansion and differentiation of RF B cells was equivalent in all hosts. Unexpectedly, by day 7, the response contracted in the WT environment, whereas dysregulated expansion continued in the deficient environments. Furthermore, MyD88 and FcR are non-redundant, as responses were larger and more prolonged in mice lacking both compared with mice deficient in either factor alone. By day 10, the AFC frequency was 40-fold larger in double-deficient compared to WT recipients. These results reveal novel regulatory roles in the EF B cell response for receptors that are typically proinflammatory. Targeting these pathways may provide a way to curtail dysregulated autoreactive B cell activation.

P3.14.34
Renal Transplant Recipients lack circulating CD19+CD24hiCD38hi Interleukin-10 producing Regulatory B-Lymphocytes

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Introduction: B-lymphocytes are commonly associated with antibody mediated acute or chronic allograft rejection. However, recent studies have demonstrated that CD19+CD24hiCD38hi regulatory B-Lymphocytes (Breg) appear to suppress immune effector mechanisms by cell-cell contact or via production of IL-10. Here, we characterized and analyzed peripherally circulating Bregs by flow cytometry in healthy subjects (n=18) and renal transplant recipients receiving a calcineurine inhibitor based immunosuppression (n=41).
Methods: Freshly isolated PBMCs from healthy subjects (HS) and renal transplant recipients receiving a CNI-based immunosuppression were isolated. Four color FACS was performed for CD19, CD24, CD38 and IL-10 either after isolation or after 72hrs co-culture in presence of PMA/Ionomycin and TLR9-ligand. Patients that previously received thymoglobuline or rituximab or exhibited acute infections (CrP>0.5 mg/dL) were excluded from analysis.
Results: The amount of CD19+B-cells among lymphocytes was 11% in HS (n=18), 3% in CsA (n=6, $p<0.05$) and 7% in tacrolimus (TAC, n=35, $p<0.05$) treated patients. Among B cells, a distinct subset of CD24hiCD38hi Breg cells was found to be ~5% in HS, 2.4% in TAC-patients and almost blunted in patients receiving CsA. After stimulation, only ~4% of Bregs in HS and even fewer in CsA/TAC patients produced IL-10 (1.2% and 1.5%, $p<0.05$). Co-culture of positively isolated mitogen stimulated CD19+ B cells in presence of CsA (1, 10 and 100 ng/mL) or TAC (1, 5 and 10 ng/mL) confirmed these results.
Conclusion: CNI-based immunosuppression reduces the amount of Bregs and inhibits their IL-10 production. Whether this affects allograft function needs to be analyzed in further studies.

P3.14.35
Endosomal TLR triggering hyperactivates B cells, induces plasma cells differentiation, class switch and immunoglobulin production in Sjögren's syndrome

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Patients with primary Sjögren's syndrome have B cell disturbances resulting in hypergammaglobulinemia and autoantibody production. Most patients are untreated, enabling studies of an unmanipulated human autoreactive immune system. Here we used vaccination as a tool to analyze immune responses in vivo. Untreated Sjögren's patients and healthy controls were vaccinated twice against the H1N1 influenza, and monitored by multiple sampling. Surprisingly, patients developed higher titers of IgG H1N1 antibodies, increased total IgG and increased autoantibody levels in response to vaccination. In addition, an expansion of CD138+ plasmablasts was observed, and up-regulation of several pro-inflammatory cytokines. To dissect the B cell hyperreactivity, immunoglobulin class switch was induced in vitro in CD19+IgD+ B cells from patients and controls. Significantly more plasmablasts and higher titers of IgM and IgG were observed in TLR9

stimulated Sjögren-patient derived cultures. Similar results were obtained by TLR7 stimulation, but not by α-CD40 or BAFF. The importance of the endosomal TLR pathways was further demonstrated by analyzing B cells from patients treated with the drug chloroquine, which targets the endosome. In these patients, no differences were observed in class switch and plasma cell differentiation compared to healthy individuals. This observation was further confirmed by in vitro treatment of IgD+ B cells with chloroquine which significantly inhibited class switch and plasma cell differentiation. In conclusion, we demonstrate for the first time that B cell activation via endosomal TLRs leads to enhanced plasma cell differentiation and class-switch in patients with Sjögren's syndrome, explaining the induction of hypergammaglobulinemia in Sjögren's syndrome patients.

P3.14.36
The role of B cells as antigen presenting cells in allergic inflammation

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Allergy is one of the leading health problems in industrialized countries, affecting around 50 million people in the United States only. The hallmark of this disorder is a strong Th2 response with upregulated levels of IL-4, IL-5 and IL-13, which leads to enhanced IgE production, cell recruitment to the site of allergen entry and exaggerated immune response leading to tissue damage. The pathological role of B cells in allergic disorders as the source of immunoglobulin E has been known for many years. In contrast, B cell capacity to regulate T helper cell responses via MHC class II dependent presentation of allergenic peptides in ongoing airway inflammation remains elusive. Using a mouse model of house dust mite induced allergic asthma, we seek to determine if B cells residing in lungs are able to stimulate effector T helper cells in the lungs to proliferate and secrete cytokines. This approach may help us determine if B cells may contribute to allergic inflammation via presentation of antigens to resting memory Th2 and/or effector Th2 cells during a secondary immune response.

P3.14.37
Anti-thrombin antibodies induced by dengue virus with both anti-thrombotic and pro-fibrinolytic activities

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Dengue virus (DENV) infection may result in severe dengue hemorrhagic fever (DHF). However, the mechanisms that cause hemorrhage in DHF are unclear. In this study, we demonstrated that antibodies against human thrombin were increased in the sera of dengue patients but not in other virus-infected patients. Thrombin cross-reacted antibodies were also found in the sera of DENV immunized mice and rabbits. To further characterize the properties of these antibodies, affinity-purified anti-thrombin antibodies (ATA) were collected from both DENV patients' sera and DENV immune rabbit's sera by thrombin and protein A/L affinity columns. We found both human and rabbit ATA bound not only to DENV, human thrombin, but also cross-reacted to human plasminogen (Plg). ATA from both sources could inhibit thrombin activity as determined by chromogenic assay, thrombin time, and fibrin formation. To our surprise, both rabbit and human ATA could also enhance Plg activation in vitro. The anti-thrombotic and pro-fibrinolytic activities of rabbit ATA were further confirmed in vivo by passive transferring into mice. Taken together, these results suggest that the anti-thrombotic and pro-fibrinolytic effects of ATA induced by DENV may contribute to hemorrhage in DHF.

P3.15 B and T cell memory

P3.15.01
***In vivo* imaging of calcium signaling in B cells of mice expressing the genetically encoded YC3.60 calcium indicator**

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B cell signaling especially through the B-cell antigen receptor (BCR) has been studied intensively by using cell lines and purified primary B cells *in vitro*. Upon antigen stimulation, BCR signaling such as calcium mobilization and MAP kinase activation is induced, which is supposed to be crucial for B cell fate such as proliferation, differentiation and apoptosis. To assess calcium signaling in B cells, synthetic calcium indicators such as Fluo-4, Indo-1 and Fura-2 are commonly used. However, they have significant drawbacks, damaging cells and showing a short half-life. It is also difficult to label the cells homogenously *in vivo* using them. To overcome these problems, we employed the genetically encoded calcium indicator YC3.60 for the analysis of B cell signaling. We established transgenic mice with the genetically encoded calcium indicator YC3.60 based on the Cre/loxP system. After crossing with various Cre lines, the YC3.60 reporter mice exhibited robust YC3.60 expression in a wide variety of immune cells. We labeled B cells by crossing YC3.60 mice to CD19-Cre mice. In YC3.60/CD19-Cre mice, most of mature B cells in various lymphoid tissues such as spleen, lymph node, and Peyer's patches expressed YC3.60 without significantly altering B-cell function. After cross-linking of the BCR, calcium mobilization could be induced in spleen B cells from YC3.60/CD19-Cre mice. By *in vivo* imaging, calcium mobilization in B cells was observed in spleen. Thus, with this system, we will be able to validate B cell activation *in vivo* under physiological conditions.

P3.15.02
A triple positive staining to capture B cells expressing anti-tetanus toxoid IgG in human blood

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Aiming to sort B cells expressing anti-tetanus toxoid IgG from human blood that could be used for heavy and light chain amplification and cloning, this work started by the preliminary detection of such cells in 10mL of human peripheral blood. After informed consent, blood was drawn from six volunteers and the mononuclear cells (PBMC) were stained using anti CD19-FITC, anti IgG-APC, and streptavidin-PerCP-Cy5.5, following analysis by flow cytometry. Tetanus toxoid (TT) conjugated to biotin (TT-bio) was included or not in the mixture. Five donors were selected based on their high anti-TT antibody titers: 12.0, 16.1, 13.3, 13.0 and 12.2 UI/mL; a sixth donor did not receive tetanus booster immunization after infant vaccination (titer 1.2 UI/mL). Despite the fact that all donors presented protective anti-TT antibody titers (>0,1 UI/mL), neither donor (1) or (6) showed detectable anti-TT producing B-lymphocytes in the blood. The other 4 donors presented variable responses, expressed as anti-TT B lymphocytes/106 PBMC as 16.13, 36.18, 11.15 and 2.82. The frequency of anti-TT lymphocytes did not correlate with frequency of total lymphocytes in PBMC, which varied between 35 to 62%. The individual variability of the immunological response denotes the importance of testing several donors, even with a small volume of blood. The possibility of detection of the desired cells by the above mentioned triple staining protocol prompted us to drawn a large volume of blood from the donors in order to start the sorting protocols.

P3.15.03

***Ex vivo* analysis of human memory B lymphocytes specific for A and B influenza hemagglutinin by polychromatic flow-cytometry**

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Understanding the impact that human memory Bcells (MBC), primed by previous infections or vaccination, exert on neutralizing antibody responses against drifted influenza hemagglutinin (HA) is key to design more protective vaccines. A major obstacle to these studies is the lack of practical tools to analyze HAspecific MBCs in human PBMCs *ex vivo*. We report here an efficient method to identify MBCs carrying HAspecific BCR in frozen PBMC samples. By using fluorochrometagged recombinant HA baits and vaccine antigens from mismatched influenza strains to block BCRindependent binding, we developed a protocol suitable for quantitative, functional and molecular analysis of single MBCs specific for HA from up to two different influenza strains in the same tube. This approach will permit the identification of the naive and MBC precursors of plasmablasts and novel MBCs appearing in the blood following infection or vaccination, thus clarifying the actual contribution of preexisting MBCs to antibody responses against novel influenza viruses. Finally, this protocol can enable the use of high throughput deep sequencing to analyze changes in the repertoire of HA^+ Bcells in longitudinal samples from large cohorts of vaccinees and infected subjects with the ultimate goal of understanding the *in vivo* Bcell dynamics driving the evolution of broadly crossprotective antibody responses.

P3.15.04

Innate signals control the binary cell fate choice between CD8+ effector and memory T cell differentiation via Notch

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The adaptive immune system must simultaneously remove the acute threat of microbial infections and lay the foundation for immunological memory. To this end, activation of naive CD8 T cells yields both short lived effector cells (SLECs), which provide immediate protection, and memory precursor effector cells (MPECs). This process involves a binary cell fate decision by bipotent precursors and is controlled by external signals. In particular, strong inflammation implies high infectious load and calls for generation of immediately protective SLECs. To unravel the mechanisms governing the cell fate choice between SLECs and MPECs, we focused on Notch, a conserved regulator of binary cell fate decisions. We find that surface expression of this receptor is induced in CD8 T cells by type I Interferons, while expression of its ligands is induced on migratory tissue derived dendritic cells upon viral infection. Genetic loss of Notch in CD8 T cells leads to an inability to generate SLECs, whereas generation of MPECs is increased. Genome wide transcriptome analysis showed that Notch controls expression of almost half the SLEC-specific gene expression program, while repressing a similar fraction of the MPEC program. Notch uses a multipronged mechanism to drive SLEC differentiation, involving induction of SLEC specific transcription factors such as Tbet and Blimp1 as well as enhancement of receptivity to IL-2. Thus, our findings identify Notch as a critical translator of the inflammatory cues, which control the decision of activated CD8 T cells to commit to the effector or the memory fate.

P3.15.05

Optimized fluorescent labeling to identify memory B cells specific for Neisseria meningitidis serogroup B antigens *ex vivo*

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Antigen (Ag)-specific memory B cells (MBC) generate anamnestic responses and high affinity antibodies (Ab) upon re-exposure to pathogens. Attempts to isolate rare Ag-specific MBC for in depth functional analysis at the single-cell level have been hindered by the lack of tools with adequate sensitivity. We applied two independent methods of protein labeling to sensitive and specific ex vivo identification of Ag-specific MBC by flow cytometry: stringently controlled amine labeling, and sortagging, a novel method whereby a single nucleophilic fluorochrome molecule is added onto an LPETG motif carried by the target protein. We show that amine-labeled or sortagged NadA, a major Ag in the meningococcal B vaccine, identify NadA-specific MBC with high and comparable sensitivity and specificity in a mouse model of vaccination. We distinguish NadA-specific switched MBC induced by vaccination from the background signal contributed by splenic transitional and marginal zone B cells.
In conclusion, we demonstrated that protein structural data coupled to sortag-technology allows the development of engineered Ags that are as sensitive and specific as chemically-labeled protein in detecting rare MBC, decreasing the possibility to disrupt conformational B cell epitopes.

P3.15.06

Modulation of heme oxygenase (HO)-1 enzyme activity by metalloporphyrins affects the antiviral T-cell response

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Adoptive transfer of virus-specific cytotoxic T cells (CTLs) can prevent reactivation of latent viruses such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) and have been demonstrated to lead to a sustainable and rapid reconstitution of antiviral immunity. *In vitro* stimulation and expansion of virus-specific T cells is required for clinical applications. In the present study we asked whether modulation of the immunomodulatory enzyme heme oxygenase-1 (HO-1), which is the inducible isoform of enzymatic heme degradation, might have effects on *in vitro* T cell activation. To this end we investigated whether (1) induction by cobalt-protoporphyrin (CoPP) or inhibition by tin-mesoporphyrin (SnMP) can affect expansion of virus-specific CD8+ T cells *in vitro*, (2) modified HO-1 activity modulates proliferation and functional activity, (3) *in vitro* application influences other cell populations within the group of PBMCs (DCs, Tregs, natural killer cells) mediating effects on proliferating T cells, (4) HO-1-modulated antigen-specific T cells would be suitable for the purpose of adoptive immunotherapy. Inhibition of HO-1 via SnMP in peptide-pulsed PBMCs results in a significant enhanced virus-specific $CD8^+$ T-cell proliferation, whereas increase of HO-1 activity by CoPP did not impaired T-cell responses. Transcription and secretion of IFN-γ in response to viral peptides is up-regulated upon inhibiting HO-1. Inhibition of HO-1 has no effect on mDCs and NK cells, but depletion of Tregs and additional application of SnMP multiplies virus-specific T-cell frequencies. Taken together we found, that SnMP-treatment may be suitable for the purpose of adoptive T-cell therapy.

P3.15.07

Pou6f1 is dispensable for mouse T cell development and virus-specific memory CD8+ T cell generation

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Pou6f1 is a POU-domain transcription factor whose paralogs control self-renewal (e.g. Oct-4) and cell fate determination (e.g. Pit-1, Brn3a). It has been shown to be selectively upregulated at the DN3a

to DP stages in thymocytes; and across different models in antigen-specific memory/memory-precursors relative to shorter-lived mature CD8+ T lymphocytes. On these bases, we hypothesized that it is required for maintaining a multipotent and/or differentiation-poised state in T cells. To test this, we quantitatively validated its differential expression in lymphocytes, and generated and characterized conditional (Cre/loxP-based) Pou6f1 knockout mice. Germ-line deletion (EIIa-Cre-driven) resulted in viable and fertile progeny with no obvious morphological or behavioral abnormalities. Mice with an early deletion in thymocytes (Lck-Cre-driven) showed normal frequency and phenotype of thymic populations (DN, DP, SP4 and SP8) and peripheral CD4+ and CD8+ T cells, according to the expression of TCRβ, CD3, and activation (CD5, CD44, CD69), differentiation (CD25, CD27, CD28, CD62L, CD122), survival (CD127) and senescence (Klrg1) markers. Likewise, specific deletion in CD8+ cells (E8I/CD8a-Cre-driven) did not affect either antigen-specific CD8+ T cell effector response (*ex-vivo* OVA-induced IFN-γ synthesis), or memory generation (pentamer binder frequency) and response upon infection with vaccinia-OVA virus. The effects on both memory formation in a competitive adoptive transfer setting and secondary responses are under study. In conclusion, this is the first description of a Pou6f1 knockout mouse. Results to date suggest that Pou6f1 may not be involved in lineage commitment of thymocytes or CD8+ T lymphocytes, or that in the current experimental models its loss is compensated.

P3.15.08
Unique features of protective cytotoxic T cell response during chronic T. gondii infection

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Protective immunity against T. gondii reactivation during the chronic phase of infection relies on the presence of immunodominant antigen-specific cytotoxic T cells. How the immunodominant cytotoxic T cell populations emerge and are maintained compared to subdominant populations is poorly understood. With the recently identified MHCI-restricted T. gondii epitopes, we were able to track various epitope-specific cytotoxic T cell populations throughout the course of infection. We discovered that the immunodominant population: 1) exhibited an unusual kinetics without a contraction phase, partially due to prolonged antigen presentation in the lymphoid organs; 2) maintained the effector functions and phenotypes without exhaustion over a long period of time; 3) was driven by different transcriptional programs in lymph nodes and in the spleen. We will discuss potential factors that cause these unique features of the protective T. gondii-specific cytotoxic T cell population. Our studies describe a novel cytotoxic T cell behavior during chronic protozoan infection that differs from other well-studied persistent infection such as LCMV and HCV.

P3.15.09
Dividing and non-dividing CD27^{low} and CD27^{high} human memory B-cells are differently induced depending on the stimulus

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Protective antibody responses depend on efficient memory B-cells activation. Heterogeneity among responding memory B cells has been shown greater than previously appreciated. According to their proliferating status and intensity of CD27 expression, different subpopulations, endowed with distinct functional characteristics, have been described in memory B-cells stimulated with CpG-ODN and interleukins.
OBJECTIVES: To evaluate the proportion of dividing and non-dividing functionally distinct CD27^{high} or CD27^{low} memory B-cells generated after stimulation with a surrogate T-dependent (CD40) or T-independent (TLR9) stimulus in the presence or absence of IL-21 and/or BCR signaling.
METHODS: B-cells from healthy donors were purified from PBMC by magnetic negative selection. CFSE-labelled B-cells were stimulated with anti-CD40 or CpG-ODN, with or without anti-IgM, in the presence or absence of IL-21. B-cell proliferation and phenotype were evaluated by flow cytometry.
RESULTS: We found that neither anti-CD40 nor anti-IgM alone or in combination induced proliferating memory B-cells. However, CpG-ODN alone induced proliferation, with a higher proportion of dividing CD27^{low} than CD27^{high} memory B-cells.
IL-21 induced equal proportions of non-dividing CD27^{low} or dividing CD27^{low} and CD27^{high} on CD40-activated memory B-cells. In contrast, IL-21 increased dividing CD27^{high} and markedly decreased dividing CD27^{low} on CpG-ODN-activated memory B-cells.
Addition of anti-IgM consistently increased the proportion of dividing CD27^{low} on memory B-cells costimulated with either anti-CD40+IL-21 or CpG-ODN.
CONCLUSIONS: Distinct proportions of dividing and non-dividing CD27$^{high/low}$ memory B-cells are generated depending on stimulus. Given the different functional capabilities of these subpopulations, this has to be taken into account when trying to bias immune responses.

P3.15.10
Distinct memory T- cell-subsets secrete IFN- and IL2 in response to CD4+ T-cell HIV-1 epitopes from a vaccine candidate

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The persistence of functional memory T cell is important to ensure a protective immunity to Human Immunodeficiency Virus (HIV) infection. Recently, we have described 18 HLA-DR promiscuous epitopes from HIV-1 consensus B sequences that have been successfully tested in HLA-DR transgenic mice. To investigate the functionality of the subsets of memory T cells that respond to these peptides among PBMC from HIV-infected patients. 14 healthy donors and 61 HIV-infected patients with CD4$^+$ lymphocytes counts higher than 250 cells/mm3. Six clinical groups were included according to the stage of infection, plasma viral load (VL) and antiretroviral therapy use (ART): long-term non-progressors (LTNP), aviremic under ART (AV-ART), viremic under ART (VI-ART), viremic without ART (VI without ART), recently infected viremic without using ART (VI-RI) and controllers. The cellular phenotype and cytokine production were evaluated by multiparameter flow cytometry. Our peptide set was able to activate central memory (TCM), effector memory (TEM) and highly differentiated effector memory (TEMRA) functional memory subsets that secrete IFN-γ and IL-2 in 100% of the HIV-patient tested. The HIV-1 peptide set also induced memory T lymphocyte subsets proliferation. TEMRA-CD4$^+$IFN-γ$^+$, total TEMRA-CD4$^+$IFN-γ$^+$, TCM-CD8$^+$IFN-γ$^+$, total TCM-CD8$^+$IFN-γ$^+$, total TEM-CD8$^+$IFN-γ$^+$, TEM-CD8$^+$IFN-γ$^+$ and TEMRA CD8$^+$IFN-γ$^+$ frequencies negatively correlated with HIV viral load in viremic patients. These data suggest that these functional memory subsets are important to control the viremia. Our results suggest a functional heterogeneity of T memory subsets in response to the set HIV-1 peptides, which may constitute a new vaccine candidate to HIV infection. Supported by DST/AIDS-Brazilian Program/Unesco

P3.15.11
Higher cytotoxic capacity in peripheral blood of sarcoidosis patients

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BACKGROUND. Sarcoidosis is a granulomatous disease of unknown etiology, mainly affecting the lungs, where greatly increased numbers of CD4+ T cells typically are seen. However, CD8+ T cells can sometimes make up the majority of cells recovered by bronchoalveolar lavage (BAL). Previous studies have extensively studied Th1 cytokine-producing T cells and established their role in the pathological process. Little is however known about cytotoxic cells in sarcoidosis. METHODS: Peripheral blood (from 17 sarcoidosis patients and 11 healthy controls) and BAL fluid (from 19 patients and 4 controls) was obtained. Flow cytometric analysis of cell subsets was performed after staining with antibodies against the surface molecules CD3, CD4 and CD8 as well as antibodies against the

cytotoxic intracellular mediators perforin, granzyme B and granulysine. The PBMC cytotoxic activity was quantified with the 51Cr release assay. RESULTS: Sarcoidosis patients had a significantly higher percentage of both perforin+ ($p < 0.001$) and granzyme B+ ($p < 0.05$) CD8+ T cells in blood. In BAL, however, a tendency in the opposite direction was seen. Higher cytotoxic activity was observed in PBMC from patients with sarcoidosis compared to controls. CONCLUSION: Cytotoxic cells, including CD8+ T cells, may be involved in the propagation or regulation of the inflammatory process in sarcoidosis, but further studies are needed to delineate their role in this disease.

P3.15.12
Interleukin-33 is dispensable for the maintenance of memory CD8 T cells

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Generation and maintenance of effector and memory T cells is tightly regulated by cytokines. We have shown recently that the alarmin interleukin-33 (IL-33) is essential for the generation of a fully functional and potent anti-viral CD8 T cell response against infection with lymphocytic choriomeningitis virus (LCMV). Moreover, IL-33-deprived cytotoxic CD8 T cells express less KLRG1 indicating a reduced frequency of short-lived terminally differentiated effector cells (SLEC), but show an increased expression of IL-7receptor (IL-7R), which is characteristic of memory precursor cells (MPECs) in this model. These results indicate, that memory generation and maintenance might be dysregulated in the absence of IL-33 signalling. Therefore, we infected WT and IL-33receptor (ST2)-deficient mice with LCMV and followed the faith of virus-specific CD8 T cells in the blood over time. Although we found drastic differences in frequencies of these cells and their expression of effector/memory-associated surface molecules at the onset of infection, these differences vanished over time and became undetectable by day 60 post infection. To exclude influence of viral antigen on memory maintenance in the absence of IL-33 signalling, we sorted TCR-transgenic P14 cells from infected WT mice and adoptively transferred them into naive WT or IL-33-deficient recipients. In both hosts, effector P14 cells turned into memory cells with similar kinetics and phenotype. These data indicate a redundant role for IL-33 in the maintenance of anti-viral CD8 T cell memory.

P3.15.13
Phenotypic and functional heterogeneity of circulating CD4+ and CD8+ T cell subsets and their age related changes

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The phenotypic heterogeneity of human CD4+ and CD8+ T cell subsets has been widely reported. Many different surface markers are currently used to identify T cell subsets, resulting in a rather confuse panorama. We analyzed the phenotype of circulating CD4+ and CD8+ T cell subsets in healthy individuals, based on the expression of CD45RO and CD27. Thus, four CD4+ and CD8+ T cell subsets were initially identified: CD45RO+CD27+ (T_{CM}), CD45RO+CD27- (T_{EM}), CD45RO-CD27+ ($T_{naïve/early}$) and CD45RO-CD27- (T_{EF}). The percentage of circulating CD4+ and CD8+ T cell subsets showed a higher frequency of circulating CD4+T_{CM} compared to CD8+ cells, and of CD8+T_{EF} cells compared to CD4+ cells. There was a significant negative correlation between CD4+ $T_{naïve/early}$ and CD4+$T_{EM,\ and\ between}$ CD8+ $T_{naïve/early}$ cells and CD8+T_{CM}. Further analysis confirmed a high heterogeneity regarding the expression CD127, CD62L, CD45RA, CD28 and CD57; the later mainly expressed by CD27- T cells. The functional evaluation of T cell subsets showed that memory T cells (T_{CM} and T_{EM}) were the main responders to the stimulation with anti-CD3 plus anti-CD28 and PPD. Finally, significant age-related changes in the frequency of T cell subsets were associated with a reduction in the frequency of $T_{naïve/early}$ and an increase in the frequency of memory T cells, mainly T_{EM}. Our results corroborate the heterogeneity of CD4+ and CD8+ T cell subsets and their age-related changes; however, their biological meaning requires careful analysis and interpretation.
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P3.15.14
Lung APCs of influenza memory mice challenged with influenza virus contain less virus compared to primary infected mice

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Aim: To study phenotype, function and localization of influenza virus APCs and virus specific memory T cells.
Methods: Mice were infected intranasally with a sublethal dose of live H_1N_1 PR8 influenza virus. After three months animals were challenged with a lethal dose of the homologous virus and three months post challenge T cells were isolated from sacrificed mice. APCs were isolated from naive and immune syngeneic mice at 36 hours post infection and virus RNA was detected by RT-PCR. Purified APCs and T cells were co-cultured *in vitro* and cytokines were determined by immunoaffinity with Gyrolab Bioaffy and with FACS.
Results: After primary infection virus RNA was detected in lungs and in mediastinal lymph nodes but not in spleen or in inguinal lymph nodes. Virus challenge of protected mice showed a low or no PCR signal in lungs and in lymph nodes similar to primary infected mice treated intranasally with homologous influenza immune serum. Challenge of immune mice rapidly induced IFN-γ containing T cells in lung and spleen and stimulation *in vitro* with $CD11c^+$ dendritic cells of virus infected lungs resulted in poor proliferation of immune $CD4^+CD44^+$ T helper cells while retaining a high production of IFN-γ, IL-17 and IL-13. The inhibited proliferation was enhanced by removal of $CD4^+CD25^+$ Regulatory T cells.
Conclusion: Anti-influenza antibodies of immune mice inhibit excess virus uptake by airway associated APC.s without affecting the pro-inflammatory cytokine response of memory T cells in lung and spleen.

P3.15.15
Single cell tracking reveals the segregation of early memory CD8+ T cells from the effector pool by slowing down cell cycle

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Upon an encounter with cognate antigen, naïve T cells undergo extensive cell divisions associated with functional and phenotype change. The kinetics and mechanisms that result in the generation of effector and memory CD8+ T cells have remained controversial. Here we made use of a novel approach to dissect the proliferative behavior of activated CD8+ T cells, that is single cell tracking of cell cycle progression of OVA-specific T cells transgenically expressing the fluorescent ubiquitination-based cell-cycle indicator (FUCCI). The naïve T cells in G0 phase express red fluorescent protein and turn to yellow and green according to progression to S/G2/M. Following infection with OVA expressing influenza virus in vivo, FUCCI/OTI T cells vigorously divided with decreasing red+ cells from 99% to 5% and increasing green+ cells up to 44.6% by day4 post infection. Unexpectedly, red+ FUCCI/OTI cells reappeared at the peak of infection with early memory phenotype. These cells had a history of vigorous prior proliferation indicated by cell trace dye dilution, suggesting they derived from the fast dividing effector T cell pool. Next, we tracked divisions of single cells trapped in microwells. Mathematical modeling revealed strong kinship in cell cycle time of progeny derived from a single cell. Strikingly, in later divisions (generation ≥8), a slow cycling subpopulation detected with smaller cell size and expression of memory markers. Collectively, we report a novel developmental process whereby at the height of the immune response T cells segregate into populations within distinct proliferative histories characteristic of terminal effector and memory T cells.

P3.15.16
Molecular mechanisms for memory B-cell development and function

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Upon immunization with T-cell dependent antigens, antigen-bound B cells proliferate extensively to form germinal centers (GC) in the peripheral lymphoid organs and then differentiate into either long-lived plasma cells (LLPCs) or memory B (Bmem) cells, both of which constitute a B-cell part of the immunological memory. However, molecular mechanisms for the development of Bmem cells remain poorly understood, partly due to the lack of an in vitro model system. Recently, we have established a culture system in which mouse naïve B cells can be extensively propagated on feeder cells expressing CD40L and BAFF to generate GC-phenotype B (iGB) cells undergoing immunoglobulin class switching. The iGB cells after primary culture with IL-4 develop into Bmem cells in vivo that elicit rapid immune responses, whereas after the secondary culture with IL-21, they develop in vivo into LLPCs that produce antibodies in the bone marrow, but not Bmem cells. The tertiary culture without cytokines partially restores the Bmem development and abolishes the LLPC development. Thus, this novel system has enabled in vitro differentiation from naïve B cells into Bmem or LLPC precursors that maturate in vivo, and therefore will be useful to study the mechanisms for the development and function of Bmem and LLPCs. In this workshop, I will discuss newly discovered molecular requirements for the Bmem cell development and function.

P3.15.17
Isolation of mouse B cells and T cell subsets in as little as 15 minutes

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Following an immune challenge, innate and adaptive immune cells undergo a series of orchestrated events culminating in an effective immune response. Within the adaptive branch, effector functions are carried out by antibody producing B cells, and CD4+ and CD8+ T cells that produce cytokines and cytolytic effector proteins which function to coordinate immune responses or directly eliminate infected cells. While most effector cells become senescent and apoptotic, some B- and T cells further differentiate into memory cells that provide long lasting immunity. The signals that regulate these processes have been widely investigated and continued efforts will help construct a definitive model for the role B- and T cells in adaptive immunity. For most of these studies, highly purified cells are required and current methods for isolating these cells from mice require either lengthy protocols or flow based cell sorting. To address these limitations, we have developed new column-free, immunomagnetic cell isolation kits to isolate untouched B cells and naïve and memory T cell subsets in as little as 15 minutes. Purities of up to 98% can be achieved using the manual EasySep™ pour-off method or using the fully automated RoboSep™ cell separator. Isolated cells can be immediately used for downstream assays and are fully functional as assessed by in vitro proliferation and cytokine production assays.

P3.15.18
AID dependent memory response in unimmunized mice

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We constructed a knock-in transgenic mouse model, which allowed the irreversible expression of EYFP in AID expressing B cells after tamoxifen regimen (AID-Cre-EYFP mice). We observed a systemic response in absence of any immunization, with EYFP+ B cells detectable at different frequency in Peyer's patches, peritoneal cavity, spleen and bone marrow up to 6 months after tamoxifen-induced labelling. All these tissues presented different proportion between plasma cells (B220- YFP+) and memory B cells (B220+ EYFP+). Around 60 to 80 % of the YFP+ plasma cells in bone marrow, but also in spleen, were IgA+, whereas the EYFP+ memory B cells were mainly IgM+ in these two tissues. Adoptive transfers with bone marrow (as source of B2 cells) and peritoneal cavity (as source of B1 cells) showed that EYFP+ B cells seemed to have a B2 origin. To fully understand this endogenous response, we are presently analyzing AID-Cre-EYFP mice on a germ-free background in order to evaluate the role of commensal microbiota in the generation of these memory responses. In the same way, we are currently crossing AID-Cre-EYFP mice with FoxP3-DTR mice to determine whether the generation of EYFP+ cells in absence of immunization could have an autoimmune origin.

P3.15.19
In vivo IL-6 inhibition using anti-IL-6 receptor antibodies modulates double negative (CD19+IgD-CD27-) B cells in Rheumatoid Arthritis patients

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Purpose. The role of IL-6 during the differentiation of B cells has been appreciated. Although phenotypic reports on double negative (CD19+IgD-CD27-) memory B cells in autoimmune disease are increasing, precise molecular analyses of their immunoglobulin (Ig) genes are sparse. Therefore, we analyzed the in vivo effect of therapeutic IL-6 inhibition using tocilizumab (TCZ) in patients with rheumatoid arthritis.
Methods. DN B cells were analyzed from RA patients at baseline and 12, 24 and 48 weeks during TCZ treatment phenotypically and using single B cell PCR to study the mutational pattern of Ig- receptors VH genes.
Results. RA patients (n=42) showed a significantly higher percentage ($P<0.0001$) of DN B cells compared to healthy individuals. These cells comprise a heterogeneous mixture of IgA, IgG and IgM expressing cells with clear dominance of IgG+ cells. Analysis of rearranged IgR sequences from healthy donors (n=3) and pre-therapy RA patients (n=7) revealed a comparable but diversified mutational pattern of DN B cells with mutated and non-mutated sequences. Under IL-6R inhibition a significantly reduced mutational frequency in their Ig-receptors at week 12 ($P<0.0001$), 24 ($p= 0.0147$) and 48 ($p= 0.0021$) was observed. In contrast, therapeutic TNF-inhibition did not produce significant changes in the mutational frequency of DN Ig-receptors.
Conclusions. Our data suggest expanded DN B-cells populations in RA which are susceptible to IL-6R inhibition by reduced mutational frequencies of single Ig-receptors. These results indicate that DN B cells have dependence on the IL6/IL6R system for differentiation in vivo which can be modulated by anti-IL6R therapy.

P3.15.20
Characterising PI16-positive T helper cells

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T helper (Th) cells play a major role in protecting the body against pathogens. Any imbalance in Th cell subsets (Th1, Th2, Th17, Th22, Th9 and T follicular helper cell) could lead to autoimmune and inflammatory diseases. Peptidase inhibitor 16 (PI16), also known as prostate secretory protein of 94 amino acid - binding protein, was discovered to be expressed on memory regulatory T cells. This study investigates the expression and function of PI16 on Th cells. In healthy adults, 5-25% of CD4+ Th cells express PI16 with over 90% having a memory phenotype. In contrast, more than 40% of PI16+ Th cells have naive phenotype in patients with scleroderma. Furthermore, PI16+ Th cells have an increased expression of chemokine receptors CCR4, CCR5 and CCR6 compared with PI16- Th cells. Transwell migration assays showed that more PI16+ Th cells migrated towards the CCR4 and CCR6 ligands (CCL17 and CCL20) compared with PI16- Th cells. After 7 day stimulation using CD3 / CD28 beads, with and without Th17 polarizing cytokines, PI16+ Th cells produce more IL-17A and less IFN-g compared with PI16- Th cells. PI16+ Th cells also have a higher expression of ROR-gt compared to PI16- Th cells. The memory phenotype of PI16+ Th cells and high expression of Th17-like chemokine receptors, increased ROR-gt expression and high production of IL-17A suggest that PI16 could play an active role at the site of infection or inflammation. Further studies are ongoing to understand the functional role of PI16 on Th cells.

P3.15.21
Functional genomic characterization of murine CD8+ CD103+ tissue resident memory T cells

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Recent research focusing on memory T lymphocytes suggests the existence of several, highly specialized tissue-resident memory T cell (Trm) subsets instructed to preferentially home to, subsequently become resident in, and rapidly respond upon recall antigen challenge in distinct organ environments.
Although there is consensus that all CD8+ Trm cell subsets are characterized by integrin alphaE (CD103) expression, they are also known to express unique markers in a tissue-specific fashion, depending on their organ residence. Nevertheless, the full spectrum of these markers is unknown, leaving much room to speculation about possible differences between Trm functions in distinct organs.
The aim of this study was a comprehensive enumeration of both consensus and organ specific biomarkers of murine CD8+ Trm cells resident in the small intestine, lung and liver. We show that pure fractions of intact, viable murine CD8+ CD103+ Trm cells can be isolated from these organs using automated tissue processing followed by a two-step MACS sorting method. Using circulating CD8+ CD62L- T effector cells as reference, we also present initial findings obtained from a whole-genome gene expression profiling study describing common features of, and organ-specific differences between these Trm cell subsets.
Our data contribute to a better understanding of memory T cell homeostasis and also may support the further development of recently introduced human therapies targeting T cell-mediated, organ-limited diseases, such as certain forms of transplant rejection, graft versus host disease, some organ-specific autoimmune, or chronic inflammatory disorders.
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P3.15.22
Identification of dominant B & T cell epitopes of E2 glycoprotein:a way towards vaccine preparation for chikungunya

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Introduction: Chikungunya virus (CHIKV) is a worldwide emerging pathogen. CHIKV is an *Alphavirus* that is transmitted to humans by virus-carrying *Aedes* mosquitoes.Growing evidence indicates that the innate immune response plays a role in controlling CHIKV infection.To date formalin inactivated virus vaccine, attenuated vaccine & chimeric vaccine have been developed. The peptide based vaccine concept is based on the identification and chemical synthesis of B-cell and T-cell epitopes having immuno-dominant functions.
Objectives: Selection and chemical synthesis of peptides of E2 protein of chikungunya virus.Competitive immunoassay of selected peptides. Direct binding assay, end point titre & IgG subclass estimation of peptide antisera. In vitro T cell proliferation by thymidine assay.
Methodology: Based on DNA star & tepitope software, peptide sequences were selected & synthesized by Fmoc chemistry. Competitive immunoassay of peptides were performed with E2 protein. Inbred mice were immunized with peptides. Direct binding assays were performed at a fixed dilution. Antibody peak titers were determeined with serial dilution of peptide antisera.
Results: Out of seventeen peptides ten peptides were showing competitive immunoassay shows high end point titre. IgG2a & IgG2b were dominant compared to other IgG subclasses.Three peptide shows higher stimulation index compare to others in vitro T cell proliferation assay.
Conclusion: These B & T cell immune-dominant sequences can be exploited for the development of a subunit vaccine against CHIKV.

P3.15.23
Comparison of four established cell culture conditions to assess antigen-specific memory B cell responses in humans and nonhuman primates

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Effective vaccines should elicit protective primary effector immune responses and immunological memory that is characterized by the ability of the immune system to respond rapidly upon subsequent encounter with a pathogen or antigen. Serum antibody levels usually wane over time after a vaccination series while memory B and T cell responses are long-lived and therefore may play a crucial role in long-term protection. Assessment of serum antibody and memory T cell is commonly employed to evaluate vaccine immunogenicity and the duration of immune response, however the measurement of memory B cell response generated by vaccination has been very limited. In this study, we evaluated four previously published cell culture conditions used for *in vitro* induction of antibody secreting cells, including: 1) PWM+SAC+CpG, 2) PWM+SAC+CpG+IL-10, 3) R848+IL-2, and 4) CD40L+CpG+IL-21. We found the combination of R848 and IL-2 to be optimal for the assessment of tetanus-specific memory B cell response in humans as well as malaria antigen CelTOS-specific memory B cell response in vaccinated rhesus monkeys. The assay was then used to investigate the kinetics of antigen-specific memory B cell response in animals after receiving three doses of the CelTOS vaccine. Peripheral blood memory B cells specific to CelTOS were detected after the 2nd dose and markedly increased after the 3rd dose of vaccine. Our results suggest that a combination of R848 + IL-2 is an optimal culture condition to assess antigen-specific memory B cell response for both humans and rhesus monkeys.

P3.15.24

Evaluation of IgA antibody, Melatonin and Cortisol levels in diurnal and nocturnal colostrum samples from healthy puerperae that delivery by vaginal and caesarean partum

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Human breast milk is rich in maternal leukocytes, immunoglobulins, and hormones, and therefore transfer immunity passively for the newborn. Here we compared the diurnal and nocturnal IgA, melatonin and cortisol levels in colostrum from healthy mothers that delivery by vaginal (n = 11) and caesarean (n = 9) partum. Melatonin levels of colostrum from mothers, who had vaginal delivery, was significantly higher at night when compared with day values (day = 4.1±0.4; night = 39.9±3.3 pg/ml, $P < 0.0001$, paired t-test). Whereas, cortisol levels of colostrum from mothers, who had vaginal delivery, was significantly higher at day when compared with night values (day = 3.0±0.5; night = 1.6±0.2 ng/ml, $P = 0.005$, paired t-test). However, melatonin and cortisol levels in colostrum obtained from mothers, who had caesarean delivery did not show a diurnal variation (day = 20.7±0.7; night = 24.6±2.3 pg/ml and day = 6.2±0.2; night = 5.7±0.5 ng/ml, respectively). On the other hand, no significant difference between diurnal and nocturnal IgA levels from mothers, who had vaginal or caesarean delivery were detected: (diurnal = 2.6±0.3; nocturnal = 2.7±0.9 g/l and diurnal = 3.2±0.5; nocturnal = 3.2±1.3 g/l, respectively). In conclusion, we show that healthy puerperae, who had vaginal delivery, present a significant day/night rhythms of melatonin and cortisol. Furthermore, melatonin and cortisol are inversely related, the peak of cortisol is observed in the morning and the melatonin peak is observed in the night. On the other hand, we observed no rise in diurnal or nocturnal IgA.

P3.15.25

High frequency of T memory stem cells precedes T cell immune-reconstitution following human bone marrow transplantation

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Bone marrow transplantation (BMT) is a potential curative approach for the treatment of haematological malignancies. Before transplantation, a chemotherapeutic conditioning regimen ablates the immune system almost completely. In this scenario, the T-cell immune-reconstitution post BMT is initially dependent on the thymic-independent peripheral expansion of donor and chemotherapy-resistant recipient T-cells. This process requires several months, during which the patients are highly susceptible to infections and disease relapse.

Recent data suggest that T-cells at the early stages of differentiation, and, above all, the self-renewing and multipotent T Stem Cell Memory (TSCM), are endowed with superior T-cell immune-reconstitution capacity in preclinical models. However, whether TSCM are involved in T-cell recovery following immunodeficiency in humans still needs to be defined.

Here we show that cells with TSCM phenotype (CD45RA+CD27+CCR7+CD57-CD95+) are present at very high frequency in the host at 7 days post haploidentical T-repleted BMT. The TSCM preferentially survive cyclophosphamide treatment, employed early post BMT to prevent graft versus host disease. Polychromatic flow cytometry-assisted sorting of T cells subsets combined with TREC quantification revealed that the TSCM derive from the differentiation of donor naïve T cells transferred with the graft. In the following weeks, a gradual repopulation of the T cell compartment by central memory, effector memory and effector cells occurs.

Our data strongly suggest that TSCM preferentially mediate T-cell immune-reconstitution in lymphopenic host. These results examine in depth the human T-cell differentiation process and will have important implications in the development of clinical strategies to improve immune recovery in the post BMT setting.

P3.15.26

Regulation of epigenetic modifications for AID-accession to the IgV-locus by histone acetyltransferase activity of GANP in germinal center B-cells

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Antigen-driven B-cells undergo immunoglobulin (Ig) variable (V)-region somatic hypermutation (SHM) that is initiated by activation-induced cytidine deaminase (AID). We have shown that germinal center-associated nuclear protein GANP forms a functional complex with AID in B-cells. AID is recruited from cytoplasm to the IgV-region locus by the help of GANP. Here, we investigate the molecular mechanism how GANP assists AID-accession to the rearranged IgV-region locus. The histone acetyltransferase domain of GANP is necessary for chromatin modification at the IgV-loci through selective binding to the terminal region of the rearranged exon with VH-DH-JH. GANP is physically interacted with active form of RNA polymerase II (Pol-II) and its stall factor Spt5. Chromatin-bound GANP modulates nucleosome occupancy for AID positioning at the IgV-locus with altered positioning of Pol-II and DSIF, presumably providing ssDNA substrate in the transcription bubbles for AID-mediated IgV-region SHM. These data suggest that GANP modifies the epigenetic status of IgV-region for induction of efficient SHM.

P3.15.27

Expression of TCR transgenic b-chain leads to contraction of T lymphocytes pools with surface phenotypes of effector and central memory cells.

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Naive T lymphocytes in mice can be distinguished from antigen-experienced T cells by different coexpression of activation markers, such as CD44 and CD62L. These markers determine different traffic of T lymphocytes in the organism, but hardly reproduce real antigenic experience of a T lymphocyte. Mechanisms maintaining homeostasis of T lymphocytes with different activation phenotypes remain largely unknown. To investigate impact of transgenic chains of T cell receptor (TCR) on formation of T lymphocytes, their peripheral survival and activation surface phenotypes, we have generated transgenic mouse strain expressing transgenic b-chain of TCR 1D1 (belonging to the Vb6 family) on the genetic background B10.D2(R101). Intrathymic development of T cells in these transgenic mice is not impaired. 70-80% of peripheral T cells express transgenic b-chain and only 20-30% of peripheral T cells express endogenous b-chains. The ratio of peripheral CD4+CD8- and CD4-CD8+ T lymphocytes stays unchanged in transgenic animals. However the percentage of T lymphocytes with the “naive” phenotype CD44-CD62L+ is significantly increased and the levels of effector memory CD44+CD62L- and central memory CD44+CD62L+ T lymphocytes are markedly decreased in both subpopulations. On the contrary, T lymphocytes expressing endogenous b-chains have the surface phenotype of activated T cells CD44+. Thus, we have shown for the first time that pools size of T lymphocytes with different activation phenotypes depends on the structure of T cell receptors.

P3.15.28
Modulation of mTOR by Respiratory Syncytial virus and its contribution to CD8 T cells memory differentiation

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Respiratory Syncytial Virus (RSV) is the most common cause of serious lower respiratory tract disease in infants and is a high priority for vaccine development. RSV-specific adaptive immune responses did not confer protection and the infection suppresses lung memory CD8 T cells. mTOR exhibits immunostimulatory effects on memory CD8 T cells. Our objective was to evaluate if RSV can modulate mTOR on CD8 T cells contributing to diminish memory T cell generation and protection. We found the UV inactivated RSV phosphorylated mTOR on Human PMBC at Ser2448 5-fold higher than the control, but it not altered the phosphorylation of Ser2481 and Thr2446. mTOR activation by RSV was completed inhibited using rapamycin, but it was partially inhibited using a PI3K inhibitor. TLRs agonists can activate mTOR. RSV F protein interacts with TLR4 and RSV G protein antagonizes the effects of RSV F protein. We found that RSV F protein slight stimulated mTOR phosphorylation and RSV G protein decreased mTOR activation. We evaluated memory CD8 T cells phenotype cultured with RSV for 4 days plus rapamycin during 24hs. The frequency of CD8+CD122+CD127+ and CD8+KLRG1^{Low}+ T cells increased around 3-fold when mTOR was inhibited comparing to cells culture with only the virus. The results demonstrated that RSV modulated mTOR activation, and it was partially dependent on TLR4 stimulation and PI3K signaling. The modulation of mTOR by RSV is probably implicated on the generation of memory CD8 T cells since the phenotype of these cells changed in the presence of rapamycin.

P3.15.29
Tsc2 controls the generation of virus-specific memory CD8 T cells

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Inhibition of the mTOR pathway with the pharmacological inhibitor rapamycin, can enhance the formation of virus-specific memory CD8 T cells. mTOR signals through two separate protein complexes, mTORC1 and mTORC2 but the role of each in CD8 T cell differentiation remains unclear. Activation of mTORC1 is inhibited by the Tsc1/2 protein complex, and genetic deletion or inactivation of Tsc1/Tsc2 leads to hyperactivation of downstream mTORC1 targets. Therefore, we sought to characterize the role of Tsc1/2-mTOR signaling in the formation of memory CD8 T cells. We attenuated Tsc2 signaling in CD8 T cells by crossing mice that express a dominant negative form of Tsc2 with OT-I transgenic mice (Tsc2-DN OT-I). We report that disruption of Tsc2 lead to impaired generation and/or maintenance of memory CD8 T cells. An increased proportion of Tsc2-DN OT-I effectors expressed KLRG1 compared to wild-type OT-I cells. Prior to adoptive transfer, we observed an increase in naïve CD44hi CD8 T cells in Tsc2-DN OT-I mice. Earlier studies reported that Tsc1-deficient mice have decreased numbers of naïve CD8 T cells and increased frequency of CD44hi expression that could be reversed with prolonged rapamycin treatment. However, rapamycin was unable to rescue naïve T cell survival. Acute rapamycin exposure selectively inhibits mTORC1 but prolonged treatment can also disrupt the assembly of mTORC2. Moreover, while Tsc2 negatively regulates mTORC1, it can bind to and positively regulate mTORC2. Taken together these data suggest the possibility that CD8 T cells rely on mTORC1 and mTORC2 signaling to control effector and memory differentiation respectively.

P3.15.30
Memory B Cell and Plasma Cell Development in Response to Vaccination

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Successful vaccination relies almost completely on the long-term production of high affinity, neutralising antibody. The plasma cells secreting this antibody typically originate in germinal centers, transient structures that also give rise to recirculating memory B cells, which in turn can supplement the number of plasma cells if required. The rate with which B-lineage cells emigrate from the GC, the duration of the migration period and the capacity of these cells to survive in a competitive environment will ultimately determine whether an immunization is successful.
We have examined factors considered important in determining attributes of humoral memory. For memory B-cells this has included the relationship between antigen dose and memory frequency, GC-origin and persistence, and the requirements of particular survival proteins during memory development. For plasma cells, the study has included analysis of their maturation, turnover in relation to production and identifying the relationship between external survival factors, survival and the survival program ultimately enacted, including the identification of the survival proteins required for plasma cell persistence. While these studies are yet to provide an holistic explanation of immunological memory, they do provide insight into its formation and maintenance, suggesting how outcomes favourable to vaccination may be achieved.

P3.15.31
Newly identified MHC class I epitopes from common human Adenovirus serotypes to improve diagnostic and therapeutic options in adoptive T-cell therapy

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The adoptive transfer of donor-derived T lymphocytes from adenovirus (ADV)-seropositive donors can safely and effectively reduce or prevent the clinical manifestation of ADV in patients with low or absent frequencies of ADV-specific T cells. So far only a few peptides derived from the major capsid protein hexon have been identified as immunodominant targets. To increase the number of immunogenic targets, more intensive ADV epitope screening is essential.
Candidate viral target epitopes from the hexon, penton, fiber and E3 proteins of the predominant serotypes 1, 2, 5 and 31 in frequent HLA alleles (A*01, 02, 03 and B*08) were preselected by reverse immunology. 27 candidate epitopes were identified and the respective nonamer peptides were synthesized. The candidate peptides were verified by IFN-γ ELISPOT and pMHC multimer staining using cells from 56 healthy donors and 9 patients with ADV infection. ADV-specific T cells against the preselected peptides were further analyzed in phenotype and function.
Functional active CD8+ T cells specific for 5/27 peptides were detected. Among these peptides, A*02_Hexon$_{TLL}$ and A*01_Penton$_{STD}$ elicited significant IFN-γ secretion in donor and patient T cells. 64% of healthy A*02-positive donors showed A*02_Hexon$_{TLL}$-positive memory T cells, while in 53% of A*01-expressing donors A*01_Penton$_{STD}$-specific T cells were found. The immunogenicity of these epitopes could be confirmed in ADV-infected patients.
In conclusion, the newly described epitopes allow multimer staining of ADV-specific T cells to follow the clearance of ADV in patients after HSCT and represent promising candidates for adoptive immunotherapy, making this approach applicable for a broader patient cohort.

P3.15.32
Histamer-based selection and separation of functionally active ADV-specific CD8+ T lymphocytes

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Multimers of soluble peptide-major histocompatibilty complexes (pMHC) allow a specific visualization, phenotype characterization and reversible isolation of antigen-specific T cells from *ex vivo* samples. Adoptive transfer of antigen-specific T cells sorted by pMHC multimers is an effective therapeutic strategy for treatment of patients with malignancies or infectious diseases after transplantation.
The reversible pMHC Histamer technology was developed enabling a specific detection and isolation of antiviral T cells from peripheral blood mononuclear cells. The HLA-A01/ADV5_Hexon Histamer was generated by coupling 6xHis-tagged pMHC molecules onto cobalt-magnetic beads. The specificity and sensitivity of the magnetic bead-based Histamer was evaluated by flow cytometry. Sorting of ADV-specific CD8+ cytotoxic T cells (CTLs) was performed by immunomagnetic separation, followed by the monomerization of the pMHC Histamer in the presence of L-histidine. Sorted T cells were analyzed in phenotype and function.
The reversible Histamer showed high specificity and sensitivity (up to 99.5%). Antigen-specific T cells were isolated by this technology with a high purity of up to 99.6%. A rapid and complete disassembly of the T-cell surface-bound pMHC Histamer followed by the subsequent dissociation of the pMHC monomers from CD8+ CTL receptors was achieved using 100mM L-histidine. The function of antiviral T cells enriched by Histamer staining did not differ from CTLs induced by standard T-cell assays.
This reversible T-cell staining procedure preserves the functionality of antigen-specific T cells and can be adapted to GMP conditions. The pMHC Histamer technology offers full flexibility and fulfills all requirements to generate clinical grade T lymphocytes.

P3.15.33
Evaluation of B cell subpopulations over time upon immunization against Streptococcus pneumoniae in HIV-1 patients - A 48 week study

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Alterations of B cell population and subsets have been confirmed during HIV infection. Ongoing HIV replication disrupts this cell population causing reduced memory and reduced naïve resting B cells leading to impaired responses to vaccines. To what extent antiretroviral agents reverse these changes remains uncertain. Evaluation of memory B cells is important in determining the capacity of the immune system in producing functional antibodies. Aim of this study was to detect changes in B cells over time post vaccination with the 23-valent polysaccharide pneumococcal vaccine in a cohort of 66 HIV-1 patients with preserved CD4+ T cell counts (>350 cells/μL). Patients were divided into treated and antiretroviral naïve. Frequency of total B cells and memory B (CD21highCD27+) cell was higher in successfully treated patients, though parallel profiles were described over time. Similar expansion was revealed in resting memory (CD27intCD21high) B cells. IgM memory (CD19+CD27+IgMhigh)B cells fluctuated in a resembling way during time irrespective of antiretroviral intake. Activated mature B cells (CD20+CD21lowCD27+) were higher in HAART naïve patients and their decline differed significantly after 4 weeks (p=0,018) and 24 weeks (p=0,035). These facts are consistent with few previous studies which suggest that loss of memory B cells cannot by restored by antiretroviral therapy since immune impairment might occur early after infection with the HIV. Memory B-cell subpopulations might predict the risk of pneumococcal disease more accurately than antibody levels.

P3.15.34
Blockade or deletion of FcγR2B (CD32) prevents FVIII-specific memory B cells response in Haemophilia A mouse model

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Formation of inhibitory antibodies to FVIII is a frequent and severe complication of replacement therapy in haemophilia A. Cross linking of B cell receptor (BCR) and FcγR2B on B cells inhibits BCR signalling leading to a suppressed antibody production. Here, we investigate the role of FcγR2B to modulate FVIII-specific memory B cell response using $F8^{-/-}$ single deficient (B6;129S4-*F8*^{tm2Kaz}/J) and $F8^{-/-}$FcγR2B$^{-/-}$ double deficient mice (B6;129S4-*F8*^{tm2Kaz}/J/B6;129S4-Fcγr2b^{tm1Ttk}/J). Anti-FVIII antibody titres and initial amounts of FVIII-specific splenocytes were similar in $F8^{-/-}$FcγR2B$^{-/-}$ mice compared to $F8^{-/-}$ mice after intravenous exposure to 4 weekly doses of 80 or 400 IU/kg human FVIII. In contrast, *in-vitro* differentiation of FVIII-specific memory B cells into antibody secreting cells (ASCs) was diminished in splenocytes from $F8^{-/-}$FcγR2B$^{-/-}$ mice upon FVIII re-stimulation over 6 days. This effect could be mimicked by blocking FcγR2B using monoclonal antibodies (mAb) or $F(ab)_2$ fragments against CD16/32 on splenocytes from immunized $F8^{-/-}$ mice thereby confirming the memory B cell defect seen in $F8^{-/-}$FcγR2B$^{-/-}$ mice. Staining with caspase 3 antibodies indicated increased rates of B cell apoptosis when FcγR2B was blocked by mAb during FVIII re-stimulation. Transfer of B220-depleted but comprising FcγR2B expressing antigen presenting cells (APCs) did not restore FVIII-specific memory B cells function in $F8^{-/-}$FcγR2B$^{-/-}$ mice. In conclusion, FcγR2B plays a crucial role for the FVIII-specific recall response, possibly by preventing apoptosis of memory B cells during antigen re-stimulation.

P3.15.35
CD8 T cell effector and memory fate decisions are differentially regulated by CD27 and 4-1BB costimulation

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Much of our knowledge of memory CD8 T cell generation has come from studies of immunity to viruses and bacteria. Translating such knowledge into universal vaccination strategies, however, has yet to be achieved. Here we have analyzed the CD8 T cell response elicited by two experimental vaccines comprising a peptide/protein antigen and an agonist that delivers a costimulatory signal via CD27 or 4-1BB. Although both vaccines primed a comparable CD8 T cell response, their capacity to generate memory differed, an effect that occurred independently of CD4 T cell help. Priming with 4-1BB agonists generated a long-lived memory cell pool, whereas memory T cells generated using CD27 agonists were short-lived. T cell transfer experiments showed that continued stimulation through CD27 or 4-1BB beyond the primary response was not required for these differences, suggesting that the effects on memory were due to differences in early activation events. Consistent with this notion we demonstrated that CD27 triggering during priming preferentially enhanced IL-2 secretion by CD8 T cells. Furthermore, CD27 activation of CD8 T cells resulted in prolonged expression of CD25 and higher expression of perforin, gp49b and the transferrin receptor, indicative of sustained IL-2 signaling and increased effector cell differentiation. Neutralizing IL-2 during priming did not affect CD27-mediated T cell expansion but had a detrimental effect on perforin expression and caused accelerated effector cell death. Our study, therefore, demonstrates that the balance between vaccine-induced effector and memory CD8 T cells can be manipulated by targeting different costimulatory receptors.

P3.15.36
Analysis of factors that induce memory T cells

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Naïve T cells recirculate between blood, lymph ducts and secondary lymphoid organs. Consequently, it should be different time that required for Ag-specific T cells, which are located in the various tissues, to populate and enter the draining lymph nodes in late time of an immune response were primed in different environments and preferred to differentiate into memory T cells. We have investigated the kinetics of both OVA specific TCR Tg (OT-1 and OT-2)T cells stimulated earlier (early-stimulated T cells)and later in an immune response (late-stimulated T cells). To ascertain above studies, naïve T cells isolated earlier from lymph nodes of OT-1/CD45.2+ mice and OT-2/CD45.2+ mice were injected i.v. into CD45.1+/CD45.2+ host mice. For immunization of mice, GM-CSF induced bone marrow derived DCs were incubated with OVA protein and LPS, and were injected s.c. into host mice. The early-stimulated T cells were developed by adoptive-transfer of Tg T cells into host mice before Ag-pulsed activated DCs were injected and the late-stimulated T cells were developed by adoptive-transfer of Tg T cells into mice 5-7 days after injection of Ag-pulsed activated DCs. The late-stimulated T cells seemed to divide slower and proliferate less than the early-stimulated T cells. Particularly, CD4+ T cells stimulated in late time found to have little-cell division. Contraction phase of the late-stimulated T cells happened slowly. These results suggest that the timing of recruitment of T cells into the draining lymph nodes affects T cells-fate that are influenced by different environments, i.e. co-stimulation molecules, soluble factors, APCs.

P3.15.37
Identification of T-lymphocyte responses and long-term immunological memory in survivors of ebolavirus infection

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Ebolaviruse (EBOV) causes hemorrhagic fever disease in humans and non-human primates, with high case-fatality rates and there is currently no licensed vaccine or approved specific treatment for human use against EBOV infection. An important parameter for vaccine development is the induction of long-term immunologic memory. To define specific elements of durable immunity that might be relevant to vaccine-mediated protection, we investigated the persistence and quality of EBOV-specific T-cell immune responses in two survivors from the Kikwit, Zaire species EBOV (ZEBOV) hemorrhagic fever outbreak, 11 years after resolution of their symptoms. Here we show for the first time, long-term persistence of ZEBOV-specific $CD4^+$ and $CD8^+$ T-cell memory and identified T-cell epitopes targets in survivors of infection. Antigen-specific $CD4^+$ and $CD8^+$ T cells comprised mainly central memory-like ($CD45RO^+$ $CD27^+$), and not terminally differentiated ($CD57^-$) cells. Furthermore, these cells exhibited a cytokine-producing profile (IL-2, IFN-γ and TNF-α) characteristic of polyfunctional T-cells. Finally, we define HLA-A02 and -A24 restricted epitopes on ZEBOV GP, the protein target of gene-based preclinical vaccine candidates. These results demonstrate that long-term immunity to ZEBOV is achievable in humans and provide a framework for rational vaccine design.

P4.01 Immunity to virus infection

P4.01.001
T-and B-cell responses to human rhinovirus infection

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Background: The infection of existing and also emerging rhinoviruses (HRV) pose severe health risks for patients with asthma or chronic obstructive pulmonary disease and is the leading cause of exacerbation of airway diseases. Here, we studied the effect of HRV1B, HRV14, HRV16 and HRV29 infection to human peripheral blood mononuclear cells (PBMC-s). We hypothesize that HRV activate inflammatory cells, what might be the reason for airway dysfunction during asthma exacerbation.
Methods: We infected PBMC-s with four different HRV-s. Using assays of thymidine incorporation, flow cytometry, in-situ hybridization, immunofluorescence, imagestream, multiplex bead-based immunoassay and qPCR, we measured PBMC activation, rhinovirus expression, cell proliferation, morphology and death rate.
Results: Different serotypes of HRV induced proliferation of plasmablasts (CD19+, CD27++ and CD38++), but not the CD4(+) and CD8(+) T-cells. Cytokine production was more characteristic to innate immune response with elevated IL6 and IL8 production. UV inactivation did block the rhinovirus effect. We also found that from CD20+ B-cells could be infected by HRV1B in vitro. We developed the in-situ hybridization method for detection of HRV1B, HRV16 and HRV29 with combination of immunofluorescence staining for aCD20.
Conclusion: Rhinovirus has the unique ability to bypass antigen presentation and directly infect human B-cells and activate human plasmablasts. This could explain the strong association of rhinovirus with exacerbation of airway diseases.

P4.01.002
Tax-1-mediated HTLV-1 replication and NF-kB activation is inhibited by the MHC-II transactivator CIITA

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Human T-cell Lymphotropic Virus type-1 (HTLV-1) is the causative agent of an aggressive malignancy of CD4+ T lymphocytes. Many evidences have shown that constitutive activation of NF-kB pathway by Tax-1 is crucial for T-cell transformation.
We demonstrated that CIITA, the master regulator of MHC class II gene transcription, inhibits HTLV-1 replication by blocking the function of the viral transactivator Tax-1. Interestingly, CIITA suppresses also Tax-1-mediated activation of the NF-kB pathway. CIITA interacts with both Tax-1 and RelA and inhibits the recruitment of RelA into Tax-1-containing nuclear bodies. It is known that both Tax-1 and Tax-2 stimulate the classical NF-kB pathway, whereas only Tax-1 induces the non-canonical NF-kB pathway. We found that CIITA inhibits also Tax-2-mediated NF-kB activation demonstrating that it acts by suppressing at least the classic NF-kB pathway.
Future studies will be focused to define the minimal region of CIITA inhibiting NF-kB activation by Tax-1 and to elucidate which step of NF[[Unsupported Character - Codename]]-KB signaling pathway is targeted by CIITA.
All these findings suggest that CIITA, a viral restriction factor against HTLV-1 replication, may also counteract Tax-1 transforming activity.
Thus, assessing the molecular basis of CIITA-mediated Tax-1 inhibition may be important in defining new strategies to control HTLV-1 spreading and oncogenic potential.
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P4.01.003
Detection of the autoimmunity in primary Epstein-Barr virus infection by indirect immunofluorescence

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Introduction: Epidemiological data suggest that the Epstein-Barr virus (EBV) is associated with several autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis. The aim of this study was to investigate the autoantibodies in primary Epstein-Barr virus infection by indirect immunofluorescence assay (IFA).
Methods: Sera from 32 subjects (F/M=14/18, median age 21±9 years) with primary acute EBV infection were analysed using IFA for anti nuclear antibody (ANA). The sera were characterized by the presence of anti-early antigen (anti-EA) seropositivity, both IgM and IgG types, and by the absence of anti-Epstein Barr nuclear antigen-1 antibodies (anti-EBNA-1) and with the negativity of IgG avidity. ANA was detected by IFA. Serum samples were processed in dilution of 1:100 using HEp-2010/liver biochip (Euroimmun AG, Germany) and conjugated with specific anti-human IgG. The fluorescence intensity was scored at x 400, semi-quantitatively from 1+ to 4+ relative to the intensity of the positive (4+) and negative control.
Results: Of the 32 individuals investigated, the screening by immunofluorescence on Hep-2010 cells revealed 12 ANA-positive sera (37.5%), with speckled, homogen, nucleolar and cytoplasmic fluorescence patterns. The percentage of positive sera was 5/12 (41.6%) in males and 7/12 (58.3%) in females.
Conclusion: Due to the limited sample, the present study should be considered as a preliminary study. Further researches should continue to investigate hypotheses of EBV as a causative risk factor in autoimmunity.

P4.01.004
Human cytomegalovirus UL141 targets the TRAIL death receptors to inhibit host NK cell responses

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Human Cytomegalovirus (HCMV) is the leading infectious cause of congenital malformation and a major cause of mortality and morbidity in the immunocompromised. Death receptors (DRs) of the TNFR-superfamily make a major contribution to antiviral immunity by promoting apoptosis and regulating immune homeostasis during infection. Consequently, signalling through DRs constitutes a key target for viral immune evasion functions. We have identified the HCMV UL141 glycoprotein as necessary and sufficient to restrict cell surface expression of the TRAIL Death Receptor 2 (TR-2). UL141 binds TR-2 to promote its intracellular retention and desensitises cells to apoptosis mediated not only by soluble TRAIL but also to TRAIL expressed on the surface of NK cells. NK cells play a critical role in control of viral infection in the immunocompetent host. UL141 was originally identified as a potent inhibitor of NK cell function by sequestering the NK-activating ligands CD155 and CD112 in the ER, thereby inhibiting NK cell degranulation mediated through ligation of the activating receptor DNAM-1. Here we show that in the context of HCMV infection, sequestration of TR-2 by UL141 is also able to inhibit NK cell mediated cell death and highlights UL141 as a pleiotropic inhibitor of NK activation and effector function.

P4.01.005
ERAP deficiency leads to absence of anti-influenza immune response to known immunodominant HLA-B27 peptide

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The role of HLA-B27 in modulating host response to infection is undefined, yet B27 confers susceptibility to arthritis. Immune response to viral infections is characterized by immunodominance (ImDc). Defining factors contributing to ImDc is difficult due to multiple MHC-I allele co-expression in humans and mice. To overcome this limitation, we generated human MHC-I transgenic (Tg) mice deficient for endogenous mouse MHC-I molecules (i.e., DKO) and express only one human MHC-I allele. To assess whether co-expression of additional MHC-I alleles influences the pattern of anti-flu CTL epitope recognition and ImDc, novel double and triple MHC-I Tg mice were established on a DKO. In flu-infected, double Tg A2/B7 or A2/B27 mice, IFN-γ ELISpot assays with the flu epitopes A2/M1.58-66 and B7/NP418-426 or B27/NP383-391 showed specific recognition of both peptides by both alleles respectively. In contrast, in flu-infected B7/B27 Tg mice a significantly reduced NP383-restricted CTL response was detected while there was no change in the response level of NP418-restricted CTL. Subsequent flu-specific studies revealed that co-expression of B7 and B27 is associated with i) a partial deletion of Vβ8.1+ B27/NP383-restricted CD8+ T cells and ii) a failure of Vβ12+ CD8+ T cell expansion following flu infection in B7/B27 Tg mice. Further studies with flu-infected ERAP deficient B27/ERAP and B7/B27/ERAP mice revealed complete ablation of the B27/NP383-391 CTL response indicating importance of ERAP in B27 peptide generation. The selective deletion of B27-restricted T cells has important implications for models defining the role that HLA-B27 plays in susceptibility to reactive arthritis and ankylosing spondylitis.

P4.01.006
NF-kappaB1 is required for virus-specific CD8+ T cell responses, and prevents excessive inflammation and lethality during chronic infection

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Background: The NF-kappaB family regulates numerous cellular functions that vary depending on cellular context and specific subunit composition. In particular, inflammation and immunity are broad processes for which different NF-κB dimers may serve opposing roles, the intricacies of which are slowly being unraveled. NF-kappaB1 knock-out (p50-/-) mice were previously shown to display no overt haematopoietic developmental nor functional abnormalities, yet given the potential for diverging roles of p50 in immune responses, we hypothesised that p50 plays an important chronic during chronic immune activation. The aim of our study was to investigate the role of p50 in chronic persistent infection.
Design: We chronically infected p50-/- mice with lymphocytic choriomeningitis virus docile strain and examined immunological and virological outcomes. Results: Upon infection p50-/- mice showed a profound defect in virus specific CD8 T cell expansion and function. The blunted adaptive response observed in p50-/- animals is associated with an overwhelming, non-specific and lethal inflammatory reaction. Intriguingly, we found that naïve p50-/- mice exhibit a previously unreported neutrophilia, and that both neutrophils and macrophages from p50-/- mice display aberrant pro-inflammatory cytokine profiles following LCMV infection, which is likely responsible for mortality in these animals. Conclusion: Although p50 is a component of the pro-inflammatory NF-kappaB dimer, its ablation also disrupts many regulatory and anti-inflammatory processes. Our findings demonstrate that p50 is a crucial regulator of inflammatory responses during chronic infection. This has important implications for infections such as HIV-1 that are associated with immune activation and counterproductive inflammatory responses.

P4.01.007
CD56+CD3+ T cells in relation to CMV infection in normal subjects and kidney transplant patients

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Background: CD56+CD3+ cells are a small subset of T cells of unknown function but with cytotoxic capacity following activation *in vitro*. In preliminary work, we found that levels were higher in normal subjects positive for cytomegalovirus (CMV) than in CMV negative subjects. Kidney transplant patients are particularly prone to reactivation of CMV infection which, as well as causing acute disease, may play a role in triggering the rejection process.
Aims: This work aims to investigate the role of CD56+CD3+ T cells and their response to CMV and other pathogens, and their possible role in triggering the immune system toward the transplanted organ.
Material and Methods: Phenotypic studies of memory and activation markers of CD56+CD3+ in normal CMV+ and CMV-. Functional analysis, consisting of the response of these cells to stimulation with CMV and SEB antigens will be tested by measuring cytokine production and cell proliferation (CFSE labelling).
Results and Conclusion: CD56+CD3+ cells are found to be higher in both healthy and post-transplant patients the same as has been found in the preliminary study. Several markers such as CD8, CD4, NKG2C and CD45RO were significantly found to be higher in CMV+ than CMV-. Moreover, CD161, CD28, CD62L and CD127 were significantly found to be lower in CMV + than CMV -. When cytokines production were compared between CMV+ and CMV- donors, IFN-γ and TNF α are significantly produced more in CD3+CD56+ cells from CMV+ than CMV-. These results indicate that CD3+CD56+ cells play a major role in response to CMV infection.

P4.01.008
Involvement of NK cells in the acute lung injury of H1N1 influenza infection

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The outbreak of 2009 pandemic swine-origin H1N1 influenza virus represented a major threat to global health. Acute lung injury, characterized by pulmonary edema, capillary leak, and lymphocyte infiltration was main cause of death. NK cells played important roles in the pathogenesis not only by direct cytotoxicity, but also by rapidly producing proinflammatory cytokines. SAα2,3Gal- and SAα2,6Gal, H1N1 virus receptors, were expressed on epithelial cell of trachea, bronchus and alveolus. Following virus challenge, the body weight was loss from 1day-post-infection (dpi) and decreased only 67% of initial weight on 7 dpi. Meanwhile, survival rate was decreased to only 27% on 7 dpi. A large number of infiltrating lymphocytes were recruited to perivascular and parenchyma areas. Total lymphocytes in lung tissue peaked at 2 dpi, and were 4-fold compared with normal control. The ratio of NK cells was increased on 1 dpi; and 80% of them were rapidly activated. The number of NK cells also rapidly rose to 3-fold of control. Especially, the activated NK were 21-fold of control. Total karyocytes in BALF increased and peaked at 3 dpi. The ratio of total NK cells in BALF was continued increase, and 92% of NK cells were activated on 2 dpi. The number of total NK cells and activated NK cells in BALF peaked at 3 dpi. Proinflammatory cytokines and chemokines, such as IL-6, TNF-α, IL-17, KC, MIP-1α, RANTES, were increased in serum and BALF. Our data demonstrated NK cells and proinflammatory cytokines played critical role in lung injury during H1N1 virus infection.

P4.01.009
Human cytomegalovirus viral IL-10 polarizes monocytes towards a deactivated M2c phenotype to repress host immune responses

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Several human cytomegalovirus (HCMV) genes encode products that modulate cellular functions in a manner likely to enhance viral pathogenesis. This includes UL111A, which encodes homologs of human interleukin-10 (hIL-10). Depending upon signals received, monocytes and macrophages become polarized to either classically activated (M1 pro-inflammatory) or alternatively activated (M2 anti-inflammatory) subsets. Skewing of polarization towards an M2 subset may benefit the virus by limiting the pro-inflammatory responses to infection and so we determined whether HCMV encoded viral IL-10 influenced monocyte polarization. Recombinant viral IL-10 protein polarized CD14+ monocytes towards an anti-inflammatory M2 subset with an M2c phenotype, as demonstrated by high expression of CD163 and CD14, and suppression of MHC class II. Significantly, in the context of productive HCMV infection, viral IL-10 produced by infected cells polarized uninfected monocytes towards an M2c phenotype. To explore mechanism, we assessed the impact of viral IL-10 on heme oxygenase 1 (HO-1), which is an enzyme linked with suppression of inflammatory responses. Polarization of monocytes by viral IL-10 resulted in upregulation of HO-1 and inhibition of HO-1 function resulted in a loss of capacity of viral IL-10 to suppress TNF-α and IL-1β, implicating HO-1 in viral IL-10 induced suppression of pro-inflammatory cytokines by M2c monocytes. In addition, a functional consequence of monocytes polarized with viral IL-10 was a decreased capacity to activate CD4+ T cells. This study identifies a novel role for viral IL-10 in driving M2c polarization which may limit virus clearance by restricting pro-inflammatory and CD4+ T cell responses at sites of infection.

P4.01.010
Quantitation of mRNA cytokine levels in ferrets following influenza infection

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After infection with influenza virus, a rapid inflammatory response is generated by the innate immune response, followed by an antigen-specific adaptive immune response that uses different effector mechanisms to help clear the virus/infection. Analysis of cytokine profiles can enable characterization of this immune response at the sites of infection. Highly pathogenic influenza viruses typically induce altered cytokine profiles compared to low pathogenic viruses (Cameron J Virol 2008, 82:11308). Thus cytokine profiling may provide valuable information on the level of the immune response as well as give insights into virulence and pathogenesis of different influenza viruses.

In vivo analysis of novel human influenza viruses often uses the ferret model as they can easily be infected with most human influenza viruses and display similar symptoms as humans. The aim of this study was to develop a high-throughput real time RT-PCR TaqMan assay to quantify a full range of cytokine mRNA profiles in the respiratory tract of influenza-infected ferrets.
Ferret-specific primer/probe sets to IL1a, IL1b, IL2, IL4, IL6, IL8, IL10, IL12p40, IL17, MCP1, TNFa, IFNg, IFNa, IFNb were designed to target cytokines of the innate and adaptive immune systems. These assays were optimized to maximize sensitivity and specificity and the multiplexing of primer/probe sets was also examined. The resulting assays now enable us to analyse the ferret's immune response during any influenza infection. Work is ongoing to determine if one or more of these assays can be used to measure the severity of an infection and the level of these cytokines following vaccination.

P4.01.011
Reproducibility of Pentamer and Streptamer staining of CMV-specific CD8+ T-cells: defining the optimal multimer concentration and incubation conditions.

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Pentamers (PM) and Streptamers (ST) are widely used to detect CMV-specific CD8+T-cells. However, the optimal staining conditions remain to be established. We analysed different concentrations of PM/ST and temperatures for PM staining, and assessed the reproducibility of both techniques.
PBMCs were isolated from six HLA-A*02:01 CMV-seropositive healthy donors. After multimeric staining of $1x10^6$ PBMCs, anti-human CD3/CD8/CD45 were added. Cells were acquired in a FACSCalibur and CMV-specific T-cell percentage was examined.
PM/ST titration: For PM, 2.5/5/10 µl of HLA-A*02:01/PMpp65$_{495-503}$ were incubated with PBMCs for 10 minutes at room temperature (RT). For ST, 0.75 µg PE-labelled Strep-Tactin were mixed with 0.25/0.5/0.75/1 µg of HLA-A*02:01/STpp65$_{495-503}$ to form the ST complex. From those, 0.1/0.2/0.3/0.4 µg were incubated with PBMCs for 45 minutes at 4ºC.
PM staining temperature: PBMCs were stained with 5µl of HLA-A*02:01/PMpp65$_{495-503}$ for 30 minutes at 37 ºC, 10 minutes at 4ºC, or 10 minutes at RT.
PM/ST staining reproducibility: Per donor, six tubes per multimer (PM/ST) were incubated with PBMCs. The percentage of variance associated with the technique was estimated using a random effects model, separately for PM or ST.
The best results for PM were obtained using 5µl (median=0.56%;IQR=0.41-0.99) during 10 minutes at RT (median=0.48%;IQR=0.13-0.93), while 0.2µg of ST complex gave the best results for ST (median=0.51%;IQR=0.44-0.88), although statistical significance was not reached (Mann-Whitney test, p>0.05). Furthermore, the percentage of variance showed low values of variability (PM:0.3%;ST:0.7%), proving the reproducibility of both techniques.
In summary, staining conditions do not seem to alter the reproducibility of PM/ST quantification of CMV-specific CD8+T-cells.

P4.01.012
KIR2DL2 overexpression influences NK cell response to CpG

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NK cells are one of the most important innate immunity effectors towards viral infections. In particular, these cells are activated by viral CpG motifs through TLR9. For this, NK cell deficiency is responsible for viral increased susceptibility. Rizzo et al. (J Neuroimmunol, 2012; 251: 55-64) reported a 40% of Multiple Sclerosis patients with NK cells unable to counteract herpesvirus infection after CpG treatment. These NK cells showed an increased expression of the inhibitory receptor KIR2DL2 and of its ligand HLA-C1. Since it has been previously demonstrated that KIR3DL2 can directly bind CpG and facilitate its entry into the cell and it is known that CpG is able to modulate KIRs expression, we evaluated the ways of uptake of CpG treatment in NK cell lines, the effect on KIRs expression and NK activity status. We confirmed the role of KIR3DL2 in CpG uptake and we observed, for the first time, the induction of KIR2DL2 expression that reduced the cytotoxic activity of NK cells towards HLA-C1+ target cells and the secretion of pro-inflammatory molecules (IFN-gamma). This modulation via KIR3DL2-CpG is mediated by specific transcription factors and could explain the KIR2DL2+ NK cell unresponsiveness to the viral infection after CpG treatment.
Our results demonstrate the mechanistic basis of the lower responsiveness to CpG of KIR2DL2 expressing NK cells, suggesting a potential role in identifying new therapeutic strategies in herpesvirus susceptible individuals.
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P4.01.013
HLA-G influences nasal polyp relapse in HPV positive patients.

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The underlying mechanisms of nasal polyp development have yet to be determined. Human papilloma virus (HPV) infection is considered as a possible candidate, as associated with nasal inverted papilloma1. HLA-G molecule, an antigen with membrane-bound and soluble isoforms that interact with immune cell inhibitory receptors, is up-modulated by intrleukin-10 (IL-10), an anti-inflammatory cytokine, and increases during viral infection as immune-escape mechanism2. HPV infection is known to influence IL-10 and HLA-G expression3, suggesting a possible implication in nasal polyps. We investigated the influence of high risk HPV infection, IL-10 and HLA-G in nasal polyp relapses. We enrolled ten non-allergic patients with nasal polyps and analysed the presence of high-risk HPV by PCR (Sacace), the expression of HLA-G and IL-10 receptor on nasal epithelial cells by immunofluorescence (87G-PE, IL-10R-FITC) and of sHLA-G and IL-10 by ELISA. Our results evidenced high risk HPV infection in 50% of polyps, of which the 80% presented polyp relapse (OR:15). Only HPV positive polyps expressed mHLA-G and IL-10R on epithelial cells. The analysis of nasal epithelial cell culture supernatants showed a correlation between IL-10 and sHLA-G secretion in HPV positive samples. No expression was observed in HPV negative polyps. These data suggest an association between HPV infection, up-modulation of IL-10 and HLA-G expression in nasal epithelial cells and polyp relapses.
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P4.01.014
Beta-amyloidogenic peptides protect against Herpes Simplex Virus-1 (HSV-1) infections

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The causes of Alzheimer's disease (AD) are debated. Experimental data and epidemiological studies suggest that Herpes Simplex Virus-1 (HSV-1) infection is a risk factor. HSV-1 stays latent in trigeminal neurons and can be reactivated under low immune status of the host. AD is characterized by deposits of neurofibrillar tangles and senile plaques which lead to cortical neurodegenerescence. Senile plaques found extracellularly in brains of patients contain the fibrillar form of beta-amyloidogenic Aβ1-40 and Aβ1-42 peptides generated by presenelin cleavage of amyloid precursor protein (APP). Interestingly, herpetic encephalitis caused by Herpes viruses affects the same brain regions as AD. The question is open whether beta-amyloidogenic peptides also possess a physiological role. In this connection, they display anti-bacterial and anti-yeast activities in vitro. We cultured fibroblast (MRC5) and epithelial (A549) cell lines in the presence of amyloidogenic peptides Aβ1-40 and Aβ1-42 added before, simultaneously or after challenge with HSV-1. DNA was extracted and viral replication quantified by real-time PCR 24 h later. Controls were scrambled peptides and antimicrobial peptide LL-37.
Results showed that peptides Aβ1-40 and Aβ1-42 inhibited HSV-1 replication, depending on their sequence of addition. For instance, they showed efficiency when added 2 h before or simultaneously to virus challenge. However, they were inefficient when added 2 h or 6 h after HSV-1. In contrast, LL-37 inhibited viral replication under all conditions. Scrambled peptides had no activity.
Our data suggest that amyloidogenic peptides Aβ1-40 and Aβ1-42 may belong to a novel class of antimicrobial peptides (AMP) that possess antiviral activity.

P4.01.015
HSV-1 infection causes reduced surface expression of MHCII and co-stimulatory molecules on infected DC and suppressed T cell activation in vitro

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Introduction: Herpes Simplex Virus 1 (HSV-1) causes a lifelong persistent infection of the peripheral nerve tissue, which can lead to life threatening encephalitis or disseminated infection in immunocompromised persons. HSV-1 developed numerous mechanisms to evade immune response. The main target for HSV-1 is the MHC class II (MHCII) processing pathway in dendritic cells (DC). We used a HSV-1 construct with Gaussia luciferase and mCherry, which can allow differentiating infected cells from uninfected (Bystanders). Results: Our studies on infected murine spleen- and bone marrow-derived DCs revealed increased apoptosis in bystander cells (mCherry negative), when compared to infected cells (mCherry positive) and a reduction in DC maturation status as based on diminished expression of MHCII, CD80 and CD86. We observed a reduced T cell activation in samples containing infected DC with ovalbumin (OVA) through OVA-specific T cell proliferation assays. We have also observed a decreased autophagic flux in bystander cells and no significant change in infected cells when compared to uninfected sample. Addition of PP242, an activator of autophagy, revealed an increased autophagic flux in infected cells, when compared to uninfected and bystanders, which can be due to synergistic effect from virus and PP242. Further studies revealed a reduction in HSV-1 viral gene expression in infected neurons when activated antigen specific T cells were present. Conclusion: HSV-1 infection of DC causes apoptosis of bystander cells and reduced DC activation status, which suppresses both CD4+ and CD8+ T cell activation.

P4.01.016
Lethal immune mediated-disease after LCMV infection in STAT1 KO mice requires interferon regulatory factor 7 and is driven by type I interferon

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STAT1 KO but not WT, STAT2 KO, IRF9 KO or IFNAR KO mice succumb to a lethal CD4+ T cell-mediated disease following systemic infection with lymphocytic choriomeningitis virus (LCMV). Here we determined the role of the key transcriptional activator of type I interferon (IFN-I) production, interferon regulatory factor 7 (IRF7), in this lethal host response by generating STAT1/IRF7 KO double mutant mice. In contrast to STAT1 KO mice, STAT1/IRF7 KO mice as well as IRF7 KO mice survived LCMV infection and STAT1/IRF7 KO mice also developed a persistent infection. LCMV infection in STAT1 KO mice was associated with the marked induction of a number of cytokines in the serum including IFN-α, IFN-β, IFN-γ, IL-5, IL-6 and MCP1 which was absent in STAT1/IRF7 KO mice which had a modest increase in MCP-1 only. In addition, in the spleen of LCMV-infected STAT1 KO mice, IFN-β and IFN-γ mRNAs were also much more highly induced when compared with WT mice, while in STAT1/IRF7 KO mice, IFN-γ mRNA induction was similar to WT animals, there was no induction of IFN-β mRNA. To elucidate further a possible role of IFN-I in lethal disease, we examined STAT1/IFNAR KO mice. In contrast to STAT1 KO mice that all succumbed, STAT1/IFNAR KO mice all survived infection with LCMV. In conclusion, lethality resulting from LCMV infection in STAT1 KO mice is dependent on IRF7 through a mechanism that likely involves IFN-I which is a key driver of a catastrophic antiviral response.

P4.01.017
Influenza vaccination leads to dramatic redistribution of lymph node resident dendritic cells into inter-follicular regions

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Multi-photon live imaging has become a powerful tool in dissecting immune response to viral antigen, though to date little attention has been paid to lymphoid-resident dendritic cell (LNDC) populations. Initially described as sessile networks within the T cell compartment, we have observed a robust activation and relocation of LNDCs within minutes of UV-inactivated Influenza A arrival in draining lymph nodes (LNs). Combining live imaging with three-dimensional fluorescent reconstruction of draining lymph nodes, LNDCs were tracked in rapid migration beginning in the T cell cortex and resolving in medullary projections within inter-follicular regions (IFRs). LNDCs become activated and acquire antigen within IFRs where viral antigen concentrates. With a slightly delayed kinetics, antigen specific naïve CD4+ T cells are identified within 12 hours of immunization into IFRs where they make direct contact with LNDC and viral antigen. Strikingly, viral specific (but not naïve) CD4+ T cells express activation markers CD69 and CXCR3 and develop a Th 1 phenotype prior to skin draining DC arrival. Together, we believe that these data elucidate the importance of the LNDC population within draining LNs, provide insight into antigen drainage and early T cell activation after viral vaccine administration, and highlight the previously unappreciated magnitude of activity within draining LNs prior to the arrival of canonical migratory DCs from the periphery.
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P4.01.018
HTLV-1 infected thymic epithelial cells convey the virus to CD4$^+$ lymphocytes

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Human T Lymphotropic Virus type 1 (HTLV-1) can lead to development of lymphoma and leukemia (ATL) or to commitment of muscular/nervous systems (HAM/TSP). It remains unknown what triggers these diseases. Although the virus has T lymphocyte tropism, it can infect other cells by cell contact and cell-free virus. Activated T lymphocytes re-circulate in lymphoid organs, including the thymus, where the thymic epithelial cells (TEC) interact with recirculating cells. Accordingly, infected lymphocytes might transmit the virus to TEC. Interestingly, TEC express molecules related to the HTLV-1 transmission (GLUT-1, CCR4, Neuropilin 1). *In vitro* experiments showed that TEC could be infected by cell line derived from ATL and HAM/TSP patients. These infections happened from cell contact and by cell-free virus derived from cell supernatants. The virus can be seen after 24h and 10 days, when virtually all cells in the culture are infected. A microarray assay was performed comparing TEC treated with non-infected and HTLV-1$^+$ cells supernatants. The results revealed an increase of chemokine and adhesion molecule gene expression confirmed by RT-PCRq. Nevertheless, no difference in non-infected lymphocyte attraction was observed in a Transwell migration model. Lastly, HTLV-1 infected TEC were capable to spread the virus through the culture and infect target lymphocytes. Together these results show that TEC can be infected by HTLV-1+ lymphocytes and cell-free virus, can infect other TEC and convey the virus to non-infected T lymphocytes.

P4.01.019
Rapid T cell activation following Epstein-Barr virus transformed lymphoblastoid cell line co-culture with peripheral blood mononuclear cells from cytomegalovirus positive but Epstein-Barr virus naïve children

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Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are common and persistent herpesviruses known to influence immune cell maturation. We have earlier shown that CMV related imprint on NK- and T cells in children is boosted by co-infection with EBV. To better understand the mechanisms involved in the synergistic effect of co-infection on the T cell compartment, peripheral-blood mononuclear cells (PBMCs) from CMV+ EBV naïve children were co-cultured with EBV+ transformed lymphoblastoid cell lines (LCLs) directly or with the supernatant derived from LCLs pre-cultured for 72 hours. PBMCs in medium only or in culture with Ramos (EBV- Burkitt's lymphoma cell line), or with Ramos supernatants were included as controls. After culture, the cytokines IL-2, IFN-γ and TNF-α, were investigated in the supernatants through ELISA. ELISA results for the co-culture experiments showed that PBMCs cultured in medium alone, in Ramos supernatants or with Ramos cells failed to stimulate cytokine production. In contrast, PBMCs cultured with LCL supernatants or with LCLs produced IFN-γ and TNF-α, the levels of which were higher when PBMCs were cultured with LCLs rather than with LCL-derived supernatant. High levels of IL-2 was seen only when PBMCs were co-cultured with LCLs. Data herein suggests that as early as 3 days following culture, CMV+EBV naïve T cells become activated and produce cytokines in response to EBV co-infection. The characteristics of the responding T cells and their molecular triggers are currently being investigated.

P4.01.020
Seroprevalence against pandemic influenza virus A/H1N1/2009 in pregnant women

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For influenza virus infection, vulnerable groups include people with morbid obesity, chronic obstructive pulmonary disease and pregnant women. It is speculated that the increased rate of morbidity and mortality in pregnant women is due to a status of "temporary deficiency", induced to avoid rejection of the product of conception. However, production of antibodies during pregnancy does not decrease and could be transferred to the product by transplacental pathway. For the A/H1N1/2009 pandemic influenza virus, antibodies response against the virus during pregnancy has not been investigated. We determined seroprevalence of A/H1N1/2009 in pregnant women during different trimesters and transplacental transfer of antibodies to umbilical cord blood (UCB), using hemmaglutination inhibition (HAI), and using serum from 249 pregnant women, 25 non-pregnant women and 5 UCB post-partum, collected from December 2009 to February 2010. Sixteen percent from pregnant and 24% non-pregnant women had antibodies against A/H1N1/2009 with titers ≥1:40. The highest prevalence was found in third trimester pregnant women (23%, titers between 1:40 to 1:5120). UCB samples have equal antibody titers as the corresponding choridecidual space. This results show that during third trimester, when morbidity and mortality rates are the highest in pregnant women infected with A/H1N1/2009, pregnant women have protective titers as those found in non-pregnant women. Differences in responsiveness facing A/H1N1/2009 does not seem to be related to the presence of protective titers of hemmaglutinating antibodies in pregnant women, but could be related to neutralizing specific antibodies against A/H1N1/2009. Also, these antibodies seem that could be transferred transplacentally.

P4.01.021
Role of CD48 in regulation of T-cell mediated immunity in HTLV-1 infection

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HTLV-1 (Human T cell lymphotropic virus type 1) infection is associated with an aggressive T cell malignancy ATLL (Adult T-cell leukemia/lymphoma), the virus also establishes lifelong persistent infection in humans resulting in a carrier state (clinically asymptomatic).
CD48 is a GPI anchored cell surface molecule ubiquitously expressed on various hematopoietic cells, its expression has been shown to be up-regulated in viral infections as a sign of ongoing breach of immunity and therefore facilitates immune response. In HTLV-1 infection, one of the factors suggested to play a role in its persistence is the increased expression of the trans-activating viral gene encoding HTLV-1 tax among others; tax protein is the dominant target antigen of the T cell response to HTLV-1 in infected individuals.
In this study, we investigate the role of CD48 in HTLV-infection, we show that it is down regulated in HTLV-1 infection; the presence of HTLV-1 tax protein also suppresses CD48 expression. On blockade using anti-CD48 AB, the effector function of T cells in terms of cytokine production improved. Thus, we propose a possible means of immune evasion by HTLV-1 virus by Cd48 down-regulation and a possible inhibitory role in HTLV-1 infection.

P4.01.022
Identification of Antiviral-related Genes Up-regulated in Response to *Bombyx mori* Nucleopolyhedrovirus

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Silkworm larvae often suffer from viral infections causing heavy losses to the economy of silk industry. Insects exhibit both humoral and cellular immune responses that are effective against various pathogens like bacteria, fungi, protozoa, etc., but no insect immune responses are effective against viral infection. To obtain genes related to insect antiviral immunity from *Bombyx mori*, the cDNA library was constructed from *B. mori* nucleopolyhedrovirus (BmNPV)-infected *B. mori*. From the cDNA library, we selected 411 differentially expressed clones, and the 5' ends of the inserts were sequenced to generate ESTs. In this work, 135 unigenes were generated after the assembly of 411 differentially expressed clones ESTs. Of these 135 unigenes, we selected 109 antiviral response-related candidates except 26 clones that high similarity with genes derived from BmNPV. Among 109 unigenes, a total of 80% had significant matches to genes from other organisms in the database, whereas 20% of the unigenes had not matched in the database. Functional groups of these sequences with matches in database were constructed according to their putative biological function. Three largest categories were control of cellular organization (52%), metabolism (20%), and protein fate (10%). The genetic information reported in this study will provide more information about antiviral-related genes in silkworms.

P4.01.023
CD8$^+$ T-cell memory: forward genetic analysis by germline mutagenesis and screening

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The development and long-term memory maintenance of antigen-specific CD8$^+$ cytotoxic T-lymphocytes (AgCD8) is genetically controlled. However, not all the genes involved in this process are known to date. To identify new memory regulators, we have undertaken an *in vivo* forward genetic approach based on germline mutagenesis and screening. Genome-wide random Ethyl-(N)Nitroso-Urea (ENU)-induced point mutations were created and a library of third generation mutant mice screened for genetic defects affecting the development of Lymphocytic Choriomeningitis virus (LCMV)-specific AgCD8 and their survival into long-term memory cells. By screening 1263 mice, we have isolated 3 mutant strains named memi, binu and alois.
We recently reported that memi, a null mutation in the nucleotide salvage enzyme deoxycytidine kinase, affects T-lymphocyte thymic development, peripheral homeostasis and AgCD8 response to LCMV infection. binu and alois mutants do not develop observable spontaneous phenotypes, but upon infection present enhanced quantity and impaired quality of AgCD8 memory, respectively. Whole-genome sequencing and identity by descent analysis of binu and alois mutants has narrowed down the list of candidates to 4 and 10 genes, respectively. binu is currently being confirmed as a mutation in a known T-cell regulator, whereas alois affects an as yet un-described novel immune gene. Although alois mutant mice retain wild-type LCMV-specific AgCD8 numbers, their AgCD8 cells present impaired memory qualities after primary and secondary infections, indicating that *alois* controls the memory potential of AgCD8. Our non-hypothesis driven approach has therefore unraveled new avenues to investigate and understand what finely tunes the quality of CD8$^+$ T-cell memory response.

P4.01.024
Cross-reactive antibodies to swine influenza viruses in adults after vaccination with trivalent inactivated influenza vaccine in Korea

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A number of human infection cases of swine influenza virus A(H3N2)v have been reported in the United States since 2011. We evaluated levels of cross-reactive antibody to swine influenza viruses (H1N1, H1N2, H3N2 and H3N2pM) isolated in Korean swine herd, and assessed whether seasonal trivalent inactivated influenza vaccine (TIV) induces seroprotection in human adult group against these swine influenza viruses. Cross-reactive antibody to four swine influenza subtypes was evaluated by hemagglutination inhibition (HI) assay and microneutralization assay (MN). The analysis included 55 paired sera collected during 2011-2012 and 2012-2013 season in adults aged >20. Seroprotection was defined as an HI titer of ≥40 and MN titer of ≥80. Vaccine-induced antibody levels were assessed with paired sera before and after seasonal TIV vaccination. The overall seroconversion (4-fold increase in antibody level) rate, by HI assay, after TIV immunization against swine influenza viruses were swH1N1 (64%), swH1N2 (62%), swH3N2 (44%) and swH3NpM (45%), respectively. The overall seroprotection rate of HI and MN were ≥56% and ≥60% following TIV vaccination. Therefore the enhorced seasonal influenza vaccination might provide protective effect to swine influenza virus human infection.
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P4.01.025
Pentamers and streptamers for the monitoring of CMV-specific CD8+ T-cell immune reconstitution after allogeneic stem cell transplantation: a comparative analysis

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Reconstitution of CMV-specific CD8+ T-cells (CTLs) is crucial for patients after allogeneic Stem Cell Transplantation (allo-SCT). The aim of this study is to evaluate the correlation between staining with two different multimers (Pentamers (PM) and Streptamers (ST)) in the monitoring of immune reconstitution.
A total of 94 PBMCs samples were isolated from 14 CMV-seropositive HLA-A*02:01 patients. To form the ST complex, 0.75 µg PE-labelled Strep-Tactin and 0.5 µg Streptamer HLA I were mixed. In addition, 0.2µg HLA-A*02:01/STpp65$_{495\text{-}503}$ or 5 µl HLA-A*02:01/PMpp65$_{495\text{-}503}$ were incubated with 1x10^6 human AB serum-blocked PBMCs for 45 minutes at 4ºC or 10 minutes at room temperature, respectively. After multimeric staining, anti-human CD3/CD8/CD45/CD4 were added and 500,000 cells were acquired in a FACSCanto II. CMV-specific CTLs response was monitored monthly during a median of 11.4 months after SCT with a median of 8 samples analysed per patient. Correlation between both techniques was determined by Rho Spearman test.
The median frequencies of CMV-specific CTLs detected by PM and ST were 31.58x10^5/L (IQR=4.04-267.24) and 42.85x10^5/L (IQR=3.52-288.31), respectively. Both techniques correlate each other ($r_{Spearman}$ = 0.982; p<0.01). Nevertheless, ST showed higher mean values than PM looking at the median value of the difference PM-ST (-1.42 [-23.5, 1.35]. The correlation between the difference PM-ST and the PM/ST mean showed that the higher CMV-specific CTLs quantity the larger differences PM-ST ($r_{Spearman}$=-0.331; p<0.01).
We concluded that both techniques may be equally useful to examine immune reconstitution, although detection levels are higher with ST staining possibly due to a larger sensitivity of this technique.

P4.01.026
Targeted PGE_2 inhibition enhances anti-influenza immunity through induction of type I IFN in Macrophages

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The 1918 Spanish Influenza virus pandemic had a major impact on the rise in popularity of non-steroidal anti-inflammatory drugs (NSAIDs). Although these drugs are used extensively for the management of Influenza symptoms, their role in anti-viral immunity is poorly understood. NSAIDs act mainly by inhibiting cyclooxygenase enzymes involved in the synthesis of all prostaglandins, including PGE_2. We now show that during Influenza infection, upregulated PGE_2 inhibited macrophage type I interferon production and apoptosis via EP2 and EP4 receptors, thereby increasing virus replication. This inhibitory role of PGE_2 was not limited to innate immunity since both antigen presentation and T cell mediated immunity were also suppressed. Inhibition of microsomal prostaglandin E-synthase 1 (mPGES-1), and thereby PGE_2, in human Mφ was protective against Flu infection. These data show that the mPGES-1/PGE_2 pathway is specifically targeted by Influenza virus to evade host anti-viral immunity and propose that specific inhibition of PGE_2 may serve as a new treatment for viral infection.

P4.01.027
Serological survey of BTV in Morocco

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The bluetongue (BT) is a non-contagious disease transmitted by blood-sucking arthropods of the genus Culicoides. The disease affects small ruminants, especially sheep which are very sensitive.
Since 2004 two serotypes circulating in Morocco BTV-1 and 4, several samples were collected during different epidemics, the strains were isolated but no thorough phylogenetic study has been established to characterize and reveal different reassortants have been able to generate from parental strains.
The first year of our study involved a serological survey of sheep and cattle to define the epidemiological situation with respect to this arbovirus, also reveal the rate of coinfection by differents serotypes.
Out of the 429 sera that were collected, 216 are from the cattle of the Gharb region, 109 were collected in 2009 from different regions of Morocco, and the rest was collected in 2012 from sheep of other regions of the country. All the sera collected were tested by neutralization using reference viruses of serotype 1 and 4 and the result is that the rate of co infection was high especially among cattle. Hence the results prove the importance of a molecular study involving BTV isolates that represents different regions of Morocco and different years since the first outbreak.
The next step is mainly based on the sequencing of BTV isolates to better understand the evolution of this virus through the years, then a study of the pathogenesis and immune response of animals would also assess whose goal is to classify different reassortants in Morocco.

P4.01.028
Prevalence of human papillomavirus subtypes in breast cancer in Moroccan woman

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Breast cancer is one of the most frequently diagnosed cancers in women in Morocco, but its aetiology remains unknown. Viruses including Epstein-Barr virus, a human equivalent of murine mammary tumour virus and human papillomavirus have been detected in benign breast tissues and breast tumours and are considered to be involved in the aetiology of breast cancer. Two clinically relevant high-risk HPV (HR-HPV) types 16 and 18 are etiologically associated with the development of cervical carcinoma and are also reported to be present in many other carcinomas in extra-genital organ sites. However, there are controversial data on the meaning of viral induction of breast cancer. Human papillomaviruses HPVs could be important risk factors for breast carcinogenesis and metastasis. Based on this hypothesis, we evaluate the prevalence of broad range of HPVs in a Moroccan breast carcinoma cases. We have analyzed 88 Snap frozen breast tumor tissues by using type-specific multiplex genotyping (TS-MPG) assays which combine multiplex PCR and a bead-based technology the multiplex genotype. Our study revealed that 20 (22.7%) of the 88 samples are HPV positive and the high-risk HPV types 16 and 18 were not detected in our samples, but 4.54% others probable/high risk types were detected.

P4.01.029
Lung CD8+ T cell impairment during acute viral respiratory infection is associated with a unique gene expression profile

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Viral acute lower respiratory infections (vLRI) generate pulmonary $CD8^+$ cytotoxic T lymphocytes (CTLs) that are functionally impaired, while virus-specific CTLs in the spleen remain functionally competent. We have shown that the inability of lung CTLs to maintain effector functions resulted in impaired viral clearance in a murine vLRI model (*J Clin Invest* 2012) and may therefore contribute to recurrent vLRI. Here we show a cell-intrinsic role for the inhibitory receptor Programmed Death-1 (PD-1) in mediating lung CTL impairment. To further define differences between impaired lung and unimpaired spleen CTLs during vLRI, we performed gene expression analysis on epitope-specific cells from the lung and spleen and naïve $CD8^+$ T cells. The expression of ~2000 genes changed as cells differentiated from naïve to effector lung CTLs. Surprisingly, >350 genes were differentially expressed between lung and spleen CTLs. The lung-specific genes encode proteins with diverse functions, including cytokine production, transcriptional regulation, trafficking, cell cycle and metabolism. Notably, several genes associated with CTL exhaustion were upregulated, including TIM-3, LAG-3, 2B4, IL-10 and BLIMP-1. We then compared lung CTLs in this study to exhausted spleen CTLs present during chronic LCMV clone-13 infection (Doering et al *Immunity* 2012) in a separate analysis. We found that lung CTL gene expression represents a unique state of $CD8^+$ T cell differentiation that shares some aspects of both early and late chronic LCMV gene profiles. These data demonstrate that impaired lung CTLs represent a unique physiologic state. Further characterization of lung-specific genes may lead to novel therapeutics against serious vLRI.

P4.01.030
The complement system has a critical role in the inflammatory reaction that follows influenza virus infection

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In this work we evaluated the functions of some of the major complement proteins in the response to influenza using mice deficient in C3, C1q and C4. We observed that all of the complement deficient strains show higher mortality and morbidity rates compared to wild type animals after a sublethal infection with influenza. In addition, complement C3 was necessary to promote the phagocytosis of the virus and/or viral infected cells by the major phagocytic cells in the lungs (alveolar macrophages, neutrophils and monocytes) at early time points after infection. We also observed a clear reduction in the number of viral positive respiratory dendritic cells in the complement deficient animals compared to B6 controls that correlate with a deficient T cell activation in the draining mediastinal lymph node. However, the levels of the pro-inflammatory cytokines IL1β, MCP1, MIP2 and TNFα in the lung of mice at day 2 post-infection were significantly higher in C3-/- and C4-/- animals compared with the control group.
From these results we can conclude that the pronounced decrease in the phagocytic activity of lymphocytes in the lungs from complement deficient animals facilitates the dissemination of the virus and increases the epithelial damage, augmenting at the same time the cytokine storm that leads to the dead of the animal. In addition, the adaptive immune responses are impaired mainly due to a deficient activation of the migratory respiratory dendritic cells that fail to activate efficiently antiviral T cell responses in the draining lymph node.

P4.01.031
Human cytomegalovirus directly supress CD4 T-lymfocyte activation and proliferation

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CD4 T cells are important regulators of the immune system and are vital for mounting a strong immune response against viral infections. Human cytomegalovirus (HCMV) is known to be a strong modulator of the innate as well as adaptive immune responses. In this study, we found that HCMV directly inhibited proliferation of CD4 T cells and rendered them unresponsive to immunological stimuli. This effect was not observed when CD4 T cells were treated with Herpes Simplex Virus-1/ 2 or measles virus. When stimulated with phytohemagglutinin, concanavalin A, or phorbol myristate acetate, HCMV-treated T cells were unable to proliferate, revealing an ability of HCMV to inhibit CD4 T cell response. Furthermore, HCMV also prevented proliferation of leukemic T-cell lines. HCMV-treated CD4 T cells expressed the activation markers CD45RO and CD69, were not apoptotic and produced decreased levels of the cytokines IL-4, IFN-γ and TNF-α, compared to untreated controls. The inhibitory effect of HCMV on CD4 T cell proliferation was not mediated by HCMV gH, gB or other immunogenic glycoproteins, since intravenous immunoglobulins or gB- or gH-specific neutralizing antibodies did not prevent the suppression of T-cell proliferation. Our observations show that HCMV inhibits CD4 T cell function with potential clinical consequences for both humoral and cell-mediated immune responses.

P4.01.032
Narrowing of influenza A virus specific T-cell receptor alpha and beta repertoire with increasing age

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Alterations in memory CD8 T cell responses may contribute to the high morbidity and mortality caused by seasonal influenza A virus (IAV) infections in older individuals. We questioned whether memory CD8 responses to this non-persistent virus, to which recurrent exposure with new strains is common. will change overtime with increasing age Here, for the first time we show that the HLA-A2-restricted IAV $M1_{58-66}$-specific Vα T cell repertoire was significantly narrowed leading to oligoclonal expansions including the usage of a single identical VA12 clonotype with age . This was the case for all 8 older donors. The VA repertoire of older individuals also had longer CDR3 regions with increased usage of alanine/glycine runs which may enhance TCR promiscuity. Collectively these results suggest that CD8 memory responses in humans to non-persistent viruses like IAV are dynamic, and become narrower with preferential retention of T cell repertoires with features of enhanced promiscuity with age.

P4.01.033
Study of humoral immune response in patients with tick-borne encephalitis against the background of virus circulation

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The aim was to identify antibodies to tick-borne encephalitis virus and its RNA in patients with acute and chronic infection. Serum of patients with acute and chronic form of encephalitis were examined by EIA to determine titers of specific immunoglobulin M (IgM) and G (IgG) and by polymerase chain reaction to determine virus RNA. A relationship between antibody formation and circulation of tick-borne encephalitis virus was detected. Simultaneous circulation of the virus and IgM was indicated in the acute form of encephalitis. The immune response of 22% of the patients was ill-defined in the presence of circulating virus in the blood. The absence of IgG may indicate the realization of the primary immune response and the beginning of the circulation of immune complexes. In late convalescence period the circulating virus in the majority of the patients (75%) was not found against the background of circulating antibodies M and G. In 25% of cases in the complete absence of free antibody, the virus was detected in blood cells. 40% of patients with the chronic form had no virus in the blood, and the IgG titer was high. In 60% of cases there was opposite situation, with low IgG the virus circulation was found in the blood, that indicated its output into the blood stream and the next stage of viremia. These results may indicate preferential localization of tick-borne encephalitis virus in the tissues of the nervous system with periodic reactivation and induction of humoral immune response to polymorphic classes of immunoglobulins.

P4.01.034
Some liquorogic features of meningeal form of tick-borne encephalitis

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The aim of our work was to study liquorologic indexes in case of meningeal form of tick-borne encephalitis.
We examined samples of cerebrospinal fluid and serum of patients with meningeal form of encephalitis. In the samples of cerebrospinal fluid we identified the level of white blood cell, protein and specific immunoglobulin M. In blood serum we identified the presence of specific immunoglobulin M and G (IgM and IgG).
It was found that the meningism occurred immediately at admission and were traced for an average 5-8 days. For meningism occurrence all patients had cytosis 125,8±30,5 cells in 1 mm^3, while relieving them - 27,1±9,4 cells in 1 mm^3 ($p<0,05$). Pleocytosis was mainly lymphocytic. The protein level in the height of meningism was 0,49±0,08, and with its disappearance it was 0,34±0,01 g/l ($p>0,05$). The geometric mean titer of specific IgM in cerebrospinal fluid was 7,37÷0,35. The increased amount of leukocytes in cerebrospinal fluid at admission was associated with high protein level (r=+0,52) and presence of specific IgM (r=+0,50) in cerebrospinal fluid. High level of protein in cerebrospinal fluid during the second study correlated with prolonged circulation of specific IgM in cerebrospinal fluid and serum (r=+0,88, and r=+0,56, respectively), reflecting the preservation of inflammations. We noted the close relationship between the level of specific IgM in serum and cerebrospinal fluid (r=+0,82).
Thus, complex study of cerebrospinal fluid in the dynamics is still an important piece of monitoring the progress of infection.

P4.01.035
EBV infection leads to activation of chronic lymphocytic leukemia (CLL) cells and worsen clinical prognosis in CLL patients

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Chronic lymphocytic leukemia (CLL) is a heterogenic disease in clinical outcome, but the reason of heterogeneity remains not understood. The role of chronic immune stimulation, leading to the activation of B lymphocytes is under investigation. Preliminary observations indicate a possibility of facilitate clonal expansion and leukemic transformation of B cells by exoantigens. The aim of the study was an assessment of the influence of Epstein-Barr virus (EBV) infection on activation of CLL cells and determination of the relationship between the presence of viral DNA and CLL clinical outcome. The assessment of the immunophenotype of peripheral blood (PB) lymphocytes, focusing on lymphocyte activation markers, such as CD25 and CD69, with the use of flow cytometric method, and the assessment of the presence of the EBV DNA with the use of RT-PCR method, was conducted in blood samples (PBMC) of 80 untreated CLL patients. Significantly higher percentage of activated B cells CD19+CD25+ and T cells CD3+CD25+ was found in PB of those CLL patients in whom DNA EBV was found in PBMC ($p<0.0000001$ and $p=0.024$, respectively). Positive correlation between the number of copies DNA EBV and the percentage of CD19+CD25+ B cells ($r=0.502$, $p=0.033$), CD19+ZAP-70+ B cells ($r=0.751$, $p=0.0003$), and CD19+CD38+ B cells ($r=0.476$, $p=0.038$).

Moreover, those patients whose lymphocyte counts doubled in less than 12 months had more DNA EBV copies (p=0.0003). Obtained results suggest that EBV plays an important role in the stimulation of CLL lymphocytes and may induce B-CLL cells proliferation, leading to the fatal outcome of the disease.

P4.01.036
Activation of type III interferon response by human paramyxovirus infection

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Human Metapneumovirus (hMPV), is a paramyxovirus considered a major respiratory pathogen responsible for up to 15% of low respiratory tract infections hospitalizations in infants and young children. It is also causes bronchiolitis and pneumonia. Currently, there is not vaccine available against hMPV. Knowledge of the critical aspects of the host immune response is crucial to understanding the pathology associated with hMPV infection. We have recently found that hMPV induce type III interferon (IFN). However, the role of this antiviral cytokine in hMPV infection is unknown. In this work, we investigate the production of IFN-lambda in vitro and in vivo using lung epithelial cells and an experimental mouse model of infection. Our data indicate that hMPV induce IFN lambda in epithelial cells in vitro and its production is dependent of viral replication. hMPV is susceptible to the activity of the three isoforms of IFN-lambda. In the mouse model, we observed that hMPV induced IFN-lambda in vivo. Moreover, treatment of mice with IFN-lambda prior hMPV infection resulted in a reduced lung virus titer and a decreased number of inflammatory cells in the lung. Overall, these findings highlight the importance of the interferon responses in hMPV infection and suggest that IFN lambda play a role in the antiviral immune response to paramyxovirus infection.

P4.01.037
Influenza virus NS1 protein counteracts ZAPS function to evade RIG-I-mediated antiviral defence

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Type I IFNs are produced in response to viral infection and are key cytokines for the activation of innate immunity. The induction of type I IFNs is triggered by the detection of viral nucleic acids through pattern recognition receptors. In particular, the cytosolic RNA sensors, retinoic acid-inducible gene-I (RIG-I) and its family members essentially mediate the recognition of a variety of viruses by host cells. These RNA sensors are frequently targeted by viral proteins for the evasion of host antiviral activities. It was previously reported that influenza virus derived nonstructured protein 1 (NS1) interacts with RIG-I to inhibit its downstream signalings. However, little is known about its detailed mechanism. In this study, we show that influenza virus NS1 protein inhibits RIG-I-mediated antiviral signaling through the counteraction against ZAPS. The association of ZAPS with RIG-I was found to be inhibited by NS1 protein. Conversely, upregulation of ZAPS levels resulted in disinhibition of the NS1 effect on RIG-I activity, and promoted the activation of IRF-3 and NF-κB pathways. Furthermore, the transgenic mice with upregulation of in vivo expression levels of ZAPS exhibited a resistance to lethal infection with influenza A virus. Thus, these findings indicate that the interaction of RIG-I and ZAPS is one of the key points, wherein NS1 inhibit RIG-I-mediated antiviral activity, which provides a mechanistic insight into innate immune evasion by influenza virus.

P4.01.038
Virus-associated activation of innate immunity induces rapid disruption of Peyer's patches in mice

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Early in the course of infection, detection of conserved pathogen-associated molecular patterns by innate immune receptors can critically shape the subsequent adaptive immune response. Here we investigate the influence of virus-associated innate immune activation on lymphocyte distribution in secondary lymphoid organs. We show that virus infection of mice leads to rapid disruption of the Peyer's patches which form the main inductive site of intestinal immune responses. In contrast, other secondary lymphoid organs such as peripheral skin-draining lymph nodes were not affected. The observed effect was not dependent on an active infectious process but was due to innate immune activation and could be mimicked by virus-associated molecular patterns such as the synthetic double-stranded RNA poly(I:C). Profound histomorphological changes in Peyer's patches were associated with depletion of organ cellularity, most prominent among the B-cell subset. We demonstrate that the disruption is entirely dependent on type I interferon signaling. At the cellular level, we show that virus-associated immune activation by IFN-α blocks B-cell trafficking to the Peyer's patches. We further identified downregulated expression of the gut-homing molecule α4β7-integrin as the underlying molecular mechanism. We observed that B-cell distribution within the intestinal lamina propria, which is also dependent on the α4β7-integrin, was not altered following virus-associated innate immune stimulation. In summary, our data identify a mechanism that results in type I IFN-dependent rapid but reversible disruption of intestinal lymphoid organs during systemic viral immune activation. We suggest that such re-routed lymphocyte trafficking may impact the development of B-cell immunity during systemic viral infections.

P4.01.039
Aged-related changes in the immune response to influenza infection

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Elderly people are more susceptible to viral infections and response poorly to vaccination. In this regard, influenza virus infection is a worldwide health problem, which aggravates in individuals over 65 years of age. Therefore, the understanding of how influenza virus infection activates the immune system is crucial to design prophylactic and immune therapeutic strategies to reduce morbidity and mortality in the elderly. Nevertheless, the immune response to influenza virus infection is complex and remains largely unknown. Using experimental data for young and aged mice, we propose a mathematical model for influenza infection to reveal the relevant mechanisms which contribute to the impaired immune responses in the elderly. Experimental data revealed different kinetics for viral replication between young and elderly. The aged mice showed 1-2 days delay for the clearance of the virus. Results suggest that a reduced viral replication in epithelial cells as well as the impaired immune mechanisms directed by CD8+T cells may be responsible for the poor immune responses in the elderly. This work provides new insights for vaccination and immune intervention in the elderly.

P4.01.040
Myeloid derived supressor cells restrain Natural Killer cell activity in CVB3 myocarditis

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Enteroviruses, especially coxsackievirus B3 (CVB3), are considered as most relevant agents of acute and chronic myocarditis. Underlying pathogenic mechanisms determining the course of enteroviral myocarditis are mainly investigated in murine models which perfectly match the different outcome of enteroviral myocarditis in humans. While the innate immunity of the host determines the first line of defense against the virus infection, the presence of ongoing inflammation indicates that acquired immunity plays an important role in the pathogenesis of chronic myocarditis.
The importance of Natural Killer (NK) cells for the efficiency of viral clearance and disease progression has been suggested a long time ago. Recently we could show that C57BL/6 mice which are resistant for chronic myocarditis enable more mature NK cells at any time of CVB3 infection in spleen and heart. NK cells from C57BL/6 mice also exert more IFN-γ and cytotoxicity and express more of the activating receptor NKG2D than those of permissive A.BY/SnJ mice. Myeloid derived suppressor cells (MDSC) are known to restrain NK cell activity in viral infections by inhibiting cytotoxicity, NKG2D expression and IFN-γ production. In order to investigate whether MDSC influence NK cell function in CVB3 myocarditis we compared expression profiles of MDSC subsets in A.BY/SnJ, C57BL/6 and NKG2D knock out mice in the course of CVB3 infection. The obvious variation in MDSC populations in these mouse strains denotes their role in the progression of viral myocarditis. Moreover, we confirmed these data by investigating the influence of MDSC on NK cells in in vitro experiments.

P4.01.041
Influenza-specific B cell response in the human challenge model for influenza A virus

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Influenza A virus is one of the major viruses causing acute respiratory infection in humans. Neutralising antibodies, measured by the haemagglutinin-inhibition assay, have been shown to be the protective correlate of influenza vaccination. Protective antibody levels might however be short-lived, because the antibody titre fades from the peak level at the first month of inactivated influenza immunisation or natural infection. Establishing new correlates of immunity, especially cellular aspects, could contribute to the evaluation of efficacy of new influenza vaccines. Studying the B cell response in adults may be useful to benchmark the response for other age extremities, in which the induction of antibody is relatively impaired and ineffective. Here, we studied the kinetics and magnitude of influenza virus-specific B cell and antibody responses, over the course of 28 days in sero-negative healthy adult volunteers experimentally infected with seasonal influenza H1N1 (A/Brisbane/59/07) virus. We demonstrated a robust strain-specific plasmablast and memory B cell responses in the peripheral blood, which correlated with the throat viral load and the duration of viral shedding, indicating an antigen-specific response. On day 28 post-infection, influenza virus-specific B cells were further identified from the circulating compartment of isotype-switched B cells. Our results clearly define the cellular basis of antibody response in human influenza infection and provide information that may assist in optimising the vaccine for the maintenance of protective antibody level in susceptible adults.

P4.01.042
Inhibition of monocyte differentiation into dendritic cells by HTLV-I-infected breast milk macrophages

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Human T cell leukemia virus type I (HTLV-I), a causative agent of adult T-cell leukemia (ATL), is transmitted from mother to child predominantly by breastfeeding. Oral HTLV-I infection early in life is associated with a subsequent risk of ATL. Although the pathogenic mechanisms of ATL remain largely unknown, previous studies have shown that monocytes from ATL patients had reduced capacity for DC differentiation. In order to clarify the mechanisms responsible for the impairment of DC differentiation, we have established an immortalized macrophage cell line (HTLV-BrMMΦ: HTLV-I-infected breast milk macrophage) from BrMMΦ by persistent infection with HTLV-I. Because HTLV-I is transmissible from HTLV-BrMMΦ to peripheral blood mononuclear cells (PBMC), HTLV-BrMMΦ is a possible source of infection in mother to child transmission (MTCT). When CD14+ monocytes were cultured with GM-CSF and IL-4 in the presence of HTLV-BrMMø, they altered the surface phenotype of immature DC and reduced stimulatory capacity of T cell proliferation. Also, the expression of CD1a, CD1b, CD11b, DC-SIGN, and HLA-DR were CD1a, CD1b, CD11b, DC-SIGN, and HLA-DR were significantly blocked in the presence of HTLV-BrMMø. The impairment of monocyte differentiation might be not due to HTLV-I infection of monocytes but due to unknown soluble factors. Since other HTLV-I-infected cells exhibited similar inhibitory effects on monocyte differentiation to DCs, we speculated that HTLV-I infection might cause the production of some inhibitory cytokines in infected cells. Identifying the factors responsible for the impairment of monocyte differentiation to DCs may be helpful to understand ATL pathogenesis (244 words).

P4.01.043
Dengue and other flavi virus specific T cell specific responses in healthy individuals in Sri Lanka

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Objective: Although infection with the dengue viras (DV) cause severe clinical disease in some individuals, it results in mild or asymptomatic infection in the majority of individuals. Therefore, we set out to determine correlates of a DV-specific protective immune response.
Methods: T cell responses to the DV NS3 peptides, Japanese encephalitis virus (JEV) and non dengue viral proteins were determined by Ex vivo IFNY ELISpot assays, in a 67 healthy volunteers. IFNY cultured ELISpot assays for serotype specific (SS) DV peptides were carried out using a previously defined panel of SS peptides for all 4 DV serotypes.
Results: SS T cell responses to at least one DV serotype were detected in 46/47 seropositive individuals but absent in all dengue seronegative(n=20) individuals. 23/46(53%) responded to only 1 DVserotype, 18/46(39.1%) to 2 serotypes and 3/46(6.5%) to 3 serotypes.
DV-NS3 specific responses were seen in 24(51.06%) and JEV responses in 18(38.3%) seropositive individuals. DV-NS3 responses were also seen in 3(15%), seronegative individuals. A significant and positive correlation was observed with the number of DV-serotypes a person responded to and ex vivo DV-NS3 responses (Spearman r = 0.65, p=0.001). A significant and positive correlation was also seen with the number of DV serotypes a person responded to and DV antibody titres (Spearman R=0.70,p<0.0001)
Conclusions: Our data suggest that this panel of SS peptides are useful in determining the past infecting DV serotypes. The frequency of DV- NS3 appear to rise with the number of DV infections a person has had.

P4.01.044

Release of interleukin-1alpha from herpes simplex virus 1 infected keratinocytes provides a functional danger signal

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Herpes simplex virus-1 (HSV-1) is a human pathogen that utilizes several strategies to circumvent the host immune response. It has been reported that HSV-1 inhibits both apoptotic and inflammatory pathways in infected cells. We here document that HSV-1 infected keratinocytes release the pro-inflammatory cytokine interleukin-1 (IL-1), hence preserving the ability of the infected cells to signal danger to the surrounding tissue. During its lifecycle HSV-1 generates double stranded RNA. We have previously reported that the double stranded RNA analogue poly(I:C) induces pyroptosis in keratinocytes and extracellular release of IL-36. In a similar manner we here show that poly(I:C) triggers release of IL-1 from keratinocytes through a mechanism involving the inflammatory caspases, caspase-1 and/or caspase-4. Inflammatory caspases are activated in HSV-1 infected keratinocytes; however, the extracellular release of IL-1 is independent of this activity. To explore the *in vivo* role of the released IL-1 we employed a mouse model involving primary infections on flank skin, local spread to sensory nerves, retrograde axonal transport to the dorsal root ganglion, and secondary shingles-like blistering around nerves infected through anterograde migration of the virus. We observed no difference in secondary lesion sizes in wild type and IL-1 receptor (IL-1R1) knockout mice. However, a trend towards increased mortality of IL-1R1 knockout mice was observed, as mice ultimately developed encephalitis and/or disseminate disease. Hence, IL-1 signaling may be an important danger signal during HSV-1 skin infections.

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P4.01.046
Elevated plasma soluble Sema4D/CD100 level in patients with hemorrhagic fever with renal syndrome: positive correlation with disease severity

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Semaphorin 4D (Sema4D/CD100), which is broadly expressed on immune cells and could be cleaved proteolytically, generating a biologically active soluble form, is considered to be involved in several aspects of immunity, and also in some diseases such as tumors, autoimmune diseases, and HIV infection. However, the detection of CD100 and its pathological significance in patients with many other acute infectious diseases have not been reported. To investigate the pathological significance of soluble CD100 (sCD100) in patients after Hantaan virus infection, we detected the level of sCD100 in plasma samples from 99 patients and 27 healthy controls and found that the sCD100 level in acute phase was higher in patients than that in healthy controls (P<0.001), and declined in convalescent phase. Moreover, the sCD100 level was negative correlated with the platelet count and positive correlated with white blood cell count, serum creatinine or blood urea nitrogen of the patients. Overall, for the first time we reported the level of plasma sCD100 in patients with acute infectious disease. These results demonstrated that plasma sCD100 might be a novel biomarker for the severity of the disease.

P4.01.047
Evaluation of the immune response of live attenuated mumps vaccines (RS-12 strain) produced with different stabilizers in guinea pigs

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Background and Aim: Mumps is a contagious disease belonging to Paramyxoviridae family. Although vaccination against mumps has been significantly beneficial, sensitivity to temperature is one of major obstacles in mumps vaccination. In the present study, two new stabilizers were used for manufacturing of live attenuated mumps vaccine, and immune response of this vaccines was evaluated after administration to guinea pigs.
Methods: Fist, mumps virus was harvested and blended with different stabilizers separately to prepare the final bulk. Then, the final product was prepared by lyophilization of final bulk. The final product subjected to quality control tests including: accelerated stability test, potency test, as well as in vivo test. For evaluation of immune response of the 3 mumps vaccines in experimental animals, vaccines were administered to guinea pigs. Furthermore mumps specific antibody level was measured in guinea pigs sera using Hemagglutination inhibition method (HI).
Results: Virus titration results of the vaccine containing new stabilizer showed that titer loss, before and after lyophilization, and after reconstitution was within WHO acceptable range. Analysis of results showed appropriate immune response in guinea pigs. Based on our findings the HI titer after administration of mumps vaccines with TD stabilizer was more than the two other stabilized live mumps vaccines.
Conclusion: increasing thermal stability of live attenuated viral vaccines such as mumps, can help to eradicate the disease, and can be useful economically. After long term stability tests, this mumps vaccine strain can be a suitable candidate for developing new mumps virus live attenuated vaccine.

P4.01.048

A NK complex-linked locus restricts the spread of Herpes simplex virus type 1 (HSV-1) in the central nervous system (CNS) of C57BL/6 mice

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Natural resistance to mortality in mice infected with Herpes simplex virus type 1 (HSV-1) is mouse strain dependent and an autosomal dominant trait. In mice infected with virus via the oral mucosa, HSV-1 spreads throughout the central nervous system (CNS) of susceptible strains but is restricted to the brainstem of resistant mice. In resistant C57BL/6 (BL/6) mice, viral restriction is mediated by natural killer (NK) cells and $CD8^+$ T-lymphocytes.
We combined mendelian analysis, studies of congenic and intra-NKC recombinant mice, along with antibody depleted mice to further examine the restriction of viral spread in the brains of BL/6 mice.
We report a NK complex-linked genetic locus, *Hrl2*, whose alleles determine the restriction of viral spread in the CNS. Mendelian analysis determined that restriction of viral spread is a dominant trait and consistent with a single gene effect. Studies with congenic mice determined that the locus maps to the NKC on chromosome 6 but is separate from previously identified loci; *Hrl1* and *Rhs1*. Studies with intra-NKC recombinants determined that the locus maps to a segment between CD69 and D6Wum34. Studies with antibody depleted mice determined the effect of this locus is mediated by NK1.1 expressing cells.
A NK complex-linked locus (*Hrl2*) restricts the spread of HSV-1 in the CNS of BL/6 mice however the precise mode of action of the locus remains unknown.

P4.01.049

The importance of cross-strain protective human TCR signatures for universal immunity against influenza

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In contrast to the current antibody-mediated and strain-specific influenza vaccines, T-cells directed at conserved internal proteins elicit broad immunity against seasonal and pandemic influenza viruses. Here, we dissected the optimal human influenza-specific $CD8^+$ T-cell populations across different HLAs, both at functional and clonal levels, to provide insights into universal immunity against influenza. We have generated a database of the influenza-specific antigenic regions restricted by the majority of human HLA-A and HLA-B alleles, and determined how conserved these regions were in influenza viruses circulating over the last century. We found that although sporadic variations can be found across many peptides, mutations 'escaping' T-cell recognition get fixed only for selected $CD8^+$ T-cell populations. Using a human single-cell multiplex RT-PCR for TCRαβ, we found that specific TCRs can recognize multiple variants derived from different influenza strains. For example, a dominant public TCR clone (TRAV27 GGSQGNL; TRBV19 SIRSSYEQ) found at high frequency within $M1_{58}{}^+CD8^+$ T-cell responses in all HLA-A*0201^+ individuals can recognize the naturally-occurring variants of the peptide. This suggests that the HLA-$A2^+$ population (30-50% global coverage) have universal T-cell immunity to any $M1_{58}$ variant, which can, at least in part, explain high conservation level for $M1_{58}$. Conversely, TCR clones within the $B7^+NP_{418}{}^+CD8^+$ populations differentially recognise distinct peptides from either the pandemic or seasonal influenza viruses. Our current analyses across other HLAs extend these findings. Thus, our studies suggest that a pre-emptive influenza vaccine that elicits TCR pools, which recognize distinct antigenic variants can provide universal immunity against any future influenza strain or subtype.

P4.01.050

T cell mediated mortality in neonatal mice upon LCMV infection

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Newborns are more susceptible to infections due to their lack of immunological memory and under-developed immune systems. Passive maternal immunity protects neonates until their immune systems have matured. We questioned if a noncytolytic virus that produces strong T cell responses in adults would also induce a response in neonates. Neonates were infected with LCMV at 5e4, 500, 50, 5 and 0.5 PFU. Surprisingly, at all doses the majority of pups succumbed to infection during the peak of the T cell response. Death was caused by T cell-dependent pathology and not viral load as 100% of T cell deficient pups survived with minimal lung and liver pathology. However, passive immunity from LCMV-immune mothers protected 100% of pups from death by helping control viral load early in infection. In adult mice high antigenic stimulation can cause over-activation of T cells leading to clonal exhaustion, where cells undergo a stepwise loss of function. Clonal exhaustion allows for survival from some high dose viral infections and results in persistent infection. We found that neonates without passive immunity that survived to day 14 did not show signs of T cell clonal exhaustion. Furthermore, surviving pups were not persistently infected, clearing virus by week 3. Neonates are commonly thought to have less functional immature immune systems, but these results show that neonates are capable of producing strong T cell responses that contribute to increased mortality.

P4.01.051

Association between IL-28B(rs12979860) polymorphisms of interferon-λ (IFN- λ) family with Hepatitis C virus infection outcome

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Background&Aim: Hepatitis C infection is a global health problem There are several factors, which is effective on developing diseases outcome in individuals infected with HCV such as, environmental condition, host genetic factors. Single nucleotide polymorphisms near the human IL-28B gene are associated with sustained virologic response.The aim of this study was to evaluate the influence of IL28B genotypes on SVR rates in a group of patients with chronic hepatitis C. Materials and methods: In this study, 133 HCV-RNA positive patients, 96 samples (72.2%) males and 37 samples (27.8%) females with mean age of 36.38±12.49 years,and 173 healthy volunteers with age, sex and geographical area matched with patients were recruited. DNA was extracted and genomic DNA were amplifed for IL-28B gene polymorphism by Tetra-ARMS-PCR.
Results: In the present study, the frequencies of the IL-28B genotypes (rs-12979860) in patients were as follows: C/C, 41.4%; C/T, 41.6% and T/T, 17.3%. Frequencies of the IL-28B genotypes in healthy control were in healthy control as follows: C/C, 42.5%; C/T, 40.6% and T/T, 16.9%. 85.5% patients with the C/C genotype achieved a sustained virologic response (SVR), compared with 44.4% with the C/T and 21.7% with the T/T genotype ($p<0.0001$).
Conclusion: There was not a significant difference in polymorphism of IL-28B between patients and controls. IL-28B (rs12979860) CC genotype was associated with a better treatment response rate. The SVR rate significantly associated with the C allele Genetic change in IL-28B gene as an independent variable in determining the efficiency of response to antiviral therapy will be important.

P4.01.052

Regulatory T cells in persistently virus infected hosts influenced immune responses against superinfection with unrelated viruses

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Expansion of regulatory T cells (Tregs) and dysfunctional T cell responses are found in chronic virus infections (e.g. HIV, HCV). It is still unclear whether Tregs from chronic infections may influence immune responses against superinfections with unrelated viruses in vivo.
Therefore we established a mouse model: mice persistently infected with Friend Virus (FV), a murine retrovirus, were superinfected with the murine cytomegalovirus (mCMV). We found that FV expanded Treg cells significantly decreased mCMV-specific T cell responses during primary mCMV superinfection in persistently FV infected mice. In addition, we detected that a primary mCMV infection led to 'reactivation' of FV resulting in functional FV-specific T cells. However, FV expanded Tregs were not influenced during mCMV infection and were rapidly able to dampen the 'newly' generated FV-specific T cell responses.
Here, we show for the first time that Treg cells expanded in a chronic virus infection played an important role during superinfection with unrelated viruses.

P4.01.053

Granzyme B mediated T-cell immunity against small DNA viruses

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Objectives. A novel role for CD4+ T cells with cytolytic potential (CD4+ CTL) is emerging. These cells appear to have a role in controlling malignancies and chronic infections. Human parvovirus B19 establishes a persistent infection, yet no data has been reported whether B19 -specific CD4+ CTLs are present. Such cells could have a role in the pathogenesis of autoimmune disorders and possibly in some thyroid malignancies associated with B19.
Methods. Cytolytic responses of human parvovirus B19 specific T cells were explored by, stimulating peripheral blood mononuclear cells (PBMC) with recombinant B19-VP2 virus like particles (VLPs). Human bocavirus 2 (HBoV2) VLPs and *Candida albicans* antigens served as control antigens. Cytolytic potential was evaluated by EIA-based quantitation of Granzyme B (GrB) from tissue culture supernatants.
Results. GrB responses induced by the B19 antigen were readily detectable and stronger than the responses with the HBoV control antigen. T cell depletion and HLA blocking experiments showed that the GrB was secreted by CD4+ T cells. Interestingly, some B19 seronegative subjects also showed GrB responses with B19 VLPs.
Conclusion. A vigorous B19-specific GrB response was found in seropositive individuals suggesting role for CD4+ CTLs in B19 immunity. Such cells could potentially function in, immune regulation and triggering of autoimmune phenomena, possibly including even some cases of SLE or rheumatoid arthritis.

P4.01.054

TLRs expression dynamics in patients with herpes-associated erythema multiforme due to immunotherapy

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Herpes-associated erythema multiforme (GAEM) is the most common form among erythemas. Long-term virus persistence in the body leads to development of secondary immunodeficient states. Defects in the mechanisms of recognition of various microbial PAMPs by TLRs expression changes may impair the immune response. The aim of investigation was to study the TLRs expression on PBMC and in the skin of GAEM-affected patients and dynamics of these indicators in the course of therapy. TLRs expression levels were determined by flow cytometry. In study of 39 GAEM-affected patients there was observed high TLRs level in their sera, which confirms the obvious role of viruses in the pathogenesis of dermatosis. In patients with disease duration of less than 6 months there were low values of TLR2 (up to 0.05%) and TLR4 (0.1%) due to increased expression of TLR3 (up to 96.7%) and TLR9 (up to 97.8%). In patients with combined GAEM HSV1 and HSV2 infection in the form of recurrent facial herpes and genital herpes the expression levels of TLR4 and TLR9 in the sera were by 4-fold greater than the values of healthy subjects. Immunovac-VP-4 therapy favored to increase the TLR2, 3,9 expression in sera. Overexpression of these TLRs may be a laboratory marker of the outbreak, the severity of infection and the effectiveness of the therapy. Along with the appointment of antiviral complex the therapy with immunomodulators restores cell cooperation in the immune response of GAEM-affected patients. This work was supported by RFBR grant №11-04-96037r_ural_a and administrative body of Perm Region.

P4.01.055

Interferon-γ regulates contraction of the influenza-specific CD8 T cell response and limits the size of the memory population

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Understanding the mechanisms of virus-host interactions and the factors that regulate memory T cell responses are important for the generation of efficient vaccines. The factors that regulate the contraction of CD8 T cell responses and the magnitude of the memory population against localized mucosal infections such as influenza are currently undefined. Using a mouse model of influenza infection we demonstrate that the absence of IFN-γ or IFN-γR1 leads to aberrant contraction of antigen-specific CD8 T cell responses. The increased accumulation of the effector CD8 T cell population was independent of viral load. Direct *ex vivo* analysis
revealed an increased amount of cell death in influenza-specific-CD8 T cells from infected wild type (WT) mice compared to IFN-$\gamma^{-/-}$ mice.
The absence of contraction was associated with an increased fraction of CD8 T cells expressing the interleukin-7 receptor at the peak of the response, indicating enhanced numbers of memory/precursor cells in IFN-$\gamma^{-/-}$ and IFN-γR1$^{-/-}$ mice compared to WT after the peak of the CD8 T cell response. Re-challenge of the infected mice with a heterologous strain of influenza enhanced the CD8 T cell recall response in the IFN-$\gamma^{-/-}$ and IFN-γR1$^{-/-}$ compared to WT mice, confirming that higher frequencies of memory precursors are indeed formed in the absence of IFN-γ signaling. In summary, we have identified IFN-γ as an important regulator of
localized viral immunity that promotes the contraction of antigen-specific CD8 T cells and inhibits memory precursor formation, thereby limiting the size of the memory cell population after influenza infection.

P4.01.056
Regulation of inducible expression of syncytin-1 following influenza A/WSN/33 virus infection

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Syncytin-1, a highly fusogenic envelope protein encoded by a human endogenous retrovirus (HERV-W) element (*ERVWE1* locus) on chromosome 7q21, is normally expressed exclusively in the syncytiotrophoblast layer of the human placenta. Whereas syncytin-1 appears crucially involved in the development and functioning of the human placenta, its ectopic expression in adult tissue has been associated to a range of pathological conditions, such as cancer, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), preeclampsia and schizophrenia. We previously reported on the transactivation of HERV-W elements, including that encoding syncytin-1 by influenza A/WSN/33 virus infection in a range of human cell-lines of non-placental origin. The mechanisms underlying the transactivation by virus infection were, however, not identified. qPCR analysis indicated up-regulation of transcripts encoding transcription factor glial cells missing 1 (GCM1) and syncytin-1 in response to influenza A/WSN/33 virus infection in both malignant cell-lines and primary fibroblast cells. Knock-down expression of GCM1 by siRNA, followed by viral infection suppressed the transactivation of the gene encoding syncytin-1 in CCF-STTG1 cells. Pyrosequencing analysis revealed high methylation levels of *ERVWE1* promoter and enhancer regions in non-placental cells in comparison with human choriocarcinoma JEG-3 cells. No alteration of methylation status was observed between uninfected and virus infected non-placental cells. Our results indicated that inducible expression of syncytin-1 following influenza A/WSN/33 virus infection in non-placental cells come from elevation of both unspliced (corresponding to retro-viral genomes) and spliced *ERVWE1* transcipts (encoding syncytin-1). Expression of syncytin-1 appear critically linked to GCM1 during both physiological and pathological conditions. Expression of the transcription factor GCM1 is required for expression of syncytin-1.

P4.01.057
Profile of NAb responses in HIV-1-infected individuals in China and epitope analysis of viral envelope from broad neutralizers

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To clarify the profile of neutralizing antibody (NAb) responses in HIV-1-infected individuals in China and the epitopes of viral envelopes from broad neutralizers, the neutralizing ability of plasmas from chronic infected individuals from Xinjiang and Guangxi Province, against pseudoviruses of subtypes B, C, CRF07_BC, CRF01_AE and B' using the TZM-b1 pseudovirus assay were analyzed. Breadth and strength of neutralization of the plasmas were evaluated. Viral species in infected individuals with broad NAb responses (broad neutralizers) were analyzed using a single genome amplification (SGA) of envelope genes and sequencing followed by bioinformatics analysis. We found that in Xingjiang Province, where most samples were derived from IDUs, 3.2% of the plasmas can neutralize four subtype B viruses and 5.3% can neutralize four subtype C viruses at inhibition higher than 80% of infection. Only 1.6% and 0.8% of the plasmas were able to neutralize the viruses of both B and C subtypes. 0.8% of the plasmas were able to neutralize all 11 viruses of three different subtypes, B, C, and CRF01_AE at higher than 80% of infection. However, in individuals from Guangxi province where most samples were derived from sexual contact infection, lower neutralization titers were found with same virus set. Unique epitopes were identified within viral envelope glycoprotein of broad neutralizers. (This work was supported by the National key project 2008ZX10001-012).

P4.01.058
Delivery of human EV71 receptor by adeno-associated virus increase EV71 infection in adult mice

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Enterovirus 71 (EV71) is responsible for hand, foot, and mouth disease in children under 6 years of age. Recently, there are two EV71 receptors in human have been identified human P-selectin glycoprotein ligand-1 (hPSGL-1) and human scavenger receptor class B, member 2 (hSCARB2) as the functional EV71 receptors. We hypothesized that delivery of hPSGL-1 by adeno-associated virus (AAV) could increase EV71 infection in adult mice. To prove this hypothesis, both green hSCARB2 and hPSGL-1 gene was cloned into AAV vector to generate rAAV- hSCARB2 and rAAV-hPSGL-1 virus. We found that oral and intravenous administration of rAAV-GFP can express highest level of GFP in the intestine and other organs. Based on this result, rAAV-hSCARB2 and rAAV-hPSGL-1 were administered by oral and intravenous route for gene transfer. The expression levels of both hSCARB2 and hPSGL-1 in the intestine and brain were analyzed using real-time PCR and Immunohistochemistry. Histopathological examination revealed that the expression of hSCARB2 and hPSGL-1 in brain and intestine were detected after 4 weeks. After EV 71 infections, we also found that both hSCARB2 and rAAV-hPSGL-1 infected mice could up-regulate cytokines secretion in brain or intestine. Moreover, the infectious EV71 viral particles were detected in the brain and intestine from the infected animals. Although the increased EV71 infection did not show neurological manifestations, this technology could be applied to innate receptor-deficient mice to increase their infection and pathogenesis in adult mice.

P4.01.059
Double-stranded RNA (dsRNA)-induced IP-10/CXCL-10 production is augmented by Fas activation

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Viral double-stranded RNA (dsRNA) is recognised by Pathogen Recognition Receptors (PRRs) including RIGI-like Receptors (RLRs) and Toll-Like Receptors (TLRs) and results in cytokine/chemokine production. Fas, a well characterised death receptor, may also mediate inflammation. Our aim was to investigate potential crosstalk between the Fas signalling pathway and anti-viral signalling pathways.
THP-1 monocyte-derived macrophages (THP1-Mø's) were stimulated with poly(I:C) (dsRNA mimetic) +/-agonistic anti-Fas antibody (CH-11). Co-stimulation resulted in a 50% reduction in IL-8, TNFα, IFNβ and IL-10, relative to poly(I:C) alone, as assessed by qRT-PCR. In contrast, IP-10/CXCL-10 production increased by 50% following co-stimulation relative to poly(I:C) alone. Results were confirmed using human monocyte-derived macrophages.
Fas-associated death domain (FADD) is recruited to Fas following Fas activation and is required for Fas signalling. Overexpression of FADD inhibited poly(I:C)-induced IP-10 luciferase production, while inhibiting FADD signalling by overexpressing the FADD death-domain augmented poly(I:C)-induced IP-10 luciferase. To further confirm the role of FADD in poly(I:C)-induced IP-10/CXCL-10 production, wild-type and $FADD^{-/-}$MEFs were treated with increasing doses of poly(I:C). IP-10/CXCL-10 production in $FADD^{-/-}$MEFs was increased compared to wild-type MEFs.
Our results indicate that activation of the Fas signalling pathway, may be necessary for optimal poly(I:C) induced IP-10/CXCL-10 production. As IP-10/CXCL-10 is a potent T-cell chemo-attractant, cross-talk between these pathways may potentially promote the recruitment of T-cells to sites of viral infection.

P4.01.060
Contribution of Hantaan virus-specific CD4$^+$ T cells to anti-viral T-cell response in humans

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Hantaan virus (HTNV), the prototype of genus Hantavirus, could cause the lethal hemorrhagic fever with renal syndrome in human. Our previous studies have shown that the HTNV-specific CD8$^+$ T-cell responses were essential to control the HTNV infection. However, the contribution of CD4$^+$ T cells to the host response against HTNV infection has not been clear. Here, we mapped the CD4$^+$ T-cell epitopes with IFN-γ ELISPOT assays, using 351 overlapping peptides spanning the entire structure protein of HTNV in a large cohort of HTNV-infected individuals. A total of 65 novel 15-mer CD4$^+$ T-cell epitopes of the HTNV were identified, among which 24 peptides were dominant target epitopes. Using intracellular cytokine staining and proliferative assays, we found that the epitopes-specific CD4$^+$ T cells showed an effector memory and differentiation phenotype (CD27$^-$ CD28$^{+/-}$CCR7$^-$CD45RA$^-$), and displayed multiple-cytokine patterns including IFN-γ and TNF-α, meanwhile the CD107a, granzyme B and perforin, suggesting the effective and cytotoxic ability against HTNV infection. Furthermore, the HTNV-specific CD4$^+$ T cells derived from more severe patients with much higher viral load displayed narrower antigenic repertoire and much weaker responses, meanwhile, an impaired proliferative capacity than those from patients with milder outcome. Taken together, these results indicate that the effective HTNV-specific CD4$^+$ T-cell response is important for virus control, which would add weight to understanding the important role of CD4$^+$ T-cell response after HTNV infection and provide a rationale foundation to speed up the process of effective vaccine development.

P4.01.061
CD200R1 is required for sustained HSV-1 infection and for HSV-induced and TLR2-dependent response

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Herpes Simplex Virus (HSV) is a highly prevalent human pathogen and is the most common cause of sporadic encephalitis, a devastating disease of the central nervous system (CNS). Herpes Simplex Encephalitis (HSE) results in tremendous damage to the CNS, which is thought to be caused by both direct viral damage to the brain and the body's immune response to the infection. CD200R1 is expressed on myeloid cells and on brain microglia. We have found that CD200R1-deficient cells are not able to support robust HSV replication, when compared to wild-type cells. HSV-1 infection of the brains of wild type but not CD200R1 knockout mice resulted in sustained virus replication suggesting that CD200R1 expression is pro-viral. CD200R1 was also important for the TLR2 response induced by HSV-1. Without CD200R1, cells are unable to secrete IL-6 upon either HSV-1 infection or upon TLR2 stimulation with lipopeptides. Our studies demonstrate that CD200R1 is an important component of both the replication cycle of HSV-1 and the innate immune response of the host to herpesvirus infection and TLR2-stimulation..

P4.01.062
α-defensin-induced MxA expression in healthy human periodontal tissue

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Although periodontal tissue is continually challenged by microbial plaque, it is generally maintained in a healthy state. To understand the basis for this, we investigated innate antiviral immunity in human periodontal tissue. The expression of mRNA encoding different antiviral proteins, myxovirus resistance A (MxA), protein kinase R (PKR), oligoadenylate synthetase (OAS), and secretory leukocyte protease inhibitor (SLPI) were detected in both healthy tissue and that with periodontitis. Immunostaining data consistently showed higher MxA protein expression in the epithelial layer of healthy gingiva as compared with tissue with periodontitis. Human MxA is thought to be induced by type I and III IFNs but neither cytokine type was detected in healthy periodontal tissues. Treatment *in vitro* of primary human gingival epithelial cells (HGECs) with α-defensins, but not with the antimicrobial peptides β-defensins or LL37, led to MxA protein expression. α-defensin was also detected in healthy periodontal tissue. In addition, MxA in α-defensin-treated HGECs was associated with protection against avian influenza H5N1infection and silencing of the MxA gene using MxA-targeted-siRNA abolished this antiviral activity. To our knowledge, this is the first study to uncover a novel pathway of human MxA induction, which is initiated by an endogenous antimicrobial peptide, namely α-defensin. This pathway may play an important role in the first line of antiviral defense in periodontal tissue.

P4.01.063
Immflammation in Influenza A H1N1 pandemic virus

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Objective: Evaluate how overproduction of proinflammatory cytokines induced by LPS might affect host immune response and clinical course of infection in C57/Bl6 H1N1 influenza A pandemic virus infected mice.
Material and methods: Four groups of mice were compared, based upon the challenge they were exposed to: 1) LPS 2) pandemic H1N1 Influenza A virus (pH1N1) + LPS 3) pH1N1 4) mock. LPS intraperitoneally inoculum dose were 200 µg/mice and pH1N1 infection dose was $2*10^5$ PFU/mice. Samples of serum, lung and spleen were collected at different time-points in order to assess levels of IL-6 by ELISA. Histopathological studies, body weight changes and clinical signs were evaluated daily.
Results: Mice infected with H1N1 and treated with LPS showed a higher percentage of weight loss and a longer recovery period related with a higher histophatological score as compared with LPS-untreated mice. Levels of IL-6 in lung of infected animals were similar within groups, large amounts of this protein was observed at day 5pi.
Conclusion: Results so far indicated that treated with LPS and infected with H1N1 showed a more virulent/pathogenic disease progression, by an IL-6 independent mechanism. Further analysis are in progress.

P4.01.064
Strategies eliciting cross-reactive CD8+ T cell immunity by increasing cross presentation of nonreplicating viral immunogens

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Studies of humoral immunity indicate that influenza virus evades antibody response directed exclusively against haemagglutinin and neuraminidase, by varying targeted epitopes and antigenic shift. Cytotoxic T lymphocytes (CTL) directed to more conserved structure proteins may result in a cross protective immunity against different influenza strains or pandemic viruses.

The annual vaccine, based on whole inactivated influenza virus is incapable to elicit a consistent CD8 influenza specific response. The ideal influenza vaccine would generate CTL that recognizes highly conserved viral peptides presented by HLA class I of infected cell. To this end the cross reactive CD8 memory response was evaluate in healthy individuals to different HLA supertype restricted epitopes derived from avian influenza virus isolated in poultry worker in northern Italy, (A/turkey/Italy/214845/2002(H7N3)). The highest CTL frequency recognized epitopes derived from matrix protein, basic polymerase complex and nucleoprotein. Several epitope-specific clones were generated and used in cross presentation assay to evaluate the recognition of peptide from dendritic cell pulsed with whole inactivated PR8, X31, H7N3 viruses. So we set up an efficient antigen presentation assay aimed to identify novel molecules or drugs improving cross presentation. Preliminary data demonstrate the ability of lysosomotrophic drug (chloroquine) to improve cross-presentation by inhibition of endosomal protease. Chloroquine may represent possible adjuvant in vaccine formulation that can recall CD8 and antibody response resulting in more vaccine efficiency.

P4.01.065
mTOR-dependent regulation of mucosal CD8 T cell immunity

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Mucosal tissues are sites of frequent pathogen exposure and major routes for the transmission of infectious diseases. Migration of virus-specific memory CD8 T cells into mucosal tissues such as the small intestine and vaginal mucosa is highly restricted to non-circulating tissue resident memory CD8 T cells. Resident mucosal memory CD8 T cells are sufficient to protect against the establishment of mucosa-acquired viral infections, and thus it is essential to develop vaccine strategies that elicit long-lived mucosal CD8 T cells. CD8 T cells residing in mucosal tissues are phenotypically distinct from those in secondary lymphoid tissues and factors critical for their formation are poorly defined. It is also unclear whether mucosal CD8 T cells possess a unique requirement for their generation and maintenance from that of circulating memory CD8 T cells. We report that unlike in the secondary lymphoid tissues, the formation of virus-specific memory CD8 T cells in the small intestine and vaginal mucosa is dependent on mTOR signaling. In addition, we demonstrate using a CD8 T cell mediated model of intestinal autoimmune disease that inhibiting mTOR results in a loss of CD8 T cells in the small intestine and protects mice from lethality. Furthermore, we will discuss data that targets mTOR signaling intrinsically within the CD8 T cells to provide mechanistic insight into how the mTOR pathway specifically controls the generation of mucosal CD8 T cells in response to viral infections.

P4.01.066
mTOR regulates protection against a lethal H5N1 influenza infection by modulating the antibody response

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Highly pathogenic H5N1 avian influenza A viruses pose a serious threat for global catastrophe. Current vaccines do not provide protection against novel influenza A viruses that have emerged through genetic reassortment across different species, and attempts to create universal influenza vaccines have, as yet, been unsuccessful. We show here that rapamycin, an immunosuppressive drug that inhibits mTOR, promoted cross-strain protection against lethal H5N1 infection when administered during immunization with a serologically different H3N2 influenza virus. Rapamycin treatment reduced germinal center formation and B cell class-switching, yielding a unique repertoire of antibodies that enhanced protection against a lethal, heterosubtypic influenza infection. These data not only establish a requirement for mTOR in B cell class-switching, but also demonstrate that a broader antibody repertoire, which provides better protection against multiple strains of influenza, can be achieved by reducing germinal center formation during the primary infection. These findings have important implications in designing universal influenza vaccines.

P4.01.067
Influence of the factors OCT3/4, SOX2, KLF4 or c-Myc on cell transformation promoted by the oncoprotein tax from HTLV-I

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Human T-cell leukemia virus type 1 (HTLV-I) infects approximately 15-20 milion people worldwide and is able to cause Adult T-cell Leukemia (ATL). The virus encodes the oncoprotein Tax wich can immortalize human T-lymphocytes and transform rodent cells in vitro. However the mechanism involved in cell tranformation by Tax are not fully understood. It has been shown that cancers are comprised of two broad categories of cells: those with high- and those with limited-proliferative potential. Cells in the former category are termed cancer stem cells. CSCs share several properties with adult stem cells, particularly the abilities to self-renew and differentiate into multiple cell types. Our new research proposal addresses a key question "Can the HTLV-1 Tax oncoprotein transform embryonic stem cells better than primary cells?" In the present study NIH3T3 cells were transfected with both Tax and individual pluripotence-associated factors (OCT3/4, SOX2, KLF4 and cMYC into CMV3.1 expression plasmid) communly used for cell reprograming. Hella cells were used as tranformed cell control. The cell growing of the transfected cells was kept on soft agar and the cell colony counting (UFC) was achieved using ImageJ software. According to the results, cell transfection using Tax plus KLF4 (mean=197±13.4) and Tax plus OCT3/4 (mean=94.7±18.0) promoted a higher UFC counting as compared to the cells trasfected with Tax (mean=21,3±8,7) or the other factors, isolately. Althouth very preliminar, the results presented here point to an interessting way for future investigations on cell transformation pathways observed in HTLV-I infection.
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P4.01.068
IDO activity by langerin-negative dendritic cells contributes to local immune suppression in the skin expressing HPV oncoprotein E7

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Chronic infection of anogenital epithelium with human papillomavirus (HPV) promotes development of cancer. Many pathogens evoke immunosuppressive mechanisms to enable persistent infection. We have previously shown that grafted skin expressing HPV16 E7 oncoprotein from a keratin-14 promoter (K14E7) is not rejected by a syngeneic, immunocompetent host. In this study we show that indoleamine 2, 3-dioxygenase (IDO) 1, an IFN- inducible immunoregulatory molecule, is more highly expressed by dendritic cells from K14E7 skin than nontransgenic control skin. Furthermore, inhibiting IDO activity using 1-D/L-methyl tryptophan promotes K14E7 skin graft rejection. Increased IDO1 expression and activity in K14E7 skin requires IFN- and iNKT cells, both of which have been shown to negatively regulate T-cell effector function and suppress K14E7 graft rejection. Further, $CD11b^{hi}$ $Langerin^{-}$ dendritic cells from K14E7 skin express high level of IFN- receptor (IFN-R). K14E7 transgenic skin recruits significantly higher number of dendritic cells, independent of IFN- and IFN-R expression. Consistent with these observations in a murine model, we found higher expression of IDO1 and IFN- but not IDO2 in the cervical epithelium of patients with HPV-associated cervical intraepithelial neoplasia (CIN) 2/3. Our data support a hypothesis that induction of IDO1 in HPV infected skin contributes to evasion of host immunity.

P4.01.069
Immune deficiency in newborns and infants with cytomegalovirus infections

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The problem of children born on infected mothers is one of the main problems in modern neonatology and pediatrics. Wide dissemination of cytomegalovirus infections in the adult population represents a high risk of becoming infected pregnant woman and newborns. According to the results of autopsies 37,5% of children died of infectious pathology and the main cause of death, accompanied or complicate the course of the underlying disease.
Aim of investigation: studying the clinical and immunological features of children with cytomegalovirus infections and to examine the effectiveness of immunotherapy. We have examined 89 infants with cytomegalovirus born from mothers with positive cytomegalovirus serological tests.
Results: It was established that the humoral type of IDS was detected by increased levels of specific IgM, IgG and IgA antibodies and activation of pro-inflammatory cytokine synthesis of IL-1, IL-6, TNF-α in serum. These patients had characteristic disturbances in cellular immunity - the quantitative deficit of T- and B-cells and increase the percentage of NK cells. After treatment by immune modulator Licopid they had normalization of peripheral blood leukocytes, revealed a significant increase in absolute and relative numbers of CD3+ and CD4+ lymphocytes, normalization of CD4/CD8 ratio and levels of IgM, IgG, IgA, which changed to the normal indexes.
Thus, it was found that the treatment by Licopid in infants with immunodeficiency leads to the reduction of the duration of disease, contributes to a more rapid improvement of children and this drug can be used as very effective immune modulator.

P4.01.070
APOBEC3 deaminases induce hypermutation in human papillomavirus 16 viral genomic DNA upon interferon-β stimulation

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APOBEC3 deaminases are interferon-inducible antiviral factors that suppress a wide range of viruses and retroelements, including human immunodeficiency virus-1, possibly by accumulating cytidine (C)-to-uracil (U) mutations in viral genomic DNA. Currently, it remains unknown whether APOBEC3 proteins are involved in the pathogenesis of human papillomavirus (HPV) infection. Here, we examined the possible role of APOBEC3 deaminases in pathophysiological processes in HPV infection. W12 cells are derived from an earlier phase of HPV16 infection, and they retain the viral episome in the nucleus. When W12 cells were stimulated by interferon-β (IFN-β), the expression of APOBEC3 proteins, including APOBEC3A, 3G, and 3F, was upregulated. IFN-β stimulation induced C-to-T and G-to-A hypermutations in the HPV16 E2 gene when uracil DNA glycosylase (UNG) was inhibited by the expression of UNG-inhibitory protein (UGI). The overexpression of APOBEC3 proteins also induced hypermutations in UGI-expressing W12 cells. Furthermore, hypermutations by IFN-β were blocked by transfection of small interfering RNA against APOBEC3G and 3A. However, antiproliferative activity or restriction of HPV16 DNA by IFN-β was not impaired by knocking down APOBEC3G and 3A. The overexpression of APOBEC3 caused the activation of DNA damage stress responders such as γ-H2AX. We found that clinical samples from pre-cancerous (CIN1) patients also accumulated hypermutation on HPV viral DNA. These data suggest that IFN-β induces both HPV hypermutations and cellular DNA damage response through APOBEC3 expression, but APOBEC3 may not require antiviral activities by IFN-β. APOBEC3 protein could be a risk factor in facilitating oncogenesis in HPV infection.

P4.01.071
Rapid method to screen for CMV, EBV, and AdV specific T cells in multiple samples

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Flow cytometric analysis of activation markers and cytokines after short-term in vitro T cell stimulation is well established for research studies to identify and characterize antigen-specific T cells. But its routine use for immune monitoring applications is hampered, because screening of a lot of samples is time-consuming, requires many manual handling steps, and analysis experience. We worked out a complete strategy to rapidly study with a semi-automated process in multiple samples the functional profile of T cells.
For simultaneous analysis of multiple human PBMC samples we established an antigen-coated 96-strip-well culture system to stimulate T cells. This flexible ready-to-use format allows screening either for single antigens or in parallel for several specificities by combining 8-well-strips possessing different antigens. To reduce time and work load, we developed a rapid and easy-to-handle intracellular staining protocol for the 96-strip-well plate and defined reagents. Without cell harvesting and washing steps, cells are fixed and stained within 50 minutes. Furthermore, we integrated an automated flow cytometric analysis process, including a hands-free measurement using pre-defined experiment settings and acquisition templates. We also applied an automated gating strategy for data analysis and a final report summarizes the results. This standardized process, including reagents, protocol, and analysis decreases inter- and intra-assay variations.
In summary, with our newly developed tools and protocols for in vitro T cell stimulation, staining of activation markers and cytokines, and automated flow cytometric analysis we have set up a fast and convenient procedure to routinely monitor antigen-specific T cell responses.

P4.01.072
Polyfunctional T cells are generated concurrent with the establishment of immunodominance hierarchy during the evolution of the T cell responses to Epstein-Barr virus

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Background: Whether immunodominance corresponds to the functions of the antigen-specific T cells during the evolution of the T cell responses towards Epstein-Barr virus (EBV) remains unclear. This study aims to characterize the functions of EBV lytic- and latent-protein-specific T cells in relation to the immunodominance hierarchy from early to persistent phase of infection.
Methods: PBMCs of ten patients with infectious mononucleosis (IM) and four with asymptomatic primary infection (AS) collected from diagnosis through 12-months post-infection were stimulated with overlapping peptides of four lytic-(BZLF1, BRLF1, BMLF1,and GP350) and five latent-(EBNA1, EBNA3A, 3B, 3C and LMP2) proteins, followed by multi-color flow cytometric assay examining the co-expression of three cytokines (IFN-γ, TNF-α and IL-2), perforin and CD107a in CD4+ and CD8+ T cells as well as cytotoxicity and proliferation assays.
Results: Both lytic and latent peptide-specific CD4+ and CD8+ T cells showed increased proportion of polyfunctional T cells capable of producing multiple cytokines simultaneously with enhanced cytotoxic and proliferative capacity over time in all IM and AS patients. The CD8+ T cell responses shifted from the lytic BZLF1/BRLF1 peptides to the latent EBNA3 family peptides and the CD4+ T cell responses from broad range to more focused reactivity towards EBNA1 peptides during the progression from early to persistent phase of infection. Importantly, the immunodominant T cell responses also demonstrated concurrent increase in their functionality.
Conclusions: Polyfunctional T cells are generated concurrent with the establishment of immunodominance hierarchy during the evolution of the T cell responses to EBV.

P4.01.073
Neuroimmunological characterization of children suffering from psychomotor retardation due to Cytomegalovirus infection

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Cytomegalovirus (CMV) infection is the most frequent congenital infection in humans. The 90% of babies asymptomatic at birth but may develop severe neurological deficits during early childhood, when the diagnosis is no longer possible. This diagnostic problem is linked to incomplete clinical characterization of this disease. From this experience we wanted to study childhood bearing psychomotor retardation, looking for the probable role of CMV infection in psychomotor development as an immunological mechanism involved in this process. 70 children from 6 to 36 month of age with disabilities of unknown cause and group controls sex/age-matched were analyzed. CMV, rubella and toxoplasmosis specific IgG were determinate in serum/cerebrospinal fluid paired samples and the antibody index (AI) were quantified. Rubella and Toxoplasmosis AI were not detected in any patients while pathologic CMV-AI was found in 24 patients with psychomotor retardation. Only, one patient from control group was positive to CMV-AI. Matched polymerase chain reaction (PCR) on filter paper used for biochemical screening that was kept from the neonatal period and AI data were available in 15 patients. PCR was simultaneously positive or negative in fourteen cases only one patients belonged a control group show PCR positive with AI negative(p=0.0037). The main clinical manifestations of the patients with congenital infection were seizure (70%), chorioretinitis(28%), hepatosplenomegaly (20%) microcephaly/macrocephaly (20%) and thymus hypoplasia (19%), humoral immunodeficiency(43%), cellular immunodeficiency 95% .IgG-CMVAI and cellular immunodeficiency should be considered as an important tool to determinate if CMV infection is involved in patients suffer from psychomotor congenital impairment.

P4.01.074
Role of the FCGR2A Polymorphism in Susceptibility to Dengue in a Venezuelan Population

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Dengue virus infection is a health public problem. Virus serotypes, viral load, antibody-dependent enhancement (ADE) of infection and IFN-gamma/TNFalpha-mediated immunopathogenesis have been suggested to contribute to the progression and severity of dengue disease. The FCGRIIA gene (CD32) displays a functional A G single nucleotide polymorphism in the region encoding its ligand-binding domain, defining two allotypes which differ in their avidity for complexed human IgG2 and IgG3. Two studies show the role of a polymorphism that affect the function of the receptor for the Fc domain of IgG, which facilitates antibody-dependent enhancement of dengue infection, show associations with to the most severe form of DF in Vietnam and Cubans. In this study we studied the association between FCGRIIA polymorphism and susceptibility to dengue infection. Whole blood was collected from patients with dengue (n= 136; DF=87 and DHF=38) and healthy individuals (n= 132). A PCR with sequence-specific primers (SSP) was applied to establish FCGR polymorphism. Frequencies were determined by direct counting and X2 test was applied to determine frequency differences between groups. Statistically significant differences were found when the control group was compared with virally infected patients (DF+DHF), or with DF clinical form. The frequency of the R/R131 genotype was increased in healthy individuals compared with the whole series of dengue (30.60% vs. 18.25%, respectively; OR=0.51; IC 95%: 0.2893-0.9020; p= 0.0098; pc=0.0294) and DF patients (30.6% vs. 17.24%, respectively; OR=0.47; IC 95%: 0.2426-0.9203; p=0.013; pc= 0.039). This data suggest that, R/R131 genotype seems to confer protection to the development of dengue infection.

P4.01.075
Poly-γ-glutamic acid, a polypeptide secreted by *Bacillus sp*., induces CD14-dependent type I interferon responses through the TLR4-MD2 complex

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Poly-γ-glutamic acid (γ-PGA) is an anionic polypeptide secreted by *Bacillus* sp. that has been shown to activate immune cells through interactions with toll-like receptor 4 (TLR4). However, its ability to induce the type I interferon (IFN) response has not yet been characterized. Here, we report that γ-PGA induces type I IFN via the TLR4 signaling pathway. The induction requires two TLR4-associated accessary proteins: myeloid differentiation factor 2 (MD2) and the pattern-recognition receptor CD14. The induction of IFN-β depends on the molecular weight of γ-PGA. IFN-β production and the expression of the type I IFN-response genes, 2'-5' OAS and ISG56, were stimulated by γ-PGA with a molecular weight of 2,000 kDa or greater. The type I IFN responses induced by γ-PGA had antiviral activity against the SARS coronavirus, as assessed using a SARS coronavirus replicon *in vitro*. Our results identify high-molecular weight γ-PGA as a novel TLR4 ligand requiring CD14 and MD2 for the activation of type I IFN responses, and suggest that the microbial biopolymer γ-PGA is a potential broad-spectrum antiviral agent protecting against virus infections that are sensitive to type I IFN.

P4.01.076
Phosphorylation dependent 14-3-3 protein interactions in innate immune response

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The innate immune system plays an essential role in protecting the host against infections. Viral RNA is the most important viral structure that is recognized by specific receptors of the host, resulting in activation of innate immune response, including antiviral interferon and pro-inflammatory cytokine production, as well as apoptosis of virus-infected cells. The 14-3-3 proteins are a family of acidic regulatory proteins found in all eukaryotes that participate in a variety of cellular processes including metabolism, apoptosis, signal transduction and cell-cycle regulation. We have previously shown that 14-3-3 signaling pathways are activated by viral dsRNA in human keratinocytes. However, the functional role of 14-3-3 proteins in viral infection has not been defined. The aim of this study is to characterize the role of 14-3-3 proteins in innate immune response using high-throughput proteomics combined with bioinformatics and functional studies. First, we have characterized the global changes in protein phosphorylation in human keratinocytes after viral dsRNA transfection. Then, we have used 14-3-3-affinity chromatography combined with quantitative proteomics to identify 14-3-3 interacting phosphoproteins during viral infection. Ingenuity Pathway Analysis shows that many signaling pathways related to apoptosis and cytokine signaling were clearly upregulated after stimulation. Network analysis shows that NFκB and p38 signaling pathways are major targets of 14-3-3 mediated signaling. Sirtuin 1 and MAPKAPK2 are two of the most central component of this signaling network, and the functional contribution of sirtuin 1 and MAPKAPK2 is currently assessed by functional studies.

P4.01.077

Indigenous chickens infected by avian paramyxovirus type 1 strain express pathways involved in innate immunity and epigenetics

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Newcastle disease is devastating to backyard poultry in Africa owned by rural farmers mostly women and youth. It's caused by avian paramyxovirus type 1- APMV-1. The disease has no effective cure and the current vaccines have no practical delivery systems to farmers in rural settings. The aim was to study the innate immune virus response genes expressed by indigenous chicken and if these play a role in providing natural immunity. Airway epithelial cells were obtained and cultured and infected with a virulent APMV-1 Kenyan strain. RNA was extracted after 36 hours and RNASeq method was used for gene expression studies. Mapping, quality control and statistical analysis was done on the raw data using the CLC genomics workbench. Comparisons of the indigenous and commercial gene expression profiles showed the nucleocapsid gene was highly expressed for the APMV-1. In indigenous chickens after the gene set enrichment analysis, the inflammatory pathway had 16 genes expressed, the immune response pathway had 10 genes, the protein kinase pathway had 9 genes and the NF kappa B cascade had 7 genes expressed. Most of the genes according to the gene ontology definition are involved in innate immunity. Pathways also involved in post-translational modifications were also expressed in indigenous poultry implying an epigenetic role towards disease response. In conclusion, indigenous chickens have expressed natural immunity that needs to be explored further. This has implications for research into diagnostic and vaccination kits that would be useful in Africa for controlling not only Newcastle disease, but other poultry diseases.

P4.01.078

Expression of retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) in the human term placenta

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Innate recognition of viruses is mediated by germline-encoded pattern recognition receptors (PRRs) that initiate signaling events, resulting in the production of type I interferon and proinflammatory cytokines. Double-stranded RNA and 5'triphosphate group of ssRNA generated during viral replication are detected by sensory molecules, named RIG-I-like receptors (RLRs). Three members of the RLRs family have been characterized so far, including RIG-I (retinoic acid-inducible gene-I), MDA5 (melanoma differentation-associated gene 5) and LGP2 (laboratory of genetics and physiology 2). RIG-I participates in the recognition of Paramyxoviruses, Rhabdoviruses, Flaviviruses and Orthomyxoviruses, whereas MDA-5 is essential for the recognition of Picornaviruses.
In this study, we investigated the RLRs gene expression in fetal and maternal parts of the human placentas during viral infection. The explants of third-trimester chorionic villi and deciduas were exposed to infection with dsDNA viruses (human cytomegalovirus, HCMV; herpes simplex virus type 1, HSV1) or ssRNA virus (vesicular stomatitis virus, VSV). RIG-I, MDA5 and LGP2 mRNA levels were assessed by real-time PCR.
Our results demonstrated that all RLRs were expressed in term human placentas with the highest level of LGP2. RIG-I and MDA5 (in fetal part) have been identified as the main mediators of VSV recognition, whereas RLRs were not involved in the identification of dsDNA viruses. The expression of LGP2 in HCMV-infected explants was decreased compared to mock-infected. The results clearly indicated that RLRs play essential role in RNA viruses' recognition in term placenta.

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P4.01.079

Immune response to Apeu virus (orthobunyavirus) infection in murine model

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Introduction and Objectives: Arthropod-borne viruses have emerged as a major human health concern. Viruses transmitted by mosquitoes are the cause of the most serious and widespread human diseases worldwide and are ubiquitous in areas where man lives. The Apeu virus (APEUV) belongs to group C arboviruses, member of Bunyaviridae family and Orthobunyavirus genus. Although APEUV has already been isolated for more than five decades, the role of the in vivo or in vitro immune response in the pathogenesis of APEUV remains unknown. The aim of this study was to investigate the infection kinetics of as well as the immune response profile triggered by APEUV infected Swiss and C57BL/6 mice. Methods and Results: The animals were experimentally infected with APEUV at different periods (12, 24, 48, 72 and 120 hours). The spleen cells proliferative response was evaluated in vitro using the MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] colorimetric assay. A cytometric bead array (CBA) immunoassay kit was used for semi-quantitative analysis of several cytokines (IL-2, IL-4, IL-6, IFN-γ, TNF, IL-17A and IL-10) in the serum of infected mice. We observed an increase ($p < 0.05$) in the secretion of pro-inflammatory cytokines (IL-6, IFN-γ, TNF) in serum of animals 72 hours post-APEUV infection. Supporting the hypothesis that the 72 hours after infection follows the peak of infection by these viruses. Conclusion: The APEUV induces an early response in Swiss mice and late inflammatory in C57BL / 6.

P4.01.080

Vaccinia and Apeu virus activation of human double-negative T cells stimulated in vitro

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The Apeu virus (APEUV), a Orthobunyavirus of group C, has been described in human infections. Zoonotic human infections caused by Vaccinia virus (VACV), a Orthopoxvirus, in Brazil reached worldwide recognition. Antiviral immune response mechanisms target CD4+ T lymphocytes (T helper), CD8+ T lymphocytes (cytotoxic) and NK cells (natural killer). Monkeys infected with SIV do not develop clinical disease and the CD4-CD8- T cell population (double-negative-DN) was responsible for compensating, the function of CD4+ cell depleted in this disease. This suggests that the DN T cells play an important role in the antiviral immune response. The aim of this work was to evaluate the activation profile of DN T cells expressing alpha-beta (αβ TCR) or gamma-delta T-cell receptors (γδ TCR) from healthy humans. The peripheral blood mononuclear cells were isolated from four donors and exposed to UV-inactivated APEUV and VACV (WR). The expression of surface markers (CD4, CD8, αβ TCR and γδ TCR) and the intracellular cytokines (IL-1β. IL-6, IL-10, IL-17A, IFN-α, IFN-β, IFN-γ and TNF-α) were assessed by flow cytometry. We observed a higher frequency of αβ DN T cells expressing IL-10, IFN-α and TNF-α in VACV stimulated cells. IFN-γ expression was higher in VACV-stimulated γδ DN T cells compared to non-stimulated cells. We did not observe differences in the expression of these markers in APEUV's stimulated cells. These results show that DN T cells from healthy humans are highly activated in vitro by VACV and no responsive to APEUV.

P4.01.081
Mkk4 is not essential for negative selection but is required for optimal T cell activation and function during acute but not chronic infection

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Background: Our immune system is continuously poised on the edge of activation and has developed numerous checkpoints to inhibit overt autoimmunity but permit swift and robust responses to bona fide infections. Mitogen-activated protein kinase kinase 4 (Mkk4) is responsible for the phosphorylation of JNK 1/2, which regulate a vast array of cellular processes. JNK1 has been reported to play an important role in negative selection but the role of Mkk4 during this process is controversial. We hypothesized that Mkk4 plays a role in immunity and particularly immune responses to infection. The aim of our study was to determine the role of Mkk4 in immune homeostasis and infection.
Design: We analysed conditionally gene-targeted mice, and infected animals with acute and chronic forms LCMV.
Results: We show for the first time that Mkk4 is not required for development and homeostasis of the T cells. However, during an acute infection, mice with a deficiency of Mkk4 in T cells showed a blunted CD8 T cell response. In contrast, during chronic overwhelming infection loss of Mkk4 did not contribute further to the inefficient T cell responses that have been observed in wild-type mice.
Conclusion: These data clearly identify an important role for Mkk4 in the initial activation and proliferation of CD8 T cells during an acute infection, which is required for the production of an effective adaptive immune response. During chronic infection, however, the overwhelming inflammatory milieu appears to override the role of Mkk4 during the production of a T cell response.

P4.01.082
Human APOBEC3 cytidine deaminases: not simply antiviral defense?

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Host-encoded restriction factors are important players in innate antiviral immunity. Human APOBEC3 (apolipoprotein B mRNA-editing enzyme catalytic polypeptide) proteins induce dC-to-dU-mutations, leading to DNA degradation or hypermutation and subsequent loss of viral genetic integrity. The APOBEC3 expression pattern in the host is ill-defined and regulated by inflammatory mediators secreted upon viral infection.
APOBEC3 deaminases have the capacity to edit host DNA, which may influence cellular genome stability and potentially play a role in the onset of cancer. We hypothesize that inflammatory processes induced by virus infections enhance the expression of APOBEC3 deaminases and may represent a novel mechanism of virus-induced oncogenesis.
In order to investigate the mutagenic potential of the different human APOBEC3 deaminases, we established stable, isogenic cells lines driving tetracycline-regulated APOBEC3 gene expression. As a readout for APOBEC3-mediated host genome mutagenesis, we use counterselection against herpes simplex virus thymidine kinase (HSV-TK): APOBEC3-mediated DNA editing leads to HSV-TK reporter gene inactivation and allows survival of ganciclovir (GCV)-resistant colonies. At first, a founder line of Flp-In-T-Rex cells stably expressing the HSV-TK reporter gene was established and functionally characterized. The integrated copy number of HSV-TK genes was determined by Southern blotting. After successful integration of APOBEC3 genes via FLPase-mediated DNA recombination, a phenotypic analysis based on DNA damage repair and cell cycle progression was performed to obtain deeper insight into APOBEC3 functions.
In addition, we profiled hepatitis virus-induced tumor samples to elucidate APOBEC3 involvement in inflammation-associated carcinogenesis. Immunohistochemical studies showed a slight increase in APOBEC3 protein expression in hepatocellular carcinoma groups.

P4.01.083
An evidence for the pathogenic role for virus-specific T cells in the mouse model of coxsackievirus B3-induced myocarditis

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A long-standing question exists as to whether the chronic inflammatory process that occurs in coxsackievirus B3 infection (CVB) is due to an autoimmune response to cardiac antigens or to a virus-specific immune response. We recently reported that myocarditis-susceptible, A/J mice infected with CVB generate cardiac myosin-specific CD4 T cells that transfer the disease into naïve recipients. We now present evidence that the virus-specific T cells persist during the chronic phase of the disease as evaluated by measuring the T cell responses based on tritiated ^{3}[H] thymidine-incorporation assay and IA^{k} dextramer staining for three immunodominant epitopes of CVB viral protein (VP)1 namely, VP1 681-700, VP1 721-740, and VP1 771-790. In an adoptive transfer protocol, we tested the pathogenic role of virus-specific T cells by transferring the CD4 T cells stimulated with viral peptides into whole body irradiated naïve mice.
The preliminary data indicate that the animals receiving the cells stimulated with VP1 771-790 but not VP1 680-700 and VP1 721-740 showed myocardial necrosis, whereas pancreases remain unaffected, suggesting that virus-specific T cells might have targeted cardiac antigens.
Using bioinformatics tools, we noted that VP1 771-790 contains sequences partially identical (30%) to a protein fragment of actin, alpha cardiac muscle 1 (ACTC1 292-311), pointing to a possibility that VP1 771-790-specific T cells can cross-react with ACTC1 292-311. Taken together, our data support the notion that the immune pathogenesis of viral myocarditis may involve generation of cross-reactive immune responses to cardiac antigens.

P4.01.084
Effects of inoculum size on memory T cell inflation during cytomegalovirus infection

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Cytomegalovirus (CMV) infection of immunocompetent individuals leads to a lifelong state of latency that is controlled in part by $CD8^{+}$ and $CD4^{+}$ T cells. The persistent phase of CMV infection is typified by the accumulation of large numbers of memory T cells that display an effector-memory phenotype. This phenomenon called memory T cell inflation is driven by low level exposure to viral antigen and induction of such responses might be useful in protection against infections and cancers. In an experimental CMV model we examined the impact of the viral dose on the outcome of memory T cell inflation. We observed that low-dose inoculum of mouse CMV, as compared to intermediate dosages, impacts the kinetics of inflationary T cell responses, whereas the kinetics of non-inflationary T cell responses was unaltered. Remarkably, already early after infection the phenotype of inflationary T cells after low dose inoculum is more central-memory-like as evidenced by increased IL-2 production and decreased KLRG-1 expression. Both after low and intermediate viral inoculum inflationary T cells did not show signs of T cell exhaustion. These results suggest that the viral dose influences the outcome of persistent CMV infection by influencing memory T cell inflation. This could have implications for the development of CMV- based vaccine strategies.

P4.01.085
Investigation of ELISA and PCR for diagnosis of Infectious Mononucleosis

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Infectious mononucleosis (IM) is an infectious disease that is characterized by fever, sore throat, lymphoadenopathy, swollen liver and swollen spleen. The etiology of IM is *Epstein-Barr virus* (EBV). Humans are the only known reservoir of EBV. The transmission of virus requires a close contact with the saliva of an infected person. After primary infection, EBV remains latent in the epithelial cells of the normal salivary glands and peripheral blood B cells and becomes reactivated. The aim of this study was to assess ELISA and Nested PCR as a diagnostic tool for IM. In this study, 50 samples were collected from the suspicious patients with EBV and 50 samples from the healthy individuals as the control. Both techniques were applied for the collected samples. The results driven from PCR showed that 76% of the patients and 14% of the control samples had EBV DNA in serum. Statistical analysis showed significant difference between the patient and the control samples (P<0.0001). O ur results suggest that serology may be more sensitive and could be performed as the initial screening test for acute EBV infection. Although, the PCR test is routinely used as an accurate method for detection of pathogens with a higher specificity and sensitivity comparing the immunoassay, but in IM, according to our data, not only ELISA seems to be the best assay for detecting antibodies against EBV but also, it is a cost effective test with less consuming time.

P4.01.086
Virus detection within leucocytes of Atlantic salmon (*Salmo salar* L.) by flow cytometry.

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Virus infections have the most serious ethical and economic impact on fish farming worldwide. No virus vaccines available on the market provide adequate protection. Infectious pancreatic necrosis virus (IPNV) is a birnavirus harming salmonids. This virus resides within the immune cells and during persistent infection replicates in adherent leucocytes, presumably macrophages. In the fight against viral infections in fish the innate cellular activities have central roles, and it has been shown that IPNV causes reductions in the levels of neutrophils in carrier fish. By using flow cytometry we have developed an assay to analyze the presence of IPNV within leucocytes of Atlantic salmon. Particularly, we have analyzed the presence of virus within B-cells and neutrophils from head kidney (primary and secondary lymphoid tissue) and from peripheral blood after *in vitro* challenge of leucocytes by IPNV. IPNV was detected within B-cells and neutrophils as well as in other leucocytes that could not be identified due to lack of cell specific antibodies. The sensitivity of the method was verified by analysis of virus within a persistent infected cells derived from the cell line CHSE-214 (Chinook salmon embryo cells). The consistence of the assay was also verified in an extensive *in vivo* challenge of salmon and subsequent detection of IPNV in leucocyte populations. Presence of IPNV within the immune cells may influence on the cells functions involved in immune response. Identification of immune cells harboring virus during acute and persistent stages of infection may give valuable information concerning immune stimulation and vaccine development.

P4.01.087
Human papillomavirus-associated induction of human β-defensins in cervical intraepithelial lesions

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Cervical cancer is the second most common malignant neoplasm in women worldwide. HrHPV (high-risk human papillomavirus) E6 and E7 oncoproteins play a key role in the deregulation of innate immunity antimicrobial peptides and proteins (AMPs). Therefore, the present study was performed to analyse AMPs expression in HPV-induced cervical lesions.
60 cervical specimens were analysed for the presence of LL-37, RNase 7, and human β-defensin (hBD)-1, hBD-2 and hBD-3 using TaqMan Real Time PCR mRNA expression. Isolated DNAs were tested for HPV presence. Moreover, HPV 16, 18, 31 and 33 DNA load was performed to evaluate possible correlations between AMPs expression and HPV types.
Our study showed that in women with normal cytology and ASCUS (Atypical Squamous Cells of Undetermined Significance), hrHPV was absent. On the other hand, 72.2% (13/18) LGSIL (Low-Grade Intraepithelial Lesion) and 93.3% (14/15) HGSIL (High- Grade Intraepithelial Lesion) patients presented infection with hrHPV in single or co-infection represented mainly by HPV16, 18, 31 and 33. hBD-2 and hBD-3 mRNA expression was significantly increased (P<0.003) in both LGSIL and HGSIL patients whereas LL-37, RNase 7 and hBD-1 gene expression did not differ significantly from normal epithelium. AMP expression correlated neither with the number of HPV types nor with the high-risk and low-risk HPV DNA loads of the quantified types.
hBD-2 and hBD-3 expression was shown to be significantly upregulated in HPV-induced cervical lesions. These findings suggest a possible biological significance in the innate immunity against these lesions but their role remains to be solved.

P4.01.088
PD-1 induces anti-viral T cell motility paralysis: the immunological synapse perspective.

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During persistent viral infection with lymphocytic choriomeningitis virus (LCMV) - as with numerous other viruses - CD8 T-cell differentiation leads to a unique subset of antigen-specific T-cells which are impaired in their ability to mount effective immune responses. This process is termed immune exhaustion. The suboptimal effector function of exhausted cells is partly due to sustained expression of the inhibitory receptor 'programmed-death-1' (PD-1). In order to gain some understanding into the molecular mechanisms governing the induction phases of T cell exhaustion during infection, using total internal reflection microscopy, we examined the behavior of LCMV-specific CD8 T cells placed on artificial planar lipid bilayers. Specifically, we compared CD8 T cells from acute and chronically infected mice (7 days post-infection) in their ability to form immunological synapses when engaging PD-1 with its ligand, PD-L1. Our results demonstrate that PD-L1 localizes to the central supramolecular activation cluster, and importantly, that PD-L1/PD-1 engagement promotes stable, mature immunological synapse formation while decreasing anti-viral T cell motility, despite reduced phosphorylation of proximal TCR signaling molecules like ZAP-70. These results corroborate in vivo two-photon microscopy data showing that immune exhaustion during viral persistence is defined by prolonged motility paralysis in the splenic marginal zone / red pulp and is restored by therapeutic blockade of PD-1 /PD-L1. We propose that motility paralysis during persistent viral infection decreases T cell scanning efficiency and facilitates engagement of regulatory pathways that negatively impact anti-viral T cell function as a manifestation of immune exhaustion induced by PD-1.

P4.01.089
Immunologic response among adults infected by different HIV-1 Subtypes who on anti-retroviral drugs in Northern Tanzania

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Background: Human immunodeficiency virus (HIV) is characterized by great genetic diversity due to its high mutations that occur during replication. HIV-1 infections are characterized by high rates of viral turnover and extensive viral diversity that has implications on disease progression, vaccine development as well as treatment response to antiretroviral drugs.
Objective: The aim of this study was to determine immune response among adults infected by different HIV-1 subtypes who are receiving ARV in Northern Tanzania
Methods and materials: A retrospective study on 63 patients from PRIOR-6 study which were characterized using peptide ELISA representing HIV-1 subtypes A, B, C, D and E. The CD4+T-lymphocyte cell counts were recorded at the baseline, at six and twelve months.
Results: All 63 samples were successfully sub typed with adherence of >92% where analyzed. HIV-1 subtype A was the most prevalent and was 30(47.62%), followed by subtype C= 23(36.51%) and subtype D 10(15.87%). Subtype D showed a higher immunological failure as compared to subtype A and C with the Hazard Ratio (HR, 5.6;) and 95% CI=1.3-23.52, P=0.02) or to the World Health Organization stage >2 of illness (HR, 4.7; 95% CI, 1.20-18.6), P=0.02 compared to A and C.
Conclusion and recommendations:HIV-1 Subtype A was the most predominant, stable and less virulent while C is increasing compared to D which is decreasing. HIV-1 Subtype D showed significantly immunological failure relative to other categories of the viruses. Further studies are needed to address mutations associated with ARV drug resistance among HIV-1 subtypes.

P4.01.090
Development of a new generation smallpox vaccines

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Vaccinia virus (VACV) strain LIVP earlier was used for smallpox vaccination in Russia. Cloned variants of VACV LIVP were isolated and compared in biological properties with parental virus. One of these VACV variants was chosen for next step of the research. Mutant variants of the selected cloned VACV with targeted deletions of one to four genes encoding virulence factors (B8R, C3L, N1L, and A56R) were constructed. Excision of one or more target genes failed to reduce reproduction of the virus in the cell cultures CV-1 or Vero. Created VACV variants could be used as new generation safe smallpox vaccines. Comparative analyses of attenuation and immunogenicity of the created VACV mutants are under study. DNA vaccine combining plasmids carrying the variola virus genes M1R, A30L, and F8L of intracellular virion surface membrane proteins as well as A36R and B7R of the extracellular virus envelope proteins under control of cytomegalovirus promoter has been constructed. This DNA vaccine induced production of a high titers of vaccinia virus-neutralizing antibodies in mice similar to those elicited by the live vaccinia virus immunization. Mice vaccinated by created DNA vaccine were completely protected against a lethal (10 LD_{50}) challenge with highly pathogenic ectromelia virus. These results suggest that such vaccine will be efficient in immunization of humans against smallpox.

P4.01.091
Plasticity of uterine Natural Killer cells during maternal cytomegalovirus infection

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Circulating Natural Killer cells constitute the first line of defense against invading pathogens. These effectors of the innate immune system recognize and kill tumor or viral-infected cells. In addition to peripheral blood and lymphoid organs, NK cells are found in the decidua basalis of the human pregnant uterus. Decidual NK cells secrete a large variety of cytokines, chemokines and growth factors but are poorly cytotoxic. We previously showed that dNK cells freshly isolated from the 1st trimester decidua basalis are able to kill target cells upon ligation of NKp46 activating receptor. This cytotoxic capacity is under the control of CD94/NKG2A inhibiting receptor. We challenged the hypothesis that upon viral infection, dNK cells receive activating signals that overcome the inhibitory signals and trigger their cytotoxic effector function. Using an ex vivo autologous system, we describe a mechanism by which dNK cells kill HCMV infected targets. We show that dNK cells engage activating immune synapse with HCMV-infected autologous targets. dNK cells stimulated with HCMV-infected targets are able to reorganize rapidly both their microtubule organizing center and their Golgi apparatus. These changes in cell morphology result in focused delivery of lytic granules and subsequent killing of target cells. Furthermore, dNK cells stimulated with infected autologous fibroblasts acquired a cytotoxic phenotype. Taken together our data provide the first evidence that dNK cells may be involved in the protection of the fetus against maternal viral infections.

P4.01.092
Susceptibility and response of human lymphocytes to dengue virus infection

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The Dengue represents one of the most important arboviruses in the world with more than 2 billion people living in endemic areas. WHO estimates about 50 million of DENV cases/year with 25000 deaths/year. Dengue infection can be asymptomatic or varies from a dengue fever (DF) to a more serious condition like dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). The occurrence of severe DENV cases could be explained by low avidity of T lymphocytes and antibodies, which generated a deficient antiviral response and contributed to high viraemia and cytokine storm observed in severe DENV cases. In this context, lymphocyte susceptibility and response to dengue virus infection would contribute to understand of dengue pathogenesis and vaccine development. Here, we demonstrated that T (CD4+ and CD8+) and B (CD19+) lymphocytes are susceptible to dengue virus infection, however, CD8+ cells presents higher susceptibility to infection. Additionally infected lymphocytes enhance the expression of CD69, an activation marker. We also demonstrated that DENV infection of lymphocytes is mediated by heparan sulfate receptors and that apoptosis of lymphocytes is not dependent of viral replication. Additionally, we have demonstrated that infection of CD8+ cells by DENV does not impact the function of degranulation with the expression of CD107a in surface of these cells, after stimuli with PHA . The next steps will be determine if infected CD4+ and CD19+ are still function after a infection with DENV. Finally, we intent to validate the *in vitro* data in patients infected with dengue virus.

P4.01.093
Severe tuberculosis is associated with a mixed (Th1, Th2, Th17) defective cytokine response to *Mycobacterium.tuberculosis* antigens in children

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Background: Assessment of cytokine/chemokine responses to *Mycobacterium tuberculosis* (M.tb) peptides in children may provide alternative infection markers, help for discrimination between latent (LTBI) and active tuberculosis (TB-disease) and explanation for TB severity status.
Methods: Fifteen cytokines/chemokines were quantified in a multiplexed microsphere-based assay following whole-blood stimulation with M.tb antigens (contained in the commercial quantiFERON kit) in 47 children (median age: 8 years). Concentrations were compared between LTBI (n=12, median age: 10 years) and TB-disease (n=28, median age: 8 years) and between non-severe pulmonary TB (n=11, median age: 7 years) and severe-TB (n=17, median age: 8 years). Seven non-infected children were simultaneously analysed as controls.
Results: Beside IFNγ, IP10 and IL13 appeared to be sensitive biomarkers to diagnose M.tb infection (area under ROC curve: 0.96, 95%CI, 0.91-1 and 0.97, 95%CI, 0.93-1.00 respectively). No studied analytes distinguished LTBI from TB disease.
TB-disease severity was associated with a decrease of M.tb antigen-induced Th1 cytokines (IL-12: p=0.09; IFNγ: p=0.07) and a clearly defective Th2 cytokine levels (IL5: p=0.02; IL13: p=0.02; IL4: p=0.08). IL17, IL10 and MCP-1 levels appeared depressed in severe TB (p=0.07, 0.09 and 0.08 respectively).
Conclusions: This study confirmed IP-10 and identified IL13 as potential biomarkers for M.tb infection diagnosis but no cytokine/chemokine for TB staging in pediatric tuberculosis. Severe pediatric TB-disease was associated with defective mixed cytokine responses rather than the presumed unbalanced Th1/Th2/regulatory cytokine network.

P4.01.095
Expression profiles of Toll-like receptors 1-10 in human placenta during viral infection

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Placenta provides an important physical and immunological barrier against infectious agents and a pregnancy-specific component of the innate immune system. Toll-like receptors (TLRs) are essential for the induction of innate immunity responses in different human tissues including the maternal and fetal interface. The objective of this study was to investigate the TLR1-10 gene expressions in the human term placentas during viral infection.
The explants of third-trimester chorionic villi and deciduas were exposed to infection with laboratory strains of HCMV, HSV-1 or VSV. Gene expression of TLR1-10 was assessed by quantitative real-time PCR using Power SYBR Green PCR Master Mix. The relative gene expression values were calculated using the comparative Ct method, and data were normalized against YWHAZ levels.
This study clearly demonstrated that the human term placenta differentially expressed functional TLR1-10, indicating that these receptors are believed to be important for placental immune responses against viruses.
We found that TLR3 mRNA levels were lower than other receptors in all examined placentas. We observed increased expression of TLR1, 9 and 10 in HCMV-infected decidua compared to unstimulated tissue. These findings demonstrated that the decidua was a critical component of the innate immunity response during HCMV infection. In contrast, infection with HSV-1 or VSV did not affect the expression of TLRs.
Our results suggest that the TLRs could be important immune regulators in placenta during viral infection.
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P4.01.096
Blimp-1 as a major transcription factor for the generation and function of cytotoxic CD4 effector T cells (ThCTL)

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CD4 T cells are best known for helper roles in immunity, but they can also become cytotoxic cells (ThCTL) that kill MHC class II$^+$ targets presenting cognate Ag. We find that a subset of CD4 effectors peaks in the lung of influenza A/PR8 (IAV)-infected mice at 8 days post-infection, expresses the NK cell-associated receptors NKG2A/C/E and has potent perforin-dependent, Fas-independent Ag/MHC class II-specific cytotoxic activity. CD4 T cells from lung, spleen and draining lymph nodes express mRNA for the NKG2A, inhibitory receptor while mRNAs for the activating receptors, NKG2C and NKG2E, are only detected in the lung. Interestingly, Ab to NKG2A/C/E receptors or their ligand, non-classical MHC class I molecule Qa-1, significantly block ThCTL-mediated target cells killing. The NKG2A/C/E$^+$ ThCTL subset co-expresses PD-1, CD27, PSGL-1, GrB and Ly6c, but not CD103, as well as high levels of transcription factors Blimp-1 and Eomes. Since it is involved in development of non-follicular CD4 effectors, we analyzed the role of the transcriptional repressor Blimp-1. Conditional KO of Blimp-1 in T cells did not affect total numbers of CD4 effector cells in IAV-infected lung, but did reduce the number of ThCTL, their expression of the many characteristic phenotypic markers and their cytotolytic function. Thus ThCTL are generated during an acute anti-viral response by a unique program involving Blimp-1 as a major transcription factor, supporting their identity as a defined CD4 subset. Moreover, their use of NKG2A/C/E provides a valuable marker and suggests a unique target specificity for this novel subset.

P4.01.097
Chronic exposure to IFNα reduces T helper-dependent antiviral CD8+ T cell responses

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Type I interferons (IFNa/b) are produced by most cells upon viral infection. They have undeniable potent natural anti-viral effect and they promote effective anti-viral immune responses. However, comparative studies on HIV and pathogenic or non-pathogenic Simian Immunodeficiency virus infections in non-human primates show correlations between immune hyperactivation, chronic IFN signature, and pathogenesis. Therefore, in the context of chronic infection, IFN I might have a negative impact on the host by maintaining the innate immune system in a chronic state of activation. In conditions mimicking a chronic viral infection by repeated exposure to IFNα, we found that virus-specific CD8+ T cell responses dependent on CD4+ T cell help were strongly reduced. The reduced ability of mice chronically exposed to IFNα to mount efficient anti-viral CD8 T cell responses was due to decreased CD4 T cell help related to a strong reduction of conventional dendritic cells. Our data suggest that chronic exposure to IFNα occurring during chronic infection may support viral persistence and/or opportunistic infections by reducing conventional dendritic cells, therefore inducing poor T helper-dependent CD8+ T cell responses.

P4.01.098

Differential cytokine and chemokine signatures produced by human innate immune cells after *in vitro* infection with West Nile virus isolates

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Background. West Nile virus (WNV) is an enveloped positive-strand RNA virus transmitted by mosquitoes. The outcome of infection varies from asymptomatic (80%) to severe disease (<1%), and fatality. The role of the immune response in the clinical outcomes is poorly understood. We hypothesize that different viral isolates may induce distinct cytokine/chemokine profiles, which may influence disease progression, risk for death, and development of specific clinical outcomes.
Methods. We determined *in vitro* the kinetics of infection of monocyte-derived macrophage and dendritic cells by different WNV isolates: WNV02 1956 (the prototype), and two other isolates presenting higher (ARC13-2006) or lower (BSL2-05) virulence than the prototype in C3H and C57Bl6 mice. The infection was performed using a MOI of 0.01.
Results. In both macrophages and dendritic cells, ARC13-2006 induced the highest levels of IL-6, IL-8 and TNF-α, and the lowest levels of CXCL10 and CCL2 of the studied isolates. Also, it induced higher levels of CCL5 than BSL2-05. In contrast, BSL2-05 induced the highest level of IL-10 among the isolates, and presented higher levels of CXCL10 and CCL2 than ARC13-2006.
Conclusion. The three WNV isolates presented distinct kinetics of infection, which were associated with distinct immune profiles. Infection of innate cells with ARC13-2006 was associated with a pro-inflammatory immune response, while infection with BSL2-05 was associated with a mixed immune profile, suggesting that the variability of the isolates may play a role in the clinical outcome of infection.
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P4.01.099

Pro-apoptotic BH3-only proteins Noxa and Bim co-operate to negatively regulate CD8+ T cells during chronic active infection

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Background: The inability of T cells to eradicate HIV-1 is attributed to a functional impairment that eventually leads to their demise. Apoptosis plays dual roles in infection. Death of infected cells is beneficial but death of uninfected T cells is deleterious. Collateral death is mediated by non-specific inflammatory responses. We hypothesize that by promoting death in infected cells and by antagonizing death in uninfected T cells we can eliminate chronic persistent infections. The aim of our study was to investigate the role of pro-survival and pro-apoptotic proteins during chronic infection. Additionally we investigate the efficacy of small drug molecules that modulate cell death.
Design: We chronically infected mice conditionally deficient in Bcl-2, Mcl-1, Bim or Noxa with LCMV docile. We specifically deleted genes in effector T cells coincident with their activation. Additionally, we used pro-apoptotic small molecules to determine effects on virological and immunological outcomes.
Results: We demonstrate a previously undiscovered role for Noxa in the attrition of antigen-specific CD8+ T cells during chronic active infection and a synergistic co-operation between Noxa and Bim. We also elucidate the role of Mcl-1 and define the therapeutic efficacy of a combination of small molecule drugs that modulate apoptosis during chronic infection.
Conclusions: These studies provide important mechanistic insights into the dual role of apoptosis during chronic active viral infection with implications for the use of novel small molecule drugs that modulate apoptosis.

P4.01.100

Differential Responses of Immune Cells to Type I Interferon Contribute to Host Resistance to Viral Infection

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Type I interferons (type I IFN) play a major role in antiviral defense. However, how they orchestrate *in vivo* the functions of distinct immune cells is not well understood. We determined that type I IFNs produced during murine cytomegalovirus (MCMV) infection differentially affect dendritic cells (DCs) and natural killer (NK) cells. Type I IFNs induce cell-intrinsic responses in DCs, activating antiproliferative, antiviral, and lymphocyte-activating gene networks, consistent with high activity of the transcription factor STAT1 in these cells. By comparison, NK cells exhibit lower STAT1 expression and reduced type I IFN responsiveness. Rather, type I IFN indirectly affect NK cells by inducing IL-15, which activates the transcription factor E2F and stimulates genes promoting cell expansion. Type I IFN cell-intrinsic responses are necessary in DCs, but not NK cells, for MCMV resistance. Thus, sensitivity to type I IFN-induced cytokines and differences in type I IFN receptor signaling program immune cells to mount distinct responses that promote viral control.

P4.01.101

The study of genes encoding interleukin-1β, 2, 8,10 expression in the rabbit infected with RHD (rabbit haemorrhagic disease)

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Interleukins (IL) play an important role in antiviral immunity, for example in infection of rabbits with RHDV, which is an etiological factor of viral haemorrhagic disease. Studies on the changes in the expression of genes encoding IL are few, and applies only to liver cells. Thus, the aim was to assess changes in the expression of genes encoding IL-1β, 2,8,10 in peripheral blood leukocytes (components of the immune system) in rabbits experimentally infected with RHD virus- strain V-411. The analysis was performed with real-time PCR, and the results were analyzed by relative quantification method by Livak, using two reference genes (HPRT and β-actin). The study was performed on 6 rabbits (4 individuals infected intramuscularly with RHDV and 2 control) and the blood was sampled from ear's marginal vein in "0" (ie. before administration of antigen and placebo), and then in 8,12,24,28,36 p.i.
Studies have shown the increased expression of the genes encoding IL-2 in the 8 and 12 h p.i. and decrease in the 24-36h p.i. A similar, but not identical picture of changes was registered in the expression of gene for IL 1β. For IL-10, the decrease of expression was registered in 8 and 12h p.i. and the increase between 24-36h. There have been no changes in the expression of genes encoding IL-8 registered.

P4.01.102

CytoDiffTM panel allowing monitoring effectiveness of the highly active antiretroviral therapy on HIV infected patients

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Background: It has been shown that the CytoDiff™ panel, a 5-color/6marker reagent, which provides a 10-part white blood cell differential allows more efficient monitoring of immune status in HIV

patients; however the ability of the CytoDiff panel for monitoring effectiveness of the highly active antiretroviral therapy (HAART) has not been tested.
The aim of this study was to analyze the leucocyte subsets provided by the CytoDiff application on a cohort of HIV patients with and without HAART.
Methods: Samples from 199 HIV infected patients and 33 normal blood donors were analyzed. The viral load, the CD4 lymphocyte count, manual differential and a detailed clinical status of these patients were obtained. Samples were stained with the CytoDiff panel (CD36-FITC, (CD2+CD294)-PE, CD19-ECD, CD16-PC5 and CD45-PC7), lysed and 20 000 leucocytes were analyzed on a FC500 Flow Cytometer (Beckman Coulter) using CytoDiff CXP software.
Results: A comparison between control group and HIV patients was performed on each leucocyte sub-population identified with CytoDiff. A significant decrease from the control group was observed for the Lymphocytes total, Lymphocytes CD16+, B Lymphocytes and Eosinophils with simultaneous increase for the CD16+ monocytes and neutrophils. HAART decreased viral load and softened these shifts in HIV infected patients. HIV infected patients with HAART exhibited higher levels of the Lymphocytes total, Lymphocytes CD16+, B Lymphocytes as well as lower levels CD16+ monocytes and neutrophils if compared with HIV infected patients without HAART.
Conclusion: CytoDiff panel can be used to monitor the effectiveness of HAART.

P4.01.103
Universal influenza vaccine requires CD4 T cells for heterosubtypic protection

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Current influenza vaccines are ineffective against new viruses and the source or strain of the next outbreak strain of influenza is unpredictable, therefore establishing universal immunity by vaccination is fundamental to limiting the impact of influenza. Virus specific CD4 and CD8 T cells are able to directly kill virus infected cells and co-ordinate local innate immune responses. Importantly, T cells are able to react against different strains and subtypes of influenza. A novel vaccine has been developed using a Vaccinia vector encoding H5N1 derived proteins and a molecular IL-15 adjuvant. The vaccine has proven effective against different H5N1 clades, and most importantly group 1 and group 2 influenza viruses, which have widely different HA and NA proteins. Here we establish the role of T cells in providing universal protection against influenza established by a vaccinia-H5N1 vaccine. The vaccine is able to induce robust immunological memory, enabling the survival of mice against many different influenza viruses, such as seasonal H3N2, 2009 pandemic H1N1, lethal avian H5N1 and H7N7 influenza viruses. Influenza specific CD4 and CD8 T cell responses are elicited by the vaccine, and recruited following viral challenge in the lung and periphery. Selective depletion experiments for T cell subsets has established an important role of CD4 T cells in heterosubtypic protection depsite low epitope sequence conservation across different influenza viruses. Unraveling how this vaccine is able to protect against different unrelated influenza strains is key to designing and improving future vaccine strategies and public health.

P4.01.104
BCG restores antiviral immune response in patients with recurrent respiratory papillomatosis by affecting dendritic cell differentiation

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Recurrent respiratory papillomatosis (RRP) is characterized by a high relapse frequency after standard surgery due to inefficient immune response to chronic HPV infection. Additional immunotherapy with ,,Calgevax" (BCG) considerably improves the outcome. The present study investigates the effects of BCG on cellular immune responses in RRP.
RRP patients (n = 17) subjected to combined CO2 laser microsurgery / BCG-immunotherapy were studied before (0), 6, 12 and 20 months after the start of immunomodulation. Percentages of circulating plasmacytoid dendritic cells (pDC), mature (CD86+) DCs, and regulatory (CD4+FoxP3+) T cells, as well as the level of in vitro PHA-stimulated Th17 effectors and Th1/Th2 cytokine secretion were determined by flow cytometry (FACSCanto II, BD), in comparison to healthy controls.
At point 0, significantly decreased pDCs and CD86+DCs levels were established (average 4.8% and 32% respectively), that approached healthy control levels after 12 months of BCG application (8.5vs.8, and 55vs.60, p >0.5 for both). Further on, 12 months of BCG immunotherapy significantly decreased the share of proinflammatory Th17 (0.4 vs. 0.69, $p<0.05$) and increased the level of circulating Treg (4.6 vs. 7.1, $p<0.05$). Finally, the initially decreased IFNγ/IL-4, and IFNγ /IL-10 ratios, were restored after 20 months of BCG application.
BCG increases the efficiency of antiviral T-cell response by stimulating the maturation and differentiation of pDCs, resulting in efficient antigen presentation, restoration of Th1/Th17 disbalance, and induction of Treg preventing the terminal differentiation of T cell clones in the settings of chronic infection.

P4.01.105
The influence of antibodies on the immune response to respiratory syncytial virus infection

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Respiratory Syncytial Virus (RSV) can cause severe bronchiolitis in young infants. Approximately 50% of severe RSV infections can be explained by currently known risk factors. Hence, the pathogenesis of disease severity is still poorly defined. Evidence suggests that maternal antibodies might have an influence on severity of infection. This study tries to identify the influence of antibodies on the immune response to RSV infection.
Previous experiments have shown that antibodies (Ig's) present in human serum (HS) prevent RSV from infecting cells. However, stimulation of human peripheral blood mononuclear cells (PBMCs) with RSV in combination with HS induced a synergistic increase in interferon-γ (IFN-γ) production. To study whether the enhanced IFN-γ production is depended on immune complexes, other HS components or infectious versus antibody-neutralized virus, we stimulated human PBMCs with RSV and BPL-inactivated RSV in combination with HS and Ig depleted HS. Measurement of cytokine levels indicates that antibodies are the main cause of this synergistic increase in IFN-γ production. In both monocyte and NK cell depleted fractions of PBMCs this synergistic response persisted. IFN-γ could already be measured after 4 hours on mRNA level and after 24 hours on protein level, possibly suggesting a direct stimulation of T cells by opsonized RSV.
This study shows that anti-RSV antibodies present in human serum enhance the induction of IFN-γ by PBMCs stimulated with RSV. These data indicate that the presence of (maternal) neutralizing antibodies during a primary RSV infection might enhance inflammation and affect disease severity.

P4.01.106
Lipopolysaccharide stimulation inhibits viral infection of human macrophages with two distinct mechanisms

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It is well known that respiratory viral infections enable commensal bacteria in the nasopharynx to disseminate to other sites in the body and cause disease. However, this is not a uni-directional interaction and several studies have shown that substantive crosstalk exists between bacterial pathogen-associated molecular patterns (PAMP)-signalling pathways and the induction of anti-viral immunity. Studies so far have been performed in murine models and it is unclear how far these findings can be extrapolated to human infections. Therefore, we use influenza A virus (IAV) and respiratory syncytial virus (RSV) to examine how bacterial ligands can affect the subsequent viral infection of human monocyte-derived macrophages (MDMs) and epithelial cells.
Human epithelial cells (A549) stimulated by a panel of different bacterial ligands did not display any significant alteration in susceptibility to RSV or IAV. However, pre-stimulation of human MDMs with lipopolysaccharide (LPS) reduced the infection rate of both RSV and IAV with approximately 80%. Interestingly, our results show that LPS protects against viral infection in two distinct mechanisms. The inhibition of IAV infection was associated with the ability of LPS to induce a type I interferon and anti-viral response, which results in a reduced transcription of viral RNA. In contrast, LPS triggered a short-term, RSV-specific response preventing RSV entry early in the infection.
Taken together, these data demonstrate the bi-directional nature of viral-bacterial interactions and suggest that the composition of an individual's nasopharyngeal flora may help determine their susceptibility to viral respiratory infections.

P4.01.107
A novel RSV genetic vaccine based on chimpanzee Adenovirus and MVA vectors is safe and highly immunogenic in animal model and fully protects cotton rats and newborn calves from RSV challenge

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Chimpanzee-derived Adenovirus (ChAd) vectors are insensitive to pre-existing immunity and have been used in heterologous prime/boost regimens with Modified Vaccinia Ankara (MVA) vectors encoding the same antigen, leading to unprecedented immune responses in humans.
We have generated ChAd and MVA vectors encoding for the F, N and M2-1 RSV antigens to induce a full spectrum of immune responses including neutralizing antibodies and T cells.
The vaccine vectors were tested in pre-clinical animal models by different regimens and routes, including mucosal delivery by intranasal spray. When tested in the cotton rat model, the vaccine completely prevented replication of the virus in the lung and in the nasal cavity. The results of lung histopathology analysis showed that the vaccine was safe and did not induce enhanced pulmonary pathology.
BRSV-seronegative calves were vaccinated intranasally (i.n.) with ChAd/RSV and boosted 8 weeks later with MVA/RSV intramuscularly (i.m.). Calves were challenged with BRSV 4 weeks after boosting. Mucosal vaccination with ChAd/RSV primed BRSV-specific IFNγ-producing cells in peripheral blood and BRSV-specific serum antibodies, which were boosted by i.m. vaccination with MVA/RSV. Following challenge with BRSV, calves were completely protected against virus replication in the nasopharynx and lungs and were completely protected against the development of pulmonary pathology.
Formal GLP toxicology study in mice showed no vaccine related safety concern and GMP grade RSV genetic vectors have been prepared and are ready to be tested in clinical trials.

P4.01.108
Molecular characterization of rotavirus detected in diarrhoeic children less than five years of age in Kano State, Nigeria

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Background: Rotavirus is the most common cause of severe diarrhoeal illness in infants and young children 0-5 years of age in both developing and developed countries.This Study was aimed at determining the prevalence and genotyping of rotavirus among these children using ELISA and RT-PCR
Methods: A total of 285 stool samples were collected from infants and children 0-5 years of age. The diarrhoeic Stools were analyzed for rotavirus antigen and the rotavirus positive stools were further subjected to VP7 and VP4 genotyping using gene specific primers.
Results: Rotavirus was detected in 36.5% of the diarrhoeic children. The infection occurred throughout the study period with higher peaks in the drier month of April 77.6% and lowest in July 12.2% (Pearson Chi Square analysis: X^2= 27.720, P<0.05, df=1). No statistically significant difference was observed (P>0.05). Three different rotavirus P-genotypes (P[8], P[4], and P[6]) were detected in this study and Mixed infection were detected. Six different G-genotypes were detected.
Conclusion: Rotavirus was found to be an important cause of diarrhoea in children 0-5 years of age in Kano, North-Western Nigeria.

P4.01.109
A Critical Role of IL-17 in Modulating B Cell Response During H5N1 Influenza Virus Infection

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Interleukin-17 (IL-17), a member of IL-17 family, plays a crucial role in mediating immune response against extracellular bacteria and fungi in the lung. Although there is increasing evidence that IL-17 is involved in the protective immunity against H1 and H3 influenza virus infections, little is known about the role of IL-17 in the high pathogenic H5N1 influenza virus infection. In this study, we found that H5N1-infected IL-17 knockout (KO) mice showed markedly increased weight loss with severer lung immunopathology and significantly reduced survival rate as compared with wild-type controls. Moreover, a substantially decreased frequency of B cells was observed in the lung of IL-17 KO mice after virus infection, which was correlated with reduced CXCR5 expression on B cells and decreased CXCL13 production in the lung tissues of IL-17 KO mice. Consistently, B cells from IL-17 KO mice exhibited a significant reduction in chemokine-mediated migration in culture. Taken together, our findings have demonstrated a critical role of IL-17 in mediating the recruitment of B cells to the site of pulmonary influenza virus infection in mice.

P4.01.110
Early growth response genes (Egr) -2 and -3 are essential for the activation of naïve B and T cell and primary response against virus infection

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T cells robustly respond to virus infection. However, the inflammatory effects of responding T cells are controlled to minimize immunopathology. Egr-2 and -3 are zinc-finger transcription factors rapidly induced in naïve B and T cells (1, 2, 3). The deficiency of Egr-2 and -3 specifically in B and T cells results in severe autoimmunity (3). It is however unknown whether they also play roles in protective immmunoresponses. We have now shown that Egr-2 and -3 are required for naïve B and T cell activation in response to virus infection. Defects in the induction of Egr-2 and -3 restrain the IL-2

production and the expansion of viral specific CD4 and CD8 T cells leading to the development of chronic infection. We found that Egr-2 and -3 are transactivated by AP-1. The induced Egr-2 and -3 are essential for the maintenance of optimal AP-1 activation and AP-1 mediated IL-2 production leading to the rapid expansion of viral specific B and T cells. In addition to the proliferation of viral specific T cells, Egr-2 and -3 control the production of pro-inflammatory cytokines of T cells at early stages of infection to limit inflammatory pathology leading to robust anti-viral effect with minimum immunopathology. The functions of Egr-2 and -3 are limited in naïve T cells in primary immunoresponses and are not involved in the development of the memory pool and secondary responses. In conclusion, in addition to the maintenance of immunohomeostasis, Egr-2 and -3 are important to primary immune responses against virus infection.

P4.01.111
PERIPHERAL BLOOD MONOCYTES IN ACUTE AND STABLE COPD HAVE IMPAIRED RESPONSES TO RESPIRATORY VIRUSES

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Patients with chronic obstructive pulmonary disease (COPD), suffer frequent exacerbations due to respiratory virus infections. Our aim was to determine if peripheral blood monocytes (PBMCs) from exacerbating and stable COPD patients had impaired immune responses when exposed to influenza (H1N1pdm09) and rhinovirus (RV-1B). Methods; we recruited 10 subjects who had been admitted to hospital with acute exacerbations of COPD and then 6-8 weeks later when recovered and 10 age matched healthy controls with no lung disease. Subjects had blood drawn and monocytes selected using Ficoll-paque centrifugation. Cells were stained with CD3, CD4, CD8, CD56, CD14, CD11c and CD303 and identified with flow cytometry. PBMCs were then exposed to H1N1 and RV-1B, expression of PD-1, PDL-1 was measured as well as release of interferon (IFN)-α, IFN-λ, IFN-γ, TNF-α and IL-1β. Results; PBMCs from subjects with COPD when acutely unwell demonstrated reduced release of IFN-α, IFN-λ, IFN-γ, TNF-α when exposed to H1N1 and RV-1B, in convalescence release of IFN-λ, and TNF-α remained impaired compared to controls. PBMCs in COPD acute and convalescent samples demonstrated increased expression of PDL-1 on plasmacytoid dendritic cells at baseline. Following exposure to virus there was increased expression of PD-1 in COPD CD4 and CD8 cells. Conclusions; PBMCS in acute and stable COPD demonstrate impaired IFN responses to virus infection with altered expression of PD-1/PDL-1.

P4.01.112
Distinct immunity between pandemic H1N1 and seasonal H3N2 influenza infections in humans

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Results from the immunopathogenetic studies of different influenza infections in humans may provide useful information concerning the appropriate measures to be taken for preventing complications and fatality of an emerging influenza infection. In this study, we investigated the innate and adaptive immune responses of pandemic (H1N1) 2009 influenza in comparison to seasonal H3N2 and other febrile infection. In a cohort of 159 patients, we studied different infection immunity between the pandemic (H1N1) 2009 and H3N2 seasonal infections. Both H1N1 and H3N2 patients had lymphopenia, but the H1N1 patients had prominent lower $CD8^+$ cells and the H3N2 patients had lower $CD19^+$ cells. Lower NK-cell perforin content and plasma bactericidal activity were prominently found in the H1N1 patients. The H1N1 patients also had higher Treg cells with augmented Foxp3 expression as well as TGF-beta 1 production but lower interferon-gamma production. The H3N2 patients, however, had higher T-bet expression with lower TGF-beta1 production. In conclusion, impaired NK-cell function and bactericidal activity associated with augmented Treg-cell function might render the H1N1 patients more susceptible to bacterial superinfection.

P4.01.113
Role of host and viral factors in negative antibody response of seronegative occult hepatitis B virus infection

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Background The lack of antibody response in seronegative occult hepatitis B virus (HBV) infection or OBI can be caused by host and viral factors. The polymorphism of human leukocyte antigen (HLA) class II gene, especially in HLA-DRB1 locus, will determine host antibody immune response whereas mutation in HBV surface (S) gene, which overlaps the HBV polymerase (P) gen, potentially affects the replication ability of HBV. This study aims to investigate the HLA-DRB1 genotypes and the HBV S gene characteristics from OBI seronegative individuals in Ternate.
Methods Host DNA were extracted by salting out method, and further analyzed for HLA-DRB1 alleles together with 9 anti-HBc positive OBI individuals as controls using LABType® SSO Typing Test (One Lambda, USA). HBV S gene of 9 OBI seronegative isolates were detected by PCR, cloned and analyzed for the presence of amino acid variation
Results HLA-DRB1*12 and *15 were the most common alleles. The distribution of these alleles was not statistically different between seropositive and seronegative OBI groups. HBV DNA was found in second round of amplification (<200 IU/mL). From 67 sequenced clones, 60 (89.6%) showed the presence of S gene mutations.
Conclusions Our results suggest that there was no significant difference in HLA-DRB1 allele distribution among OBI seropositive and seronegative groups. The lack of antibody response in OBI seronegative is more likely due to the low level of HBV DNA. This could be caused by mutations in the S gene and its overlapping P gene, which may affect HBV replication.

P4.01.114
Viral dsRNA and ssRNA analogues directly activate mast cells to cysteinyl leukotriene generation and modulate their IgE-dependent releasability

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Mast cells (MCs) are particularly abundant at anatomical sites close to external environment and express functional Toll-like receptors (TLRs) by which detect invading bacteria. Recently, a few reports indicated that MCs also possess cytoplasmic TLRs, including TLR3 and TLR7/8, that recognize by-products of viral replication, i.e. dsRNA and ssRNA, respectively. Accordingly, MCs might be involved in anti-viral defense or/and in patomechanisms of viral-related diseases. Therefore, the study aim was to examine MC response to TLR3 and TLR7 agonists, i.e. poly(I:C) and R848, respectively. Due to MC central role in allergic reactions, we also investigated effect of TLR3- and TLR7-depended activation on IgE-mediated MC response. Experiments were performed *in vitro* on freshly isolated fully mature rat peritoneal MCs (rPMCs). We used western blot and flow

cytometry to determine TLR3 and TLR7 protein expression, histamine release assay to examine degranulation, and ELISA tests to study cysLT and CXCL8 synthesis. Our results indicated that rPMCs constitutively express TLR3 and TLR7 proteins and selectively respond to poly(I:C) and R848 stimulation. TLR3- and TLR7-mediated MC activation led to dose- and time-dependent cysLT synthesis without degranulation and CXCL8 generation. Moreover, MC co-stimulation with poly(I:C) and anti-IgE amplified cysLT secretion, whereas MC priming with R848 resulted in decrease of anti-IgE-induced degranulation. Our results suggest that dsRNA and ssRNA viruses could activate MCs to release of potent proinflammatory mediators and modulate IgE-mediated allergic processes. This work was supported by the National Science Centre in Poland (Grant No. 2011/03/N/NZ6/03528) and by the Medical University of Łódź (Grant No. 502-03/6-164-01/502-64-005).

P4.01.115

Secondary *Streptococcus pnuemoniae* infection impairs the immunity to influenza virus by down-regulating the adaptive immune response

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Background: During pandemic and seasonal influenza, secondary bacterial infections often enhance mortality. Given the clinical importance of the co-infection and increasing risk of influenza outbreak, it is important to understand how the immune system would be affected in dealing with the dual infections.
Methods: Mice were sequentially infected with influenza virus and *Streptococcus pneumoniae*. Survival and weight lost were recorded. The lungs from infected mice were examined for virus titre, bacterial cell counts. The spleens and lymph nodes from infected mice were examined for cellular immune response against the pathogens.
Results: Co-infected mice lost their body weight significantly and had 100% morality, whereas mice infected with either influenza or pneumococcus alone lost their body weight transiently and all recovered from the infections. Co-infected mice had significantly higher virus titre, bacterial cell counts in their lung when compared to the mice infected with either of the pathogens alone. When compared to single influenza infection, secondary pneumococcal infection significantly accelerated disease kinetic of influenza to cause lymphopenia, and suppressed the virus specific IgG response by down-regulating follicular B cells and follicular T helper cells. The suppression of the humoral immunity was critical to cause excess mortality as adaptive transfer of “immune serum” against influenza after secondary pneumococcal infection rescued co-infected mice from death.
Conclusion: In the co-infection,secondary pneumococus suppressed the adaptive immunity to the initial influenza virus, which impaired the control of influenza and caused excess mortality. This study contributes to the understanding of the pathogenesis of the co-infection.

P4.01.116

Irreversibly skewed development of B cells towards terminal stages during HIV-1 infection and resulted in their functional impairment

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Background: HIV-1 infection results in the abnormalities of B cells. Here we defined the skewed differentiation of B cells and their functionality in HIV-1 chronically infected subjects.
Methods: We employed flow cytometry to determine the differences in the frequencies and the phenotypes of B-cell subpopulations among32 HIV-1 viremic subjects naïve to antiretroviral therapy (ART), 34 ART successfully treated patients and 12 HIV-seronegative donors. Furthermore, HIV Env specific or influenza HA specific antibody secreting cells (ASCs) were quantified.
Results: $CD19^+$ B-cell counts were significantly less in the chronically HIV-1 infected than the HIV-seronegative group ($p=0.05$), which was manifested in naïve, immature, resting memory B cells and plasmablasts($p<0.01$, $p=0.02$, $p=0.02$ and $p<0.01$, respectively); In contrast, the frequencies of tissue-like memory B cells, defined as dysfunctional B cells, was elevated in chronically HIV infected subjects ($p<0.01$). Activation markers CD38 and CD95 expressing B cells increased in chronically HIV infected subjects ($p<0.01$ and $p<0.01$, respectively) whereas Bcl-2 decreased comparing to HIV-seronegative subjects ($p<0.01$). Successful ART only partially restored the skewed development of B cells. Furthermore, B cells from HIV infected subjects showed impaired responses to influenza HA ($p=0.03$) and the suppression of HIV replication by ART led to the decreased HIV-specific ASCs ($p<0.01$).
Conclusions: HIV infection resulted in the skewed differentiation of B-cell subpopulations towards terminal stage, which was accompanied by a functional impairment; ART only partially restored the abnormality. It remains a challenge to revoke B cell functionalities.

P4.01.117

Immunological pressure by monoclonal antibodies targeting glycoproteins of the 2009 pandemic influenza A(H1N1) virus drives quasi-species with increased virulence in mice

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In 2009, a pandemic influenza virus (H1N1pdm09) emerged and was spread to world-wide. A panel of monoclonal antibodies (D319, D360, D383, D430, I38-7), specific for the H1N1pdm09 virus hemagglutinin (HA) or Neuraminidase (NA), was generated from B-cells of the H1N1pdm09-infected mice. To indentify the antibody-binding sites (epitopes), the mAbs were used for the selection of escape-mutants which were generated by cultivation of a H1N1pdm09 strain (A/Korea/01/2009) with excess of the mAbs in embryonic eggs. The sequence-analysis of escape-mutants revealed 10 amino-acid positions recognized by the mAbs. The positions were distributed in the epitopes belonging to antigenic sites Sa (172, 173, 178 and 180), Sb (201 and 203) and novel epitopes (56, 144 and 146) in HA and 272 in NA. The escape-mutants with G172E and N173D in HA showed significantly reduced reactivity to mAb-D383 and sera from H1N1pdm09-infected ferrets. The escape mutants with D144E, N146D, T201N and A203D in HA were proved to have drastically lower reactivity to mAb-D360 and -D430. The escape mutants having D144E in HA were revealed to have weak reactivity to human sera from vaccinated individuals against H1N1pdm09 and enhanced binding avidity to host cells through RDE avidity assay, compared with mother strain and other variants. Furthermore, the escape mutant viruses showed increased virulence in mice, compared with the mother strain. Collectively, these results demonstrate that immunological pressure by monoclonal antibodies targeting H1N1pdm09 can drive the virus to increased virulence with altered host-cell binding properties. This work was supported by #2012-N43003-00 (intramural research fund)

P4.01.118

Adaptive Immunity Restricts Replication of Novel Murine Astroviruses

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The mechanisms of astrovirus pathogenesis are largely unknown, in part due to a lack of a small-animal model of disease. Using shotgun sequencing and a custom analysis pipeline, we identified two novel astroviruses capable of infecting research mice, murine astrovirus (MuAstV) STL1 and STL2. Subsequent analysis revealed the presence of at least two additional viruses (MuAstV STL3 and STL4), suggestive of a diverse population of murine astroviruses in research mice. Complete genomic characterization and subsequent phylogenetic analysis showed that MuAstV STL1 to STL4 are

members of the mamastrovirus genus and are likely members of a new mamastrovirus genogroup. Using Rag1−/− mice deficient in B and T cells, we demonstrate that adaptive immunity is required to control MuAstV infection. Furthermore, using Stat1−/− mice deficient in innate signaling, we demonstrate a role for the innate immune response in the control of MuAstV replication. Our results demonstrate that MuAstV STL permits the study of the mechanisms of astrovirus infection and host-pathogen interactions in a genetically manipulable small-animal model. Finally, we detected MuAstV in commercially available mice, suggesting that these viruses may be present in academic and commercial research mouse facilities, with possible implications for interpretation of data generated in current mouse models of disease.

P4.01.119

Hepatitis C virus-infected cells induce down-regulation of natural killer cell activating receptors through modulation of receptor traffic

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Natural killer (NK) cells play an important role in the defense against virus infections. We previously reported that cell-to-cell contact with hepatitis C virus (HCV)-infected cells reduces functional capacity of NK cells. Decreased cytotoxicity is correlated with down-regulation of NK cell activating receptors, NKG2D and NKp30, on NK cells. In this study, we investigated whether the traffic of the activating receptors to or from NK cell surfaces is modulated by the interaction of NK cells with HCV-infected cells. HCV-permissive human liver cell lines were infected with HCV virions and co-cultured with primary human NK cells. Degranulation of NK cells and surface or total (surface and intracelluar) expression of NKG2D and NKp30 were measured. Redirected degranulation assays proved that down-regulation of NKG2D and NKp30 was direct cause of the decreased cytotoxic capacity of NK cells. Considerable amount of both receptors were found in cytoplasm as well as on plasma membrane, suggesting that they recycle from cytoplasm to plasma membrane. After cell-to-cell contact of NK cells with HCV-infected cells, both surface and total expression of the activating receptors diminished. It implied that receptor traffic in the NK cells contacted with HCV-infected cells was modulated by degradation of intracellular pools of the receptors. Suppression of protein degradation using chemical inhibitors resulted in restoration of NKG2D and NKp30 expression on the surface of NK cells. In conclusion, the interaction between NK cells and HCV-infected cells modulated the traffic of NK cell activating receptors and thereby suppressed functional capacity of NK cells.

P4.01.120

The roles of Interferon regulatory factor members in virus infection sensitized IDO immunomodulation

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IDO is an important intracellular heme protein enzyme in tryptophan metabolism with immunoregulatory and antiviral function. It has been recognized as an antimicrobial biostatic defense through tryptophan deprivation. IDO expression has been reported in flavivirus infection. The role of IDO in virus infection might play roles in the inhibition of growth of micro-organisms and has profound effects on T cell proliferation and immune tolerance. We have demonstrated that interferon regulatory factors could induce IDO expression in interferon-gamma induced THP1 cells to regulate T cell immune response. The expression level of IDO was decreased in hepatitis C virus replicon cell lines which knockdown of interferon regulatory factors. IDO activity in culture supernatant of interferon regulatory factors knockdown cell lines were decreased in chemical method by spectrophotometer analysis. However, the expression levels of viral non-structural proteins were not changed. These results give hint that interferon regulatory factors are responsible for IDO induction at hepatitis virus C infection.

P4.01.121

Immunogenicity of two measles-mumps-rubella vaccines in Iranian pre-school children, a randomized controlled trial

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Objectives: The prevalence of measles, mumps and rubella (MMR) diseases has declined dramatically over recent years as a result of universal immunization programs. The present study was undertaken to compare the immunogenicity of two MMR vaccines administered to Iranian preschool children.
Methods: In this randomized, double-blind and multicenter prospective study, 820 children aged 6 years were injected with either a local MMR vaccine (MMR-Local, n=383) or a commercial vaccine manufactured by GlaxoSmithKline (MMR-GSK, n=437). All subjects received MMR vaccine at 1 and 6 years of age, following the national vaccination schedule of Iran. Blood samples were collected before and one month after the 2nd vaccine dose. Immunogenicity of the vaccines was assessed by ELISA using commercial kits.
Results: The geometric mean titers (GMT) of the antibodies induced against measles and rubella by MMR-Local were 1.2 and 5.6 IU/ml and those of MMR-GSK were 1.5 (p=0.029) and 97.9 IU/ml (p<0.001), respectively. The GMTs of antibodies produced against mumps were 99.2 RU/ml for MMR-Local and 98.9 RU/ml for MMR-GSK vaccines, respectively. No significant differences were observed between the two vaccines components regarding the GMT obtained for samples collected before administration of the 2nd vaccine dose.
Conclusion: Immunogenicity against mumps was similar for the two vaccines, but immunogenicity of the local vaccine against rubella and to a lesser extent measles was significantly less efficient than that of the MMR-GSK. This difference could be attributed to the virus strain or the preparation or formulation protocol of the local vaccine.

P4.01.122

HCV-specific CD8+ T-cells are abundant in HCV-seronegatives but show private TCR repertoires

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Introduction: Presence of virus-specific CD8+ T-cells has been frequently detected in uninfected individuals, but detailed characteristics have been poorly defined. This study aimed to detect phenotype, T-cell receptor (TCR) repertoire and functionally characterize T-cells specific for the immunodominant HCV NS3-1073 epitope in HCV seronegative individuals (HCV-SNs) with different risk factors of HCV exposure.
Results: NS3-1073-specific CD8+ T-cells were frequently detected in HCV-SNs by ex vivo tetramer enrichment and showed a highly variable memory phenotype (CD45RA- 5-42%). After in vitro expansion, significant HCV NS3-1073-specific T-cells were detected in 31.8% (21/66) of HCV-SNs. Unexpectedly, no differences between

the different risk groups were observed. The TCR α and β repertoire of sorted NS3-1073 specific CD8+ T-cells demonstrated a high variability between individuals. Some HCV-SNs showed highly oligoclonal TCRs while others were rather diverse. Of note, some TCR β repertoires included CDR3 motifs could also be found in patients with acute HCV infection. Importantly, detection of NS3-1073-specific T-cells in one HCV-SN correlated with rapid and strong response to the peptide vaccine IC-41 that includes the NS3-1073 epitope.
Conclusion: Our data show that in HCV uninfected individuals memory T-cells specific for NS3-1073 are abundant and display private TCR specificities. One mechanism for this phenomenon can be T-cell cross-reactivity as previously shown for this epitope. The oligoclonal TCRs and the similarity of CDR3 motifs between HCV-SNs and acute HCV patients support this hypothesis. Thus, the presence and variability of pre-existing cross-reactive memory T-cells may help to explain the varying responses to HCV infection and vaccination.

P4.01.123
MAP kinase phosphatase 5 targets IRF3 to regulate type I interferon production in response to influenza infection

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MAP kinase phosphatase 5 (MKP5), one member of MAPK phosphatase (MKP) protein family, plays critical roles in both innate and adaptive immune responses. However, the role of this protein in anti-viral immune responses has not been well studied. To investigate the role of MKP5 in innate immune response against influenza, wild-type (WT) and MKP5 knockout (KO) mice were infected with A/Putero Rico/8/34 (PR8, H1N1) viruses. We found that viral titers in the lung of MKP5 KO mice are significantly lower on day 3 and day 5 post-infection than those in the lung of WT mice, which is associated with increased expression of IFN-beta in the lung and IFN-beta concentrations in the BAL from KO mice compared with those from WT mice. Macrophages from MKP5 KO mice produced significantly higher amount of IFN-alpha and IFN-beta in response to PR8 virus infection than cells from WT mice. The increased expression of type I interferon in KO cells is associated with increased IRF3 phosphorylation and nuclear translocation compared with WT cells in response to influenza infection. Immunoprecipitation and *in vitro* binding assays demonstrated that MKP5 interacts with IRF3 *in vitro* and *in vivo*. Our study demonstrated that MKP5 regulates IRF3 to control type I interferon production in response to virus infection.

P4.01.124
2009 H1N1 infection primes for strong immunological memory in human nasal-associated lymphoid tissue that offers cross-reactive immunity to H1N1 and avian H5N1 viruses

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Background: Influenza is a highly contagious mucosal infection in the respiratory tract. 2009 pandemic H1N1 (pH1N1) virus infection resulted in substantial morbidity and mortality in humans. Little is known on whether immunological memory develops following pH1N1 infection and whether it provides protection against other virus subtypes. Methods: Enzyme-linked immunosorbent spot assay was used to analyze hemagglutinin (HA)-specific memory B cell responses after virus antigen stimulation in nasal-associated lymphoid tissues (NALT) from children and adults. Results: Individuals with serological evidence of previous exposure to pH1N1 showed significant cross-reactive HA-specific memory B responses to pH1N1, seasonal H1N1(sH1N1) and avian H5N1(aH5N1) viruses upon pH1N1 virus stimulation. pH1N1 virus antigen elicited stronger cross-reactive memory B cell responses than sH1N1 virus. Intriguingly, aH5N1 virus also activated cross-reactive memory responses to sH1N1 and pH1N1 HAs in those who had previous pH1N1 exposure, and that correlated well with the memory response stimulated by pH1N1 virus antigen. These memory B cell responses resulted in cross-reactive neutralizing antibodies against sH1N1, 1918 H1N1 and aH5N1viruses. Conclusion: 2009 pH1N1 infection appeared to have primed human host with B cell memory in NALT that offers cross-protective mucosal immunity against not only H1N1 but also aH5N1 viruses. These findings may have important implications to future vaccination strategies against influenza. It will be important to induce and/or enhance such cross-protective mucosal memory B cells.

P4.01.125
Impact of vaccination route and chosen adjuvant on immune responses and host-pathogen interplay during influenza infection.

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Influenza causes an acute infection in the host and initiates a cascade of immune reactions activating almost all components of the immune system. Most of the initial innate response, including cytokine release, influx of granulocytes or natural killer cells and cell activation, is responsible for the acute onset of the clinical symptoms. On the other hand, innate immunity is an essential prerequisite for the development of adaptive immune responses leading to disease resolution.
In this study, we evaluated the immunological response to influenza virus A/PR/8/34 (H1N1) infection of naïve Balb/c mice in comparison to virus pre-exposed or differently immunized mice. Using a panel of different vaccination routes and adjuvants, we characterized the impact of different vaccination-induced immune responses during actual pathogen encounter, and how it contributes to disease resolution or pathology.
We immunized Balb/c mice systemically or mucosally with an influenza subunit vaccine (HA/NA proteins from A/California/7/2009/H1N1) formulated with i) MF59 to induce a mixed Th1/Th2 response, ii) a combination of MF59 and CpG to get a more Th1-prone response and iii) LTK63 to obtain a Th1/Th17 polarized response. We assessed antibody titers and individual T helper profiles after vaccination and dissected the complex host-pathogen interplay at different timepoints after infection. To that end, we determined ex-vivo CD4 T helper responses in the lung, characterized differential lung cell composition and cytokine environments.
Our studies should help to better understand how immune responses and ultimately vaccination success can be steered via different administration routes and adjuvant formulations.

P4.02 Liver viral infections

P4.02.01
A deficient translocation of CD3ζ, ZAP-70 and Grb2 to lipid raft, as a hallmark of defective adaptive immune response during chronic hepatitis B infection

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Hepatitis B is considered to be a worldwide public health problem. An immunosuppressor microenvironment has been proposed to contribute to viral persistence during chronic disease. Understanding the intracellular signaling cascade in T-cells from HBV infected patients, will contribute to unravel the mechanisms that control the development of immune response during Hepatitis B. We analyze lipid rafts formation and early activation signals in chronically HBV infected patients, compared to naturally immune subjects (NIS).

Patients show: 1) diminished GM1 clustering, 2) A deficient lipid rafts recruitment of CD3ζ/ ZAP-70/ Grb2, and 3) these proteins do not merge with GM1 within the lipid rafts. Finally, immunoprecipitation assays proved that ZAP-70 does not associate to CD3 ζ. These results show for the first time, defects regarding early key events in T-cell activation, in chronically infected HBV patients, which may contribute not only to understand HBV immune tolerance, but to reveal new potential therapeutic targets to control the infection

P4.02.02
HCV genotype 3 infected patients: Resistant and the relapsers

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Hepatitis C virus (HCV) has infected about 7 million Pakistani. Standard interferon in combination with ribavirin is the widely used approved therapy for HCV genotype 3 (~75-90% prevalence in Pakistan). A study was conducted to determine the relapsers and resistant to interferon treatment in HCV genotype 3 infected patients. Institute's Ethical Committee approval was granted for the study. All the patients included in the study were those who received recombinant IFN-α plus ribavirin with 24 weeks therapy duration. Serum markers i.e., liver function tests (LFTs), HCV viral load, genotyping, pre-biopsy tests and liver histopathology were taken into consideration. The investigation showed that 20% of the patients were non-responder to the interferon treatment and 28% of the patients among responders were relapsers. There is a need to conduct studies taking into consideration viral as well as host factors that can help us evaluate the reasons behind relapse/ non-response to interferon therapy.

P4.02.03
Possible routes of transmission among HCV infected Pakistani subjects

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In Pakistan, the viral hepatitis infections are mounting day by day especially HCV. The route of transmission and the risk factors associated are critical to evaluate the factors resulting in high infection prevalence. We conducted a study, where after taking the consent from the patients, data was gathered in order to figure out the possible routs of transmission in HCV patients. Hence 168 HCV patients from 22-65 years (divided into 3 groups i.e., group I: 21-35 yrs, group II: 36-50 yrs and group III: 51-65 yrs) were included in the study. About 46% of the patients (55 females and 22 males) were in group I, 45% (35 females and 41 males) in group II and 9% (8 females and 7 males) in group III. About 40% of the patients had undergone surgery, 51% visited the dentist for treatment, 58% practiced unsafe sex, 54% of the patients had ear piercing, 20% had blood transfusions, 2 % had needle stick injuries, 1% was drug user, and 43% were used to shave from barbers. There was only one case of tattooing among the patients and only one male shared the blades. So the dental treatment, surgery, unsafe sex, ear piercing and blood transfusions are the major risks of HCV transmission. There is a need to prevent HCV by educating and brining awareness in our society and also focusing on HCV management practices.

P4.02.04
Surface receptors and cytokines in CD4+ T cells upon HCV core transduction

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Regulatory T cells have been identified as a specialized T cell subset that suppresses auto-reactive cells to maintaining immunological tolerance and inhibit autoimmunity. The role of Treg in some viral infections, particularly Hepatitis C virus infection, range from suppressing T-cell responses directed against the virus to down-regulating immune responses causing liver damage. Treg play a prominent role in maintaining the delicate balance between an immune response that is sufficiently robust to clear the infection and the immunopathological consequences of sustained immune activation and inflammation.
As there is evidence indicating that HCV can be present in cells either than the hepatocyte, including CD4+ T lymphocytes, we have analyzed the effect of expressing HCV-core as a single viral protein in CD4+ primary lymphocytes. Previously, we and others have shown that HCV-core induces suppression when expressed in the CD4+ tumor T cell line Jurkat, the NK cell line YTS, or when added to CD4+ T cell cultures.
Materials and methods: CD4+ primary lymphocytes were transduced with lentiviral supernatants expressing an HCV-core-GFP expression cassette.
Results: HCV-core transduced CD4+ T cells acquire a regulatory phenotype upregulating several molecules such as Foxp3, CTLA4 or PD1; expressing low levels of IL-7R (CD127) and suppressing the response of bystander T cells.
Conclusions: Our results add a new element to understanding Treg populations in the context of Hepatitis C virus expression and give the opportunity to explore a mechanism to generate CD4+ T cells with regulatory activity by the expression of a single viral protein.

P4.02.05
Cytokine profiles are modulated through the STAT family and define distinct clinical courses during hepatitis A virus infection

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The hepatitis A virus (HAV) infection is the major cause of acute liver failure and liver transplantation in pediatric patients. Although the incidence of hepatitis A has fallen since the introduction of vaccines, it remains as a public health problem especially in developing countries. The clinical spectrum of HAV infection ranges from mild or asymptomatic liver disease to acute liver falilure. However, the reasons for this variability are not clearly defined yet.
In this study, a statistically robust approach was used to retrospectively analyze cytokine expression in type A virus-infected children, allowing us to develop a structured model that can generate biological testable hypothesis. The resulting model suggested that modulation of the activity of Signal Transducers and Activators of Transcription proteins (STATs) occupied a central role during HAV infection, leading us to compare the degree of STATs phosphorylation in peripheral blood lymphoid cells from pediatric patients with minor and intermediate HAV-induced liver damage. Interestingly, we found that TGF-beta and IL-8 overexpression related to HAV-induced minor liver disease was associated with increased STAT-3 phosphorylation. In contrast, overexpression of IL-6, TNF-alpha and MCP-1 related to intermediate liver disease, correlated with increased STAT-3 and -5 phosphorylation. Our data suggests that different members of the STATs family may interplay to adjust the balance between immune response and infection by modulating cytokine profiles, playing an essential role in the differential progress of HAV-induced pathology. This work was partially funded by the 127229, INFR-2012 and 849 grants from the CONACyT and COECyTJAL to FNA.

P4.02.06
Transcriptome profile of HCV-specific CD8 cells in early HCV infection

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Background and aims: In chronic HCV infection, virus-specific T cells are dysfunctional, and reconstitution of their function may represent a rational strategy to improve available therapeutic options.
Different co-stimulatory pathways may be involved in T cell exhaustion, but a comprehensive analysis of their role in HCV-related CD8 cell dysfunction is still lacking. Aim of our study was to perform a transcriptome analysis of HCV-specific CD8+ T cells, in order to obtain a genome-wide repertoire of misregulated genes potentially involved in T cell dysfunction, to be used as a knowledge basis for the development of a tailored, chronic HCV therapeutic approach based on their correction.
Results: Differential gene expression signatures distinguishing HCV- and FLU-specific CD8 cells sorted from acutely HCV infected patients with self-limited or chronically evolving infection were revealed by transcriptome (oligo-microarray) analysis. A significant difference in the expression profiles of 499 misregulated genes also emerged from the comparison of HCV-specific CD8 cells from patients with different evolution/outcome of infection (self-limited vs. chronic). This gene set is enriched in pathways related to apoptosis, cell stress, negative regulation of proliferation and cytokine production.
Conclusions: Ongoing analyses and data-mining within the above described experimental framework will provide novel insights into HCV infection pathogenesis, which will deepen our understanding of the molecular mechanisms underlying HCV persistence. A detailed analysis of differentially expressed genes will allow the identification of new molecular targets for the restoration of a functionally efficient anti-viral CD8 response as well as novel predictors of disease evolution.

P4.02.07
The role of Foxp3+ regulatory T cells in the inhibition of an adaptive immune response in a persistent HBV mouse model

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Worldwide more than 400 million people suffer from chronic HBV infection and have a high risk factor to develop liver cirrhosis and hepatocellular carcinoma. We applied adenoviral genome transfer (AdHBV) to overcome the species-barrier of HBV infection and successfully developed an immune-competent murine model for persistent HBV infection (Huang et al., Gastroenterology 2012). The forkhead box P3 transcription factor (Foxp3) has been shown to influence several adaptive immune responses, for example in the context of LCMV, where protective roles for regulatory T cells (Treg) have been uncovered (Rowe et al., Immunology 2012).
Here we analyse the role of Foxp3+ Treg on HBV persistence using Foxp3.LuciDTR-4 mice. The mice display 95% Treg depletion following injection of diphtheria toxin (DT) (Suffner et al., J. Immunology 2009). In AdHBV-infected and DT-treated Foxp3.LuciDTR-4 mice we observed a dramatic decline of the serum HBsAg at d10, whereas the control groups remained positive for HBsAg. They also showed an increase in HBs190-97 -specific CTLs, which suggests a strong cytotoxic function of these HBs-specific CD8+ T cells in liver and spleen. Additionally, the serum ALT level of those mice peaked at d7 by 4-fold. During persistent HBV infection, Treg seem to play a role in the inhibition of HBs-specific CTL responses. Further analysis for revealing the detailed mechanism of how Treg act on tolerance induction towards HBV has to be investigated.

P4.02.08
Monoclonal antibodies to various epitopes of HBs antigen inhibit HBV replication

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Antibodies against the 'a' determinant of hepatitis B surface antigen (HBsAg) are able to neutralize circulating HBV particles. It has been reported, that single mutation within this region coincide with the emergence of mutants escaping the immune response. Since multiple immunodominant epitopes are present within this region, we investigated whether a single mutation may account for virus escape from humoral immunity.
Nine murine HBsAg specific monoclonal antibodies (MAbs) were produced. Reactivity of all antibodies with 11 recombinant escape mutants of HBsAg was assessed by ELISA. HepaRG cells were used as an *in vitro* model to evaluate viral neutralization capacity of MAbs. Viral infection markers, cccDNA and HBeAg, were analyzed by real time PCR and ELISA, respectively.
The MAbs were classified into 4 subgroups based on their pattern of reactivity to the mutant HBsAg. Accordingly, 3 MAbs react with all mutants, 3 MAbs failed to recognize the T126N and Q129L mutants, 2 MAbs did not bind to the D144A mutant and finally 1 MAb did not react with the T143K, D144A and P142S mutants. All MAbs, however, were able to inhibit the establishment of HBV infection dose dependently.
Our results indicate that antibodies against different epitopes of the 'a' determinant are able to neutralize HBV. Since the antibody response to HBsAg is normally directed against multiple epitopes within the 'a' determinant, it seems that mutations within a single or a limited number of epitopes can hardly result in viral escape. Therefore, cellular mechanisms may contribute to the HBV immune escape.

P4.02.09
Impact of cytokines and apoptosis genes polymorphisms on the outcome of hepatitis C virus infection in Tunisian hemodialysed patients

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Introduction: The aim of this study was to investigate the possibility of a significant relationship between the spontaneous clearance or the persistence of HCV infection and cytokines, and apoptosis genes polymorphisms in Tunisian hemodialysed patients.
Methods: The polymorphisms of genes: IL-1 (-889 IL-1α, -511 and +3954 IL-1β, IL-1-Ra), IL-18 (-137 and -607), IL-12p40 (-1188) and Apo1/Fas (-670) were determined by PCRRFLP, PCR-SSP and PCR-VNTR, at 100 healthy blood donors and 100 hemodialysed patients infected by the HCV. The patients were classified in two groups: G1 included 76 active chronic hepatitis patients (positive RNA-HCV) and G2: 24 hemodialysed having eliminated spontaneously virus (negative RNA-HCV).
Results: The frequency of genotype association [-37GC/-607CA] IL-18 is statistically higher among G2 patients (41,7%) compared to that at G1 hemodialysed (15,8%) (p=0,008, OR: 0.26, 95%CI: [0.10-0.73]). We found also, a significant increased frequency of AA genotype of Apo1/Fas gene in G2 patients (41,6%) than in G1 (17,5%) (p=0,026, OR=3,49, 95% CI [1,13-10,69]). Adjustment for known covariates factors (age, gender and genotypes) confirmed these univariate findings and revealed that the genotype association GC-CA of (-137and-607) IL-18 gene and AA genotype of Apo1/Fas gene were associated to the clearance
of HCV (p=0.041 and p=0.017 respectively).
Conclusion: The two genotypes GC-CA of (-137and-607) IL-18 polymorphism and the AA genotype of Apol/Fas gene seem to influence the outcome of the HCV infection in Tunisian hemodialysed patients.

P4.02.10

Immunogenetic factors of chronic hepatitis C and hepatocellular carcinoma development observed in atomic-bomb survivors

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A series of host immune responses are thought to be involved in the course of disease development after infection, presuming that individuals' course of infection depends in part on inter-individual variation of immune responses. In this study, we examined association of *NKG2D* gene haplotypes with persistent HCV infection and hepatocellular carcinoma (HCC) development. First, we conducted a case-control study of persistent HCV infection by comparing the frequencies of *NKG2D* haplotypes between 160 cases with persistent HCV infection and 106 HCV clearance persons (controls) within a cohort of atomic-bomb survivors and found that the frequency of *HNK1/HNK1* haplotype (18%) in the HCV clearance group was about 2-times higher than that in the persistent HCV infection group (8.8%) (*HNK1* and *LNK1* alleles being associated with high and low NK activity, respectively). Second, a case-cohort genome association analysis of HCC was conducted with 115 HCC cases and a sub-cohort of 2,132 persons in the same cohort. When the subjects were divided into three radiation dose categories (non-exposure, <0.7Gy≥0.7Gy) combined with *NKG2D* haplotypes, relative risk (RR) of HCC was the highest in atomic-bomb survivors who have *HNK1/LNK1* or *LNK1/LNK1* and exposed to the highest dose (≥0.7Gy) (RR = 4.6, 95% CI: 1.1-19.2), relative to the reference category (*HNK1/HNK1* and non-exposure). These results suggest that *NKG2D* haplotypes affected the binary decision of HCV clearance or persistent infection in atomic-bomb survivors following HCV infection, and that radiation exposure as well, not only *NKG2D* haplotypes, was involved in development of HCC.

P4.02.11

Prevalence of GBV-C/HGV and HCV among HIV infected populations in South Iran

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Introduction: Individuals infected with the human immunodeficiency virus (HIV) are often co-infected with other pathogens, especially hepatitis viruses. HCV and GBV-C/HGV are blood-borne viruses that are highly prevalent among HIV/AIDS patients.
Methods: One hundred and fifty eight HIV-infected patients (98 men and 60 women) were included in this study. Patients were categorized into four different high risk groups: intravenous drug users (N=109), hemophiliacs (N=13), homosexual (N=8) and heterosexuals (N=28). RNA extraction from 100 µl of serum or plasma was performed with the RNX plus kit. Detection of GBV-C/HGV-RNA and HCV-RNA were performed by using reverse transcription and nested PCR.
Results: GBV-C/HGV-RNA and HCV-RNA were found in 40 (25.31%) and 58 (36.7%) patients, respectively. The prevalence of GBV-C/HGV and HCV RNA in intravenous drug users, hemophiliacs, homosexual and heterosexuals group was 57.1% (N = 56), 11.22% (N = 11), 3.03% (N = 3) and 28.57% (N = 28), respectively.
Discussion: According to our data the prevalence of GBV-C and HCV in HIV infected case was different in different groups; therefore we may be concluded that the route of HIV transmission may affect the prevalence of HCV and GBV-C/HGV co-infections in HIV infected individuals.

P4.02.12

Serum IP-10 profiles over time in treatment-experienced hepatitis C patients treated with a HCV NS3/4A protease inhibitor in combination with peginterferon and ribavirin

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Pre-treatment IP-10 (CXCL10) levels in hepatitis C virus (HCV) infected patients have been associated with treatment outcome upon peginterferon (pegIFN)/ribavirin (RBV) therapy, similar as *IL28B* genotype. Here we assessed on- and post-treatment changes in IP-10 serum levels in pegIFN/RBV treatment-experienced HCV-infected patients treated with pegIFNα-2a/RBV (n=65) or with a HCV NS3/4A protease inhibitor (PI) (simeprevir) together with pegIFNα-2a/RBV (n=195). Samples collected pre-treatment, at week 2 and 4 after treatment start, and 24 weeks after end of treatment were assessed.
Pre-treatment IP-10 levels were significantly lower in patients that relapsed during previous pegIFN/RBV treatment as compared to prior null and partial responders. After treatment start, serum IP-10 decreased over time. While serum IP-10 levels lower than at baseline were maintained in sustained virologic response (SVR) patients, in non-SVR patients the initial on-treatment IP-10 decline was reversed 24 weeks post-treatment. The treatment-induced IP-10 decrease versus baseline mirrored on-treatment HCV RNA declines and was observed already 2 weeks after initiation of PI/pegIFN/RBV therapy, whereas noticeable only after 4 weeks or later in subjects treated with pegIFN/RBV alone.
In line with earlier studies in treatment-naïve pegIFN/RBV treated patients, HCV RNA and IP-10 levels in treatment-experienced patients returned to baseline in patients who did not clear their HCV infection. Together with the faster decrease of IP-10 in patients treated with PI/pegIFN/RBV as compared to pegIFN/RBV alone, these findings suggest that on- and post-treatment changes in IP-10 levels in HCV-infected patients reflect alterations in the host immune response driven by the dynamics in viral load.

P4.02.13

Infectious diseases: Viral infections prevalence among gastroenterology patients

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Infectious diseases burden is mounting day by day. A number of viral infections are common in nowadays. In order to evaluate the infectious diseases and the viral hepatitis infections, a study was conducted to determine the proportion of the viral hepatitis infection among the patients that visit gastroenterology. The serum markers were evaluated and diagnosis was performed to figure out the ratio of different infectious diseases prevalent. The data was gathered from July to December 2012 among 550 patients. About 29% of the patients were infected with HCV infection, 6% HBV infection and 0.5% HBV and HDV infection. The blood borne viral hepatitis infections constitute 35.5% of all the infections. HIV infection was found in 3% of the patients, malaria 33%, enteric fever 10%, brucellosis 2%, tuberculosis enteritis 7%, tuberculosis ascites 1% and Giardiasis 8%. The viral hepatitis infections are the most prevalent infectious diseases among gastroenterology patients. There is a need of intervention to control viral hepatitis infectious.

P4.02.14

TLR-2 promotes both mouse hepatitis virus (MHV) replication and inflammatory responses in hepatocytes leading to fulminant hepatitis

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Acute viral hepatitis results from an inefficient innate immune response to clear the virus and a delayed immune adaptive response. Murine hepatitis virus (MHV) infection represents a unique animal model to identify new escape mechanisms in liver of innate immune responses. The objective of this study was to identify early disorders in TLRs, helicases, cytokines and chemokines favoring the development of a fulminant hepatitis. Groups of C57BL/6 WT and TLR2-/- mice were infected with highly hepatotropic MHV3 and/or weakly hepatotropic MHV-A59 viruses. Histopathological analysis of liver and mRNA expression levels of viral nucleoprotein, viral sensors, interferons, cytokines and chemokines assessed by RT-qPCR were done in the first 3 days of infection. The results showed that liver damages, viral replication and mRNA levels of TLR-2, TLR-3, RIG-1, MDA-5, IL-33, IFN-β, CXCL1, CXCL9, CXCL-10, CXCL-11, CCL3, CCL5, IL-6 and TNF-α increased higher or appeared sooner in MHV3-infected WT than in MHV-A59 and TLR2-/- mice. To address the role of hepatocytes in TLR2-dependent viral replication and innate immune factors, *in vitro* viral infections were performed on FL83B cells. The results showed that viral replication and mRNA levels of TLR-2 and IL-6 occurred sooner than those of other innate immune parameters. Moreover, blockade of TLR-2 by siRNA decreased IFN-β, TNF-α, CXCL-1, CXCL-10 and CCL-2 expression in infected hepatocytes and also inhibited the viral replication of MHV-A59, and at a lesser extent of MHV3, suggesting that TLR-2 signaling promotes simultaneously the viral replication and the production of innate immune factors in hepatocytes, exacerbating viral hepatitis.

P4.02.15

The altered phenotype of $CD4^+CXCR5^+$ T cells and B cells is associated with the chronic hepatitis B virus infection

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Both germinal center and blood derived $CD4^+CXCR5^+$ T cells have been established as a T helper cell subset (T follicular helper cells, Tfh) specialized for providing help to B cells. However, the role of $CD4^+CXCR5^+$ T cells and B cells in chronic hepatitis B virus infection (CHB) is poorly defined. In this research, we examined the frequency and phenotype of $CD4^+CXCR5^+$ T cells and B cells in CHB patients (n=65) and health control (HC) (n=34) by flow cytometry. Our results showed that the general frequency of $CD4^+CXCR5^+$ T cells and B cells was not changed in peripheral blood of CHB patients when compared with HC. Although the expression of surface molecules essential for helper functions like ICOS, CD40 was significantly increased in the CD4+CXCR5+ T cells of CHB patients, the expression of ICOSL was significantly decreased in both memory B cells and plasmablastes of CHB patients versus HC. The expression of cytokines IL-4, IL-21 and cytokine receptors IL-6R, IL-21R was also increased in blood $CD4^+CXCR5^+$ T cells isolated from CHB patients. In CHB patients, the frequency of blood $CD4^+CXCR5^+$ T cells was significantly correlated with, while the $CD40^+CD19^+$ B cells was negatively correlated with serum ALT and AST. Our results indicated that the altered phenotype of $CD4^+CXCR5^+$ T cells and B cells might be associated with the lack of protective antibody responses and liver injury in persisted HBV infection. The function of $CD4^+CXCR5^+$ T cells and B cells in chronic hepatitis B virus infection need to be further investigated.

P4.02.16

Interleukin-6, tumor necrosis factor-α and interleukin -10 polymorphisms are associated with the cytokines serum levels of hepatitis C virus infection in Brazilian Population

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We investigated the effects of polymorphisms in IL-6, TNF-α and IL -10 in chronic hepatitis C patients newly diagnosed and before they start the treatment in Manaus, Amazonas, Brazil. A total of 116 individual were genotyped for the genes encoding IL6 (-174), TNFα (-308) and IL10 (-1082) and the IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL17A were measured. We examined the relationships between polymorphisms variations and the cytokines profile. For the IL6(-174) SNP : the cytokines serum of IL-2 , IFN- γ , TNF-α and IL-10 were higher in subjects carrying the GG allele than GC /CC and IL-6 and IL-17A were higher in subjects carrying the GG/ GC than CC. For the TNFα (-308) SNP the cytokines serum of IL-2, IFN- γ, TNF-α, IL-10 and IL-6 were higher in subjects carrying the GG allele than GA. The cytokines serum of IL-17A was higher in subjects carrying the GG and GA. It is important to say that the AA allele was not found in this population. For the IL10 (-1082) SNP the cytokines serum of IL-2 and IFN- γ were higher in subjects carrying the AA allele than AG and GG. The cytokines serum of IL-10, IL-6 and IL-17A were higher in subjects carrying the AA and AG than GG. We conclude that the relationship between the variations of IL6, TNFα and IL10 could possible modify the cytokines serum levels and also changes the dynamics of the TH17- TH1 profiles.

P4.02.17

HHV-8 and hepatitis infections in pemphigus

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Background: Viral infection was widely associated with autoimmune diseases. In pemphigus, associations with human herpes virus 8 (HHV-8) and hepatitis C virus (HCV) were reported suggesting that these viruses may be involved in the pathogenesis of pemphigus. The aim of this study was to analyse the prevalence of HHV-8, HCV and HBV infections in pemphigus.
Methods: We enrolled 62 pemphigus foliaceus (PF), 52 pemphigus vulgaris (PV), 27 bullous pemphigoid (BP) patients and 50 controls. Anti-HHV-8 antibodies were tested by indirect immunofluorescence Kit. Serological markers of HBV infection and anti-HCV antibodies were analysed by ELISA kits.
Results: Anti-HHV-8 antibodies were observed in 12 (19%) PF, 16 (30%) PV, 12 (44%) BP patients and 18 controls (36%) with no significant difference. HCV infection was rare among patients with no significant difference with controls. High frequency of anti-HBc (41%) antibodies was observed in the PF group compared to controls (p=0.055). Inversely, a vaccinated statue was more prevalent in controls than in patients (p=0.053 and p=0,004 for PV and PF patients respectively).
Conclusion: In this case-control study we found that anti-HHV-8 and HCV infections are not associated with pemphigus. The high frequency of anti-HBc antibodies, as well as the protective effect of vaccination, suggest the involvement of HBV in pemphigus pathogenesis.

P4.02.18
Epitope mapping and identification of mutated hepatitis B surface antigens by mouse monoclonal antibodies

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Selection of hepatitis B virus (HBV) by host immunity may give rise to variants with amino acid substitutions at or around the immunodominant "a" determinant of the hepatitis B surface antigen (HBsAg), the main target of antibody neutralization and diagnostic assays. The aim of this study was to generate and characterize murine monoclonal antibodies (MAbs) against HBsAg mutants and their application for identification of these antigens.
Stable hybridoma cells were established from BALB/c mice immunized with recombinant HBsAg of the “adw” subtype and cloned by limiting dilution. Specificity of MAbs was studied by indirect ELISA and Western blotting. Topology of the epitopes was analyzed by competitive ELISA.
2C5 and 2A7 hybridoma clones producing MAbs specific for HBsAg were established. Both MAbs recognized overlapping linear epitopes within the "a" determinant. Reactivity with a panel of mutated HBsAgs revealed that two MAbs loose their binding activity upon amino acid substitution at position M133H or P142S or D144A of the HBsAg.
Our findings indicate that the amino acids at positions 133 and 142 and 144 are crucial for expression of the epitope(s) recognized by our MAbs. These MAbs are useful serological tools for identification and epitope mapping of the mutant HBsAgs.

P4.02.19
The diverse roles of necroptosis and inflammatory signaling during chronic overwhelming infections

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Background: Pattern recognition receptors, TNF-alpha (and other death receptors), and IL-1 are involved in inflammation, apoptosis, necroptosis and pyroptosis signaling. Two members of the receptor interacting protein kinase (RIPK) family RIPK1 and RIPK3 along with caspase-8 are important signaling molecules in the aforementioned processes that determine inflammatory and cell death outcomes. Chronic overwhelming infections, such as HIV-1 and hepatitis B (HBV), are characterized by a non-specific deleterious inflammatory responses that cause death of bystander T cells in HIV infection, or bystander hepatocytes in HBV infection, yet infected cells are less sensitive to cell death. We hypothesized that RIPK3 and caspase-8 may conspire in the development of a pro-inflammatory milieu and death signaling of uninfected cells. The aim of our study was to investigate the role of RIPK3 and caspase-8 in chronic overwhelming infection.
Design: We utilized conditionally gene targeted animals and compound mutant animals and two infection models. We chronically infected animals with LCMV or used a highly novel HBV chronic infection model.
Results: Contrary to other studies, which defined a role for RIPK3 in vaccinia, murine cytomegalovirus and S. enterica acute infection, we found that RIPK3 alone and RIPK3 together with compound loss of caspase-8 plays no role in outcomes to chronic LCMV infection. In contrast we describe a highly significant phenotype in HBV infected mutant animals that impacts on infectious outcomes.
Conclusion: We have identified infection specific and cell specific roles of RIPK3 and caspase-8 that provide potential therapeutic insights with implications for chronic HBV infection.

P4.02.20
Human APOBEC3 Cytidine Deaminases: not simply antiviral defense?

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Host-encoded restriction factors are important players in innate antiviral immunity. Human APOBEC3 (apolipoprotein B mRNA-editing enzyme catalytic polypeptide) proteins induce dC-to-dU-mutations, leading to DNA degradation or hypermutation and subsequent loss of viral genetic integrity. The APOBEC3 expression pattern in the host is ill-defined and regulated by inflammatory mediators secreted upon viral infection.
APOBEC3 deaminases have the capacity to edit host DNA, which may influence cellular genome stability and potentially play a role in the onset of cancer. We hypothesize that inflammatory processes induced by virus infections enhance the expression of APOBEC3 deaminases and may represent a novel mechanism of virus-induced oncogenesis.
In order to investigate the mutagenic potential of the different human APOBEC3 deaminases, we established stable, isogenic cells lines driving tetracycline-regulated APOBEC3 gene expression. As a readout for APOBEC3-mediated host genome mutagenesis, we use counterselection against herpes simplex virus thymidine kinase (HSV-TK): APOBEC3-mediated DNA editing leads to HSV-TK reporter gene inactivation and allows survival of ganciclovir (GCV)-resistant colonies. At first, a founder line of Flp-In-T-Rex cells stably expressing the HSV-TK reporter gene was established and functionally characterized. The integrated copy number of HSV-TK genes was determined by Southern blotting. After successful integration of APOBEC3 genes via FLPase-mediated DNA recombination, a phenotypic analysis based on DNA damage repair and cell cycle progression was performed to obtain deeper insight into APOBEC3 functions.
In addition, we profiled hepatitis virus-induced tumor samples to elucidate APOBEC3 involvement in inflammation-associated carcinogenesis. Immunohistochemical studies showed a slight increase in APOBEC3 protein expression in hepatocellular carcinoma groups.

P4.02.21
Polarized effector function of NK cells in chronic hepatitis C is partially dependent on the IL28B genotype

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Background: IFN-a-induced expression of TRAIL on NK cells correlates with HCV-RNA decline during interferon alpha-based therapy. A polymorphism close to the IL28B genotype has been identified to be the most important factor in determining treatment response. However, the role of the IL28B genotype in type-I-interferon-dependent regulation of NK cells function is unknown.
Methods: NK cells from healthy controls and chronic hepatitis C (CHC) patients were studied in vitro after IFNa-stimulation. Upregulation of TRAIL, CD107a and intracellular cytokine production were investigated by flow-cytometry after co-cultering with K562 or Huh7.5 cells. NK cells were also co-cultured with Huh7.5-JFH1-(Luc-NS3-5B) replicon cells to assess antiviral effector functions. The IL28B SNP rs12979860 was determined by melting curve analysis.
Results: NK cells of CHC patients showed a marked polarization towards cytotoxicity in response to IFNa stimulation with significantly higher TRAIL upregulation but lower IFNg and TNFa production. Co-cultering HCV replicon cells with resting NK cells had modest antiviral effects while IFN-a-stimulated NK cells significantly reduced HCV replication. Cytokine production but not TRAIL expression was higher in healthy subjects carrying the IL28B-CC allele. In contrast, CHC patients with an IL28B-CT or -TT genotype showed higher interferon-gamma expression. The percentage of interferon-gamma-positive NK cells after IFNa-stimulation correlated with ALT levels only in IL28B-TT-patients.

Discussion: Higher cytokine production of NK cells in IL28B-CC healthy individuals may reflect better early effector functions supporting spontaneous HCV clearance in acute hepatitis-C. In contrast, the increased activity of NK cells in IL28B-TT CHC patients may contribute to the activity of liver disease.

P4.02.22
Programmed hepatocytes cell death associated with FLIP dowregulation and Bid activation in response to extracellular PreS1/2

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Chronic hepatitis B virus (HBV) infection involves liver damage and cirrhosis, resulting in continuous cell injury and death. Cirrhosis are considered to be the most important pre-cancerous etiological factor associated with hepatocellular carcinoma (HCC). During HBV infection, hepatocytes exhibit changes in death receptor expression and in their susceptibility to death. These changes are observed not only in infected cells but also in bystander cells. Because the viral surface protein is extensively secreted and it is found in considerable amount in soluble particles, we explored the possible role of soluble preS1/2 in hepatocyte (HepG2) death modulation. We observed an increase of cell death induced by preS1/2, which was associated with down-regulation of FLIP and activation of caspase 8, caspase 9 and BID. Additionally, hepatocytes exhibit a sensitization to death mediated by the Fas receptor. These results, may contribute to understanding the role of envelope proteins (preS1/2) on pathogenesis of HBV infection

P4.02.23
Detection of hepatitis B virus variants in Tunisian patients

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The HBV genome is organized in four major overlapping open reading frames (ORF): S, C, P and X.
In this study we looked for genetic variations in genes S and P of HBV isolates from Tunisian patients. HBV serological markers were detected by ELISA and viral DNA was amplified by PCR. DNA sequencing was performed using Big Dye Terminator technology. Serologic data from 41 patients showed all patients except one were positive for HBsAg. The over all prevalence of HBeAg was 21.9%. PCR results indicated that 48.7% of the patients were positive for HBV DNA including the HBeAg positives. Ten sequenced amplicons showed all corresponding isolates to be of the D genotype. A characteristic profile of point mutations at the DNA level was found for each isolate and a 9 bp deletion in one isolate. It is noteworthy that some of these mutations were significant in ORF S but silent in ORF P and vice versa. Amino acid substitutions in the major hydrophilic region of the small S protein were identified in three isolates. Another point mutation was observed at the preS2 start codon in the middle protein . Several mutations were identified in the P protein of all isolates, most of them being located in the spacer domain. Four mutations were located in the conserved RT domains and two in the conserved motif. In conclusion, we describe for the first time a set of mutations located in highly conserved regions of both the polymerase and surface antigen proteins.

P4.02.24
The Cytotoxic T Lymphocyte Response to HLA-Cw08-restricted Envelope Epitope in Resolved Hepatitis B Infection

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The effective immunity is important strategy for HBV viral clearance particularly CTL which powerfully contribute to control HBV in acute and self-limited patient but not in chronic infection. Here, we firstly identified HLA-C-restricted CTL epitope of HBV antigen in acute patient using a panel of overlapping synthetic peptides covering the entire amino acid sequence of whole HBV antigens of genotype C to stimulate and analyze HBV-specific CTL response. CTL response to 10-residue peptide corresponding to amino acids 171-180 of envelope antigen restricted by HLA-Cw*08:01 molecule was characterized which presented responsiveness 53% of all patients with resolved HBV infection to this epitope and the cross-activity of this CTL response occurred between HBV genotype B and C. The comparative specific CTL response against Env171-180 versus the known-HLA-A or -B-restricted epitopes indicated that HLA-Cw*08:01-restricted Env171-180 CTL response had mostly frequency and magnitude compared with single well-characterized epitope stimulation as well as pooled overlapping HBV peptides. It is suggested that Env171-180 is likely HBV immunodominant epitope which is probably a candidate for immune-based therapy in defective immune response patient with chronicity.

P4.02.25
Association of MICA variants and hepatitis B virus induced hepatocellular carcinoma

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Hepatitis B virus infects approximately 350 million people worldwide and is the leading cause of liver disease including hepatocellular carcinoma. Host genetics has been postulated to play an important role during HBV infection and HCC progression. The human major histocompatibility complex class I chain-related gene A (MICA) modulates the NK and T cell mediated immune responses through NKG2D receptor and is associated with several autoimmune, cancers and infectious diseases. The aim of this study is to determine the effects of MICA polymorphisms during HBV infection.
Ten functional MICA polymorphisms including the microsatellite were genotyped in 552 patients infected with HBV and 418 healthy controls by real-time PCR, sequencing and capillary-based electrophoresis. The serum soluble MICA levels (sMICA) were measured by ELISA and correlated to MICA variants and liver enzyme levels.
The MICA rs2596542G/A promoter SNP and non-synonymous substitutions MICA-129Met/Val, MICA-251Gln/Arg, MICA-175Gly/Ser, triplet repeat microsatellites and respective reconstructed haplotypes were associated with HBV-induced HCC and HBV persistence. The minor allele rs2596542A, MICA-129Met, and MICA-251Gln contributed to an increased susceptibility to hepatocellular carcinoma from liver cirrhosis. Therefore, MICA polymorphisms could play a crucial role in tumor surveillance and disease susceptibility during HBV infection. The soluble sMICA levels were elevated significantly in patients compared to healthy controls. The studied MICA variants were observed to influence the sMICA levels. The liver enzymes such as alanine amino transferase (ALT), aspartate transaminase (AST), total bilirubin and direct bilirubin were positively correlated to sMICA levels suggesting sMICA as a biomarker for liver injury.

P4.02.26
Enhancement of anti-HCV T cell immunity by immunization with an adenoviral vector encoding a TLR4-targeted NS3 protein

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Induction of T cell immunity against hepatitis C virus (HCV) has been proposed as a useful strategy to treat this infection. To enhance the immunogenicity of a vaccine against HCV we designed the recombinant adenovirus AdEDA-NS3 encoding a fusion protein containing HCV NS3 antigen bound to the extra domain A (EDA) of fibronectin, a moiety which targets antigens to TLR4 expressing cells. Immunization of C57BL/6 mice with AdEDA-NS3 induced stronger CD4 and CD8 T cell responses against NS3 than those induced by an equivalent adenovirus (AdNS3) encoding untargeted NS3 or by the recombinant EDA-NS3 protein plus adjuvants poly(I:C) and antiCD40. These responses were higher in magnitude and included polyfunctional T cells which cross-reacted with NS3 belonging to other HCV genotypes. Similar enhanced responses were also obtained in outbred mice. Interestingly, immunization with AdEDA-NS3 not only conferred a higher protection against infection with a vaccinia virus encoding NS3, but also induced more potent responses in transgenic mice expressing HCV polyprotein in the liver. These results show the superior immunogenicity of AdEDA-NS3 and suggest that it could be useful in vaccination protocols against HCV.

P4.02.27
Fulminant hepatitis caused by virus infection was controlled by BTLA signaling

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Fulminant viral hepatitis (FH) remains an difficult clinical problem in which the underlying pathogenesis is still unclear. Here we report that the B and T lymphocyte attenuator (BTLA), a member of CD28 superfamily, appears to be determining factors in the pathogenesis of FH mediated by the murine hepatitis virus strain 3 (MHV-3). We found that the production of the procoagulant molecule fibrinogen-like protein 2 (FGL2), the liver damage as well as the mortality was significantly reduced in MHV-3 infected BTLA-deficient ($BTLA^{-/-}$) mice. This effect was due to a rapid loss of infected macrophages following infection, thus reduced levels of TNF-α and FGL2 in these mice. An important role of TNF-α was demonstrated as the treatment of MHV-3 infected $BTLA^{-/-}$ mice with TNF-α which significantly increased the mortality and by the finding that FGL2 levels, tissue damage and mortality was dramatically decreased in TNF-$\alpha^{-/-}$ mice following MHV-3 infection. In addition, adoptively transferred $BTLA^{+}$ macrophages into resistant $BTLA^{-/-}$ mice resulted in an increased mortality after MHV-3 infection, while blocking BTLA signal prolonged the WT mouse survival time. Those findings indicate that BTLA-/- macrophages have stronger proinflammatory factors including IL-6, IFN-γ and TNF-α secretion in early infection, thus resulted in an upregulation of Fas and cell apoptosis. Our combined results demonstrate that maintaining macrophage survival by BTLA signaling is a decisive factor for the development of immunopathological damage induced by MHV-3. Targeting BTLA may be a novel strategy for the treatment of fulminant viral hepatitis.

P4.02.28
P300, but not PCAF, collaborates with IRF-1 in stimulating TRIM22 expression, that is independent on its histone acetyltransferase activity

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TRIM 22 plays an important role in IFNs-mediated antiviral activity. We previously demonstrated that IRF-1 was crucial for constitutive and IFNs-induced TRIM22 expression. Here, we further investigated the molecular mechanisms of IRF-1 in promoting TRIM22 expression. Using in vitro DNA affinity binding assay and in vivo chromatin immunoprecipitation assay, we found that IFN-γ stimulation significantly enhanced the binding of p300 and PCAF, but not other co-activators such as GCN-5, SRC-1 and ACTR, to the 5' extended IFN-stimulating response element-containing region of TRIM22 promoter together with IRF-1. Overexpression and knockdown analysis demonstrated that it was p300, but not PCAF, that functioned as a transcriptional co-activator of IRF-1 in IFN-γ-mediated TRIM22 production. Further study revealed that p300 mainly participated in the IRF-1-mediated TRIM22 expression by recruiting RNA polymerase II to TRIM22 promoter, and this effect was independent on its histone acetyltransferases activity. Our study indicated that p300 might serve as a bridge between IRF-1 and the basal transcriptional apparatus in TRIM22 induction.

P4.02.29
Intrahepatic CXCR3-associated chemokines and circulating autoantibody profiles in patients chronically infected with hepatitis C virus

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The liver injury in hepatitis C virus (HCV) infection is thought to be mainly due to immunological disorders and attacks of inflammatory factors rather than a direct cytopathic effect of the virus itself. To investigate whether intrahepatic inflammation and immunological disorders are correlated in HCV-infected patients, we used a real-time RT-PCR-based array and a slide-based autoantigen array to analyze intrahepatic inflammation gene profiles in liver biopsy specimens from HCV-infected (n=16) and uninfected (n=8) individuals and their circulating autoantibody profiles, respectively. Compared with uninfected individuals, HCV infection markedly altered expression of 59.5% of 84 inflammation-related genes tested. Among these genes affected, the CXCR3-associated chemokines (CXCL9, CXCL10, and CXCL11) were among the most up-regulated genes. Patients chronically infected with HCV have 2-fold or greater increase in circulating autoantibodies of IgG and IgM to 28 and 23 of 84 autoantigens tested, respectively, than that in uninfected individuals. The antinuclear antibody IgG titers correlated significantly with the levels of intrahepatic CXCR3-associated chemokines ($p = 0.002$). Given that CXCR3-associated chemokines are involved in the development of autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus, these chemokines may also play a key role in the pathogenesis of HCV-related liver disease through enhancement of autoimmune responses.

P4.03 HIV pathogenesis and immunity

P4.03.01
The MHC class II transactivator CIITA inhibits HIV-1 replication in monocytic cells

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We previously showed that the MHC-II transactivator CIITA inhibits HIV-1 replication in human T cells by competing with the viral transactivator Tat for the binding to Cyclin T1 of P-TEFb.
Here we analyzed the possible anti-viral function of CIITA in the U937 promonocytic *Plus* and *Minus* clones, characterized by efficient or inefficient capacity to support HIV-1 replication, respectively. We found that U937 *Minus* cells express MHC-II molecules on the cell surface whereas *Plus* cells do not. This correlates with CIITA expression restricted to *Minus* cells. Importantly, we show that Tat-dependent HIV-1 LTR transactivation is reduced in *Minus* cells compared to *Plus* cells. The expression of exogenous CIITA in *Plus* cells inhibits Tat-mediated activation of the viral LTR reverting the *Plus* phenotype into a *Minus* "like" phenotype. Accordingly, HIV-1

replication was significantly reduced in *Plus*-CIITA transfected cells compared to *Plus* cells.
Recently, the host factor TRIM22 exclusively expressed in *Minus* cells was shown to inhibit basal HIV-1 transcription. We found that CIITA does not induce TRIM22 expression in *Plus*-CIITA transfectants. Nevertheless, the two factors interact and co-localize *in vivo,* suggesting that they might cooperate to inhibit HIV-1 replication.
Overall our findings confirm that CIITA acts as a viral restriction factor against HIV-1 in both T cells and monocytes and may explain previous observations on the refractory state of monocyte-macrophages to HIV-1 replication when stimulated with INF-g, the most potent activator of both CIITA and TRIM22 expression.
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P4.03.02
Antinuclear antibodies in HIV infected Turkish patients

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Background: Antinuclear antibodies (ANA) can be observed in human immunodeficiency virus (HIV) infected patients. Although it has been reported that ANA positivity rate was high in HIV infected patients, several other studies have not detected ANA in the presence of HIV infection.
Objective: The aim of this study was to investigate ANA positivity in HIV infected patients.
Methods: Forty four HIV infected patients with positive HIV RNA were enrolled in the study. Indirect immunoflorescence assay (IFA) was used for detection of ANA in the sera of the patients.
Results: Of the 44 HIV RNA positive HIV infected patients, only 1 (2.2%) was found to have ANA positivity, with a cytoplasm filamentous pattern.
Conclusion: HIV infected patients have less commonly ANA positivity compared to the normal population.

P4.03.03
HLA-A68 and HLA-B15 Alleles are Correlated with Poor Immune Response to Highly Active Antiretroviral Therapy among Omani AIDS Patients

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Background: A proportion of Omani AIDS patients do not immunologically respond to HAART and their CD4 cells never hit the normal level despite viral replication is fully controlled by the treatment. This variation in the immunological response has been attributed to some host genes including those which encode Human Leukocyte Antigens (HLA). **Study Objectives:** To determine the frequency of HLA Class I and II alleles among good and poor AIDS responders to HAART and to investigate whether an association exists between a particular HLA allele and immunological response to treatment.
Methods: A total of 102 Omani healthy individuals and 81 Omani AIDS male and female patients, attending Sultan Qaboos University Hospital (SQUH), aged between 22.5 and 69 years, were HLA genotyped using SSP-PCR technique. The frequencies of HLA alleles were compared between AIDS patients and the healthy individuals as well as between “good” and “poor” immunological responders to HAART. Viral load measurement and CD4 cells counts for AIDS patients were performed according to standard techniques. The Statistical Package for Social Sciences (SPSS version 19) was used for data processing and analysis.
Results & Conclusion: Only two alleles, HLA-A68 and HLA-B15, were found to be significantly responsible for more than 50% of the poor response to HAART. Both alleles belong to the HLA class I and none of them were encountered among the prevalent HLA alleles among
Omani healthy individuals.

P4.03.04
HIV infection is associated with altered monocyte subset proportion and responsiveness to LPS stimulation

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Introduction: HIV+ individuals are at increased risk of inflammatory age-related disease despite effective suppression of viremia with combination antiretroviral therapy (cART). While monocyte subsets play individual roles in responding to inflammatory stimuli, the effect of therapy on their function and potential to contribute to chronic inflammation is not well defined.
Methods: We evaluated the inflammatory state of the three monocyte subsets (classical, intermediate and non-classical) from cART naïve, viremic and virologically supressed (VS) HIV+ donors by measuring basal and LPS-induced (10 ng/mL, 4 h) levels of IL-6 and TNF by whole blood intracellular cytokine staining via flow cytometry and compared results to age-matched HIV- controls. Soluble signalling components (LPS, LBP, sCD14), inflammatory markers (Neopterin, CXCL-10) and surface TLR-4 were also measured.
Results: All three subsets from HIV+ donors (viremic and VS) showed increased basal and LPS-induced IL-6 and TNF levels in comparison to uninfected donors ($p<0.05$ for all). Viremic HIV+ donors also displayed an increased proportion of inflammatory intermediate and non-classical monocytes in comparison to controls ($p<0.01$ for both); however this was attenuated with therapy. Plasma neopterin, LPS, LBP, sCD14 and CXCL-10 were all elevated in HIV+ groups ($p<0.05$) with a positive association between intermediate monocytes and plasma levels of CXCL-10 observed in viremic donors ($p=0.06$). Surface TLR-4 expression was also increased on intermediate monocytes of both HIV+ groups ($p<0.01$).
Conclusions: Monocytes from HIV+ donors show increased basal intracellular IL-6 and TNF levels and a heightened response to LPS, which may contribute to chronic inflammation and related inflammatory diseases,.

P4.03.05
Poor handling of bacterial antigens relate to functionally defective myeloid dendritic cells in advanced stage of HIV infection

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HIV-1 infection is associated with functional impairement of dendritic cells. The exact mechanism has not been elucidated so far. We aim to study the role of some negative regulatory factors, during HIV1 infection.
Dendritic cells in the whole Blood from therapy naïve, on ART and healthy subjects, were immunophenotyped for maturation and activation markers at baseline and after *ex vivo* stimulation with LPS. Monocytes derived DC stimulated with either LPS or HIVgp120. The expression of SOCS1 & HO1 was measured by qPCR. The culture supernatants were analyzed for cytokines.
The baseline levels of DCs ($CD11c^{+}HLA^{-}DR^{+}$) expressing CD83, CD80 and CD86 was similar in all the groups. The treatment naïve patients with advanced disease had significantly lower percentage of cells expressing CD83, CD80, CD86 and did not present the antigen well as compared to early stage patients & healthy controls upon stimulation with LPS. The DCs from ART treated patients depicted functional reconstitution. HIV-1 antigen induced an increased expression of SOCS1 & HO1 genes in moDC from healthy donors. The gp120 stimulated cultures had suppression of all the cytokines IL12p70, IL10, IL1β, IL6, IL8, and TNFα.
The results indicate a maturation defect in dendritic cells of HIV1 infected ART naïve patients in advanced stage of disease. Upon ART initiation, there was functional reconstitution. Virus triggered increased expression of SOCS1 & HO1 could be responsible for the functional impairment of these cells during HIV1 infection. Further studies evaluating expression profile of more regulatory genes in patients will elucidate the mechanisms involved.

P4.03.06

No association between TNF-α genetic variants and lipodystrophy in a population of HIV-infected patients from South of Spain

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Introduction: Some HIV-infected patients undergoing highly active antirretroviral therapy develop adipose tissue redistribution and metabolic abnormalities called lipodystrophy syndrome. The frecuency of lipodystrophy is approximately between 35-50% and host genetic factors might confer particulary susceptibility. Tumor necrosis factor alpha (TNF-α) is thought to be involved in the pathogenic and metabolic events associated with HIV-1 infection. We assessed whether carriage of the TNF-α gene promoter single nucleotide polymorphism (SNP) is associated with lipodystrophy and metabolic derangements in HIV-1-infected patients.
Material and methods: A case-control study was conducted for HIV-patients. Clinical assessments for lipodystrophy by phisical examination and DEXA were obtained. The patients were classified into 2 groups: patients with lipodystrophy and patients without lipodystrophy (control group). We compared the allelic frequency between the case and the control group to determine the possible association of TNF-α associates lipodystrophy.
We studied 277 patients with HIV infection. 186 had lipodystrophy and 91 without LD that were considered as controls. DNA was isolated from anticoagulated peripheral blood mononuclear cells using standard techniques. The data were analyzed using the chi-square test or Fisher exact test, the magnitude of associations was estimated by odds ratio by the Woolf-Haldane method.
Results: Distribution analysis of the alleles at the TNF-α -308 and -238 variants showed no statistically significant between both groups of patients with LD compared to those with no LD.
Conclusion: Although greater numbers of patients are needed the TNF-α variants seems no confer susceptibility for lipodystrophy in patients with HIV in a population from south of Spain.

P4.03.07

Increased immune activation and susceptibility to HIV-1 of $CD4^+$ T cells in children born to HIV-1 infected women

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Uninfected infants born to HIV-1-infected women have lower counts of $CD4^+$ T cells in infancy than those born to uninfected mothers. Although the exact mechanisms are unknown *in-utero* exposure to maternal HIV-1-infection could shape $CD4^+$ T cell responses and chemokine receptor expression affecting T cell homing and numbers in the circulation. We assessed chemokine receptor expression on cord blood $CD4^+$ T cells of HIV-1-exposed and healthy infants. $CD4^+$ T cells derived from cord blood had a naive phenotype and chemokine receptors associated with inflammatory responses (i.e. CCR3, CXCR6, CCR5) were virtually absent in healthy controls and HIV-1-exposed infants. *In-vitro* culture of cord blood $CD4^+$ T cells with i.e. IL1[[Unsupported Character - Symbol Font ]] or IL-2, IL-7 or IL-15 increased expression of inflammatory response-associated chemokine receptors. $CD4^+$ T cells from HIV-1-exposed infants had an increased expression of inflammatory response-associated chemokine receptors compared to healthy controls (CCR3 $P<0.01$, CCR8 $P=0.03$), whereas CCR7, largely expressed by naïve cells, was lower than in controls ($P=0.01$). Furthermore, $CD4^+$ T cells of HIV-1-exposed infants were significantly more susceptible to in-vitro infection with a R5 HIV-1 strain after prestimulation for 4 days with cytokines.
In sum, $CD4^+$ T cells of HIV-1-infants have an enhanced state of activation, with an increased expression of inflammatory response-associated chemokine receptors, likely affecting the homing of $CD4^+$ T cells. One of the additional clinical implications of the primed immune system in children born to HIV-1 infected women is an increased susceptibility to HIV-1 postnatally, i.e. via breastfeeding.

P4.03.08

Phenotypic and functional characterization of CD4 stem-cell like memory cells in rhesus macaques

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The progressive depletion of central-memory CD4 T cells (CD4+ TCM) is a key pathogenic event during HIV infection, and differentiates pathogenic from non-pathogenic SIV infections, in which CD4+ TCM are relatively resistant from direct virus infection. Recently, several studies have identified a novel subset of memory T-cells with stem cell-like properties (TSCM). These cells are the least differentiated subset of memory T-cells and exhibit increased longevity and proliferative potential when compared to other T-cell memory subsets. Currently, little is known about CD4+ TSCM in rhesus macaques (RM).
In this study, we used multi-parametric flow cytometry and applied an established set of phenotypic markers (i.e., CD45RA, CCR7, CD28, CD27, CD122, LFA-1, CXCR3, and CD95) to identify and characterize CD4+ TSCM both in healthy and SIV-infected RM. CD4+ TSCM can be readily identified in the blood and lymphoid organs of rhesus macaques, while they are rare in mucosal tissues. These cells express variable levels of the SIV co-receptor CCR5, the proliferation marker Ki-67, and the co-inhibitory molecule PD-1. When we monitored the frequency of CD4+ TSCM cells during SIV infection we observed that their level does not decrease and that they maintain a normal proliferative potential. In addition, we observed a significant inverse correlation between total CD4 T cells and the percentage of CD4+ TSCM cells. The persistence of proliferating CD4+ TSCM during chronic HIV and SIV infection suggests that these cells may be involved in the preservation of reservoir of latently infected cells, which represents a key barrier to HIV eradication.

P4.03.09

Association of DC-SIGNR genotypes with dendritic cells in HIV-1 infection

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Objectives: - Dendritic Cell-specific intracellular adhesion molecule 3 grabbing non-integrin related (DC-SIGNR) is a C-type lectin receptor and variation in its repeat region may affect HIV-1 transmission. The aim of this study was to assess peripheral blood dendritic cell (DC) frequencies and (DC-SIGNR) genotyping in healthy individuals and HIV-1 infected individuals and correlate with different clinical parameters from north India.
Methods: Blood from 30 seronegative healthy individuals and 30 patients infected with HIV-1 from North India were collected. Peripheral blood DC frequencies were determined by flow cytometer and repeat region polymorphism in DC-SIGNR was performed by PCR.
Results: There was a significantly lower number of DCs and their subsets in patients infected with HIV-1 compared to healthy individuals. A significant positive correlation of DCs and their subsets with CD4+ T cells and negative correlation with HIV-1 viral load was

found. A salient finding of this study was the association of the heterozygous 7/5 DC-SIGNR genotypes with higher percentage of DCs and their subsets and higher CD4+ T cell counts and lower viral load compared to the homozygous 7/7 DC-SIGNR genotypes in patients infected with HIV-1.
Conclusions: This is the first study to assess the DC subsets and its association with DC-SIGNR polymorphism in healthy individuals and HIV-1 infected patients and suggests the protective role of 7/5 DC-SIGNR genotypes in HIV-1 infection.

P4.03.11
Antiphospholipid antibodies in perinatally HIV infected children

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Search for predictors of fast progressing of HIV infection continues. Objectives are to evaluate clinical importance of measuring levels of antiphospholipid antibodies (APA) in serum of HIV-infected children. Materials and methods. 73 children born from HIV-infected mothers were included into study. Mean age of children in study was 2.5 years. Information contents for using of study results were received from parents. Measuring of cofactor-independent IgG anti-cardiolipin APA (Cf-I-APA) was made by ELISA. Results. In clinical stages I or II of disease APA levels were lower than in stages III-IV but remained significantly higher in comparison with HIV non-infected children (12.3+2.3 U/mL; 31.2+14.2 U/mL; 3.5+1.0 U/mL, respectively, p<0.002).During the course of ART, APA levels normalized, but this was not observed in children with stages I-II of diseases which did not received ART (8.2+1.8 U/mL and 17.2+1.8 U/mL respectively, p<0.07). APA levels increased significantly before diminishing of CD4 lymphocytes. During viral load APA levels became higher. Conclusions. Increase of Cf-I-APA levels appears before clinical signs of HIV-infection and diminishing of CD4 level. Measuring of these levels may serve as additional criterion for optimal choice of time for ART beginning. Increased Cf-I-APA levels become lower while clinical state becomes better but even when CD4 levels normalize after administration of ART they are detectable, that's why APA levels may be considered as additional criterion of effectiveness of ART. Measuring of Cf-I-APA levels may be used in health care as sign of fast progression of HIV infection in perinatally infected children.

P4.03.12
Mother-to-child anti-Nef antibody passage may protect HIV-1 vertically-infected children from AIDS disease

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HIV-1 Nef protein has been strongly associated with uninfected T-cell death (bystander effect). This protein exerts a cytotoxic effect upon CD4+ cells though some authors found no correlation between Nef concentration in plasma and T-cell counts. On the other hand, we have previously observed that high titers of anti-Nef antibodies were found in plasmas of long-term non-progressor children but not in typical or rapid-progressors. The aim of this preliminary study was to evaluate the protective effect that may have the pre-existent anti-Nef antibodies at acute or primary infection. Twelve children who had been clinically assisted in the Garrahan Hospital from Buenos Aires were retrospectively included in the study. Plasma samples at 1-3 months post-birth were evaluated for anti-Nef IgG. The children were then classified into 3 groups according to the anti-Nef antibody levels: IgG- (anti-Nef-IgG titer <50) n=2, IgG+ (anti-Nef-IgG titer range 200-7500) n=7 and LTNPsimil (anti-Nef-IgG >11000) n=3. We observed that the children of the last group remained AIDS-free for more than 10 years while the children that had low titers or no anti-Nef Antibodies progressed to AIDS within 2 years post-birth. Our preliminary data and results suggest that pre-existent anti-Nef antibodies may have a protective effect, preventing AIDS disease progression in HIV-1 vertically infected children. Although this is a preliminary study, our observations would be of great interest for a therapeutic vaccine design as well as alternative therapies development.

P4.03.13
Inflammatory status in residual immunodeficiency under cART in HIV-infected patients

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Current combined antiretroviral treatments (cART) for HIV infection, indefinitely maintaining undetectable viral loads in most patients, have successfully decreased AIDS-related diseases and mortality. However, a higher prevalence of age- but non-AIDS-related diseases has been revealed as a new clinical outcome in the HIV infection management. High levels of systemic inflammation, usually accompanying age-related morbidities, have been reported in viremic HIV-infected patients. However, the effect of a long-term suppressive cART remains still unknown. In this study, we have analyzed systemic inflammatory levels (sCD40L, IFNγ, TNFα, IL1α, IL1β, IL6, IL8 and IL17) in a cohort of viremic HIV regarding 1) age-matched, 2) elderly and 3) elderly at risk of death uninfected healthy controls. Besides, we have longitudinally analyzed the effect of a suppressive cART on these inflammatory markers. Our results showed that, while IL6 and IL8 levels were similar in HIV-infected patients and healthy elderly individuals, most of the analyzed markers showed a worse profile in HIV-infected patients than elderly subjects at risk of death. In addition, at least 96 weeks of suppressive cART are needed to normalize some of these markers (IL6, IL8 or IFNγ) while sCD40L, TNFα or IL1α remained altered even after two years of suppressive cART. Altogether our results show that systemic inflammation in HIV-infected patients remains altered even under long-term suppressive cART. Moreover, inflammation patterns are different to those observed in uninfected elderly individuals, suggesting that immune alterations of HIV-infected patients should not be considered strictly as premature immunosenescence but residual immunodeficiency under cART (RIA).

P4.03.14
Evaluation of Monocytes and Natural Killer cells activation, inflammatory parameters in Virologically Suppressed HIV infected Maraviroc treated patients

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Background: Monocytes and Natural Killer (NK) cell activation contribute to complications on HIV infection. The effect of maraviroc (MVC, antiretroviral drug) on immune activation in virological suppressed HIV-infected patients (pts) needs to be further characterized.
Methods: In a multicenter cross-sectional study were enrolled HIV infected pts receiving antiretroviral therapy (ART)≥ 6 months with or without MVC, defined respectively MVC group and not MVC group. Peripheral blood NK and monocyte phenotype and activation were studied by flow cytometry and correlated with inflammatory and metabolic parameters.
Results: Overall, 68 pts with HIV-RNA ≤50 copies/ml were included, 43 in MVC group (63%) and 25 in not MVC group (37%). In a univariate analysis in MVC group CD38+ mean fluorescence intensity (MFI) were reduced and PDL1 MFI increased on inflammatory

monocytes (iM) (p=0.0032 and p=0.0447). BMI was positively correlated with iM percentage (%) (p=0.034), Total cholesterol was negatively correlated with NK HLA-DR frequency (p=0.034); d-dimer was positively correlated with HLA-DR MFI on iM and pM (p=0.05 and 0.04). CD38 and PDL1 MFI on iM were negatively correlated (p=0.0001). Only in not MVC group, we found a positive correlation between PCR and %NK CD69+(p=0.032), d-dimer and %NK HLA-DR (p=0.05). In a multivariate analysis minimum arterial pressure, HCV-RNA and %TCD4 correlated with MVC use (p=0.004, p=0.016 and p=0.026).
Conclusion: Monocytes and NK activation persists in virologically suppressed HIV infected individuals. Reduction of iM activation might impact on atherosclerotic plaque formation/remodelling. Modulation of persistent immune-inflammation in HIV-infected pts is feasible using different treatment.

P4.03.15
Analysis of NKG2D ligand expression and release during HIV-1 infection: implication for NKG2D-mediated immunosurveillance

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Human cells may respond to viral infection, tumor transformation or other stress by expressing on their membrane ligands for activating receptors present on cytotoxic NK and T cells, thus eliciting recognition and elimination by the immune system. Work from our and other laboratories has shown that, upon infection with HIV-1, CD4+ T cells express ligands for the NKG2D activating receptor, becoming susceptible to lysis mediated by NK cells that are all NKG2D+. However, some viral proteins (Nef, Vpu, Vif) contrast the cell surface expression of NKG2D ligands, hence protecting infected cells from NKG2D-mediated cytotoxic responses. Here we investigated whether HIV-1 infection also causes the release of soluble NKG2D ligands (sNKG2DLs) in the extracellular environment. In vitro experiments show that HIV-1 induces the release of soluble NKG2D ligands in a manner that relies on the activity of cellular matrix metalloproteinases (MMP). Moreover, we found that naïve HIV-infected patients displayed increased plasma levels of sNKG2DLs and reduced NKG2D expression on NK and CD8+ T cells. In individual patients, uptake of antiretroviral therapy (ART) resulted in the drop of sNKG2DLs and recovery of NKG2D expression. Of note, sNKG2DLs in patients' plasma down-regulated NKG2D on NK and CD8+ T cells and impaired NKG2D-mediated cytotoxicity of NK cells. Thus, NKG2D detuning by its soluble ligands may promote HIV-1 immune evasion and compromise host resistance to opportunistic infection but ART and, possibly, MMP inhibitors, have the potential to avoid such immune dysfunction. This work was supported by grants from Italian Ministry of Health and Bristol-Myers Squibb.

P4.03.16
Presence of HIV-1 broadly neutralizing antibodies during pregnancy in relation to mother-to-child transmission blocking

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Introduction: HIV vaccine research is shifting towards the need to generate high titer antibodies capable of neutralizing primary isolates of multiple HIV-1 clades and conferring sterilizing immunity. There are strong arguments in favor of a beneficial role of broadly neutralizing antibodies in prevention of HIV infection if these antibodies exist prior to exposure.
Methods: Archived plasma samples obtained from HIV1-C infected mothers recruited into a Prevention of Mother to Child Transmission study, conducted in Botswana were used in the assays. Plasma collected at screening, recruitment and delivery, was analyzed for HIV-1 broadly neutralizing antibodies and the responses were related to mother-to-child transmission at delivery. Panels of well-characterized HIV reference strains were used to assess plasma ability of HIV-1 neutralization using TZM-bl cells with a tat regulated luciferase reporter gene. Results were expressed as fifty or ninety percent inhibitory dose, defined as the highest plasma dilution causing fifty or ninety percent reduction in relative luminescence compared to virus control wells after subtraction of background luminescence.
Results: Both transmitters and non-transmitters show presence of broadly neutralizing antibodies at fifty percent HIV-1 infection inhibition, but moderately on the HIV-1C subtypes. However, non-transmitting mothers show significantly higher neutralization at ninety percent virus inhibition, across different HIV-1 clades at delivery, compared to the transmitting mothers.
Conclusion: Broadly neutralizing antibodies are present during pregnancy and there is potential of their role in reducing transmission at delivery. However, there is need to understand further the immunological environments that support viral neutralization and their dominant effect.

P4.03.17
A more pro-inflammatory environment is generated in nervous cells cultures in the simultaneous presence of HIV-1 and Toxoplasma gondii, even with lower parasite replication

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Background: HIV-1 and *Toxoplasma gondii* (*Tx*) can invade central nervous system (*CNS*) and affect its function. To study CNS's pro-inflammatory/neurotoxic response, we evaluate production of cytokines and nitric oxide (*NO*) in primary cultures of nervous cells in the simultaneous presence of HIV-1/*Tx*.
Methods: We used primary cultures of *Sprague-Dawley* rat-fetal brain cells, cultured in 24 wells plaques ($2.5x10^5$ cells/well), in RPMI-10%FCS (Group A-*Control*), or in the presence of $1.9x10^9$ copies/ml of $HIV\text{-}1_{IIIB}$ (Group B-*HIV*), of 1.000 viable tachyzoites/well of *Tx*-RH strain (Group C-*Tx*) or in the simultaneous presence of HIV/*Tx* (Group D-*HIV/Tx*), for 3, 5 and 7 days. IL-1α, IL-6, IL-10, TNF-α and *NO* levels were determined in culture supernatants; nervous cells and free tachyzoites were counted after trypsin treatment. ANOVA or Kruskal-Wallis tests were used, as appropriate.
Results: The presences of only HIV-1 or *Tx* were associated with significantly lower nervous cell counts (Groups B and C vs. A). Significantly higher levels of IL-1α, IL-6, IL-10, TNF-α and *NO* were associated with HIV-1-stimulation (Group B vs. A). *Tx*-infection alone (Group C) didn't induce significant production of any cytokine or *NO*. But when HIV/*Tx* were co-culture, production of IL-1α was significantly enhanced, IL-10 and TNF-α were significantly lower (Group D vs. B), while nervous cells and free tachyzoites counts were significantly lower (Group D vs. B and C).
Conclusion: Simultaneous presence of HIV/*Tx* was associated with a more pro-inflammatory environment and more nervous cell destruction, suggesting enhancement of neurotoxicity (as could be in co-infected patients), even with lower parasite replication.

P4.03.18
Enhancement of NK polyfunctional cells upon TLR-7/8 activation is dependent of CD62L expression in HIV-1 exposed non-infected individuals

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Introduction. Natural killer cells (NK) exert a crucial role in the immune response to certain infections and transformed cells. In humans, two subtypes of NK cells are described, CD56brigth and CD56dim. Moreover, NK cells express Toll-like receptors (TLR) that help in the pathogen recognition stimulating cytokine production and cytotoxic pathways. The NK cell has been related as protection in exposed-uninfected individuals (ENI). To better understand the role of NK cells, is important to evaluate their ability to secrete several factors followed TLR-7/TLR-8 stimulation. Aim. To determine the polyfunctional NK profile by TLR-7/8 stimulation in ENI and HIV-1-

infected partner. Methods. ENI and HIV-1-infected (INF) partner (n=10/each) were from Instituto de Infectologia Emilio Ribas of São Paulo-Brazil. Peripheral blood mononuclear cells were stimulated for 6 hours with agonist CL097 (TLR-7/8 - 10 µg/mL) or PMA (30 ηg/mL) + ionomycin (0.3 µg/mL) and NK subtypes were assessed by flow cytometry. Results. Increased polyfunctional (CD107a, IFN-γ, TNF-α, IL-10) response in CD56brigth and CD56dim cells upon CL097 stimulation were observed in ENI when compared to healthy controls group (HC). It was also observed that polyfunctional profile of these cells was dependent on the expression of CD62L. Conclusion. These data suggest that high polyfunctional NK responsiveness for TLR-7/8 activation in exposed-uninfected individuals may represent an important mechanism of resistance for HIV-1 infection.

P4.03.19
Diminished immune response of M1 and M2 macrophages derived from HIV+ patients

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Even 30 years past from the recognition of AIDS as a worldwide epidemic caused by HIV, many questions remain unanswered. Besides of huge CD4+ T cells commitment, classical (M1) or alternatively activated (M2) macrophages derived from HIV+ patients exhibit phenotypic changes, what can be associated with the immune impairment. Our aim was to evaluate the immune response against different PAMPs, developed by macrophages obtained from HIV+ patients.
This project was approved by ethical committee from FMRP-USP. All clinical samples were obtained from HIV+ treatment-naive patients, assisted at HC-FMRP-USP. Control group was recruited from volunteer blood-donors of Blood Bank of Ribeirão Preto - FMRP-USP. Mononuclear CD14+ cells were isolated from peripheral blood samples and plated using M1 or M2 driven medium. After six days, LPS or β-Glucan were added to cells during 24 hours and the supernatant was collected. Cytokines and chemokines from cells culture and plasma samples were quantified. Analysis of mRNA expression was performed to confirm cell phenotype.
The purity of cells in the culture was upper than 93%. Our preliminary data have shown higher levels of inflammatory chemokines on plasma and M1 supernatant samples from HIV+ patients. The CCL3 expression seems to be better indicator of cell classical activation under M1 conditions.
Taken together, our results indicated an inflammatory disorder caused by HIV infection, which may be associated with prolonged macrophage activation on M1 pattern, resulting on exhaustion of the immune response against pathogens and allowing the occurrence of opportunistic infections.

P4.03.20
NetFCM: A tool for Automated Flow Cytometric Data Analysis

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Multiplexed methods such as multiparametric flow cytometry (FCM) are important to elucidate the comprehensive pattern of a healthy and perturbed immune system. Advances in the field of FCM allows for the detection of up to 18 parameters on individual cells, yielding output data of increased dimensionality and complexity.
To tackle the problem of increased complexity, we developed NetFCM, an automated method driven by machine learning methods to analyse multiparametric flow cytometric data. The tool uses information from multidimensional datasets to classify and cluster the samples into populations.
The framework of the data analysis is divided into phenotypic characterisation of the cells and functional characterisation of the samples. The phenotypic characterisation is based on an automated classification of the cells based on a flexible sequence of algorithms. The functional characterisation of the samples is an unbiased statistical classification and clustering of the datasets based on the expression of a chosen set of markers on the cells within the individual datasets.
To test the validity of NetFCM, peripheral blood mononuclear cells collected from 23 HIV infected individuals were stimulated with saline solution, overlapping HIV Gag-p55 peptides and overlapping CMV-pp65 peptides. The FCM data was then analysed using NetFCM with the goal of collecting pure CD4+ and CD8+ T-cell populations as well as identifying IFNγ and TNF responses. The method was able to classify cells, identify positive antigen-specific responses and categorise the datasets into meaningful clusters in similarity to manual gating strategies.

P4.03.21
Divergent immunological and virological profile between HCV viremic HIV-1-infected injection drug users and former blood donors in China

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Background−Whether HIV-1/HCV-coinfected injection drug users (IDUs) and former blood donors (FBDs) in China present different immunological and virological characteristics has never been described.
Methods− HCV detectable HIV-1-infected Chinese, 61 IDUs and 87 FBDs, were recruited from a chronic HIV-1-infection cohort. The levels of $CD4^+$T cells, HIV-1 and HCV RNA load, immune activation, and CCR5/CXCR4 expression on T cells and their ligands in blood were detected using flow cytometry and molecular methods.
Results− Significantly higher HIV-1 and HCV plasma load and HLA-DR expression on T cells were observed in coinfected-IDUs compared with coinfected-FBDs after adjustment of age, sex, $CD4^+$T cell counts. The percentages of $HLA\text{-}DR^+CD4^+$T cells and $CD38^+CD8^+$T cells were associated independently and negatively with $CD4^+$T cell counts, while the frequencies of $CD38^+CD4^+$T cells were associated independently with HIV-1 load in HIV-1/HCV-coinfected subjects after adjustment for age, gender and serum aminotransferase. Furthermore, significantly lower frequency of $CCR5^+CD4^+$T cells, higher level of RANTES and lower level of SDF-1 in serum were found in coinfected-IDUs in comparions with coinfected-FBDs. The frequency of $CXCR4^+CD4^+$T cells was similar in coinfected IDUs and FBDs although the two groups showed higher level than that of NCs. In addition, injection drug use was associated independently with high levels of HIV-1 and HCV load in HIV-1/HCV-coinfected subjects with HCV viremia.
Conclusion− Our findings in Chinese HIV-1/HCV-coinfected IDUs and FBDs underline the urgency of helping coinfected injection drug users to cease drug use and the potential benefits to reduce T cell activation during antiretroviral therapy.

P4.03.22
Immunomodulatory effects of HIV-1 gp120 on human dendritic cells: role of STAT3/IL-6 axis

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The ability of HIV-1 to exploit the host immune responses to its own advantage is critical in the pathogenesis of AIDS. Since dendritic cells (DCs) are key players in the induction of immune responses, the modulation of their functional activities represents a strategic mechanism for HIV-1 to evade immune surveillance. In this study, we report that exposure of immature monocyte-derived DCs (MDDCs) to recombinant gp120 transcriptionally activates IL-6 expression and promotes its secretion in a concentration and time-dependent manner. Activation of MAPK p38 is involved in gp120 triggered IL-6 over-expression as p38 specific inhibitors markedly reduced IL-6 secretion. IL-6 over-expression induces at later time points accumulation of the tyrosine phosphorylated form of STAT3. Blocking IL-6 biological activity resulted in a dramatic reduction of STAT3

activation suggesting that gp120 might interfere with the STAT3/IL-6 axis. Consistently with this hypothesis, blocking STAT3 activation markedly decreases IL-6 secretion. Reconstruction of the signaling pathway triggered by gp120 in MDDCs unraveled a biphasic activation of STAT3, triggered early on by gp120 and leading to IL-6 secretion which, at later time points, induces a second round of STAT3 activation. Moreover, the possible involvement of cellular microRNAs in the regulation of this process will be discussed. Overall, these results indicate that DCs may contribute to the up-modulation of IL-6 found in HIV infected individuals. Furthermore, chronic activation of STAT3 in DCs may provide an explanation for the impairment of DC functions.

P4.03.23
Early appearance of an age-related, activated monocyte phenotype in young HIV+ individuals

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Background: Despite effective combination antiretroviral therapy (cART), HIV^+ individuals display chronic inflammation and an increased risk of inflammatory, age-related conditions such as cardiovascular disease, frailty and dementia. Monocytes are critical regulators of inflammation but the effect of chronic HIV infection on their activation and function remains unclear.
Methods: Monocyte phenotype and function was determined in fresh whole blood via a cross-sectional study of young (median age [range] 30 [19-45]) HIV^+ males and young (median age 28 [20-45]) and elderly (median age 72 [65-84]) uninfected males. Plasma markers of innate immune activation were measured in samples from an expanded cohort of HIV^+ and seronegative individuals (n=188).
Results: We have previously characterised age-related changes to monocytes; here we show that many of these changes occur prematurely in young, viremic HIV^+ individuals including an increased proportion of inflammatory $CD16^+$ monocytes (p=0.004), altered monocyte phenotype (altered expression of CD11b, CD115 and CD62L, p<0.05 for all), impaired monocyte phagocytosis (p=0.005), shortened telomeres (p=0.05) and increased concentrations of innate immune activation markers CXCL10, sCD163 and neopterin (p<0.0001 for all). Importantly, many of these changes persisted in virologically suppressed HIV^+ individuals receiving cART. Regression analysis revealed that HIV^+ individuals exhibit levels of CXCL10, sCD163 and $CD16^+$ monocytes similar to those in uninfected individuals aged between 5-7 years older.
Conclusions: Age-related changes to monocytes may occur up to 7 years earlier in HIV^+ individuals. These findings may help identify the mechanisms driving the premature development of inflammatory, age-related diseases in the HIV^+ population.

P4.03.24
Lymphocytes TCD4+ and viral load in patient with premiere of aids that receive antirretroviral treatment

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Introduction: The infection for HIV is a health problem; 40 million people are infected in the world. The AIDS premiere is a form of presentation of the illness in which the clinical manifestations of aids are presented as the beginning of the infection, in people without previous serology HIV; it is characterized by waste syndrome, opportunists infections, neoplasy and neurological alterations.
Objectives: The behaviour of the lymphocytes values T CD4+ was studied and of viral load in patient with aids premiere and therapy antirretroviral, to the beginning and one year after the antirretroviral treatment with Cuban generic. Results: 55 patients those were studied which had to the beginning of the treatment lymphocytes values inferior T CD4+ to 200 cell/µL and viral load bigger than 55 000 cp/µL after a year of therapy, the values of cells T CD4+ increased more than 262 cell/µL and the viral load diminished at levels non detectable in the evaluated patients. The lymphocytes values T CD4+ first floor and the high viral load associated with opportunists illnesses, being the most frequent the Neurotoxoplasmosis and the pneumonia for Pneumocystis jirovecii. The mortality associated to the aids in those bigger than 50 years was bigger in people than they presented more than an illness opportunist. Conclusions: People with aids premiere one year after initiate the antirretroviral treatment presented increase of the lymphocytes T CD4+ and decrease of the values of viral load what evidences the benefits of the antirretroviral treatment with Cuban generic.

P4.03.25
Expression of CX3CR1 drives pro-inflammatory M-DC8+ ("slan-DC") monocyte and NK cell accumulation into HIV-1-infected patient spleens

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$M\text{-}DC8^+$ or slan (6-sulfo Lac NAc glysosylation of PSGL1) monocytes are known to be present in active lesions from patients suffering from evolutive Crohn´s disease or psoriasis. In the peripheral blood mononuclear cells from viremic HIV-1 infected patients, we had found an accumulation of these inflammatory monocytes, which were mostly responsible for the hyper-responsiveness to LPS of the PBMC from viremic HIV-1 infected patients (Dutertre Blood 2012). We addressed whether these cells were also in high numbers in the lymphoid organs from HIV-infected patients.We studied mononuclear spleen cells from 10 patients (6 HIV^+ with idiopathic thrombocytopenic purpura, 4 HIV^-) and spleen cryosections from 17 patients (9 HIV^+ including 4 untreated by antiretrovirals, 8 HIV^-). We quantified and localized monocytes, NK cells and TNFα by 12-color flow cytometry and immunohistofluorescence, and performed mRNA microarray analysis.
$M\text{-}DC8^+$ ($DD2^+$) and $CD16^+$ NK cells were in higher numbers in HIV-infected than in uninfected patients. They expressed the CX3CR1 chemokine receptor, and fractalkine mRNA was overexpressed in spleens from HIV-infected compared to uninfected patients.
Following HIV chronic infection and destruction of $CD4^+$ T lymphocytes beneath the intestinal epithelial barrier, which becomes permeable to bacterial products, induced cytokines stimulate $MDC8^+$ monocyte differentiation, and chemokines attract them to lymphoid organs, where circulating bacterial products stimulate TNF-α secretion, hence immune system activation, further $CD4^+$ T cell activation and infection, direct intestinal epithelial damage. Therefore, $M\text{-}DC8^+$ monocyte depletion might interrupt this vicious cycle and restore homeostasis and viral control.
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P4.03.26
Change of cellular immunity in patients with HIV infection and tuberculosis.

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Introduction. Tuberculosis and HIV infection - diseases that are mutually aggravate each other. Clinical presentation and course of tuberculosis in patients with HIV infection depends on its stage and determines the depth of the T-cell immunodeficiency.
Objective of the study is to determine the changes of cellular immunity in patients with HIVinfection, combined with tuberculosis.
Methods. We examined 37 adult patients aged 24 to 50 with HIV infection in the third and fourth stages, combined with tuberculosis. Determined absolute numbers and percentages of white blood cells, lymphocytes, CD3+, CD4+, CD8+ lymphocyte populations, the ratio of CD4/CD8 by flow cytometry.
Result. In the HIV - infected patients with tuberculosis were found significantly reduced the percentage and absolute number of lymphocytes, the absolute number of CD3 + lymphocytes compared with the control group (p <0.001). Especially significant changes

showed the reduction of lymphocyte phenotype CD4 + (p <0.001). But opposite changes of the relative and absolute CD8+ T cells in HIV-infected patients in combination with tuberculosis were found. Indicators of the relative amount of CD8+ T cells were significantly higher, and rates of the absolute number of CD8 + T cells were significantly lower than the control group (p <0.001). CD4/CD8 level was 4 times low compared with the control group, which was the most reliable indicator of immunodeficiency and imbalance lymphocyte subpopulations.
Conclusions. Revealed violations of cellular immunity in HIV-infected patients in combination with tuberculosis may condition the activation of tuberculosis and burdening the course of HIV infection.

P4.03.27
Interleukin-7 facilitates HIV-1 transmission to cervico-vaginal tissue ex vivo

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Background: Interleukin (IL)-7 is one of the most prominent cytokines in semen of healthy fertile men and its concentration is increased in semen of HIV-1-infected individuals. IL-7 plays a central role in T cell development and homeostasis, however, despite evidence of elevated IL-7 levels in semen, little is known on its role in HIV-1 transmission.
Methods: Human cervico-vaginal and lymphoid (tonsillar) tissues were infected ex vivo using 2 laboratory-adapted or 4 primary isolated HIV-1 variants and cultured in the presence of IL-7 5 or 25 ng/mL. We evaluated HIV-1 replication by measuring HIV-1 p24gag released into culture medium. We assessed CD4+ T cell death by measuring cell depletion, and we evaluated apoptosis by measuring the expression of the apoptotic marker APO2.7 and of the anti-apoptotic factor Bcl-2. Finally, we evaluated cell proliferation by measuring the expression of Ki-67.
Results: Compared with untreated tissues, tissues treated with IL-7 showed: (I) enhanced replication of HIV-1 in a dose and time dependent manner; (II) higher number of HIV-1-infected CD4+ T cells; (III) increase in Bcl-2 expression; (IV) decrease in the fraction of CD4+ T cells expressing APO2.7; (V) reduction in CD4+ T cell depletion; (VI) increase in the fraction of CD4+ T cells expressing Ki-67. Conclusion: IL-7 exerts a protective effect on HIV-1-infected and uninfected CD4+ T cells in the early stages of infection by preventing their death and inducing their proliferation. In vivo an elevated concentration of IL-7 in semen may increase the efficiency of HIV-1 transmission to the female genital mucosa.

P4.03.28
Cytokines found to be elevated in HIV elite controllers reduce HIV replication in vitro

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Background: Soluble mediators are a major form of communication for the immune system, and HIV infection causes changes in a number of soluble biomarkers. Inflammatory cytokine responses are seen in individuals with uncontrolled viremia and are ameliorated by natural control of the virus or by reduction in viremia after HAART treatment.
Methods: Multiplex assays were used to measure 73 cytokines, chemokines, and growth factors in samples from elite controllers (EC), HAART responders (HAART), untreated HIV non-controllers (NC), and HIV-uninfected (NEG) subjects from the WIHS and SCOPE cohorts. For infectivity studies CD8-depleted PBMC were infected in the presence or absence of cytokines at 0.5µg/mL (50ng/mL for IL-21) individually or in combination, cultured for 6 days and measured for p24. Infections were controlled for cell number and MOI. Significant differences were interpreted as p<0.05 and FDR<0.1.
Results: IL-21, SDF-1, CCL21, XCL-2, CCL27 and HCC-1 were significantly elevated in the serum of EC compared to NEG or HAART and not in NC. Infections with 81-A in the presence of SDF-1, HCC-1, CCL27 and combination of all six elevated cytokines showed significant suppression of virus replication (42%, 73%,66%, 85% respectively, p<0.05). Infections with NL4-3 in the presence of SDF-1, CCL21, HCC-1, and combination also showed suppression of replication (69%, 36%, 54%, 97%, respectively, p<0.05). Cell viability was not affected by cytokine concentration.
Conclusions: These cytokines may be critical in creating the immune environment responsible for elite control of HIV. They illustrate an effective cytokine profile that may help inform future immunotherapy for HIV infection.

P4.03.29
DRB1*01:01 gene mutation study in the second exon in patients with HIV/AIDS

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Introduction. Many studies related to HIV/AIDS cases demonstrate the protective activity of the DRB1*01:01 allele, namely, AIDS-related complex developed more slowly in patients with this gene in comparison to the patients in which this allele was not detected. With the recent developments, individuals who would have needed more time to develop AIDS, it developed in less than 6 years. The second exon forms the binding site for the HLA class II to the peptide and presents it to the immune system. The aim of the study. Find out whether DRB1*01:01 ongoing point mutations in the second exon affect the operation of this protective alleles in HIV patients. Methods. A study of 200 HIV patients. DNA was isolated using the Qiagen QIAamp DNA kit, second exon nucleotide sequence was determined by "Big Dye Terminator mix". Statistical analysis was performed by using MS excel, *StatCalc* programs (p≤0.05). The OR was calculated - Wolf's method. Results. The incidence of nucleotide polymorphism in the DRB1*0101 gene of the second exon, the missense mutations of this exon were found in 200 patients: in the 9th codon - 27% of cases, 14th codon - 36%; 87th codon - 28% (p <0.001). In one sample a STOP-codon was found (13th codon). Balanced relationships between nucleotide transversions and transitions have been detected (OR 0.05, 95% CI0.00-0.053). Conclusions. After the assessment of the risk and ratio of the protective alleles, it could be possible to determine for each individual which patient is predisposed to faster development of AIDS.

P4.03.30
Effect of HAART on reconstitution of memory versus naive T cells during tuberculosis treatment in HIV/active pulmonary tuberculosis individuals

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Although HAART has improved survival of HIV-infected patients, it can lead to acute and progressive side effects and high costs can limit affordability of these drugs. Therefore, stopping of HAART in HIV/TB patients for some time can reduce the above hindrances. It is not clear how the interruption of HAART would affect reconstitution of naive and specific memory T cell populations in these patients.
Participants were randomised to receive HAART/TB treatment (intervention arm n =39) or tuberculosis treatment (control arm n =37). CD45RO and CD62L cellular markers were used to differentiate naive from memory T cells on CD4 and CD8 T cells. Plasma HIV-RNA copy levels were measured using Amplicor quantitative

restriction transcriptase polymerase chain reaction assay (PCR). Samples were analysed at baseline, 3, 6 and 12 months time points.
HAART concurrently with TB treatment caused significant changes in CD8+ naive and memory T cells unlike TB treatment alone. These observations were supported by positive correlation between viral load and CD4+ naive plus the negative correlation between viral load and CD8+ naive T cells under HAART/TB treatment. Overall, HAART caused relative decrease in naive and an increase in memory CD4+ T cells.
Concurrent HAART and tuberculosis treatment gradually restores CD8+ naive T cells in adults HIV-1 co-infected with TB with CD4+ T cell counts >350 cells/mm^3. For better recovery of naive and memory CD4+ and CD8+ T cell populations both HAART and anti-tuberculosis treatment should administered to HIV patients co-infected with tuberculosis without interruption.

P4.03.31
The Human Leukocyte Antigen-G 3'UTR 14-bp deletion is associated with increased CD4 T-cell recovery during HAART in European HIV-infected women

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Background: Human leukocyte antigen G (HLA-G) is involved in the modulation of immunological responses and might therefore assist viruses in escaping the host immune response to HIV. Homozygous carriers of the 14-bp deletion (-14) polymorphism (rs16375) in *HLA-G* have significantly higher soluble HLA-G protein expression (sHLA-G). Further, substantial gender difference in sHLA-G levels has been found in an earlier experiment. We aimed to determine the influence of rs16375 on HIV-RNA in treatment-naïve individuals and on CD4 T-cell recovery during highly active antiretroviral treatment (HAART).
Methods: The Danish HIV Cohort Study is a prospective, nationwide, population-based study of 6000 HIV-infected individuals in Denmark. From this cohort we genotyped 1363 Caucasian individuals (1178 men and 185 women) for rs16375 using a competitive allele-specific PCR system. Log-transformed mean HIV-RNA in treatment-naïve individuals and CD4 cell recovery during HAART were compared between carriers of the +14-bp allele and homozygous carriers of the -14-bp deletion by a general linear model analysis with adjustment for age, calendar period, and CD4 T-cell concentration at start of HAART (where appropriate). Analysis was stratified on sex.
Results: Among women the homozygous *HLA-G* -14-bp genotype was associated with increased CD4 T-cell recovery during HAART as compared with +14-bp allele carriers (P = 0.017). We found no significant associations with mean HIV-RNA for the rs16375. No significant associations were seen among HIV-infected men.
Conclusions: These results suggest that the homozygous *HLA-G* -14-bp genotype in women is associated with increased CD4 T-cell recovery during HAART, but not associated with viral load.

P4.03.32
Incomplete immune recovery of naïve CD4 T-cell subsets after antiretroviral treatment in HIV-1 infection

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Purpose/Objective: Our goal was to investigate how HIV-1 infection affects phenotype and function of different naïve CD4+ T-cell subsets and the role of ART in immune reconstitution.
Materials and Methods: We performed phenotypic analysis of CD4+ recent thymic immigrants (RTE) and CD4+ central naïve T cells (CN) from PBMCs in 25 HIV-1 healthy controls (HC), 18 untreated HIV-1+ viremic progressors (VP), 10 untreated HIV-1+ viral controllers (VC) and 18 ART-treated HIV-1+ individuals (10 of whom were longitudinally followed). T-cell proliferation was assessed using CFSE proliferation assays; apoptosis was assessed using 7-AAD viability staining. Statistical Analysis included paired t-test and one-way ANOVA.
Results: Identifying CD3+ CD4+ CD45RO- CCR7+ CD31+ T-cell as RTE and CD3+ CD4+ CD45RO- CCR7+ CD31- T-cell as CN, we found that the correlations between age and these subsets were disrupted in HIV-1 infection and could not be restored after ART. Cross-sectional and longitudinal analysis of our age-matched cohort revealed increased expression of the immune activation marker CD95, increased cell turnover-rate (identified by Ki-67+ expression), and increased proliferative senescence (identified by CD57+ expression) on both subsets in HIV-1 infection which could not be restored after initiation of ART. Despite remaining impaired proliferative function in response to IL-7, ART reduced apoptosis of RTE and CN, which might account for the restoration of cell numbers of RTE and CN in HIV-1 infection following ART.
Conclusions: Our data indicate an incomplete immune recovery of RTE and CN after ART in HIV-1 infection.

P4.03.33
Dissociation of the nuclear pore complex during viral infection

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HIV-1 infection induces changes in assembly of various cellular organelles, which causes translocation of cellular components into virions. The nuclear pore complex (NPC) is targeted during traverse of reverse transcribed viral cDNA through the nuclear envelope (NE). Here we investigate the change of GANP, a component of mammalian transcription-export 2 complex, in HIV-1 infection. GANP is associated with various nucleoporins (Nups) and is co-localized at the NE. HIV-1 infection altered the localization of GANP from other Nups. Knockdown of Nups causes the reduction of GANP expression, suggesting functional interaction of GANP with NPC composition. HIV-1 infectivity was increased by knockdown of GANP, presumably by the failure to regulate the HIV-1 mRNA export in a Rev-dependent mechanism. Importantly, GANP overexpression significantly augments virus production in addition to the increased Rev-dependent HIV-1 mRNA export while it does not alter HIV-1 transcription. These data suggest that HIV-1 virus causes disassembly of the host factor GANP from the NPC assembly and hijacks its mRNA export function for production of virions in the infected cells.

P4.03.34
The origin of emerged HIV after terminating anti-retroviral therapy (ART)

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Even if HIV successfully disappears in the blood by ART, it immediately emerges after interruption of ART. Comparing the third variable region of envelope protein (V3 loop)-sequence of HIV-1, we confirmed that CD4+ PBMC was not the origin of emerged HIV after interruption of ART. Next, we thought that the gut was a candidate for the origin of emerged HIV, we performed endoscopic biopsies for ART-patients to obtain ileum samples and analyzed whether there were some HIV-replicating infected cells by flow-cytometry and determined the HIV-1-V3 sequences from extracted nucleic acid if the samples were positive for HIV-1 p24 antigen by flow-cytometry analysis. To our surprise, even if viral load in the blood was undetectable level and p24 antigen was not observed among PBMCs by ART, proviral DNA as well as p24 antigen could be detected in the ileum samples from the same patient. It should be noted that among HIV-1-infected CD4+ cells, Vα24+ NKT cells and CD11c DCs also

seem to be the major population for p24-positive cells involved. Moreover, we transmitted HIV to NKT cell line and CD4+ conventional T cell line in the culture medium containing the anti-HIV drug in vitro. After washing the drug, we confirmed that HIV emerged more immediately from NKT cells than from CD4+ conventional T cells. These result suggested that NKT cells in the ileum was the most likely candidate of the origin of emerged HIV.

P4.03.35
Viral load impact in HIV cellular immune response from children and adolescents

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Objective: To evaluate cell-mediated immune response to Human Immunodeficiency Virus (HIV) in 33 infected children and adolescents in long term antiretroviral therapy. Design: Cross-sectional study. Methods: HIV-infected children and adolescents were recruited at Pediatrics Out-Patients Unit of Hospital de Clinicas in UNICAMP, Sao Paulo, Brazil and separated in two groups: 20 patients with controlled HIV viral load (VL < 50 RNA copies/mL) and 13 viremic patients (VL > 1,000 RNA copies/mL). Patients with clinical or laboratorial signs of infections of TB, CMV, hepatitis B and C, syphilis or toxoplasmosis were excluded. Aldrithiol inactivated HIV-1-specific lymphoproliferation by flow cytometry, IL-2, IL-4, IL-5, IL-10, IFN-γ and TNF-α concentration by CBA and TGF-β1 concentration by ELISA were performed on PBMC cultures. Nonparametric tests were used ($p < 0.05$). Results: Cell proliferation and was not different between patients groups, neither for HIV ($p = 0.161$) nor for PHA-stimulated cultures ($p = 0.439$). There was lower IL-2 secretion in cultures with HIV from patients with VL > 1,000 RNA copies/mL (medians 33.42 and 4.82 pg/mL for controlled and viremic patients, respectively, $p = 0.001$). IFN-γ concentration correlated to proliferation and to IL-2, IL-10 and TNF-α production in HIV-stimulated PBMC cultures from patients. Age had no association to HIV-specific proliferation or cytokine production. Conclusions: Although HIV-1 lymphoproliferation was not different between groups, there was a reduction in IL-2 production to HIV in viremic patients, showing reduced functional activity to HIV in these patients. Financial support: FAPESP (process: 2010/16513-9) and CNPQ.

P4.03.36
CCR5 expression reduces T-tropic HIV-1 strain infection by forming CD4/CXCR4/CCR5 oligomers

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The HIV-1 envelope glycoprotein gp120 regulates the initial attachment of viral particles to target cells through its association with CD4 and the chemokine receptors CXCR4 or CCR5. Using several resonance energy transfer techniques, we detected that CD4, CXCR4 and CCR5 form homo- and heterodimers as well as CD4/CXCR4/CCR5 hetero-oligomers. We report that the oligomerization between CD4 and these coreceptors reduces T-tropic HIV1 infection. FRET and BRET experiments showed that CCR5 coexpression in CD4/CXCR4 cells altered CXCR4 homodimer and CD4/CXCR4 heterodimer conformation. As a result, $gp120_{IIIB}$-induced viral fusion to host cell membranes was reduced in CD4/CXCR4/CCR5 cells, as was infection by the X4 T-tropic HIV-$1_{NL4.3}$ strain in human CCR5-expressing $CD4^+$ T cells. When coexpressed in these oligomers, CCR5 blocked $gp120_{IIIB}$ binding to CD4/CXCR4 cells and consequently abrogated actin cytoskeleton rearrangement promoted by $gp120_{IIIB}$, indicating a mechanism that prevents Ttropic HIV infection. Receptor oligomerization is a dynamic process controlled by receptor expression levels that influence the first step in HIV-1 infection, and might be a target for new therapeutic approaches for AIDS intervention.

P4.03.37
T-lymphocyte/macrophage activation and HIV production upon toll-like receptor (TLRs) stimulation in naive and HAART-treated HIV+ patients according to CD4 lymphopenia

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Background: Chronic exposure to gut-derived microbial components represents a driver of immune activation and affects both HIV/AIDS pathogenesis and HAART response. We evaluated the role of *in vitro* TLR stimulation in inducing macrophage/T-cell activation and HIV production in untreated and treated HIV+ patients with different CD4 count.
Methods: PBMCs from HIV+naive (NA; 12 with High Counts, HC: CD4>500; 11 with Low Counts, LC; CD4≤500) and HAART-treated (HT; HIV-RNA <40cp/mL; 20 Full Responders, FRs: CD4≥350; 16 Immunological Non Responders, INRs: CD4<350) were matured into Monocyte-Derived Macrophages (MDMs). PBMCs/MDMs were cultured with CD3/CD28, LPS, LTA, PGN, ssRNA, IFNg. HLA-DR/CD38 on CD8/CD4 and HLA-DR/CD69 on CD14+MDMs were analyzed (flow cytometry). HIV-1 p24 and sCD14 were measured in supernatants (ELISA).
Results: TLR stimulation did not result in cellular activation and HIV production changes in HIV+ naive patients.
Interestingly, among HT, while INRs showed significant increase in CD38+CD8+ following TLR stimulation (LPS p=.0008; LTA p=.009; PGN p=.037; ssRNA p=.0003; IFNg p=.004; CD3/CD28 p=.0004), FRs increased CD38+CD8+ after ssRNA (p=.0006) and CD3/CD28 (p=.0005) exposure. Only ssRNA stimulation accounted for a significant rise in CD69+MDMs in INRs (p=.02) and FRs (p=<.01) and p24 release, resulting significant from PBMCs in INRs (p<.01) and FRs (p=.03) and from MDMs in FRs (p=.01).
Conclusion: TLR stimulation accounts for T-lymphocyte activation in INRs, whereas viral challenge alone seems to potently activate MDMs in HAART patients irrespective of CD4 reconstitution. Our data confirm the central role of TLR stimulation by microbial-derived bioproducts in sustaining T-cell activation, with partial impact on HIV production.

P4.03.38
Platelet activation and activation of cell death pathways in platelets isolated from HIV/AIDS patients

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The acquired immunodeficiency syndrome (AIDS) is a pandemic threat. Around 1.700.000 deaths globally were registered only in 2012, accordingly with the Joint United Nations Programme on HIV/AIDS (UNAIDS). New clinical data emphasizes long term complications of HIV infection such as cardiovascular disease and thrombotic events. Platelets have a paramount role on thrombus formation and the mechanisms behind HIV-1 induced platelet abnormities are still elusive. The aim of this work is to build up this literature by characterizing platelet activation and cell death pathways in platelets isolated from HIV/AIDS patients. Platelets were isolated from platelets-rich-plasma (PRP) of 18 HIV/AIDS patients and 30 healthy volunteers. P-selectin (CD62) was analyzed by flow cytometry (BD FACScan Excalibur) as a marker of platelet activation and degranulation. Mitochondrial dysfunction and cell death pathways were analyzed by mitochondrial membrane potential, caspase-9 activity, phosphatidylserine exposure and reactive oxygen species production. We observed that, despite virological suppression achieved by HAART, platelets derived from HIV/AIDS patients exhibited marked signs of intrinsic apoptotic pathway activation (decreased mitochondrial membrane potential, increased phosphatidylserine exposure, increased reactive oxygen species production and increased caspase-9 activation). Moreover, we demonstrated that platelets isolated from HIV/AIDS patients are less responsive to thrombin stimulus (P-selectin expression and RANTES production) than control platelets. Our results suggest that platelet activation in HIV infection initiate cell death pathways in platelets, which might be an important mechanism of HIV-associated thrombocytopenia. Moreover, platelet dysfunction might correlate with HIV infection towards AIDS.

P4.03.39
Differential capacity of CCR5- and CXCR4-dependent HIV-1 strains to replicate in primary cord blood CD4+ T cells. A transcriptome analysis

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HIV-1, the causative agent of the acquired immunodeficiency syndrome (AIDS), infects $CD4^+$ cells via interaction with CD4 and either CCR5 or CXCR4 coreceptors, but only the former has pandemic proportions and is efficiently transmitted among individuals, either sexually or mother to child, whereas the latter emerges only in the late phase of the infection in association with an advanced state of immunodeficiency. On the basis of a model system in which only CCR5-dependent (R5), but not CXCR4-dependent (X4) virus efficiently replicates in cord blood derived $CD4^+$ T cell lines, we searched for gene expression profiles and differences among Mock (Control), R5 and X4 phenotype experimental groups. The transcriptome of cord blood $CD4^+$ T cells established from 6 independent donors was examined at different time points (8, 24, 48, 72 h) after infection by isogenic NL4-3 (X4) and NL-AD8 (R5) viral strains. To detect differentially expressed genes profiles involved in entry of R5 and X4 phenotype viral into T cells, we performed statistical analysis of multi-series time-course gene expression data. Approximately 6 and 73 genes were selectively mobilized by R5 and X4 HIV-1 infection respectively, while 21 genes were modulated by both strains vs. control. Most genes were regulated in the same manner by both strains vs. the Mock condition, suggesting that the asymmetric replication profiles of the two viruses might not be due to a differential capacity of the two viruses to mobilize discrete sets of genes, but by other post-transcriptional mechanisms.

P4.03.40
Adaptation of Simian/Human Immunodeficiency Chimeric Virus (SHIV89.6p) replication in Baboon by in vivo serial viral passage; a preclinical disease model

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Background: Simian-human immunodeficiency virus (SHIV) is widely used in HIV research to assess the efficacy of AIDS vaccines and anti-HIV microbicides using the macaque animal model. The objective of this study was to generate a baboon-adapted SHIV to allow similar studies to be conducted in the baboon, which is already a well-characterised model at IPR for human diseases.
Objectives: To ensure pathogenicity and increase the virulence of stock SHIV89.6P through in vivo serial passage.
Methods: To achieve this, SHIV89.6P, a macaque-adapted lentivirus, was serially passaged in vivo in three juvenile baboons via direct intravenous (I.V.) injection of undiluted cell culture supernatant in the 1st baboon and i.v inoculation of heparinised whole blood and bone marrow (BM) from SHIV-inoculated baboon into subsequent baboons.
Results: SHIV was recovered from all the three animals through co-culturing blood and BM mononuclear cells with CEMX174 and HUT78 cell lines. In addition, viral antigens were readily detected in the plasma obtained at day 14 post-inoculation (75, 172 and 2,287 pg/ml for the 1st, 2nd, and 3rd passages respectively). Titration of the inoculum in CEM X174 cells yielded higher viral titres, ranging from 10-6 to 10-6.5 tissue culture infectious doses at 50% (TCID-50), compared to the original inoculum at 10-2.5 TCID-50. Measurement of the viral Gag (SIVp27) contents in these inocula confirmed that the baboon-generated SHIV inoculum was several-fold higher (>10 µg/ml) than the initial virus stock (0.03 µg/ml).
Conclusions: These observations indicate a baboon-adapted SHIV89.6P. This can thus be utilized for future studies using the model.

P4.03.41
Chemokine and chemokine receptor expression in HIV-infected individuals with tuberculosis on ATT and ART

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Background: HIV-infected individuals with pulmonary tuberculosis (HIV+TB+) on ATT and ART frequently experience deterioration despite improvement in CD4 counts and viral suppression. The pathogenesis of such phenomenon indicative of IRIS is speculative.
Aim: To characterize chemokine and chemokine receptors expression associated with combined administration of ATT and ART.
Methodology Thirtyone HIV+TB+ patients initiated on treatment were studied at three time points (baseline, 2 months of ATT and 1 month of ART). Twenty normal healthy individuals (HIV-TB-) were recruited as controls. Expression of CD38, CXCR4 & CCR5 positive T cells along with plasma and antigen stimulated (p24 & PPD) chemokine production (MIP-1a, MIP-1β and RANTES) were investigated.
Results: HIV+TB+ patients had significantly reduced expression of CXCR4(21.1$\pm$ 1.7 vs 36.2$\pm$ 1.5; 12.6$\pm$1.5vs34.2$\pm$1.4) and CCR5(5.5$\pm$0.7vs12.0$\pm$0.9 ;11.7$\pm$1.6vs18.0$\pm$1.3) expressing CD4 and CD8 T cells compared to controls. The plasma levels of all the three chemokine were higher in patients, MIP-1α (58$\pm$13vs12.7$\pm$6.7pg/ml), MIP1-β(201.6$\pm$21.2vs44.3$\pm$18.3pg/ml) and RANTES(4909.6$\pm$444.3 vs 2454.6$\pm$324.5 pg/ml). After 1 month of ART, 58% patients had viral suppression. Two months of intensive ATT significantly reduced chemokine levels compared to baseline,MIP-1α (31.4$\pm$4pg/ml) and MIP1-β(186.4$\pm$18.9) but reversed immediately after start of ART, MIP-1(63.6$\pm$10.9) and MIP-1β (228.2$\pm$23.6). The levels of stimulated chemokines followed a similar trend.
Discussion: ART tends to normalize chemokine receptor expression irrespective of complete viral suppression. The knowledge of both chemokine and receptor expression at later time points would provide much clearer information. However the present study demonstrates the relevance of β-chemokine to evaluate the exuberant immunological response indicative of IRIS in this group of individuals.

P4.03.42
DNA sensing and innate immune activation during HIV infection

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Background: The role of innate immunity during HIV infection remains poorly understood. It is important to understand HIV immunopathogenesis and chronic immune activation. Immune cells express various pattern recognition receptors (PRRs), of which the cytosolic DNA sensors recognize foreign DNA. An important question is whether sensing of proviral HIV-DNA or DNA from opportunistic pathogens is compromised in HIV infection.
Materials and methods: Expression levels of the DNA sensors (IFI16, DDX41, and cGAS) and downstream signaling molecules (STING and IRF-3) were measured by qPCR on whole blood from 20 highly active antiretroviral treatment (HAART) naïve HIV patients, 20 HAART responders, 20 HAART non-responders, and 20 healthy controls. Furthermore HIV-DNA was transfected into PBMCs and levels of the interferon-inducible genes ISG56 and CXCL10 as well as TNF-α were measured by qPCR.
Results: We found significant differences between HIV infected individuals and controls in the response to HIV-DNA as measured by CXCL10 expression. This difference was specific since the NF-κB/TNFα pathway was not differentially induced. Finally, we demonstrated differences between patients and controls in expression of the DNA sensor IFI16, and a significant correlation between HIV viral load and IFI16 mRNA expression was observed in HAART naïve patients.
Conclusion: Our study demonstrates significant differences between HIV-infected individuals and controls in the ability to recognize and respond to DNA and mount an antiviral innate immune response. This

finding may play a role in the immunopathogenesis of HIV infection and may have potential implications for efforts to develop a vaccine or cure for HIV.

P4.03.43
Characterization of HIV-specific lymphocytes as a tool for patient management

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HIV infection is known for its heterogeneous manifestation in which the immune system plays a key role in regulating long-term balance between HIV and the host. We have carried out a follow-up study in different groups of HIV-1 infected patients, treated and untreated with antiretroviral therapy, analyzing the functional gag-specific $CD4^+$ and $CD8^+$circulating T-cells stimulated *in vitro*. The results of this study showed that subjects who control chronic infection, with or without antiretroviral therapy (virologic controllers and virologic responders) had higher levels of polyfunctional IFN-γ/IL-2 producers gag-specific CD4 and CD8 T cells, compared to subjects who had a progression of viral infection, treated with or without antiretroviral therapy (virologic non controllers and virologic non responders), that showed a predominance of single IFN-γ producers gag-specific T-cells. Moreover, we analyzed cytokines levels in the sera of HIV patients, by the Luminex platform and we found that all patients showed a state of immune-activation even though non responder patients had higher levels of inflammatory cytokines and low level of IL-10 compared with responders.
Overall, our results suggest that analysis of polyfunctional CD4 and CD8 T cells in different cohorts of HIV-infected individuals, may be of help to find a correlation between immunological and clinical status of these patients and to identify possible markers that help clinicians to predict progression of HIV disease and to define the most opportune time to initiate, modulate or interrupt therapeutic measures.

P4.03.44
Differential regulatory T cell activity in HIV-1-exposed seronegative individuals.

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The potential role of regulatory T cells (Tregs) in protection from HIV infection remains unclear, although it has been hypothesized that by dampening general T cell activation, Tregs could limit the pool of HIV-susceptible cells but they could also limit the HIV-specific response. To address this question, we analyzed samples from 129 HIV-exposed seronegative individuals (HESN) that are part of a HIV-serodiscordant couples cohort. To assess T cell and Treg function, we measured the proliferation of T cells in response to HIV peptide pools by a CFSE-based proliferation assay. The HIV-specific proliferative response rates were low for both CD4 and CD8 T cells, and surprisingly, the overall CD4 T cell proliferation response rate was not increased when Tregs were removed from cell preparations prior to culture. Of the 20 individuals that had HIV-specific CD4 T cell proliferative responses, 8 of them had Tregs that could functionally suppress this proliferation, whereas 12 of them had Tregs unable to suppress. When subjects were thus identified based on Tregs able to suppress or not suppress CD4 T cell proliferation, we found a correlation between T cell activation and Tregs frequencies. Additionally, subjects with Tregs unable to suppress proliferation had a statistically significant increase in HIV-specific production of MIP1[[Unsupported Character - Symbol Font ]][[Unsupported Character - Symbol Font ]] by CD4+ T cells. Interestingly, CD4+ T cell autocrine production of MIP-1[[Unsupported Character - Symbol Font ]] has been shown to be protective from HIV infection.

P4.03.45
Development of a new multi-epitope HIV-1 tat/env/pol/gag vaccine candidate: a preliminary study on immunogenicity

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Background: Multi-epitope vaccines may be promising HIV-1. In this study, a multiepitopic recombinant protein containing various HIV-1 antigens was expressed in E. coli BL21D3 and its immunogenicity was evaluated in BALB/c.
Methods: HIVtop4 sequence spanning the junction of six amino acid fragments ($Gag_{158-186}$, $Pol_{150-190}$, $ENV_{296-323}$, $ENV_{577-610}$, Tat_{1-20} and Tat_{44-61}) was designed based on computer analysis. Synthesized nucleotide sequence was cloned into pET23a plasmid and Expressed in BL21 *E. coli* cells by addition of 1 mM IPTG during 3 hrs culture, Then due to C-terminal fusion of 6xHis-tag, was purified by IMAC and confirmed with western-blotting. Groups of BALB/c mice (n=6) were immunized with of 20 µg of candidate vaccine adjuvanted in Complete Freund's adjuvant, Montanide ISI-70 and Alum. Two weeks after last immunization lymphocyte proliferation was measured with Brdu, IL-4 and IFN-γ cytokine with ELISA, total antibody and IgG1, IgG2a isotypes with indirect ELISA methods.
Results: Immunization led to a significant increase in the proliferative responses of lymphocytes, IFN-γ cytokine and total antibody titer with poly-isotypic form in comparison with the control groups. However Vaccine formulation in alum adjuvant resulted in the highest humoral response and formulation in Freund's adjuvant resulted in highest level of lymphocyte proliferation and IFN-γ cytokine release.
Conclusion: In this study we concluded tat, env, pol, gag with adjuvants (Montanide, Alum and CFA) can be considered as a candidate vaccine against the HIV virus.

P4.03.46
Sub-optimal CD4 T-cell recovery in HIV-1 subtype C patients on antiretroviral therapy: A search for predictive biomarkers and baseline characteristics

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Background: Despite suppressive antiretroviral therapy (cART) some 15-30% of treated HIV infected patients fail to achieve optimal CD4 T-cell reconstitution. Sub-optimal CD4 recovery has been associated with unfavourable outcomes for patients on cART. We assessed markers of immune activation, microbial translocation and patient baseline characteristics for associations with sub-optimal CD4 T-cell recovery post cART initiation.
Methods: This was a retrospective case control analysis based on the Adult Antiretroviral Treatment and Drug Resistance study, in Botswana. Cases had CD4≤ 200 cells/µl at 12 months post ART initiation and virologic suppression within 6 months. Microbial translocation (sCD14) and immune activation (interferon-gamma) markers were quantified using ELISA assays on a subset of plasma samples (30 cases and 30 controls matched by gender). Analyses of associations with sub-optimal response were based on Mann-Whitney Test, univariate and logistic regression.
Results: 21% virologically suppressed patients had sub-optimal CD4 recovery (51 from 249). Median age was 33.39 years and 69.9% were female. Baseline CD4 count <100cells, haemoglobin and aspartate transaminase were associated with sub-optimal CD4 recovery (adjusted OR (aOR) =3.03 95%CI [1.65, 5.57], p <0.001; aOR=0.81 [0.67, 0.99], p=0.038 and aOR=1.03 [1.00, 1.05], respectively). sCD14 levels were significantly different between cases and controls, p=0.0011, at 12 months.
Conclusion: Low baseline CD4 T-cell count, haemoglobin, aspartate transaminase and sCD14 levels are predictive of suboptimal CD4 T-cell recovery in this HIV-1 subtype C infected cohort. These characteristics are potentially useful in identifying patients who need frequent clinical monitoring to minimise unfavourable outcomes associated with poor CD4 T-cell recovery.

P4.03.47

CD4+ T cells lacking SAMHD1 expression are highly proliferative in vivo and decrease during HIV-1 infection

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Although it is well established that SAMHD1 is a restriction factor for HIV-1 in macrophages and resting $CD4^+$ T-cells, it needs to be determined whether SAMHD1 levels are modulated during HIV-1 infection.
Here we assessed SAMHD1 expression in $CD4^+$ T-cell subsets from antiretroviral therapy treated and non-treated HIV-1 infected individuals as well as in healthy controls, and addressed whether SAMHD1 expression is modified during T cell activation and proliferation.
Our results revealed that in healthy individuals (n=12) while the vast majority of T-cells express SAMHD1, a small subset of $CD4^+$ cells (11.025.36%) displays lower levels. Phenotypic characterization showed that memory $CD4^+$ T-cells, notably effector/memory $CD45RA^-/RO^+CCR7^-CD27^+CD28^+$ and terminally differentiated $CD45RA^+/RO^-CCR7^-CD27^-CD28^+$ subsets contain higher proportions of $SAMHD1^{low}$ cells (15.227.31% and 23.3610.37% respectively) as compared to naïve cells (7.084.27, $p<0.05$ and $p<0.001$ respectively). Importantly, both ART-treated (n=10) and non-treated (n=8) HIV-1 infected individuals displayed lower frequencies of $SAMHD1^{low}$ cells (5.423.90% and 5.473.09% respectively, $p<0.05$ as compared to controls). Moreover, $SAMHD1^{low}$ $CD4^+$ T-cells exhibit higher proportions of cycling $KI67^+$ cells as compared to $SAMHD1^+$ cells (6.155.24% vs 3.901.36% for treated and 27.7424.18% vs 5.484.46% for non-treated HIV-1 infected individuals; 2.202.24% vs 1.980.76% for controls). We confirmed these results by *in vitro* stimulation (anti-CD3/CD28) where dividing $CD4^+$ T-cells exhibited lower SAMHD1 expression as assessed by both RT-PCR and flow-cytometry.
Our results show that memory/cycling $CD4^+$ T-cells exhibit low levels of SAMHD1 which might explain their preferential productive infection and thus provide new insight in the mechanism of $CD4^+$ T-cell depletion, hallmark of HIV-1 infection.

P4.03.48

The HIV Tat protein favors the activation of CD8 T cells thus contributing to HIV-related immune dysfunctions

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Tat is a regulatory protein produced very early after HIV infection, necessary for viral gene expression, cell-to-cell virus transmission and disease progression. Tat can be released extracellularly and, upon release, enter uninfected T cells displaying several functions, only partially characterized. A large number of studies show an immunomodulatory effect of Tat on CD4 T Lymphocytes and professional APCs, demonstrating how Tat increases their activation and the secretion of pro-inflammatory cytokines and proposing a contribution of Tat to the chronic immune activation described during HIV infection.
CD8 T cells from HIV infected subjects are also characterized by several dysfunctions, including an increased activation. However, whether Tat can modulate also the CD8 T cell response is not clear.
To characterize the role of Tat on CD8 T cell activation, PBLs form healthy donors were stimulated with anti-CD3/CD28 in the presence or absence of Tat protein. Both CD4 and CD8 T cells activated in the presence of Tat showed an enhanced pro-inflammatory cytokine production compared to T cells activated without Tat. Furthermore, memory CD8 T cells from EBV positive donors were stimulated with EBV peptides in the presence or absence of Tat. EBV-specific CTLs generated in the presence of Tat exhibited enhanced effector functions compared to CTLs generated without Tat. The same effect was observed on naïve CD8 T cells primed with CD8 peptide epitopes.
Taken together, these results suggest that the Tat protein of HIV enhances the stimulation of CD8 T cells thus contributing to immune activation during the course of HIV.

P4.03.49

HIV-1 Nef downregulates CD1a lipid-antigen presentation in dendritic cells via hemopoietic cell kinase (HCK) and p21 activated kinase 2 (PAK2)

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We previously reported that Nef significantly down-regulated CD1a expression in immature dendritic cells (iDCs). Here we show that Nef down-regulates CD1a lipid-antigen (Ag) presentation, which is due to the association of Nef with p21-activated kinase 2 (PAK2), and that SH3-protein hemopoietic cell kinase (HCK) specifically expressed in immature dendritic cells (iDCs), is crucial in this process, as analyzed using a CD1a antigen presentation assay with a series of mutated *nef genes* using iDCs. The Nef-PAK2 intermolecular interaction was further confirmed by the colocalization of Nef and PAK2 and a protein fragment complementation assay, which revealed that HCK significantly augmented the intermolecular interaction of Nef with PAK2. These results suggest that iDC-specific HCK plays a critical role in the assembly of the Nef/PAK2 complex, which may be another mechanism of immune-evasion by HIV-1, and that HCK may be a therapeutic target to control HIV-1-infected iDCs, one of the possible reservoirs of HIV-1 even after anti-retroviral therapy (ART).

P4.03.50

Correlation between c-reactive protein and CD4+ cell count in HIV-infected and HIV/PTB co-infected patients at the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria

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The objective of this study was to determine the correlation between CRP and CD4+ cell count in HIV-infected patients at the University of Maiduguri Teaching Hospital; and to compare with HIV/PTB co-infected patients as well as HIV-seronegative healthy age- and sex-matched controls. A hospital-based cross-sectional comparative study was undertaken. One hundred and twenty consecutive HIV-infected patients comprising 60 HIV+ only and 60 HIV/PTB co-infected patients and 60 apparently healthy HIV-seronegative age- and sex-matched controls were recruited. The Pearson's product moment correlation coefficients (r) for the correlation between CRP and CD4+ cell count were -0.596 (p=0.000) for HIV+, -0.365 (p=0.004) for HIV/PTB patients and +0.147 (p=0.263) for healthy controls. Whereas, *r* for the correlation between CRP and HIV-1 viral load were +0.023 (p=0.859) for HIV+ patients and +0.097 (p=0.463) for HIV/PTB patients. The diagnostic usefulness of the CRP measurement as a surrogate for CD4+ cell count showed sensitivity, specificity and positive predictive values of 80.6%, 72.4% and 75.8% for HIV+ patients and 88.9%, 63.6% and 66.7% for the HIV/PTB patients. This study found a strong linear negative correlation between CRP and CD4+ cell count in HIV-infected patients as well as those with HIV/PTB co-infection. Therefore, serum CRP level of >10mg/L in HIV-infected patients, can be used to predict low CD4+ cell counts in resource-poor locations lacking the facility for measuring CD4+ cells.

P4.03.51

Manipulation of rapid apoptosis for HIV-1 gp160-specific murine CD8+ CTLs by free antigenic peptide in vivo

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We have previously reported that the cytotoxic activity of murine CD8+ CTLs specific for HIV-1 gp160 envelope protein was markedly inhibited in vitro by brief exposure to a free epitope peptide P18-I10 (RGPGRAFVTI) using the epitope-specific CTL line (LINE-IIIB) or a

clone (RT-1) (JEM, 183:879, 1996). We have also shown that recently stimulated P18-I10-specific murine CTLs rapidly fell into apoptosis in vitro after brief exposure to peptide P18-I10 (JI, 169:6588, 2002). Here, we examined whether similar inactivation or apoptosis of recently stimulated CTLs occurred in vivo by exposure to the free epitope peptide using T cell receptor (TCR) transgenic mice (Tg-RT-1) (JEM, 195:991, 2002) expressing αβTCR genes of CTL clone RT-1. When the Tg mice were inoculated with recombinant vaccinia virus expressing HIV-1 IIIB gp160 genes (vSC25) followed by injection of P18-I10 epitope peptide, apparent reduction in the number of CTLs determined by flow cytometry using H-2Dd/P18-I10 pentamer was observed within few hours. Most of the H-2Dd/P18-I10 pentamer-stained cells were positive for Annexin V and apoptosis was confirmed by microscopic analyses. Moreover, when mice were pretreated with immunosuppressive agents, such as cyclosporin A (CsA) or tacrolimus (FK506), induction of apoptosis by P18-I10 was significantly inhibited and CTL cytotoxicity was maintained. These results suggest that the rapid loss of virus-specific CD8+ CTLs might occur in vivo through apoptosis in the early stages of viral infection when activated CTLs may encounter viral epitope(s) released from virus infected cells attacked by CTLs and we can prevent the loss by pretreatment with immunosuppressive agents.

P4.03.52
Immunological, hematological and biochemical parameters are changed in asymptomatic HIV-infected patients, treated or not with antiretroviral drugs

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In HIV-infected even during antiretroviral therapy (ART) and absence of symptoms, the imbalance in immunological, hematological and biochemical factors can potentially contribute to progression to AIDS or other comorbidities. We aimed to analyze some of these parameters in 58 asymptomatic HIV-patients: ART-naïve (HIVn, n=18); virologic failure on ART (HIVf, n=18); on ART and complete viral suppression (HIVc, n=22). Quantification of viral load and CD4+ T cells were done by branched-DNA and flow cytometry, respectively. Sandwich immunosorbent assays were performed to quantify cytokines as IFN-g, TNF-a, IL-10 and IL-6. Other results were collected from medical records. Tukey-Kramer test and negative binomial and gamma distributions were calculated ($p<0.05$). Comparing three groups, we found the lowest levels of IL-10 in HIVc. The HIVf presented the lowest concentration of IFN-g and the highest levels of TNF-a and IL-6. To lymphocytes, mainly CD4+ T, HIVf presented lower counts than other groups. Triglycerides, high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) were higher in treat-patients (HIVc and HIVf) than HIVn. Total cholesterol was elevated in HIVc when was compared to HIVn, without difference to HIVf. An intriguing result was the higher aspartate-aminotransferase(AST) value found in HIVn than HIVc. When we checked the normal references ranges, the most common changes in the studied population were observed to neutrophils, total leukocytes, hematocrit, creatinine and metabolic exams. Thereby, therapeutic advances have contributed to the slower evolution to AIDS, however the laboratory changes observed in HIV+ deserve to be better understood in order to seek a greater -and better- longevity.

P4.03.53
Can cytokine profiles in HIV patients be useful in clinical practice?

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HIV infection causes an accentuated dysregulation on cytokine production. These changes have been extensively studied in patients on antiretroviral therapy (ART). The aim of this study was to characterize the cytokine profile in 58 asymptomatic HIV-patients, according to different answer to ART. The following groups were enrolled: naïve to ART (HIVn, n=18); on ART but presenting incomplete viral suppression (HIVi, n=18); on ART and presenting complete viral suppression (HIVc, n=22). Quantification of viral load and CD4+ T cells were done by branched-DNA and flow cytometry, respectively. Sandwich immunosorbent assays were performed to quantify cytokines as IFN-g (Th1 profile), TNF-a (Th1 and Th17), IL-6, IL-17 (Th17) and IL-10 (Th2 and Treg). The asymmetric variables were analyzed by negative binomial and gamma distributions ($p<0.05$); normal variables were analyzed by ANOVA followed by Tukey ($p<0.05$). The HIVc group presented the lowest concentrations of IL-10 and IL-17. The HIVi group presented intermediate levels of IL-17, the lowest levels of IFN-g and the highest of IL-6 and TNF-a; a positive correlation between these last two cytokines was observed. HIVn showed the highest level of IL-17. This cytokine was the only one that differentiated the 3 groups (HIVn > HIVi > HIVc). These findings suggest the cytokine profile can be useful in clinical practice to characterize the immunological status of asymptomatic patients.

P4.03.54
Nef-HIV-1 dimerization domain plays a key role in p22-phox association and ROS modulation

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Several mechanisms have been proposed to explain how HIV could modulate ROS generation and some data shows that HIV proteins have been implicated in oxidative stress during infection. HIV-1 Nef is a regulatory protein with pro-oxidant properties, and we recently show a specific association between Nef and p22-phox, a membrane component of the NADPH oxidase complex. In this work we explored which domain is critical to mediate this association. Due to a hypothetical predictive model of protein-protein association between p22-phox and Nef, showed low values of free energy between the molecules, in particular, residues VRGE (126-129) from p22-phox corresponding to an intracellular portion of the protein and residues RRQDI (105-109) from Nef, demonstrated the highest probability of interaction, we decided to map this association by cloning different truncated form of Nef-HIV-1, including membrane-binding, SH3, Pak1, dimerization and Pak2 domains, and perform immunoprecipitation and functional assays. We observed that dimerization domain (which contain the RRQDI sequence) is critical for ROS production (empty vector suspension: 5%±3: Nef-HIV-1 wt: 19,8%±8; truncate which contain dimerization domain: 16,4%±8 SD; $p< 0.05$) and p22-phox association. These results shows that dimerization domain play a key role in modulate ROS production due to their association with p22-phox , and probably is implicated in modulate oxidative stress during HIV infection.

P4.03.55
Genital epithelial cells interferon-alpha production is associated with HIV-1 infection in Beninese female commercial sex workers

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Interferon-alpha (IFN-α) is an important antiviral molecule of the innate immunity. HIV-1-infected commercial sex workers (CSWs) from Benin had higher levels of IFN-α in the genital mucosa when compared to those found in HIV-uninfected CSWs (p=0,039). Epithelial cells are involved in innate immune responses, by sensing pathogens via Toll-like receptors (TLRs), and are one of the first cells to encounter HIV in the genital tract. Specific aim: To define the role of epithelial cells in the production of IFN-α in the female genital tract during HIV infection. Methodology: Cervico-vaginal lavages (CVLs) and cytobrush samples from cervix of 30 HIV-uninfected and 50 HIV-1-infected CSWs were analysed. TLR7-9, interferon regulatory factor (IRF)-7 and IFN-α proteins expression patterns by genital epithelial and myeloid cells were determined by FACS. TLR7-9, IRF-7 and IFNA-17 gene polymorphisms involved in IFN-α signalling pathway were genotyped by direct DNA sequencing. Results: Genital epithelial cell TLR-7 and IFN-α expressions were higher in the HIV-1-infected

CSWs compared to those in HIV-uninfected CSWs (p=0.029, p=0.032). In contrast, IRF-7 epithelial cell expression was lower in the HIV-1-infected CSWs compared to the HIV-uninfected CSWs (p=0.005). Two IRF-7 linked genetic variants (rs1061502, rs12290989) were (p=0.041) associated with IFN-α genital levels in the HIV-1-infected group and with IFN-α intracellular expression in the HIV-1-infected and HIV-uninfected CSWs, (p=0.022, p=0.053). Conclusion: Epithelial cells are major contributors of IFN-α production in the genital mucosa and its production could be genetically-determined in CSWs from Benin.
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P4.03.56
Membrane-binding domain from NEF-HIV-1 increases FOXP3 transcription factor expression on human monocytes

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It has been shown that during human immunodeficiency virus (HIV) several mechanism are involved to evade the immune response, including a promotion of the regulatory immune response by increase regulatory T cells (Treg) development. Foxp3 a transcription factor which is considered the most important Treg marker, are also been detected in monocytes/macrophages, and it is associated with their inhibitory activity. Due to monocytes/macrophages are susceptible to HIV-1 infection and are a major viral reservoirs, we explore if Nef-HIV-1 (complete protein) and four different truncate form including membrane-binding, SH3, Pak1, dimerization and Pak2 domains) could modulate Foxp3 on peripheral blood monocytes. We analyzed Foxp3 expression in monocytes from 10 healthy individuals, in prescence of suspension purified from empty vector transformed bacteria, Nef-HIV-1, and four different truncate form. A curve dosis/time was performed, and samples were analyzed on a flow cytometer. We observe that Nef-HIV-1 and only their membrane-binding domain (250ng/ml during 24h), are responsible to increase Foxp3 expression in human monocytes (empty vector suspension: 12%±3: Nef-HIV-1 wt: 26%±5; Nef-HIV-1 membrane-binding: 18%±2 SD; p< 0.05). Our result suggests that Nef-HIV-1 increase Foxp3 expression in monocytes and membrane-binding domain is important to Foxp3 modulation. These results could be important to try reverse the suppressive microenvironment created by HIV to protect themselves in their viral reservoirs and a possible therapeutic target.

P4.03.57
The role of immunological tolerance in humoral responses to the 2F5 epitope of HIV-1

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Accumulating evidence suggests that the generation of neutralizing HIV-1 antibody may be proscribed by immunological tolerance. A majority of monoclonal human antibodies, including 2F5, that neutralize multiple clades of HIV-1 are polyreactive and bind avidly to unknown components of mammalian cells. Knock-in mice expressing the VH and VL regions of 2F5 (2F5 KI) are characterized by a central defect in B-cell development that indicates the presence of a tolerizing autoantigen(s) mimicked by the 2F5 epitope of HIV-1 gp41.
We now demonstrate that the 2F5 antibody and its unmutated ancestor avidly bind human kynureninase (KYNU), a conserved enzyme of tryptophan metabolism, via the H4 domain that shares the 2F5 epitope within HIV-1. In mice that express KYNU, B cells with 2F5-like BCR are lost during maturation in the bone marrow, as determined by binding to the 2F5 epitope. To compare the B cell repertoire of 2F5 KI mice before and after central tolerance, we use a single B-cell culture that permits maturation of autoreactive cells normally deleted in vivo. The pre-tolerance compartment (small pre-B) reacts to HIV-1 gp41 and KYNU, whereas the mature, post-tolerance B cells are purged of gp41- and KYNU-reactivity, and contain extensive light-chain editing. Opossums naturally carry an H4 alteration in KYNU that resulted in a fully active enzyme completely unreactive with the 2F5 antibody. Immunization of opossums with HIV-1 gp140 resulted in extraordinarily high HIV-1 gp41-titers.
Identification of an epitope shared by most mammals and HIV-1 provides direct evidence that immunological tolerance impairs humoral responses to HIV-1.

P4.03.58
Differences in the profile of antiviral response in mothers infected by HIV-1 and newborns

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Introduction: A high percentage (65-80%) of infants from mothers infected by HIV-1 is uninfected even in the absence of antiretroviral therapy. This finding emphasizes the importance of evaluating the immunological characteristics in the maternal-fetal interface, mainly related to the innate immune response. The purpose of this study was to evaluate the RNA and protein expression of antiviral factors in HIV-infected mothers peripheral blood mononuclear cells (PBMC) and umbilical cord blood (CB), compared with uninfected mothers-CB.
Methods and Results: The results show that expression of APOBEC3G and 3F, TRIM-5 and 22, MxA, IFN- and STING were increased in CB and PBMC from infected mothers (n =15) compared to control mothers (n =14). Expression of APOBEC3G, TRIM-22, MxA and tetherin was decreased in newborn compared to the healthy adult, moreover, the expression of these factors in CB from HIV-infected mothers were similar to those found in infected mothers group. In placental tissue it was observed decreased expression of APOBEC3G protein in decidua and TRIM-5 in villi tissue of HIV-infected mothers compared to control mothers, however this difference was not observed in the mRNA expression.
Conclusion: This data shows that HIV infection alters the expression of APOBEC3G and TRIM-5 at the post-translational stage, in placental tissue, contrariwise to the enhanced expression in PBMC from HIV-infected mothers. Despite of the immunological immaturity in the neonatal period, the maternal HIV infection generated a similar profile of antiviral factors expression in newborn, probably due to a complex factor interaction related to the pregnancy and HIV-1 infection.

P4.04 Immunity to bacterial infection (excluding mycobacteria)

P4.04.01
Mice oral immunization against Vibrio cholera: Zot and Ace recombinant proteins used as a subunit vaccine

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Cholera is an acute diarrheal disease which is characterized by discharge of voluminous rice watery diarrhea, caused by toxigenic Vibrio cholerae strains. Accessory Cholera Enterotoxin (Ace) is the third toxin of V.cholerae, increases transcellular ion transport and contributes to diarrhea. The second important toxin of V.cholera, Zonula occludens toxin (Zot), is a single polypeptide chain which binds to a receptor on intestinal epithelial cells and increases mucosal permeability by affecting the structure of epithelial tight junctions. This

property makes Zot as a promising tool for mucosal drug and antigen delivery. Ace & Zot proteins from V. cholerae strain 62013 were cloned, over-expressed and purified in Escherichia coli and their biological activity were determined on rabbit ileal loops. In the current study, we used the combination of these proteins in female BALB/c mice to evaluate the efficacy of oral administration and immunization. Following immunization, antibody responses against Zot and Ace proteins were assessed in serum and feces after 7 days. Mice were also challenged with toxigenic V.cholerae 61013 and its shedding was monitored during two weeks in feces. Immunization with both proteins induced significant Zot & Ace specific IgA responses in feces, but not IgG in serum. Mice were immunized with both of Zot & Ace proteins showed dramatically decreased toxigenic V.cholerea shedding compared to those received single protein or negative control. These results demonstrate the future perspective for the use of these proteins as a new recombinant subunit vaccine to prevent colonization and shedding of toxigenic V.cholera.

P4.04.02
Antibodies against outer membrane proteins of Moraxella catarrhalis and nontypeable Haemophilus influenzae cross-react with various pathogens of human respiratory tract

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Outer membrane proteins (OMPs) of bacteria Moraxella catarrhalis and nontypeable Haemophilus influenzae are the most immunogenic antigens of these respiratory tract pathogens. The goal of the study was to quantitatively and qualitatively assess the reactivity of anti-OMPs antibodies against cross-reactive and surface-exposed epitopes of heterologous strains as well as other important pathogens of respiratory tract.
Antisera were obtained from six groups of mice, each immunized with isolated different Moraxella catarrhalis OMPs (anti-OMPMc sera) or nontypeable Haemophilus influenzae OMPs (anti-OMPNTHi sera). The titers of cross-reactive antibodies were measured by whole-cell ELISA whereas their specificities with bacterial OMPs was confirmed by Western-blot. The studied pathogens were: Pseudomonas aeruginosa including cystic fibrosis (CF) and non-cystic fibrosis (non-CF) strains, Burkolderia cepacia (CF, non-CF), Acinetobacter baumannii, Haemophilus parainfluenzae and additionally Candida albicans. The pathogens were chosen on the basis of in silico analysis of their surface-exposed antigen homology.
We found that among analyzed anti-OMPMc and anti-OMPNTHi sera, the several were medium or highly cross-reactive. The cross-reactive titers in anti-OMPMc sera were in the range 1:200 - 1:1500 that was 500 - 1000-fold lower than titers for homologous strains. The cross-reactivity of anti-OMPNTHi sera were in the range 1:2000 - 1:11000 that was 4 - 20-fold lower than for homologous counterparts. The reactivity of some cross-reactive antibodies was confirmed with OMPs of tested strains.These results indicate that IgG antibodies induced against OMPs of M.catarrhalis and H.influenzae recognize cross-reactive surface-exposed epitopes in multiple respiratory pathogens. The biological role of this phenomenom will be confirmed in antibody-dependent assays.

P4.04.03
Functionality of cross-reactive antibodies against Moraxella catarrhalis

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Antibody-dependent immune response against respiratory human pathogen Moraxella catarrhalis is critical for its effective elimination. The goal of the study was to characterize the broad-spectrum effectiveness of cross-reacting antibodies produced in response to whole cells (Mc) or purified outer membrane proteins (OMPs) of this pathogen.
Antisera were obtained from mice immunized with bacteria (anti-Mc sera) or OMPs (anti-OMPMc sera). The titers of cross-reactive antibodies were measured by whole-cell ELISA whereas their avidities by ELISA elution assay with sodium thiocyanate. The following functional methods were used: (1) the antibody-dependent bactericidal assay, (2) the opsonophagocytic assay with human THP-1 cell line, and (3) the blocking adhesion assay with human A549 epithelial cell line.
There were high titers of cross-reactive antibodies both in anti-Mc and anti-OMPMc sera. The titers of anti-OMPMc sera were higher than that of anti-Mc sera. The cross-reactive IgG produced exclusively to OMPs had stronger avidity comparing to those elicited against Mc. Additionally, these antibodies played a pivotal role in complement-mediated killing of heterologous M. catarrhalis isolates. The positive relationship between the total titer of complement-fixing murine subclasses IgG2a and IgG2b and the bactericidal titer was found for both anti-Mc and anti-OMPMc sera. The opsonophagocytic potency as well as reduction of bacterial attachment to human epithelium was much stronger for cross-reactive anti-OMPMc than cross-reactive anti-Mc antibodies.
The presence of cross-reactive antibodies with bactericidal, opsonophagocytic and bacterial adhesion blocking potency may be additional source to control host-Moraxella catarrhalis interaction especially when the host immune status is prone to a new infection.

P4.04.04
Secondary bacterial pneumonia following flu infection: Pathogenesis and protective immunity

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Seasonal and pandemic influenza (IAV) has cost us millions of lives and inestimable amounts of money. Studies now indicate secondary bacterial pneumonia is responsible for most flu-associated fatalities. Nonencapsulated-nontypeable *Haemophilus influenzae* (NTHi) is a growing clinical concern in the context of IAV co-infections due its high antigenic variability, increasing antibiotic resistance, vaccine unavailability, and insufficient knowledge. In this study we have developed a highly susceptible murine model to IAV/NTHi co-infections. Less than 10 CFU of NTHi kill all IAV-infected mice 4 days post-bacterial infection (pbi), due partially to NTHi uncontrolled growth. Over 10^6 CFU are found in lungs of co-infected mice in contrast to the undetectable levels in single infected-mice 4 days pbi. By day 6 pbi, co-infected mice become bacteremic before succumbing to infection. To elucidate this interaction and move towards the discovery of vaccines, we utilized the novel High-throughput Insertion-Tracking by Deep-Sequencing (HITS) assay to identify virulent factors essential during IAV/NTHi co-infections. We validated HITS results *in vivo* and identified one mutant strain, NTgalU, as a potential vaccine candidate. NTgalU-immunized mice are protected against an IAV/NTHi challenge, whereas 100% of NTHi-immunized mice perish after challenge. NTgalU immunization potentiates the cellular immune response both quantitatively and functionally with >20% more $CD4^+$ and $CD8^+$ T cells and about 30% more IFN-g production. Finally, naïve mice receiving memory lymphocytes from NTgalU-vaccinated mice are protected against an IAV/NTHi co-infection. Future studies include the assessment of more virulent factors useful toward vaccine development against other pulmonary bacterial co-infections.

P4.04.05
Induction of Mast Cell Extracellular Traps by intracellular bacteria

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Mast cells (MCs) are involved in various immunological phenomena, as allergies and infectious diseases. Extracellular and intracellular bacteria are able to activate MC. A mechanism by which MCs

respond to bacterial infections is by the production of Mast Cell Extracellular Traps (MCETs), which are formed by chromatin covered of granule proteins with antimicrobial activity, in a process dependent on the production of ROS. Although extracellular bacteria induce the production MCETs, it is unknown whether intracellular bacteria induce this process. In the present study we evaluated the production of MCETs by Mycobacterium tuberculosis (Mtb) and Listeria monocytogenes (Lm). MCs were exposed to PMA, Mtb or Lm at different times. MCETs formation was evaluated by fluorescent microscopy. Morphological changes were assessed by transmission electron microscopy and production of ROS was evaluated with the NBT reduction. MCs showed a fast release of DNA when stimulated with PMA or Lm. In contrast, Mtb produced a slower release of DNA. PMA-induced MCETs showed presence of histone and tryptase. Moreover, these cells had changes in the nuclear envelope and the DNA outside the cell nucleus when stimulated with PMA or Lm. Interestingly, the MCETs showed antimicrobial activity against Lm, but not against Mtb. Finally, MCs showed significant production of ROS when challenged with PMA or Lm. Our results suggest that the intracellular bacteria tested are capable to induce the production of MCETs, and have antimicrobial activity against Lm but not Mtb.

P4.04.06
Plasmin Cleaves Human complement C3b and C5 Proteins in the Presence of Leptospira interrogans Proteins: A New Role of LigA and LigB for complement Immune Evasion

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The spirochete Leptospira interrogans is a highly invasive pathogen of worldwide public health importance. The ability of Leptospira to evade the complement system is crucial for a successful infection. An important immune evasion strategy employed by pathogens is the acquisition of plasminogen and its conversion to plasmin, which is able to cleave the complement components C3b and C5 on the pathogen's surface. Leptospiral immunoglobulin-like (Lig) proteins are multifunctional molecules that interact with extracellular matrix components and host complement regulatory proteins. We therefore decided to evaluate the interaction of these proteins with plasminogen as a complement evasion mechanism. Both C- and N-terminal portions of LigA and LigB genes were cloned, expressed and purified as recombinant proteins in E. coli. We demonstrate that the Lig proteins were able to bind plasminogen, which was activated to plasmin by addition of human urokinase type plasminogen activator (uPA). Lig-plasminogen interactions were significantly inhibited by the lysine analog aminocaproic acid, suggesting that the lysine-binding sites of the plasminogen molecule play a role in the binding. We also show that plasminogen bound to Lig proteins was converted to active plasmin and this enzyme was able to degrade fibrinogen, C3b and C5. These cleavages inactivate C3b and C5, preventing progression of the complement cascade and blocking the three complement pathways. The identification of these leptospiral ligands is of great relevance since they may represent targets for immune interference. We conclude that Lig proteins are multifunctional molecules that contribute to leptospiral adhesion and immune evasion.

P4.04.07
A murine air pouch model to study group A streptococcal pathogenesis and protective immunity

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Group A Streptococcus (GAS) is a major human pathogen worldwide, responsible for both local and systemic infections. In this work we apply the air pouch model to study GAS infection, in particular the role of a well-known virulence factor – the chemokine-degrading protease SpyCEP. Dorsolateral air pouches were inflated in CD1 mice by subcutaneous injection of 3 ml air on day 1 and day 4. On day 6, 3348 wild type or 3348*ΔspyCEP* knock out strains were injected into the pouch. At 2, 4 or 24 h after infection an air pouch lavage was performed. Lavage samples were processed for the parallel analysis of bacterial content, cell recruitment and chemokines. Mice infected with 3348Δ*spyCEP* showed a tendency to have more neutrophils and monocytes compared to mice infected with the wild type 3348 strain. Concomitantly, the chemokines KC, LIX, and MIP-2 were drastically increased in mice infected with the SpyCEP knockout strain, and growth of this mutant strain was reduced compared to the wild type. We extended the application of the air pouch model to evaluate protective immunity. Mice were immunized with adjuvant only, M protein, or a combination of GAS antigens (including SpyCEP), and samples were collected and analyzed 24 hours post infection. We observed that vaccinated groups showed significant bacterial growth reduction compared to the group immunized with adjuvant only. Taken together, our data suggest that the air pouch model promises broad application in the study of immune system response after streptococcal infection.

P4.04.08
Investigation of innate immunity genes on the japanese oak silkworm, *Anthereae yamamai*

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Japanese oak silkworm, *Antheraea yamamai*, is a large moth in the family and distributed throughout China, Korea, and Japan. Japanese oak silkworms cocoon is superior to that of the domestic silkworm in gloss, durability, and wrinkle. The main targets of our investigation were innate immune-related genes and effect molecules, the key components of humoral defense reaction in the japanese oak silkworm, *A. yamamai.* The expressed sequence tag analysis was used for screening of genes that are induced to injected bacterial lipopolysaccharide(LPS). Analysis of 1180 cDNAs resulted in identification of 31 immune-related genes including pathogen recognition, modulation and putative antimicrobial peptides. In addition, we compared acidic extracted hemolymph protein profiles between control and immune-challenged *A. yamamai* larvae using two-dimensional gel electrophoresis(2-DE). Protein spots showing differential expression were identified by MALDI-TOF and MS/MS spectra. Subsequently, three of them were estimated to be attacin antibacterial peptides with high coverage of sequence. These attacin peptides were intensively induced after an immune-challenge with LPS.

P4.04.09
An inhibitor of sirtuin 1 and sirtuin 2, cambinol, impairs MAPK signaling, inhibits inflammatory and innate immune responses and protects from septic shock

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Background: The histone deacetylase family comprises HDAC1-11 and Sirtuins (SIRT)1-7. SIRTs are involved in the control of metabolism and cell proliferation, and in the development of oncologic, cardiovascular and neurodegenerative diseases. Cambinol was recently identified as a compound inhibiting SIRT1/2 with anti-tumor activity *in vivo*. As HDAC1-1 inhibitors are potent anti-inflammatory drugs, we questioned whether cambinol also modulates inflammatory and innate immune responses *in vitro* and *in vivo*.
Methods: Macrophages, DCs, splenocytes, whole blood and PBMCs were stimulated with LPS, Pam3CSK4, CpG, TSST-1, SEB, *E.coli* and *S.aureus* before measuring activation of NF-kB and MAPK pathways, production of cytokines, NO and CD40, and proliferation. Mice were challenged with lethal doses of LPS or *K.pneumonia*.
Results: Cambinol inhibited the expression of cytokines, NO and CD40 by mouse and humans immune cells. Unexpectedly, selective inhibitors of SIRT1 (EX-527, CHIC-35) and SIRT2 (AGK2, AK7) used alone or in combination had no impact on cytokine production by macrophages, suggesting that cambinol acts by targeting more than just SIRT1/2. Cambinol impaired stimulus-induced phosphorylation of

MEKs and MAPKs. In line with these results, cambinol treatment reduced TNF blood levels (*P* = 0.04) and improved survival to endotoxic shock (from 8% to 46%; P < 0.001) and acute *K.pneumoniae* pneumonia (from 13% to 60%; P = 0.013).
Conclusions: Cambinol has powerful anti-inflammatory activity and protects from septic shock. Pharmacological inhibitors structurally related to cambinol have promising therapeutic potential for the treatment of pathologies characterized by acute and chronic inflammatory responses.

P4.04.10
Study of the impact of Sirtuin 2 knockout on innate immune responses and sepsis

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Background: Sirtuins (SIRT1-7) are NAD-dependent histone deacetylases. Sirtuins are the center of great interest due to their role in influencing metabolism, ageing and age-related diseases. The impact of sirtuins on immune responses is poorly described, and nothing is known about the role of SIRT2. The aim of the study was to analyze the influence of SIRT2 knockout on immune parameters in vitro and in vivo.
Methods: Thymic (DN1-4, DP, SP) and splenic (DN, SP, naïve and memory T cells; immature and mature B-cells; cDCs; pDCs) populations were analyzed by FACS. Bone marrow derived macrophages and DCs and splenocytes were stimulated with LPS, Pam3CSK4, CpG, *E.coli*, *S.aureus*, TSST-1, SEB, anti-CD3+CD28 before measuring cytokines, SIRTs, CD40, and proliferation. Mice (n=6-16) were challenged with LPS, TNF/D-gal, *E.coli*, *K.pneumonia*.
Results: SIRT2 is the most expressed sirtuin in myeloid cells. SIRT2-/- mice born at expected Mendelian ratio, develop normally, and express normal proportions and absolute numbers of thymocytes, and splenic T-cells, B-cells, DCs and granulocytes. SIRT2-/- macrophages, DCs and splenocytes express cytokines and CD40 (RNA and protein), and proliferate like SIRT2+/+. SIRT2-/- and SIRT2+/+ mice respond similarly (cytokine levels, bacterial counts and mortality) to non-severe and lethal septic and toxic shock.
Conclusions: SIRT2 knockout has no dramatic impact on the development of immune cells and on innate immune responses *in vitro* and *in vivo*. Considering that SIRT2 may participate to control metabolic homeostasis, we are currently assessing the impact of SIRT2 deficiency on innate immune responses under metabolic stress.

P4.04.11
Immune-response in lesional skin of secondary syphilis

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Tissue immune response is very important in several bacterial and viral infections determining granulomatous reactions (mycobacteria), local immunodeficiency (HHV8-HIV) or disregulation (HCV-HSV). Few data are known about the cutaneous host immunoresponse during infection by Treponema pallidum (TP), the causative agent of syphilis . In our study, by using several monoclonal antibodies (Mabs) specific for lymphoid subsets, monocytes-macrophages and an anti TP polyclonal antibody, we analyzed the characteristic of the infiltrate of 5 cases of secondary syphilis lesional skin. TP bacteria were mainly localized in the epithelial sheets, even if some TP were also detected in the dermis (perivascular areas). The infiltrate was characterized, as expected, by large number of plasmacytes, as detected by CD138, CD79a and MUM-1. Plasmacytes showed active in situ proliferation, as demonstrated by MIB-1/Ki-67 proliferation marker. An abundant epidermotropic T-lymphocytes infiltrate consisting of the CD8+ subset was easily demonstrated in the superficial dermis, where several macrophages (M1 and M2) were also detected. Unexpectedly the staining of dendritic cells by using anti CD1a Mab evidenced the quite complete absence of Langerhans cells, both in the epidermis and in the dermis. These data suggest the presence of a characteristic immune-disregulation in the skin affected from TP infections (abundant plasmacytes and CD8+ lymphocytes and absence of dendritic Langerhans cells).

P4.04.12
The role of neutrophil extracellular traps (NETs) in the host defense against Gram-negative sepsis (melioidosis)

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Introduction: Neutrophil extracellular traps (NETs) have emerged as a central part of innate host response to bacteria. Neutrophils release extracellular fibers that ensnare bacteria and, ultimately, kill their target. Major components of NETs include extracellular DNA-histone complexes (nucleosomes) and neutrophil-elastase. We studied the role of NETs in *B. pseudomallei* infection (melioidosis), which is an important cause of sepsis in SE-Asia.
Methods: We assessed circulating nucleosomes and neutrophil-elastase (HNE-A1AT) in 43 septic melioidosis patients and 83 controls. Isolated human neutrophils were stimulated *in vitro* with *B. pseudomallei*: NET formation was quantified by measuring nucleosome, HNE-A1AT and cleaved DNA release. NET function was investigated *in vivo* in mice infected with *B. pseudomallei* and treated with DNAse to degrade the DNA backbone of NETs. Mice were sacrificed post infection to assess bacterial loads, inflammation and pathology.
Results: Nucleosome and HNE-A1AT levels were elevated in melioidosis patients, and gradually normalized in those who survived. In line, human neutrophils stimulated with *B. pseudomallei* induced NET formation and showed increased levels of elastase and cleaved DNA. In mice, disruption of intravascular NETs by intravenous DNAse administration resulted in decreased nucleosome plasma levels compared to controls at 24 and 72 hour after infection but no differences were seen on bacterial loads and cytokine release.
Conclusion: *B. pseudomallei* induce NET formation by human neutrophils which is reflected by strongly increased levels of NET related-components in melioidosis patients. Surprisingly, disruption of NET production leads to a decrease in circulating nucleosomes but not to defective clearance of *B. pseudomallei*.

P4.04.13
Protease Activated Receptor 4 contributes to host defense in Streptococcus pneumoniae induced pneumonia

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BACKGROUND: Streptococcus (S.) pneumoniae is a common causative pathogen of pneumonia and sepsis. Pneumonia and sepsis are associated with enhanced activation of coagulation, resulting in the production of several host derived proteases at the primary site of infection and in the circulation. Serine proteases cleave protease activated receptors (PARs), which form a molecular link between

coagulation and inflammation. PAR4 is one of 4 subtypes of PARs and is widely expressed by multiple cell types in the respiratory tract implicated in pulmonary inflammation, by immune cells and by platelets. In mice, PAR4 is the only thrombin receptor expressed by platelets.
AIMS: To determine the contribution of PAR4 to the host response during pneumococcal pneumonia.
METHODS: Pneumonia was induced by intranasal inoculation with S. pneumoniae in PAR4-deficient (par4-/-) and wild-type mice. Mice were sacrificed after 6, 24 or 48 hours. Blood, lungs, liver and spleen were collected for analyses.
RESULTS: After 48 hours of infection, higher bacterial loads were found in the lungs of par4-/- mice ($P < 0.05$), accompanied by higher histopathology scores and increased cytokine levels ($P < 0.05$). At 24 hours post infection par4-/- mice displayed lower plasma and lung thrombin-antithrombin complex (TATc) levels, whereas they showed higher TATc concentrations at 48 hours. Of interest, par4-/- mice displayed a trend towards lower plasma platelet factor 4 levels at this latter time point, indicative of decreased platelet activation in spite of the apparently increased thrombin formation.
CONCLUSION: Our findings suggest that PAR4 contributes to antibacterial defence during murine pneumococcal pneumonia.

P4.04.14
Streptococcus pneumoniae serine protease HtrA, but not SFP or PrtA, is a major virulence factor in pneumonia

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BACKGROUND: Serine protease orthologs expressed by a variety of bacteria have been found of importance for virulence. Previous studies have identified two serine proteases in S. pneumoniae, HtrA (high-temperature requirement A) and PtrA (cell wall-associated serine protease A), that contributed to virulence in models of pneumonia and intraperitoneal infection respectively.
OBJECTIVE: To identify additional S. pneumoniae serine proteases and determine their role in virulence in vivo.
METHODS: The S. pneumoniae D39 genome was searched for genes encoding putative serine proteases. Mutant D39 strains lacking serine proteases were constructed by in-frame insertion deletion mutagenesis. Pneumonia was induced in C57Bl/6 mice by intranasal infection with wild-type or mutant D39 (50.000 or 500.000 colony forming units); mice were euthanized after 6 or 48 hours and blood and organs were collected for analyses.
RESULTS: The S. pneumoniae D39 genome contains three putative serine proteases: HtrA, Subtilase Family Protein (SFP) and PrtA. After low dose infection, D39ΔHtrA showed reduced virulence, as reflected by a strongly increased bacterial clearance in lungs, distant organs and no detectable bacteria in the blood. Notably, pneumococcal burdens were also modestly but significantly lower in lungs after infection with D39ΔSFP. After high dose infection, reduced virulence only remained for D39ΔHtrA; D39ΔPrtA induced significantly less lung inflammation without influencing bacterial loads.
CONCLUSIONS: These data confirm the important role for HtrA in S. pneumoniae virulence. SFP may facilitate S. pneumoniae growth after low dose infection. In contrast to its reported role in intraperitoneal infection, PrtA does not contribute to virulence in pneumococcal pneumonia.

P4.04.15
Th9 and Th22 cytokines profiles expressed by TCD4+ lymphocytes activated with different Aggregatibacter actinomycetemcomitans-serotypes

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Objective: Periodontitis represents a heterogenic group of periodontal infections elicited by bacteria residing at the subgingival biofilm. Although bacteria may cause direct tissue destruction, their pathogenicity relies mainly on the activation of Th1 and Th17 lymphocytes. This biofilm is constituted by a broad variety of bacterial species, only a limited number has been associated with the periodontitis aetiology, among them, Aggregatibacter actinomycetemcomitans.
Based on the antigenicity of LPS O-polysaccharide, distinct serotypes have been described. The aim of this study was to determine the Th9 and Th22 master-switch genes and cytokines expression on TCD4+ lymphocytes stimulated with a, b and c A. actinomycetemcomitans serotypes.
Method: Using different multiplicity of infections of a, b and c of A. actinomycetemcomitans serotypes, the mRNA expression and secretion levels for IL-9, IL-17F, IL-22 and expression levels of AHR and SPU-1/PU.1 on stimulated TCD4 lymphocytes were quantified by PCR.
Result: On TCD4 lymphocytes, A. actinomycetemcomitans b serotype induced higher levels of cytokines compared with the other strains. In addition, b serotype induced higher levels of Th9 and lower levels of Th22 cytokines, and at lower MOI than TCD4 lymphocytes stimulated with the other strains.
Conclusion: These results demonstrate a different potential of O-polysaccharide A. actinomycetemcomitans serotypes to induce Th9 or Th22 differentiation on TCD4+ T lymphocytes and a higher capacity of b serotype to trigger Th9 cytokines.

P4.04.16
Undernutrition and birth season influence the antibody response to B. pertussis in children of Northern Senegal

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Background:
While vaccines elicit a protective response in most recipients, studies suggest that environmental and nutritional factors can influence the strength of the individual response to immunization and to subsequent natural infectious challenges.
Methods:
We conducted a longitudinal survey in Senegal to assess the individual response to B. pertussis, a respiratory disease against which Senegalese children are vaccinated before the age of one. A cohort of 203 children aged 1 to 10 from four villages of the Senegal River Valley was followed-up for one year. During that period, four visits have been made to the villages to assess the immunological and the nutritional status of the children and to determine risk factors involved in the modulation of their humoral immune response to B. pertussis toxin.
Results:
A multivariate model has demonstrated that birth season and nutritional status appeared to modulate humoral response to pertussis toxin. Moreover, response to B. pertussis was dependent on age, village and time of visit.
Conclusions:
These results are consistent with the hypothesis that environmental and nutritional factors modulate children's response to pertussis following natural infection or vaccination.

P4.04.17
Immuno-microbiological aspects of chronic apical periodontitis

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Microbiological research of 24 root canals discharge at patients with chronic apical periodontitis was conducted. In assessing the persistence of opportunistic microbial species identified a number of cultures of obligate and facultative forms, of which dominated gram-positive species. Thus, in 62.9% of cases identified members of the genus Staphylococcus, including most types of coagulasa-positive met (St. aureus, intermedius et hyicus). In 70.4% of cases were found members of the genus Streptococcus, among which the most met Str. pyogenes and enterococci. Of gram-negative Neisseria species predominated (62.5%), the family Enterobacteriaceae (Proteus mirabilis et Citrobacter diversus) represented 25%. Growth of Lactobacillus sp. detected in 33% of cases, and Corynebacterium sp. - in 11%. Anaerobes (Prevotella, Fusobacterium, Peptococcus etc.)

isolated in 29.6%. At this patients found that the total protein concentration in root canals at maximum value observed in radicular cyst and minimal - in chronic periodontitis. During exacerbation of chronic periodontitis, the total protein concentration in the discharge of the root canal rises slightly, while the number of white blood cells is significantly increased (mainly granulocytes). Thus, it can be assumed that chronic apical periodontitis is caused predominantly by bacteria of the genus Staphylococcus and Streptococcus, and chronic inflammation is supported by the presence of a number of leukocyte that produce massive amounts of protein molecules (globulin).

P4.04.18
Thrombospondin 1 expression in THP-1 cells stimulated with periodontopathic bacteria

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Periodontitis is an inflammatory disease infected with periodontopathic bacteria, causing tissue destruction and bone loss around teeth. Recently periodontitis is reported to have a relationship to systemic disease such as cardiovascular disease, diabetes mellitus and preterm birth. Porpyromonas gingivalis (P.gingivalis) is one of major peridontopathic bacteria and is detected in not periodontitis region but a blood vessel. LPS of P.gingivalis was reported to activate THP-1 cells, a human acute monocytic leukemic cell line, through TLR2 and to induce inflammation in periodontitis. To identify P.gingivalis LPS effect in THP-1 cells, DNA array was used. In DNA array analysis, we focused high thrombospondin 1 (TSP-1) expression in THP-1 cells. TSP-1 is a multifunctional extracellular matrix protein and generally elevated in inflammatory processes and modulates the immune response at sites of inflammation. To detect TSP-1 expression in periodontitis, inflammatory granulation tissues were collected from periodontitis region and real time RT-PCR was performed. In these inflammatory periodontal tissues, TSP-1 mRNA were enhanced in comparison to healthy sites. In vitro experiments, P.gingivalis LPS increased TSP-1 mRNA expression in THP-1 cells dose and time dependent manner. Pam2CSK4(TLR2 ligand) and E.coli LPS (TLR4 ligand) also increased TSP-1 mRNA expression in THP-1 cells. These data suggested that P.gingivalis LPS was involved in TSP-1 introduction in inflammatory periodontal region via TLR2 receptor.

P4.04.19
Development of an in vitro infection model for the evaluation of viable, macrophage-engulfed Pseudomonas aeruginosa

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Pseudomonas aeruginosa, an increasingly multi-drug resistant human pathogen, is now one of the top three causes of opportunistic infection and there is much interest in identifying novel therapeutic targets for treatment. Though both *P. aeruginosa* virulence determinants and the macrophage responses to bacterial pathogens have been well characterised over the last several decades, most recent work acknowledges the complex interaction between the human host and the pathogen as an on-going dialogue of virulence factors adapting to the continuum that is the immune response. Therapeutic target identification is now moving beyond isolated constituent stressor studies to a more complex map of crosstalk between host and pathogen in the presence of naturally produced immune compounds in physiologically correct conditions. To address this, we have developed an *in vitro* infection model for assessing *P. aeruginosa* responses to challenge by immunocompetent murine macrophages. Furthermore, this method was adapted to examine the global transcriptional events taking place during macrophage exposure. This new technique allowed for the evaluation of individual infection parameters side-by-side in a high-throughput fashion and has the potential flexibility to be adapted for evaluation of the *P. aeruginosa* response to challenge by other phagocytic cell types (e.g. neutrophils); as well as to gauge, in tandem, how the eukaryotic host acts in response to the bacteria by employing established immunological assays. The method thus contributes a valuable tool for understanding of the greater biological process encompassing the establishment of disease.

P4.04.20
Protective immunity of intranasally infected mice with Brucella Melitensis

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The α-proteobacterium Brucella is a gram-negative intracellular bacterium responsible for brucellosis that causes abortion and infertility in animals and undulant fever in humans. If untreated, the human brucellosis can become chronic with severe clinical complications. Although eradicated in most of developed countries, brucellosis remains a worldwide public health problem.
The intraperitoneal inoculation of mice is the most used experimental model to study Brucella infection. However, natural infections are generated by contaminated food intake or aerosol. Bacteria have to cope with effectors of the mucosal immune system, which are bypassed by intraperitoneal inoculation. Thus, we have developed an intranasal infectious model in mice, using a B.melitensis strain expressing the fluorescent mCherry protein.
We have focused our study on the identification of essential signaling pathways and cells controlling the infection. IL-12 dependent IFNγ producing T CD4+ (Th1) lymphocytes played a dominant role during the infection, when compared to CD8+ T cells and B cells. We also characterized, by immuno-histofluorescence, the main alterations due to the infection in lung, spleen and liver.
Finally, we have analyzed the secondary immune response against B. melitensis in intranasally vaccinated mice. In order to determine the importance of specific antibodies, we have compared the ability of vaccinated wild-type and B-lymphocytes deficient mice to control infection. Our preliminary data suggest a major role of circulating antibodies in the control of secondary infection.
Globally, our results in intranasal infectious model could improve our ability to generate a protective vaccine against brucellosis and others infectious pathologies due to intracellular bacteria.

P4.04.21
DAP12 impairs host defense in pneumococcal pneumonia

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Background: Streptococcus pneumoniae is the most common cause of community-acquired pneumonia. DNAX activation protein of 12 kDa (DAP12) is a transmembrane protein that functions as a signal transduction adaptor molecule that associates with a variety of receptors on NK and myeloid cells. DAP12 can either activate or inhibit immune responses.
Aim: To determine the role of DAP12 in pneumococcal pneumonia.
Methods: Pneumonia was induced in DAP12 knockout (KO) and wild-type (WT) mice by intranasal inoculation with viable S. pneumoniae. Pulmonary DAP12 expression was studied using qRT-PCR. Lungs, spleen, liver and blood were harvested at 6, 24 and 48 hour post-infection for quantitative cultures. Cytokines, chemokines and MPO were measured in lung homogenates. Responsiveness towards S. pneumoniae of DAP12 KO versus WT alveolar macrophages was tested in vitro.
Results: Alveolar macrophages lacking DAP12 produced more TNF-α than WT cells upon stimulation with S. pneumoniae in vitro, suggesting a role for DAP12 in the initiation of innate immunity against this pathogen. WT mice demonstrated enhanced pulmonary DAP12 expression during pneumococcal pneumonia. DAP12 KO mice showed prolonged survival relative to WT mice which was accompanied by decreased bacterial loads in lungs, blood and distant organs at 24 and 48 hours post-infection. In addition, at 24 and 48 hours after infection pulmonary inflammation was decreased in DAP12 KO mice as reflected by lower inflammation scores and reduced chemokine and MPO concentrations.

Conclusion: DAP12 impairs host defense during pneumococcal pneumonia.

P4.04.22
TREM-1 improves host defense in pneumococcal pneumonia

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Background: Streptococcus pneumoniae is the most common cause of community-acquired pneumonia. Triggering receptor expressed on myeloid cells 1 (TREM-1) is an activating receptor that is primarily expressed on myeloid cells. Through an unknown ligand TREM-1 enhances Toll- and NOD-like receptor signaling.
Aim: To determine the role of TREM-1 in pneumococcal pneumonia.
Methods: Pneumonia was induced in TREM-1/3 knockout (KO) and wild-type (WT) mice by intranasal inoculation with S. pneumoniae. TREM-1 expression was investigated using qRT-PCR and FACS. Lungs, spleen, liver and blood were harvested at 6, 24 and 48 hour post-infection for quantitative cultures. Cytokines and MPO were measured in lung homogenates. Responsiveness towards S. pneumoniae of TREM-1/3 KO versus WT macrophages and the phagocytic capacity of TREM1/3 KO and WT neutrophils and macrophages were tested in vitro.
Results: WT mice demonstrated enhanced TREM-1 expression during pneumococcal pneumonia. TREM-1/3 KO mice were more susceptible to pneumococcal pneumonia, as reflected by a reduced survival and increased bacterial loads in lungs, blood and distant organs at 24 and 48 hours post infection. At 6 hours post-infection, pulmonary TNF-α, IL-1β and MPO levels were lower in TREM-1/3 KO mice despite equal bacterial loads, pointing to an impaired initiation of the host response. Macrophages lacking TREM-1/3 produced less TNF-α and KC than WT cells upon stimulation with S. pneumoniae and showed impaired phagocytosis in vitro. TREM-1/3 KO neutrophils showed no defect in their capacity to phagocytose S. pneumoniae.
Conclusion: TREM-1 contributes to protective immunity during pneumococcal pneumonia most likely through its expression on macrophages.

P4.04.23
Immune Evasion by Staphylococcal Superantigen-Like Protein 10

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Staphylococcus aureus is a versatile pathogen that remains the most common cause of hospital acquired infections world-wide. The rapid spread of antibiotic resistant *S. aureus* strains through human communities calls for a better understanding of its pathogenicity.
Staphylococcal superantigen like protein 10 (SSL10) is a highly conserved 26 kD protein that acts as an immune evasion molecule targeting multiple aspects of the host immune system. A strong affinity to immunoglobulin G (IgG) has been observed. In contrast to protein A and Sbi (Staphylococcus aureus binder of IgG), two other IgG binding proteins produced by *S. aureus*, SSL10 shows a strict specificity to the IgG γ1 subclass (IgG1) and the binding is limited to human and non-human primate species. IgG1 is the most abundant IgG subclass in circulation and is one of the major contributors for complement fixation. Interestingly, the SSL10/IgG1 interaction was shown to involve the Cγ2 domain of IgG1, in proximity to the C1q and Fcγ receptor recognition sites, highlighting the importance of SSL10 in *S. aureus* evasion from IgG-mediated immune attack. We have identified an interface on SSL10 that plays a crucial role in mediating its binding to IgG1. Mutations made to this binding site provide important insight on how SSL10 contributes to bacterial survival, in particular the impact on IgG1-mediated phagocytosis and complement activation.
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P4.04.24
State of cellular immunity in children with Recurrent Respiratory Tract Infections before and after vaccination Pneumo23 and Influvac

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One of the actual tasks of Pediatrics is still the problem of Recurrent Respiratory Tract Infections in children (RRTİ).Common indicators of impaired immune response in RRTİ in children, is an imbalance of T- and B-cells. The purpose of the study is to investigate the state of cellular immunity CD3; CD4; CD8; CD19; CD16/56; IRI and cytokine system sickly children and selection of appropriate therapies, correcting violations.
We observed 56 children in RRTİ group without clinical manifestations of disease at the time of the study and during the previous month.
Discussion: Results of the study of immune status showed that in the majority of cases (91.7%) in children with RRTI were identified various immunological disorders, worn both alone and combined character. The absolute value of the level of CD4+ in children with RRTI was reduced by approximately 49.6%, compared with healthy children. The absolute value of the level of CD8+ in the examined children was reduced more than 2-fold, the absolute value of CD19+ study group was reduced two times in comparison with the control group. Lymphopenia and reduction of all types of lymphocytes were found in the analysis of cellular immunity in children with RRTI, neutrophils, both in quantitative and functional composition, is not much different from the parameters of healthy children. The immune status of sickly children, characterized by suppression of specific cellular immunity parameters in combination with the increased expression of activation markers and concentration of CD16/56+, which, should be regarded as triggering compensatory mechanisms regulating immunological interactions.

P4.04.25
Long-term protective immunity of multi-antigen outer-membrane protein vaccine against Orientia tsutsugamushi

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Scrub typhus is an acute, febrile, mite-borne infection in humans caused by Orientia tsutsugamushi. Since no vaccine is available at present, development of the vaccine is an urgent issue. In the current study, we identified the effectiveness of the combination of three outer-membrane proteins (22, 47 and 56 kDa) of Orientia tsutsugamushi on enhancing the protective immunity by using single, double or triple antigenic proteins. BALB/c mice were immunized intranasally three times with two-week interval. Humoral and cellular responses were performed one week after the last immunization. The production of TNF-α, IFN-γ, IL-17, and IL-2 in CD4+ T cells, and antigen-specific antibody increased in all immunized groups. Challenge was performed with lethal dose of Orientia tsutsugamushi via intraperitoneal route, four (early) and twenty four (long-term) weeks after the last immunization. In early challenge experiment, all immunized mice survived but the control and adjuvant only group all died by day 7. In the long-term challenge experiment, PBS and adjuvant-treated groups had the survival rate of 0% and 25%, respectively, while 100% survived in all three groups treated with single antigenic protein 22, 47, or 56kDa. Double antigen-immunization groups using the combination of 22/47 kDa, 22/56 kDa, and 47/56 kDa, and the triple antigen-immunization group, 22/47/56 kDa had the survival rate of 87.5%, 100%, 62.5%, and 100%, respectively. In summary, the current study shows that the vaccine strategy using the combination of double and triple antigen enhanced

the survival rate, vaccine efficacy, and also highly-effective long-term protective immunity against Orientia tsutsugamushi infection.

P4.04.26
Evaluation of antimicrobial effects of Silver, Zinc and Copper nanoparticles on different Bacillus species spores and vegetative cells

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Antimicrobial features of three metal nanoparticles were investigated on four species of Bacillus inclusive: B.cereus PTCC(1247),B.subtilis PTCC (6633), B.polymyxa PTCC(1020), B.stearothermophilus ATCC(7953). The average size of these nanoparticles was between 10-40 nm and in general they had a circular shape. The germicidal effect of nanoparticles were accomplished anchored in the test disc diffusion and review of sensitive region and the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for vegetative cells then observed that Bacillus species spores and vegetative cells were more sensitive than silver nanoparticles. As ascertained frae scanning electron microscopy. The study also revealed that the B.subtilis greatest sensitivity to these nanoparticles are measured up to other Bacillus. So that the MIC of B.subtilis on the silver, zinc and copper nanoparticles was measured 5 ppm, 750 ppm and 40 ppm, respectively. These bacteria spores are inhibited at 350 µg/ml concentration vicinity of silver nanoparticles and the time of zero. However, the nanozinc and nanocopper had no effect on Bacillus spores and all of them have been able to grow at the highest concentration of nanozinc.

P4.04.27
Differential immune cell responses of infected lung tissues in mice strains with different susceptibility to *Legionella pneumophila*

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Legionella pneumophila is an intracellular bacterium of host phagocytic cells that causes a severe pneumonia. *Naip5* gene was previously reported to be associated with susceptibility to *L. pneumophila* infection. To investigate difference in innate immune responses between the susceptible A/J and the resistant BALB/c mice, dynamics of immune cells and gene expression patterns of lung tissues were examined at 4, 8, 24, 48hr postinfection (pi) to *L. pneumophila* by flow cytometry and microarrays, respectively.
On FACS analyses, A/J mice showed a marked influx in neutrophils and a remarkable decrease in alveolar macrophages ($CD11b^-$ $CD11c^+$) at 4hpi, followed by influx of dendritic cells and $CD11b^+CD11c^-$ macrophages at 24 hpi. BALB/c mice showed similar, but lesser degree of cellular influx. In apoptosis assays using annexin V, A/J mice showed significantly increase of apoptotic alveolar macrophages (49% vs 13.5%) and dendritic cells (50.2% vs 25.4%) at 4hpi compared to BALB/c mice. The transcriptional profiling of the apoptosis-related genes showed the genes encoding Tnfsf13b, Casp1 and Bak1 were significantly induced only in A/J mice, whereas the genes encoding Tnfrsf12a, Tnf, Birc5 and Casp4 were more up-regulated in A/J than BALB/c mice. Particularly, the induction of genes involved in macrophage apoptosis namely CCL5 and CCR5 was significantly expressed only in A/J mice.
In conclusions, this study indicates that apoptosis of alveolar macrophages and dendritic cells, as well as influx of immune cells differ between A/J and BALB/c mice, as an important host defense mechanism at the early stage of *Legionella* infection.

P4.04.28
Poly(I:C) sensitization induces a cytokine imprinting mechanism in dendritic cells and aggravates septic peritonitis

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Viral infections inducing Type I IFN were shown to be detrimental, while IL-12p40 mediated protective effects in mouse models of polymicrobial peritonitis. However, the specific contribution of IFNβ which is the first Type I IFN expressed after pathogen recognition remains unknown. Therefore we analyzed the influence of a virus infection mimicked by poly(I:C) stimulation on the disease outcome in WT, IFNβ-/- and IFNAR-/- mice.
IFNAR-/- mice showed significantly increased resistance compared to WT mice while IFNβ-/- mice exhibited intermediate resistance in the colon ascendens stent peritonitis (CASP) model. Poly(I:C) prestimulation did not change the outcome in IFNAR-/- or IFNβ-/- mice but sensitized WT mice to CASP which was associated with a dysregulated cytokine profile and reduced peritoneal granulocyte numbers. Using the IFNβ/YFPxIL-12p40/GFP double reporter mouse model we showed that conventional DCs are responsible for the production of IFNβ and IL-12p40 after CASP surgery with or without poly(I:C) pretreatment.
We recapitulated the viral followed by bacterial challenge in vitro and sorted poly(I:C) stimulated GM-CSF BM-DCs according to their reporter allele expression and restimulated with different TLR ligands. Poly(I:C) restimulated cells exhibited a fixed phenotype only expressing the cytokine they were sorted for initially, while CpG and LPS restimulated cells showed more plasticity. This fixed cytokine expression phenotype was confirmed in vivo by transferring sorted poly(I:C) stimulated cells into mice followed by CASP surgery. This study reveals a functional imprinting in DC cytokine expression after primary poly(I:C) stimulation interfering with the generation of optimally adapted immune responses to secondary bacterial challenge.

P4.04.29
Cathepsin X inhibitor decrease the production of cytokines and monocyte microparticles on THP-1 macrophage-like cells stimulated with *Helicobacter pylori*

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The infection with *H. pylori* induces higher levels of cathepsin X in macrophages of the gastric mucosa. Active cathepsin X has been shown to regulate β-2 integrin-dependent adhesion and phagocytosis, which is crucial for effective antigen presentation and initiation of T cell dependent immune response. Microparticles have been considered as cellular debris for a long time. However recent findings associate them with cellular stimulation, activation, and apoptosis. Our hypothesis suggests that cathepsin X inhibitor could suppress microvesiculation and activation of macrophages. This process could result in the insufficient immune response to the infection with *H. pylori*. Macrophages like cells were differentiated from THP-1 cell line treated with PHA for 24h. The cells were than stimulated with *H. pylori* strains***, isolated from 20*** dyspeptic patients. The *in vitro* secretion of IL-1b, IL-6, IL-8, IL-10, IL-12, TNF and concentration of microparticles were measured with flow cytometry. Higher concentration of microparticles were observed in the cases of THP-1 macrophage like cells, which were treated with *H. pylori* alone than in the cases of the untreated cells ($p<0.01$). Cathepsin X inhibitor decreased the amount of microparticles compared to THP-1 cells stimulated with *H. pylori* alone ($p<0.05$). The *H. pylori* stimulation alone as well as the stimulation in combination of cathepsin X inhibitor increased the production of IL-8, IL-6, IL-10, TNF, IL-12 compared to the negative control. However, we observed lower production of IL-1β and IL-6 by the THP-1 cells stimulated with *H. pylori* in combination with cathepsin X inhibitor ($p<0.01$).

P4.04.30
Mechanisms underlying bacteria-infected macrophage cell death-mediated IFN-γ production by innate lymphocytes

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We have previously reported that the B6HO3 T-cell hybrdioma with a CD244+CD11c+ phenotype and subsets of innate lymphocytes respond to dying macrophages infected with bacteria by producing IFN-γ in both IL-12p70-dependent and independent manners and that approximately 50% of the IFN-γ-producing innate lymphocyte expressed the adhesion molecule CD11c (J. Immunol. 176:7576,2006; Scan. J. Immunol. 71:199,2010; Front. Immunol. 2:26,2011). We refer to this phenomenon as bacteria-infected macrophage cell death (BIMD)-mediated IFN-γ production, and here we explore the mechanisms underlying this phenomenon. We found that cellular conjugate formation between B6HO3 cells and bacterial infected macrophages allows the low amounts of IL-18 secreted by the infected macrophages to exert its influence on the interface of the conjugates, thereby inducing BIMD-mediated IFN-γ production by B6HO3 cells. Similarly, the innate lymphocytes that were able to adhere to macrophages and hence included abundant CD11c-positive NK, αβ T, and γδ T cells, had greater capability to exhibit BIMD-mediated IFN-γ production than non-adherent lymphocytes. Innate lymphocytes from C57BL/6 mice, which are prone to be biased towards Th1 responses, exhibited stronger BIMD-mediated IFN-γ production than those from BALB/c and DBA mice. Macrophages that were activated by IFN-γ beforehand and then infected with bacteria produced soluble factors that could inhibit BIMD-mediated IFN-γ production. Taken together, we propose that specific subsets of innate lymphocytes may form cell conjugates with resident tissue macrophages to monitor macrophage cell 'danger' caused by bacteria, thus protecting an individual from bacterial infection through the macrophage activation by BIMD-mediated IFN-γ production at early stages of infection.

P4.04.31
Mechanisms of NK cell-macrophage Bacillus anthracis crosstalk: a balance between stimulation by spores and differential disruption by toxins

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Bacillus anthracis, the etiological agent of anthrax, is a spore-forming bacterium that kills through a combination of acute bacterial infection and toxemia.
We evaluated how B. anthracis spores are recognized by the innate immune system and we determine strategies that are employed by bacteria to subvert and evade this response.
We demonstrated that IFN-γ production by NK cells in response to B. anthracis spores is dependent on macrophages, contact-dependent and IL-12, IL-18 and IL-15-dependent, where IL-12 regulates both NK cells and macrophages activation.
B. anthracis toxins similarly disrupted IFN-γ production by spore-stimulated splenocytes, but the mechanism of inhibition was different for each toxin. LT acted at the macrophage and NK cell levels, whereas ET acted at the macrophage level. Both ET and LT directly altered the innate ability of NK cells to exert their natural cytotoxicity function in vitro and in vivo. Thus, ET subverting action leads to dissociation in NK cell function.
Our in vivo data indicate that non-toxigenic capsulated B. anthracis, administrated subcutaneously, induced recruitment of NK cells and macrophages into the lymph node draining site of infection, where interaction between these cells occurs, probably allowing performing their surveillance function in containing infection at an early stage. Additionally, capsulated strain of B. anthracis producing only one toxin, ET or LT, impaired the NK cell and macrophage recruitment into the lymph node draining site of infection.
Our findings are important for understanding the initial host control mechanisms of anthrax and the bacterial evasion strategies to overcome innate host defences.

P4.04.32
Autophagy and cell death pathways in Shigella-infected MoDCs: a difficult choice

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The immune system responds to infection in a variety of ways, including activation of pathways that promote survival to eliciting programmed cell death. The initial response to infection is geared toward eradicating or containing the infection; this can be achieved by activation of inflammatory pathway, recruitment of immune cells to the site of infection, production of antimicrobial peptides and cytokines. If the condition is unresolved, cell death signaling pathways are engaged to eliminate the infected cells from the organism. These mechanisms might not be mutually exclusive. This complexity of regulatory mechanisms is observed in the invasive process of *Shigella* spp., an enteric pathogen that causes bacillary dysentery in humans. In epithelial cells, *Shigella* triggers NF-κB via peptidoglycan sensing by the PRR Nod1, thereby inducing the secretion of CXCL8. This pathogen is equally able to induce various types of cell death in different cell populations. Apoptosis, necrosis and pyroptosis constitute the principal mechanisms by which programmed host cell death occurs.
Here, we have investigated on the molecular mechanisms and cell effectors governing the balance between survival and cell death in monocyte-derived dendritic cells (MoDCs) infected with *Shigella.* Our results show that *Shigella* induces a rapid cell death of MoDCs, with hallmarks of both necrosis and apoptosis. We also demonstrate that autophagic pathway is engaged by infected-MoDCs, *via* LC3 lipidation. By chemically blocking the autophagic process, the rate of cell death of *Shigella*-infected MoDCs increased. Put together, our data highlight that MoDCs respond to *Shigella* infection by modulating cell death and autophagy.

P4.04.33
Group B Streptococcus and Streptococcus suis differently modulate bacterial interactions with dendritic cells and natural killer cells.

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Presently, the mechanisms underlying innate and adaptive immune responses to encapsulated bacteria have not been fully elucidated. Group B Streptococcus (GBS) and Streptococcus suis are well encapsulated streptococci that can cause meningitis and septicemia. Their capsular polysaccharide (CPS) is considered the major virulence factor and previous data suggest that they selectively modulate the functions of dendritic cells (DCs). However, neither the role of Natural Killer cells (NKs) nor the effects of DCs on NK activation is clearly defined in the context of infection by streptococcal species. Here, we infected single or co-cultures of mouse bone marrow-derived DCs and splenic NKs with GBS or S. suis. The role of CPS was evaluated by using non-encapsulated mutants. We observed that NKs alone failed to directly respond to bacterial infection and that DCs are required for NK activation. Under co-culture conditions, copious amounts of IFN-γ are produced by NKs during GBS infection. The production of IL-12p70, CXCL9 and CXCL10 are significantly increased in presence of NKs compared to DCs alone. Interestingly, S. suis-infected co-cultures produced higher levels of IFN-γ, with no change in IL-12p70 production and a decrease in CXC10 release. Transwell insert experiments showed that cytokine production by DCs and NK cells depends on both direct contact and soluble factors. The CPS does not seem to play a major role in these interactions. Together, these results demonstrate that GBS and S. suis modulate DC-NK crosstalk functions differently which may have important consequences in the development of adaptive immune response against these pathogens.

P4.04.34
Immunoregulative mechanism of the Leydig cell in the infection by expression of IL-1, IL-6, TGF-β, Fas and FasL mRNA

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To investigate the immune regulative mechanism of Leydig cells in the local infection of rat's testis. Methods: Ureaplasma Urealyticum(UU) was injected into rat's bladder, which mimicked an ascending infectious way, and at the same time culture medium was injected into rat's bladder as control. The rats were sacrificed at at the first, second and third week after injection respectively. Then pathological changes of in testis were analyzed by histological examination. At the same time Leydig cells were separated from rat's testis. The comparison of levels of IL-1, IL-6, TGF-**β**, Fas and FasL mRNA expression among the three groups was made by RT-PCR. Results: As compared with control group, the levels of IL-1, IL-6, TGF-**β** mRNA expression for UU supernatant and living UU groups increased; and levels of Fas and FasL mRNA expression decreased and increased respectively after UU infection. Conclusion: During anti-infective immunity, rat's Leydig cells may regulate immune function of the testis by changing the levels of IL-1, IL-6, TGF-**β**, Fas and FasL mRNA expression and may contribute to maintain immune privilege of the testis.

P4.04.35
Differential inflammasome activation in human macrophages between Salmonella Typhimurium and Salmonella Typhi

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Salmonella Typhi caused Typhoid fever, a systemic inflammatory response syndrome (SIRS) in humans but S. Typhimurium causes only gastroenteritis. When infecting humans, S. Typhi uses Vi capsule to evade host immune system and survive in macrophages to circulate in bloodstream. After proliferating in bone marrow, liver, and spleen, S. Typhi reenters into bloodstream, causing SIRS, and intestine, causing hemorrhage and perforation. S. Macrophages are the important reservoir for S. Typhi during the infection. The interaction of macrophages with S. Typhi and S. Typhmurium was studied. S. Typhi, rather than S. Typhimurium, induced less inflammation when infecting epithelial cells by virtue of Vi. But when infecting THP-1 macrophages in MOI=100, we found that within an hour S. Typhi with or without Vi caused more cell death than S. Typhimurium. It was confirned to be caspase-1-mediated pyroptosis. The IL-1β secretion induced by S. Typhi was also significantly higher than S. Typhimurium. We constructed a mutant library of S. Typhi to search early cell death related genes. We found that SPI-1 mutants induced less cell death as well as caspase-1 activation than the parent strain. Because S. Typhi existed in liver before reentering into intestine, we also determined the growth of the two serotypes in LB with or without bile, and found that the growth of S. Typhi was more rapid than S. Typhimurium in LB with bile. We concluded that S. Typhi is more adapted to bile environment than S. Tyhpimurium to induce caspase-1-mediated pyroptosis in macrophages, presumably by hyper-expressing SPI-1 genes.

P4.04.36
Oral infection with *Brucella abortus* 2308 induces systemic activation of B cells, but a downregulation of CD86 in intestine B cells

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B-cell activation induces the transcription of MHC II genes, the expression of receptors for cytokines, and the expression of costimulatory molecules such as CD40, CD69, CD80 and CD86. Since the 1970's it was reported the interaction of B cells with *Brucella* spp., and it was used for diagnosis of some B-cell malignancies. Recently, it was reported the probable regulation of the immunity anti *Brucella* through the production of TGF- and IL-10 by splenic B cells. The objective of the work here reported is to analyze the activation of B cells in the intestine of mice orally infected with *B. abortus*, since this is the natural route of infection in humans. BALB/c mice were orally infected with *B. abortus* 2308-GFP. At different time points, a group of mice were euthanized and the cells from Peyer's patches and mesenteric lymph node were stained with antibodies to costimulatory molecules coupled to fluorochromes. Mice inoculated with PBS were included as controls. Cells were analyzed by flow cytometry. CD40 and CD80 were expressed at one hour p.i. and peaked at 24 h p.i. At this time 24% of B cells were infected con *B. abortus*. The percentage of responder cells was higher than the apparently infected ones; this result suggests that the B cells are getting infected with a very low number of bacteria, however enough to activate them or some B cells may be activated by neighboring infected cells, such as dendritic cells.
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P4.04.37
The mechanism behind insufficient Streptococcus pyogenes clearance by autophagy in endothelial cells compared to epithelial cells

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Streptococcus pyogenes (Group A *Streptococcus*, GAS) is an important human pathogen and its interaction with blood vessels is critically important with respect to serious diseases such as bacteremia or multi-organ failure. Although GAS internalizes into non-phagocytic cells to escape immune surveillance and get nutrients, our previous reports show that invading GAS induces autophagy and is efficiently killed within lysosome-fused autophagosomes in epithelial cells. In the present study, we found that GAS can survive and multiply in endothelial cells, including human microvascular endothelial cells (HMEC-1) and human umbilical vein endothelial cells (HUVEC). Since we found that canonical starvation-induced autophagy is normal in endothelial cells, we examined selective autophagy and the formation of autophagosomes specific to GAS. As results, we found that the recruitments of ubiquitin and several core Atg proteins essential to autophagosome formation against GAS are significantly reduced in these cells compared with epithelial cells. Furthermore, we observed reduced autophagosome formation surrounding GAS in endothelial cells. In contrast, while in epithelial cells autophagosomes could directly surround GAS-containing damaged endosomes prior to GAS escape into the cytoplasm. Our data suggest that the high efficient clearance of GAS is achieved by selective-autophagy targeting to GAS-containing damaged endosomes and cytoplasmic GAS in epithelial cells, but that the deficiency in the selective targeting machinery of autophagosome formation in endothelial cells results in GAS multiplication and persistence within this cell type.

P4.04.38
Detailed antibody-profile of Helicobacter pylori infected individuals revealed by peptide array screening

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Helicobacter pylori infection is a cause of gastric ulcer and gastric cancer. A number of virulence factors are associated with risk of gastric cancer, including cagA, vacA and babA.
We used a peptide array screen to investigate the detailed pattern of antibody-responses to H. pylori proteins. Antigens were selected

based on a surface proteomic screen, in addition to antigens previously shown to be relevant based on different proteomic methods. Thus, 330 antigens were selected, and arrays of 10-mer peptides with a sequential 5 amino acid overlap covering this entire proteome were printed using the PepPERPrint technology. The arrays were incubated with sera from uninfected individuals as well as with sera from patients infected with H. pylori. The detailed pattern of antibody responses were detected using Cy3-conjugated anti-Ig antibodies.
The results showed that out of the 330 antigens tested, there were peptide domains with a strong differential antibody response in the H. pylori infected subjects in 125 antigens. Thus, in these 125 antigens there were a stretch of at least 3 consecutive peptides with a response higher in Hp+ vs Hp- individuals (f-test, cut-off $p < 0.00001$). The antigen with the strongest differential response in Hp+ indiviudals was cagA.
In conclusion, a screen using peptide arrays with consecutive overlapping peptides allowed detailed identification of the antibody response to H. pylori antigens. This analysis confirmed that cagA is a very strong immunogen, and showed that there is a strong antibody response to a large variety of different surface-associated H. pylori antigens.

P4.04.39
Mechanism of interleukin (IL)-17A-mediated enhancement of protective immunity against *Listeria monocytogenes* infection

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IL-17A participates in protective immunity against *Listeria monocytogenes* (Lm) infection and the major IL-17A-producing cells were T cell receptor (TCR) γδ T cells rather than Lm-specific Th17 cells. Mechanism of the IL-17A-dependent protection against Lm infection was addressed in the present report. On day 9 of Lm infection, IL-17A-deficient (Il17a-/-) mice showed impaired protection although Lm-specific protective Th1 and Tc1 cells were normally induced. Interestingly, naïve Il17a-/- mice adoptively transfer with primed CD4+ T cells from Lm-infected WT mice showed higher bacterial burden in the spleen and liver when compared to naive WT mice transfer with the primed CD4+ cells. Therefore, it is possible that IL-17A produced by TCRγδ T cells co-operate with antigen-specific Th1 cells to mount optimum protective immunity. However, IL-17A did not show any cooperative effect with IFN-γ when Lm-infected macrophages were cultured with the cytokines. In contrast, IL-17A induced expression of IL-22 in the infected liver, and IL-17A suppressed bacterial burden of Lm-infected hepatocyte line in combination with IL-22. The results suggest that IL-17A enhances protective immunity through induction of IL-22 and enhancement of protective innate response of non-hematopoietic cells.

P4.04.40
PKF, a secreted serine protease, allows Acinetobacter baumannii to evade innate immune responses

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Acinetobacter baumannii has emerged as a hospital adapted multidrug-resistant pathogen. We previously have identified a serine Protease required for resistance to complement Killing and suppression of biofilm Formation (PKF). We wanted to determine if PKF affects the survival of *A. baumannii* within the host and if expression of PKF is regulated. We used a mouse model of lung infection to examine the survival of a PKF mutant during infection. A quantitative biofilm assay was used to examine biofilm formation at different temperatures. Real-time PCR was used to assess PKF expression at different temperatures. Additionally, recombinant exogenous PKF was used to examine biofilm formation. We recovered significantly higher numbers of an *A. baumannii* PKF mutant at 24 hours from the lungs of infected mice as compared to the wild-type. At 37^0C there was a six-fold increase in the level of *pkf* transcription compared to bacteria grown at 26^0C. Biofilm formation was significantly increased at growth temperatures ranging from 25^0C to 33^0C as compared to 37^0C. Recombinant PKF failed to disperse biofilm once it had formed, but significantly suppressed biofilm formation. Also, recombinant PKF degraded complement components resulting in increased survival of *A. baumannii* in human serum. Bacteria can sense their environment and respond by regulating gene expression. As a result, *A. baumannii* can form biofilm at room temperature which may aid in the environmental persistence of the bacterium. Once in the host, increased expression of PKF appears to protect *A. baumannii* from the innate immune response.

P4.04.41
Effect of the stimulation with different serotypes Aggregatibacter actinomycetemcomitans in the activity of the lymphocytes TCD4+

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Objective: In *Aggregatibacter actinomycetemcomitans*, six serotypes have been described based on O-polysaccharide-LPS immunogenicity: a-f. These different serotypes induce distinct dendritic cell and T-cell responses. In particular, b serotype of *A. actinomycetemcomitans* induce a Th1- and Th17-type phenotype and immune response. The aim of this study was to determine the frequency of cellular proliferation either periodontal disease or health.
Methods: $CD4^+$T lymphocytes were isolated from chronic periodontitis patients and healthy subjects. T cells were activated with autologous dendritic cells primed with different *A. actinomycetemcomitans* serotypes at different multiplicity of infection. To determine the frequency of $CD4^+$T lymphocytes activated in response to the different bacterial serotypes, limiting dilution analysis was performed and cell proliferation was quantified. T lymphocytes exposed to non-induced dendritic cells were used as control. Statistical analyzed was performed by assuming a Poisson single-hit model.
Results: Lower frequencies of T lymphocytes able to respond to a and c serotypes were detected on periodontitis patients as compared with healthy individuals. The frequencies of T lymphocytes able to respond to b serotypes were higher on periodontitis patients than controls.
Conclusion: Serotype b of *A. actinomycetemcomitans* is associated with the host immune response induced during chronic periodontitis.

P4.04.42
A neutrophil elastase inhibitor improves LPS-induced acute lung injury in burn-injured mice

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Neutrophil elastase derived from activated neutrophils is well known to cause acute lung injury (ALI), particularly in septic patients. Complications of septic ALI severely worsen the prognoses of burn-injured patients; therefore, treatment is crucial in order to improve the typical poor outcomes. We herein investigated the efficacy of a neutrophil elastase inhibitor in treating pulmonary dysfunction following LPS-induced ALI in burn-injured mice. The mice received 20% full-thickness burn injuries and were challenged three days later with LPS. The mice were treated with sivelestat, a neutrophil elastase inhibitor, one hour before and six hours after the LPS challenge. The control mice were treated with saline instead of sivelestat. Although the burn injuries severely decreased survival following the LPS challenge, treatment with sivelestat remarkably increased survival. The burn injuries markedly enhanced pulmonary microvascular permeability, the MIP-2 levels in the pulmonary tissue and plasma and neutrophil pulmonary accumulation following the LPS challenge, resulting in deterioration of ALI. Notably, treatment with sivelestat suppressed the LPS-induced increases in vascular permeability, the MIP-2 levels and neutrophil accumulation in the lungs, thereby potently improving LPS-induced ALI following burn injury. Burn injuries can induce LPS hypersensitivity via augmentation of MIP-2 production, neutrophil accumulation in the lungs and pulmonary

microvascular permeability, thereby leading to the development of ALI and worsening the outcome. Sivelestat is effective for improving pulmonary dysfunction and survival following LPS challenges in burn-injured mice, suggesting its role as an effective therapeutic tool against the development of ALI following burn injury.

P4.04.43
Citrullination and proteolytic processing of chemokines by Porphyromonas gingivalis

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Chemokine activity is regulated at multiple levels including posttranslational modification (PTM), including proteolytic processing and citrullination. Oral infection with the bacterium *Porphyromonas gingivalis* can lead to a group of inflammatory disorders of the periodontal pocket, called periodontitis, characterized by periodontal tissue destruction, loss of alveolar bone and eventually tooth loss. *P. gingivalis* peptidylarginine deiminase (PPAD), an enzyme responsible for modification of arginine to citrulline, and bacterial proteases belonging to the gingipains are considered to be potential virulence factors. Since chemokine processing may be particularly important in microbial defense mechanisms, we investigated whether PTM of chemokines by these enzymes represents a way by which *P. gingivalis* regulates the host's inflammatory response. Therefore, different *P. gingivalis* strains were incubated with the chemokines CXCL8 or CXCL10 and PTM of these chemokines was investigated by ELISA and Edman degradation. For multiple strains, CXCL8 could be recovered after incubation, however, no significant citrullination of CXCL8 by PPAD could be detected for any of the tested strains. Some strains, i.e. Pg1, Pg2 and K14, were more efficient in degrading CXCL8. In contrast to the difference in cleavage efficiency measured for CXCL8 incubated with *P. gingivalis* strains, no difference in processing of CXCL8 for the strains supernatants could be seen. Sequencing of the CXCL8 incubated with supernatant or bacteria showed that CXCL8 is processed into its more potent 5-77 and 9-77 forms. In conclusion, this study demonstrates PTM of CXCL8 by gingipains of *P. gingivalis* and strains differences particularly affect the activity of the bacterial membrane-associated proteases.

P4.04.44
Intracellular crosstalk in Wnt5a expression by LPS

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Objectives: Periodontal diseases are infections that are caused by specific gram negative micro-organisms that eventually lead to loss of supporting structures of teeth, including resorption of the alveolar bone. Wnt signaling molecules play important roles in bone biology, apoptosis and chronic inflammation. Recent studies have suggested an association of these molecules with various disorders including cardiovascular diseases, rheumatoid arthritis and osteoarthritis. We previously reported that Wnt5a mRNA expression was up-regulated in chronic periodontitis tissue. In addition, we demonstrated that the expression of Wnt5a mRNA induced by LPS of P.gingivalis, an important member of the periodontopathic bacteria, was higher than the expression induced by E.coli LPS. In this study, we investigated the intracellular cross talk in Wnt5a mRNA expression by LPS from E.coli or P.gingivalis .
Methods: Human monocytic cell line THP-1 cells were stimulated with LPS from E.coli (TLR4 ligand) or P.gingivalis (TLR2 ligand). To identify the involvement of NF-κB or PI3K/Akt/mTOR signaling pathway in Wnt5a mRNA expression following stimulation with LPS, inhibition assay, luciferase reporter assay and western blot were performed.
Results and Conclusion: The expression of Wnt5a mRNA induced by P.gingivalis LPS was higher than the expression induced by E.coli LPS. The activated proteins of the NF-κB signaling are different between the stimulation by E.coli LPS and P.gingivalis LPS. The inhibition assay and luciferase assay showed crosstalk of NF-κB and PI3K/Akt/mTOR. Our study suggests that induction of Wnt5a by P gingivalis is regulated by crosstalk signaling between NF-κB and PI3K/Akt/mTOR.

P4.04.45
The transformation of subpopulations CD64+CD16-CD32+CD11b+, CD64+CD16+CD32+CD11b+, CD64-CD16+ CD32+CD11b+ of monocytes in premature newborn with congenital pneumonia

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Monocytes (MON) of different subpopulations, expressing or not expressing CD64 and/or CD16, differ in their phagocytic activity and the ability to generate reactive oxygen species ROS (E.Grade-Griebenow et al.,2000). We had studied 3 subpopulations of Mon in very preterm infants with congenital pneumonia associated with respiratory distress syndrome(RDS). Clinical-immunological study was carried out on 1-2 days of life of those patients. One-moment expression of surface molecules of membrane MON, - CD64, CD32, CD16, CD11b,- by flow cytometry, the level of the density of molecules expression by measure of the MFI had detected. We observed 11 deeply premature infants , control group-10 healthy full-term newborn. Patients were randomized into 2 groups: group 1-with severe congenital pneumonia, group 2- with very severe congenital pneumonia. We had shown for the first time the presence of subpopulations CD64+CD16-CD32+CD11b+, CD64+CD16+CD32+ CD11b+, CD64-CD16+CD32+CD11b+ MON in full-term newborns and the different transformation of the phenotype of these subpopulations in both groups of deeply premature neonates with congenital pneumonia associated with RDS. We detected some inadequate response to bacterial invasion in patients of group1, and mark transformation of different subpopulations MON associated with greatly aggravates the manifestation of the disease in group2. The detection of the subpopulation CD64-CD16+CD32+CD11b+ MON with measure of receptors density may be useful as diagnostic marker of severity of the inflammatory process in deep preterm infants.

P4.04.46
Gestational hypothyroidism and its effects on the offspring immune response against infection: Evaluation in a murine model of pneumococcal pneumonia

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Gestational hypothyroidism affects fetus neurodevelopment and leads to cognitive deficiencies. In rodents, this condition also affects lung development and produces differences in the amount and type of immune cells present in the spleen. Preliminary data from our laboratory showed that gestational hypothyroidism increases the intensity of an autoimmune disease at the central nervous system of the progeny. We think that the offspring gestated under hypothyroidism will have alterations in the performance and/or activation state of the immune cells during an infection. To evaluate

this, we have chosen to study the immune response against the pathogen *Streptococcus pneumoniae* in mice gestated in hypothyroid mothers (MMIo mice) or in control mothers. Mice were infected intranasally to cause pneumonia and the bacterial loads in lungs and brain, the immune populations present in lungs and bronchoalveolar lavage, the cytokine profiles, and lung histopathology were analyzed. We have found that both groups of mice showed a similar response against the infection, with MMIo mice displaying a tendency to be more resistant. Interestingly, all infected mice showed a marked decrease of the resident lung T- and B-cell populations. Our work explores for the first time the correlation between gestational hypothyroidism and the functionality of the offspring immune cells during infection. Grant Support: Fondecyt 1110604, Fondecyt 1100926 and Millennium Institute on Immunology and Immunotherapy P09/016-F.

P4.04.47

Specific pulse-width mild electrical stimulation induces p53 activation via p38 MAPK and ameliorates LPS-induced inflammatory cytokine response

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p53 is activated by diverse cell stresses such as DNA damage, oncogene activation and heat stress. Studies have shown that p53 works as a suppressor of inflammatory responses in virus or bacteria infected cells. Hence, functional activation of p53 could be beneficial for treatment of inflammatory diseases. p53 activation methods such as irradiation, hyperthermia and DNA-damaging reagents have the risk of cell damage. Therefore, a novel method of p53 activation without cell damage would be advantageous. In this study, we focused on mild electrical stimulation (MES) as a p53 activator, and determined whether MES can potentiate p53 function. HCT116 cells were treated with MES using electrical stimulation generator (Duration: 0.01~10 msec, Voltage: 1 V/cm, Pulse number: 55 pulse per sec). Protein extracts or RNA were analyzed for phospho-p53 and p53 target genes expression after MES treatment. p53 binding to p21 promoter was assessed by ChIP assay. MAPKs inhibitors were used to clarify the MES-induced p53 phosphorylation pathway. Peripheral macrophages (pMφ) from p53+/+ or p53-/- mice pre-treated with MES and LPS-induced inflammatory cytokines expression were assessed by quantitative-RT-PCR. Our results show that specific MES conditions can phosphorylate p53 and potentiate transcriptional function via activation of p38 pathway. Furthermore, MES treatment ameliorated LPS-induced upregulation of inflammatory cytokines such as IL-6, IL-1βand TNFαin p53+/+ mouse-derived pMφ but not in p53-/- pMφ. Our findings suggest that MES is a novel p53 physiological activator and MES might be a new therapeutic strategy for inflammatory diseases.

P4.04.48

***Legionella pneumophila* thymidine auxotroph mutant promote a protective immunity dependent on the bacterial Dot/Icm secretion system and Myd88 signaling pathway against Legionella pneumophila *flaA*$^-$**

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The importance of the priming of the lung environment by past infections is being increasingly recognized. Exposure to any given antigen can either improve or worsen the outcome of subsequent lung infections, depending on the immunological history of the host can provide an important novel therapy for emerging infectious diseases. To gain insight in the molecular mechanisms related to protection during previous bacterial infection we used a mouse model of *Legionella pneumophila* (*L.p.*). Mouse were infected via intranasal (i.n) with thymidine auxotroph mutants of *L.p.* (*thyA*$^-$, which fail to replicate *in vivo*) for 10 days (day -10) before challenge with virulent bacteria (*L.p flaA*$^-$). At day 0 mice were infected with *L.p. flaA*$^-$ (i.n), after 2 or 4 days, lungs were harvest, crushed and plated on CYE and CFU were evaluated. We show that C57BL/6 pre-stimulated mice became significantly resistant to infection. Additionally we evaluated TLRs-/- as well as IL-1R-/- and these mice were also protected. Moreover this protection required T4SS and MyD88 signaling. To evaluate if this protection requires neutrophils C57BL/6 mice were depleted with anti-Ly6G. We found that anti-Ly6G depletion at day 0 promoted a partially reduction on protection. Additionally, we observed a partially involvement of $CD4^+$ T cells to due a deficient bacterial clearance $MHCII^{-/-}$ mice. We concluded mice pre-exposed to bacteria became more resistant for re-infection. This process requires bacterial virulence (T4SS), MyD88 signaling, neutrophils and $CD4^+$ T cells, which leads to generation of a strong and durable response. Financial support: CAPES, INCTV, CNPq and FAPESP.

P4.04.49

A critical role for Interferon-gamma but not IL-17 in staphylococcal superantigen-mediated toxic shock syndrome

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Superantigens (SAg) elaborated by Staphylococcus aureus cause a robust immune activation resulting in profound elevation in systemic levels of various cytokines. This leads to multi organ failure and death. However, the precise roles of the two major pro-inflammatory cytokines IFN-γ and IL-17, in the pathogenesis of toxic shock syndrome are not known. As HLA-DR3 transgenic mice mount a superior response to SAg compared to conventional mice, IFN-γ-sufficient (DR3.IFN-γ+/+) and -deficient (DR3.IFN-γ-/-) mice were challenged intraperitoneally with 50 µg of staphylococcal enterotoxin B (SEB). Mice were treated with 100 µg of isotype control or neutralizing αIL-17 antibodies (R&D systems). Sera were collected at 3, 6, 12, 24, 48 and 72 hours for 23-plex cytokine/chemokine assay (Bio-Rad). Animals were killed at 24, 48 and 72 hrs for experimental analyses.
SEB-challenged, isotype control DR3.IFN-γ+/+ mice had significantly elevated serum IL-17 and IFN-γ along with marked inflammation in lung, liver, kidney, heart and small intestines. SEB-challenged, αIL-17 treated DR3.IFN-γ+/+ mice had little IL-17, but significantly elevated IFN-γ in serum. Multi organ inflammation was not mitigated. SEB-challenged isotype control DR3.IFN-γ-/- mice had significantly elevated IL-17. Lung, liver, kidney and heart displayed marked inflammation. However, the small intestines were devoid of inflammation. αIL-17-treated, SEB-challenged DR3.IFN-γ-/- mice had significantly lower IL-17. They still had marked inflammation in lung, liver, kidney and heart but not in the small intestines. In conclusion, our study suggests that IFN-γ but not IL-17, plays an important role in pathogenesis of TSS. Therapeutic approaches targeting IFN-γ should be explored in treatment of TSS.

P4.04.50

Evaluation of the immune response against *Brucella abortus* in splenectomized mice

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Introduction: The influence of the spleen in control of intracellular bacterial infections remains unclear. Brucellosis is a zoonosis caused by the facultative intracellular bacteria of the genus *Brucella*. Thousands of new cases of human brucellosis are diagnosed each year worldwide. In Brazil, bovine brucellosis is endemic throughout the country, compromising the quality of cattle. *Brucella abortus* has a tropism for the spleen and induces an immune response mediated by cytokines such as IFN-γ and TNF-α. The objective of this work was to investigate the immune response against *Brucella abortus* in splenectomized mice.
Methods and Results: C57Bl/6 mice were divided into a sham-operated control group (SH) and splenectomized group (SP). Thirty days after surgery, animals were intraperitoneally infected with 10^6 bacteria/mouse of *Brucella abortus* (S2308). Twenty one days after infection, *B. abortus* colony-forming units (CFU) were evaluated in macerated liver. Cytokine production (IFN-γ, IL-12, TNF-α, IL-6, IL-

10) was analyzed in liver (ELISA). A higher number of *B. abortus* CFU was observed in liver of splenectomized mice (Ln = 3.8 ± 0.1) in comparison to the sham-operated group (Ln = 3.4 ± 0.1). IFN-γ and IL-12 production was lower in liver of SP mice (600.3 ± 75.9 and 1303 ± 99.3 pg/ml, respectively). In addition, SH animals presented a greater production of TNF-α, IL-6 and IL-10 in liver in comparison to the splenectomized group ($p<0.05$).
Conclusion: Splenectomy reduces the capacity of C57Bl/6 mice to control *B.abortus* infection which correlates with a lower cytokine production in splenectomized mice.
Financial support: CAPES, CNPq, FAPEMIG.

P4.04.51
Physiological significance of the sodium/proline transporter putP of Helicobacter pylori

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Helicobacter pylori is recognized as one of the most prevalent human pathogens. It is the causative agent of type B gastritis and gastric carcinoma. It uses several mechanisms to manipulate the immune system to persist long-term within the gastric niche manipulating phagocytosis and the subsequent oxidative burst. A genome wide search predicted that the genes encoding L-proline transport (putP) and metabolizing proteins (putA) were essential for gastric colonization (Kavermann H, et al. in 2003). Furthermore L-proline is present in high amounts in humans infected with H. pylori, can be used as energy source and is recognized as scavenger of reactive oxygen species (Nagata et al., 2003). This research focuses on the physiological role of L-proline and L-proline-specific systems for H. pylori. First characterization of the putative proline transporter in H. pylori was performed. The gene HpputP from strain P12 complemented an E. coli putP mutant. Functional analyses with HpPutP reconstituted in proteoliposomes demonstrated that transport activity depends on an electrochemical sodium gradient. Analysis of proline transport in H. pylori wild type showed accumulation of extracellularly applied L-proline and deletion of the putative transporter gene inhibited transport completely. Kinetic parameters were similar to those of EcPutP and complemented mutants were able to restore transport. The mutants had an altered energy status; flaA gene expression was impaired and phenotypic characterization demostrated the absence of flagella. Therefore proline availability could have very important implications during infection, and HpputP may represent a new drug target

P4.04.52
Protection from severe bacterial infection by injections of cytokine-gene transfected cells

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In hospital-aquired infections in immunocompromized hosts by multiple-drug resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas aeruginosa,* recovery from infection by chemotherapeutics treatment are very difficult. Since these microorganisms are weak pathogenicities, encouragement of immune responses to normal level in these hosts is important therapeutic strategy. One of procedures to improve host resistancy against bacterial infection, injections of immunostimulating cytokines will be effective. Several reports showed some cytokine induced anti-bacterial effects, but high concentration of cytokines and maintenance of long period were required to exert these effects.
In this study, we prepared specific cells which harboring cytokine genes, such as IL-2, IL-6 or IL-12 and secret cytokines. These cells will produce cytokines constantly *in vivo* and enhance macrophage activity, humoral immunity and cellular immunity. After inoculations of these bacteria in *beige* mice, model mice of Chediak-Higashi syndrome, cytokine-secreting cells were injected in mice, survival period and growth of bqacteria at injected region were traces. In this paper, we will summarize our experimental results.

P4.04.53
The scavenger receptor CD36 downmodulates the early inflammatory response while enhancing bacterial phagocytosis during pneumococcal pneumonia

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CD36 is a scavenger receptor which exhibits pleiotropic functions including: adhesion to thrombospondin, inhibition of angiogenesis, transport of long chain fatty acids and clearance of apoptotic cells. Additionally, it has been implicated in the host immune response since it acts as a co-receptor for Toll like receptor 2 (TLR2) and plays a role in *Staphylococcus aureus* infection. However, its role in other Gram positive bacterial infections is unclear. Here using mice deficient in CD36 we sought to examine the role of CD36 in pneumococcal pneumonia, a major cause of morbidity and mortality worldwide. We show that CD36 is expressed on both alveolar macrophages and respiratory epithelial cells. Early in infection $CD36^{-/-}$ mice have an exaggerated inflammatory response compared to WT littermate controls. *In vitro* studies utilizing $CD36^{-/-}$ primary cells confirm the enhanced early inflammation in response to *S. pneumoniae* and its LTA, demonstrate that *S. pneumoniae* binds to cells via its phosphocholine residues and suggest a role for CD36 in reducing inflammation induced by the phosphocholine residues of pneumococcal LTA. Later in infection, although $CD36^{-/-}$ mice exhibit impaired bacterial clearance, due to a decreased capacity of $CD36^{-/-}$ macrophages to phagocytose *S. pneumoniae*, there are minor effects on mortality compared to WT littermate control mice. These data show that CD36 contributes to the pulmonary host response during S. *pneumoniae* infection by virtue of its ability to act as a phagocytic receptor and as a modulator of the early innate immune response.

P4.04.54
Identification of functional polymorphisms of the macrophage migration inhibitory factor (MIF) gene associated with morbidity and mortality in pneumococcal meningitis and sepsis

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Background: The cytokine MIF is an important regulator of innate immune responses. Elevated MIF levels in septic patients correlate with disease severity and outcome. We examined whether functional MIF polymorphisms (-794 CATT-5/8 microsatellite and -173*G/C SNP) were associated with morbidity and mortality in patients with pneumococcal meningitis, and assessed the impact of anti-MIF therapy in a mouse model of streptococcal sepsis.
Methods: MIF polymorphisms were genotyped in 404 patients with community-acquired pneumococcal meningitis and 329 non-related proxies. MIF levels were measured in CSF of 426 patients and in human whole blood stimulated with *S.pneumoniae*. The effect of anti-MIF IgG was assessed in a mouse model of *S.pneumoniae* pneumonia.
Results: Frequencies of CATT-5/8 and -173*G/C alleles were similar in patients and controls. In patients with pneumococcal meningitis, carriage of the CATT-7 and -173*C high MIF expression alleles was associated with sepsis biomarkers, unfavourable outcome (P=0.005 and P=0.003) and death (OR 2.27 and 2.6, P=0.03 and P=0.01). CSF MIF levels were 2-fold higher in non-survivors than in survivors ($P<0.001$). MIF concentrations increased 7.5-fold in *S.pneumoniae* stimulated whole blood (P=0.001). In mice with pneumococcal sepsis, anti-MIF antibodies reduced bacterial counts, TNF and IL-6 concentrations in blood and BAL, and mortality (from 75% to 46%, P=0.03).
Conclusions: Functional MIF polymorphisms affecting MIF expression and CSF levels are associated with severity and outcome in adult patients with pneumococcal meningitis. Conducted in a homogenous population of septic patients, this study shows that genotyping may help identifying patients who could benefit from mediator targeted treatment strategies.

P4.04.55
Phagocytic activity in antibiotic and bacteriophage therapy of peritonitis caused by Escherichia coli

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Worldwide spread of polyresistant pathogenic bacteria has become a global medical problem. Phage therapy is one of alternative ways of antimicrobial treatment. Bacteriophages provide elimination of bacteria in vivo by both direct bactericidal activity and activation of phagocytosis.
Objective: Assessment of phagocytic activity in antibiotic and bacteriophage treatment of peritonitis caused by Escherichia coli in mice.
Material and methods: Eight groups of mice took part in the experiment. Each group included 10 animals. Healthy mice comprised the control group. The remaining animals had peritonitis caused by intraperitoneal injection of Escherichia coli. Gatifloxacin (G), amikacin (A) and bacteriophagum coliproteicum (BCP) were used for antimicrobial treatment. Mono (G, A and BCP) and combined (G+A, G+BCP, A+BCP and G+A+BCP) therapies were used. Sick animals received the medicines intraperitoneally in daily doses during 5 days. Phagocytosis was assessed by rate of activation, phagocytic index and completeness of phagocytosis on the 1st, 3rd and 5th days of the therapy.
Results and discussion: Parameters of phagocytosis in both monotherapy with G and A, and combined therapy with G+A and G+A+BCP were below the control values during the whole period of treatment ($p \leq 0.05$). The animals receiving BCP therapy revealed high phagocytic activity exceeding the control values ($p > 0.05$). Parameters of phagocytosis in combined therapy with G+BCP and A+BCP were equal to the control values.
These results prove bacteriophage to activate phagocytosis. Combinations of G with BCP and A with BCP can be recommended for treatment of peritonitis caused by Escherichia coli.

P4.04.56
Infection with invasive bacterial pathogens triggers distinct Nod-dependent autophagy and cytokine responses

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Autophagy plays a critical role in cellular metabolism as a starvation response but is also activated during infection as an intracellular defense mechanism. Indeed, these two roles for autophagy are linked, since infection with the invasive bacteria *Shigella flexneri* or *Salmonella typhimurium* triggers amino acid starvation, which contributes to autophagy activation. Nod1/2 are intracellular sensors of peptidoglycan fragments that drive cytokine responses. In addition, Nod1/2 promote autophagy of both *Shigella* and *Salmonella* through their interaction with the autophagy protein, ATG16L1, and ability to activate p38 MAPK signaling. Here, we examined the role of Nod1/2 in the autophagy response to *Shigella* and *Salmonella* and demonstrate that they play distinct roles during infection with these pathogens. We demonstrate that anti-*Shigella* autophagy occurs in two distinct and separable phases comprised of induction and targeting, with Nod1/2-dependent recruitment of ATG16L1 helping to overcome *Shigella's* evasion of the targeting machinery. In contrast, Nod1/2 contribute to the induction of autophagy through downstream signaling during *Salmonella* infection but are dispensable for targeting, as a result of *Salomonella*'s replication in a membrane-damaged vacuole that drives targeting through complementary mechanisms. Finally, during infection with cyto-invasive bacteria, the interaction of ATG16L1 with Nod1/2 functions to negatively regulate the pro-inflammatory cytokine response. Therefore, there is a complex interaction that occurs between Nod1/2, the autophagy system and ATG16L1 to promote autophagy of intracellullar bacteria and modulate levels of pro-inflammatory cytokines. Since Nod2 and ATG16L1 are strongly linked to Crohn's disease, understanding the interplay between these proteins may provide insight into disease pathogenesis.

P4.04.57
Selective criteria and important features of protective monoclonal antibodies to tetanus toxin

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Completely characterized, high specific monoclonal antibodies (MoAbs) with proven protective capacity could be used as the effective therapy of tetanus. The aim of our study was to elucidate the relationship between binding characteristics of MoAb and protectiveness it provides. Five MoAbs specific for tetanus toxin (TeNT) were evaluated for its fine epitop specificity, affinities of interaction with TeNT and capability to ensure protection in vivo.
Among selected antibodies, MoAb51 and MoAb62 recognize TeNT with the highest affinity (Ka>1010M-1). Bindings of MoAb26, MoAb41 and MoAb71 to TeNT are significantly weaker Ka<109M-1. Target epitopes of MoAb26, MoAb41, MoAb51 and MoAb71 are found to be in ganglioside-binding site or in its close proximity. Recognition of TeNT by MoAb62 is not influenced by TeNT-gangliosides interaction. In mice treated by tetanus toxin pre-incubated with MoAb51 slight transitional clinical symptoms of tetanus are marked, but at the end of follow up period survival was 100% without any signs of tetanus. In addition, MoAb51 confers full protection against tetanus, even administrated 2 or 6 hours after TeNT injection. Protection by MoAb26, MoAb41 and MoAb71, was partial, while MoAb62 does not provide any protection.
Since binding of TeNT to gangliosides on neuronal cells is crucial step for further intoxication, binding of MoAbs to ganglioside-binding site or some close epitops on TeNT is assumed to be critical for protection. Our results are in accordance with this assumption but also provide clear evidence that only MoAbs that recognize TeNT with high affinity (Ka>1x1010M-1) could provide full protection against tetanus.

P4.04.58
Th1-Th17 cells mediate pelvic pain induced by uropathogenic Escherichia coli

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Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is characterized by pelvic pain in men and has an etiology that is not known but may involve microbes and autoimmune mechanisms. We developed an infection model of chronic pelvic pain in NOD/ShiLtJ (NOD) mice with a clinical Escherichia coli isolate (CP-1) from a patient with chronic pelvic pain. We investigated pain mechanisms in NOD mice and compared it to C57BL/6 (B6) mice, a strain resistant to CP-1-induced pain. Adoptive transfer of CD4+ T cells, but not serum, from CP-1-infected NOD mice was sufficient to induce chronic pelvic pain. CD4+ T cells in CP-1-infected NOD mice expressed IFN-γ and IL-17A but not IL-4. Adoptive transfer of ex-vivo expanded IFN-γ or IL-17A-expressing cells was sufficient to induce pelvic pain in naïve NOD recipients. Pelvic pain was not abolished in NOD-IFN-γ-KO mice but was associated with an enhanced IL-17A immune response to CP1 infection. Furthermore, we show that regulatory Foxp3+ T cells (Treg) cells are necessary for the fateful development of pain in mice with prostatitis. In agreement with these animal model studies, clinical samples isolated from patients with CP/CPPS show higher levels of IL-17 compared to control patients. Taken together, our data demonstrate that Th1, Th17, and Treg cells play a vital role in the development of pain in chronic prostatitis.

P4.04.59
Anti-alpha toxin mAb promotes both innate and adaptive immune response in a CA-MRSA USA300 mouse dermonecrosis model

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During a S. aureus skin and soft tissue infections (SSTI), an effective host response is initially orchestrated by IL-1β and IL-17 resulting in neutrophilic abscess formation and bacterial clearance. Recent studies have shown γδ T cells along with Th1 and Th17 cells play an important role in mounting this response. Alpha toxin (AT), a key virulence determinant in *S.aureus* SSTI, plays a role in tissue damage, but its effect on the immune system is unknown. We previously described that prophylactic administration with 2A3, an AT neutralizing human monoclonal antibody, resulted in significant reduction in disease severity in a CA-MRSA mouse dermonecrosis model. Reduction in disease severity in mice that received 2A3 correlated with increased proinflammatory cytokine (e.g. IL-1β, IL-17, IFN-γ) and chemokine levels leading to neutrophil influx and abscess formation not seen in animals that received an irrelevant control IgG, R347. To understand how AT modulates the T cell responses, intra-cellular IFN-γ and IL-17 were measured in different T cell populations. Single cell suspensions, in presence of 2A3 or R347, were analyzed from skin lesions 3 and 7 days post infection. At day 3 post challenge, 2A3 prophylaxis increased IL-17$^+$ γδ T cell numbers. After 7 days infection, IL-17$^+$ γδ T cells as well as Th17 and Th1 cell numbers were significantly higher in presence of 2A3 (respectively p= 0.003 ; 0.029; 0.0096).
Together, these results suggest AT neutralization with 2A3 promotes an early innate immune response through IL-17$^+$γδT cells, and later an adaptive Th1 / Th17 response.

P4.04.60
A novel polarized ex-vivo organ culture model to study gut inflammation and response to probiotics

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In recent years, the importance of a properly balanced gut flora for homeostasis and wellbeing has been greatly highlighted by many reports. In the light of recent findings, the use of probiotic strains as nutritional supplements has been exceedingly gaining ground in the last decade. As the mechanisms with which probiotics modulate innate and adaptive immunity start to become clearer, these have repeatedly been suggested as potential treatment for a wide variety of diseases, especially inflammatory bowel disease (IBD). However, even though the benefits of probiotic treatment for conditions like atopic dermatitis are well established, very limited clinical benefit has been obtained on IBD treatment. This could because of the lack of suitable models on which to obtain valid pre-clinical data, as it has been reported that although many treatments seem promising when tested on mouse or rat models of colitis, only a very small percentage of these translate to significant clinical benefit.
We recently described a newly developed model for the culture and apical stimulation of whole human intestinal mucosal explants. Polarized stimulation proved to be important for obtaining valid data, and we showed that the tissue was only viable if incubated in an O_2 chamber and that it did not survive culture if a thick layer of medium on the apical side prevented gas exchange. We used the new set-up to test three different Lactobacilli strains, none of which appeared to be benign on inflamed IBD mucosa.

P4.04.61
Study of the development of actinomycetoma during Nocardia brasiliensis infection in mice with partial genetic blockade of eNOS

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Introduction: Nocardia brasiliensis infection, is the primary cause of Actinomycetoma in Mexico. This disease is characterized by the development of chronic granulomatous lesions containing numerous foam cells, which act as reservoirs for bacteria. Nitric oxide (NO) plays an important role in regulating blood pressure and in the process of angiogenesis as well as in anti-infectious immune response.
Objective: Study the development of actinomycetoma during Nocardia brasiliensis infection in mice with partial genetic blockade of endothelial nitric oxide synthase (eNOS).
Methodology: We compared BALB/c and C57BL6 wild type (WT) mice, with the C57BL6 heterozygous for eNOS -/+ mice. The mice were inoculated with N. brasiliensis (0.1ml 1x106 bacteria) in the footpad. They were followed up for 250 days during infection, serum was obtained for measurement of the production of IgG antibodies against N. brasiliensis and the bacterial burden was determined in blood.
Results: By the 10th day, all the mice presented the acute inflammation. On the day 30, the WT and BALB/c started developing the actinomycetoma until their sacrifice; in none of the mice with incomplete genetic blockade for eNOS, the actinomycetoma had developed until the time of their sacrifice. Even though they showed similar antibody levels to WT and BALB/c, the bacterial burden in blood was negative for all mice.
Conclusion: The incomplete genetic blockade of endothelial NO synthase (eNOS) protects mice against the development of actinomycetoma caused by Nocardia brasiliensis infection.

P4.04.62
Neutrophilic granulocyte subtype recruitment in human peritonitis

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Background: Human neutrophilic granulocytes (PMN) are distinguished into two subpopulations by the NB1 molecule (CD177). NB1 presents the serine protease Proteinase 3 on the PMN surface. Recent in vitro data have suggested a role of the NB1/PR3 complex in cell migration. We here studied neutrophil subtype recruitment in acute peritonitis in humans in vivo.
Methods: Blood and peritoneal fluid from patients treated with peritoneal dialysis presenting with acute peritonitis was analyzed by immunofluorescence and flow cytometry for NB1 and integrin expression at presentation and during follow-up. Patients without evidence of infection served as controls.
Results: NB1 was up-regulated on migrated NB1+ neutrophils. However, the proportion of NB1/CD177+PMN was very similar in every patient in blood and peritoneal fluid in patients with acute peritonitis and controls. There was no evidence of transfer of NB1 to NB1- cells. Differential cell death might obscure a migration advantage of either PMN type. However, in acute peritonitis, no significant difference in the proportion of dead cells among NB1+ and NB1-PMN was observed. Also, re-assessment of blood and peritoneal PMN after treatment again revealed very similar proportions of NB1+ among peritoneal and blood PMN.
Conclusion: Our results argue against a cell specific role for NB1/CD177 in inflammatory neutrophil recruitment in vivo.

P4.04.63
Suppression of IFN-γ signaling by Src homology-2 domain-containing phosphatase 2 during Helicobacter pylori infection

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Helicobacter pylori (*H. pylori*), a gram-negative spiral bacteria, infection not only induces gastric inflammation but also increase the risk of gastric tumorigenesis. Interferon (IFN)-γ has antimicrobial effects; however, *H. pylori* infection elevates IFN-γ-mediated gastric inflammation and may suppress IFN-γ signaling as a strategy to avoid immune destruction through an as-yet unknown mechanism. This study was aimed at investigating the mechanism of *H. pylori*-induced IFN-γ resistance. Post-infection viable clinical isolates of wild-type *H. pylori* strain HP238 decreased IFN-γ-activated signal transducers and activators of transcription 1 and IFN-regulatory factor 1. HP238 caused an increase in the C-terminal tyrosine phosphorylation of Src homology-2 domain-containing phosphatase (SHP) 2. Pharmacologically and genetically inhibiting SHP2 reversed HP238-induced IFN-γ resistance. The cytotoxin-associated gene A (*CagA)* isogenic mutant strain $HP238^{CagAm}$ failed to induce IFN-γ resistance, indicating that CagA regulates this effect. Notably, HP238 and $HP238^{CagAm}$ both caused SHP2 phosphorylation; however, imaging and biochemical analyses demonstrated CagA-mediated membrane-associated binding with phosphorylated SHP2. CagA-independent generation of reactive oxygen species (ROS) contributed to *H. pylori*-induced SHP2 phosphorylation and IFN-γ resistance when CagA was concurrently present. This finding not only provides an alternative mechanism for how CagA and ROS co-regulate SHP2 activation but may also explain their roles in *H. pylori*-induced IFN-γ resistance.

P4.04.64
Protective immunity against invasive *Streptococcus pneumoniae* infections by tissue-specific memory Th17 cells

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Pneumonia caused by *Streptococcus pneumoniae* (Sp) remains a leading cause of serious illness and death in children and elderly worldwide. Current vaccine is highly effective in preventing colonization by inducing serotype-specific antibodies. However, there is an increasing prevalence of infection by serotype strains not included in the vaccine; this highlights the need for a universal vaccine that protects against all serotypes. The first step in developing a universal vaccine is to elucidate the immune mechanism that can provide broad protection against different serotype of Sp strains. We found that intranasal immunization of mice with Sp resulted in a strong CD4+T cell response in the lung that consisted of mostly Th17 cells but also IFN-gamma producing Th1 cells. These immunized mice were protected against lethal challenge with a different serotype strain of Sp and cleared bacteria from the lung by day 2 post-challenge. Adoptive transfer of T cells from immunized mice also provided protection against a heterologous challenge, with a dominant Th17 recall response in the lung from donor memory T cells. Furthermore, immunization of mice with *Listeria monocytogenes* (LM) provided protection against a recombinant Sp strain expressing a CD4 epitope from LM (LLO190), as shown by decreased CFU in the lung, and elevated LLO190-specific Th17 response. Our results suggest that memory Th17 cells may play a key role in providing broad protective immunity against invasive Sp infection in a serotype independent manner.

P4.04.65
Differential inflammasome activation by Salmonella strains expressing mutant forms of the NLRC4 ligands FliC and PrgJ

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The Nod-like receptor NLRC4 is activated in response to *Salmonella* infection and, in combination with the adaptor protein ASC, recruits caspase-1 to form an inflammasome complex. Inflammasome assembly leads to secretion of active IL-1 and IL-18 and drives pyroptotic macrophage cell death. NLRC4 recognises FliC, the major flagellin subunit of *Salmonella*, in combination with NAIP5 and NAIP6 and also PrgJ, the rod protein of the SPI-1 type three secretion system (T3SS), in combination with NAIP2. Conserved residues at the C-termini of these bacterial proteins are required for their recognition by NLRC4. However, it is not known whether these residues are critical for function, or whether they can be mutated to abolish NLRC4 activation whilst retaining bacterial motility and SPI-1 T3SS activity. We have generated Salmonella strains lacking the *fliC*, *prgJ* and minor flagellin *fljB* genes, either alone or in combination. Constructs have been produced to express mutant forms of FliC and PrgJ in appropriate mutant backgrounds. The effect of these mutations is currently being assessed in bacterial motility and protein secretion assays, and in macrophage infection experiments to measure host cell death and cytokine secretion. This study will provide further insight into the structure-function relationship of NLRC4 ligands.

P4.04.66
The effect of dietary oils on *citrobacter rodentium* induced colitis

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BACKGROUND/AIM: Increasing evidence suggests that high dietary intake of n-3 fatty acids attenuates inflammation in chemically induced colitis, but data evaluating the effect of dietary oils on bacterial-induced colitis is lacking. The aim of this study was to determine the effect of fish oil, canola oil, and safflower oil on *C. rodentium*- induced colitis.
METHODS: Female 6-8 week-old C57BL/6 mice were fed diets varying only in fat composition with % energy from fat, 20% safflower oil (SO), 20% canola oil (CO), or 18% fish oil plus 2% safflower oil (FO) for three weeks before and 10 days after *C. rodentium* inoculation. The distal colon was assessed for histological damage score, bacterial count, macrophage and neutrophil recruitment evaluated by immunohistochemistry and levels of chemokines and cytokines assessed by real-time PCR and ELISA.
RESULTS: While bacterial burdens were similar between groups after infection, the FO group was the least susceptible to *C. rodentium*-induced colitis; histological damage score was lowest in the FO group (2.6 ±0.8) followed by CO (5.0 ±1.1) and SO (6.6 ±0.8). Moreover, the FO group demonstrated a significantly reduced inflammatory cell infiltration. Cytokines measurement indicated that the FO diet reduced critical chemokines (MCP1, MIP2 and KC) and cytokines (IL-6, IFNγ, IL-17A) and increased IL10 mRNA level compared to all other groups.
CONCLUSION: Our results indicate that in *C. rodentium*-induced colitis, the intake of fish oil was associated with a modulating effect on the host mucosal immune response in the absence of an effect on initial bacterial colonization.

P4.04.67
A mouse model of shigellosis by intraperitoneal infection

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While *Shigella spp.* causes bacillary dysentery by invading colon epithelium and promoting a strong inflammatory response in humans and non-human primates, adult mice are resistant to oral *Shigella* infection.
In this study, we found that intraperitoneal (i.p.) challenge with virulent *Shigella (S.) flexneri* 2a (YSH6000) resulted in diarrhea and severe body weight loss in adult B6 mice. Of note, virulent *S. flexneri* 2a could invade and colonize not only systemic tissues but also the serosa and lamina propria region of the large intestine. In addition, severe host cell death, epithelium shedding, barrier integrity and goblet cell hyperplasia were also found in the large intestine around 24 hours post i.p. *Shigella* infection. Microarray analysis showed that expression levels of chemokines (i.e., CCL4, CCL7, CXCL1, and CXCL10) and proinflammatory cytokines (i.e., TNF-α, IFN-γ, IL-1α

and IL-6) were dramatically increased in the large intestine post i.p. challenge. Furthermore, it was found that monocytes play a critical role in attenuating diarrhea and providing protective efficacy against *Shigella* i.p. infection. Most importantly, mice pre-vaccinated with avirulent *S. flexneri* 2a (SC602) showed effective protection against i.p. challenge with YSH6000. Taken together, i.p. challenge with virulent *S. flexneri* 2a can provoke bacillary dysentery and severe pathogenesis in adult mice, which could be applicable for understanding the induction mechanism of bacillary dysentery and for evaluating *Shigella* vaccine candidates.

P4.04.68
Production and characterization of neutralizing monoclonal antibodies directed against tetanus toxin fragment C

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Clostridium tetani causes life threatening infectious disease by production of tetanus toxin. Tetanus toxin (TeNT) is composed of light (LC) and heavy chain (HC) polypeptides with a molecular weight of 150 kDa. The TeNT HC is constituted of an N-terminal domain which is important for LC translocation and a C-terminal toxin receptor binding domain, also known as fragment C. Despite extensive investigations on epitope specificity of tetanus toxin antibodies, the immunodominant neutralizing epitopes of the toxin are poorly defined. Here, we describe the generation and characterization of four monoclonal antibodies (MAbs) specific for TeNT. Our data demonstrated that all MAbs bind to tetanus toxin and toxoid. Subfragments binding analysis showed that 2 MAbs react with fragment C, one with both fragment C and LC and one with LC, of which the former two MAbs were able to neutralize toxin activity in vitro. Sequencing of the immunoglobulin heavy chain variable region genes revealed rearrangement of various gene segments in all hybridoma clones. Individually of the clones was also confirmed by a competition assay which showed recognition of distinct epitopes by these MAbs. Our results suggest the importance of TeNT fragment C in terms of immunogenicity and toxin neutralization activity.
Key words: Tetanus toxin, Monoclonal antibody, Fragment C, Toxin neutralization

P4.04.69
Ephedrine hydrochloride inhibits PGN-induced inflammatory responses by promoting IL-10 production via PI3K/Akt/GSK3β pathway

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The approaches to control the inflammatory responses and reduce the mortality rate of sepsis are still not very effective in clinics and new potential drugs are needed to be confirmed to be useful in the induction of anti-inflammatory response. Ephedrine hydrochloride (EH) is a compound widely used for cardiovascular diseases, especially to treat hypotension caused by anesthesia or overdose of antihypertensive drugs. In this study, we reported that EH also plays an important role in the control of inflammatory response. It increases interleukin 10 (IL-10) and decreases proinflammatory cytokine (IL-6, TNF-α, IL-12, and IL-1b) expression in response to gram-positive cell wall component peptidoglycan (PGN) in primary peritoneal macrophages and Raw264.7 cells. The anti-inflammatory role of EH was also observed in experimental peritonitis mouse model induced by intraperitoneally PGN injection. Phosphatidylinositol 3-kinase (PI3K)/Akt pathway was found to be responsible for EH-enhanced IL-10 production and EH-decreased IL-6 expression. Therefore, our results illustrated that EH can help to maintain immune equilibrium and diminish host damage by balancing the production of proinflammatory cytokines and anti-inflammatory cytokine after PGN challenge. EH may be a new potential anti-inflammatory drug in treatment of severe invasive gram-positive bacterial infection.

P4.04.70
Anaphylatoxin C5a promotes kidney infection caused by uropathogenic E. coli

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Previous studies have shown that most uropathogenic E coli strains are complement resistance, and complement depletion and deficiency of C3 protected mice from urinary tract infection (UTI). However, the mechanisms by which complement contributing to UTI remain to be elucidated. In this study, we tested a hypothesis that excessive C5a generated during infection contributes to the pathogenesis of UTI.
Using a well-established murine model of ascending UTI leading to kidney infection, we found that deficiency of C5aR or blocking C5aR significantly protected mice from kidney infection at 6h -72h after bladder inoculation of E. coli. Compared with wild type mice, C5aR-/- mice exhibited a lower rate of kidney infection (31% vs 79%), reduced bacterial load in the infected kidney and tissue damage, and lowered cytokine/chemokine production in kidney tissue. C5aR antagonist treatment protected mice from the kidney infection with a similar protection level as that observed in C5aR-/- mice. Chimera studies showed that the absence of C5aR on renal or circulating cells attenuated kidney infection, suggesting that C5aR on both renal and circulating cells contribute to the infection. *In vitro*, C5a stimulation significantly increased cytokine/chemokine production by macrophages and renal tubular cells, and impaired neutrophils functions (uptake, reactive oxygen species activity), in the presence of LPS or E coli.
These data demonstrate that C5a/C5aR signaling is a critical pathogenic factor in the ascending kidney infection. Mechanistic studies suggest that C5aR mediated macrophage and renal tubular cell activation/inflammation, and neutrophil functional impairment contribute to the pathogenesis of the infection.

P4.05 Immunity to mycobacterial infection

P4.05.01
Mycobacterium tuberculosis secretory protein MPT 70 induces production of pro-inflammatory cytokines and nitric oxide in macrophages

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Secretory proteins of Mycobacterium tuberculosis play important role in pathogenesis as well as in immunity against the pathogen rendering them as promising candidates for development of vaccines and diagnostics. In the present study, we show that M. tuberculosis secretory protein MPT 70 binds to the surface of murine macrophages and stimulate secretion of proinflammatory cytokines IL-12p40, TNFα, IL-6, IL-1β but not anti-inflammatory cytokine IL-10. Pre-treatment of macrophages with MPT 70 resulted in increased NO release upon subsequent exposure to M. tuberculosis whole cell lysate or LPS, a potent inducible NO synthase (iNOS) activator thereby sensitizing macrophages for LPS-induced NO production.

MPT 70 also synergized with IFN-γ for NO production in a dose dependent manner suggesting that MPT 70 may have a pro-inflammatory function. MPT 70 was found to mediate these effects through ERK1/2 and p38 MAPK signaling pathways as TNFα released by MPT 70 and MPT 70- IFN-γ- stimulated macrophages as well as NO induced by pre- MPT 70- LPS- stimulated and MPT 70- IFN-γ- stimulated macrophages, are partially dependent on both p38 and ERK1/2 pathways as revealed by specific kinase inhibitors. MPT 70 increased p-ERK1/2 and p-p38 expression in macrophages. Also, pre-treatment with MPT 70 further heightened LPS- induced ERK1/2 and p38 phosphorylation and thus magnifies LPS effects. The study suggests that MPT 70 might play a protective role against M.tb infection by tilting the macrophage effector functions towards pro-inflammatory response and can have crucial implications in providing protection against tuberculosis.

P4.05.02
Regulation of granuloma formation in response to infection with Mycobacterium tuberculosis

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A scourge of human kind throughout the recorded history, tuberculosis (TB) continues to be a leading cause of death across the globe. Major challenges in TB control are to understand the biology of latent TB, and to identify the very early sequence of events after bacilli gains access to the lungs. In lieu of this, we chose to study the immune responses generated against Mycobacterium tuberculosis in in vitro granuloma model. Our initial studies have reflected the differences in individual manifestation of the disease in terms of resistance or susceptibility against H37Rv. Low MOIs lead to the development of granuloma-like structures which are dynamic and could be maintained for several days. We observed the differences among individuals in terms of the ability to clear infection, extent of granuloma formation and foamy macrophage appearance, and also, in the levels of many Th1/Th2 cytokines and chemokines.
A multivariate regression analysis showed some positive and negative cytokine correlates of granuloma formation, and/or resistance and susceptibility for TB. Supporting the redundancy of human system, we have found some groups of different patterns of immune responses against the pathogen. In other words, there appears to be multiple signatures for different groups of people. The next question we are addressing is whether these signatures could be of any use to diagnostics or personalized medicines. Since many meta-analysis studies have already shown polymorphism linkage to the TB susceptibility, currently, we are investigating whether these patterns are governed at genetic level or not.

P4.05.03
Role of Oxidative stress associated anemic prevalence among Leprosy Patients.

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Introduction: Leprosy is a chronic disease. A constellation of reactive oxygen species (ROS) capable of damaging cellular constituents generated in excess during the chronic, inflammatory, neurodegenerative disease process of leprosy due to prooxidant shift results from changes in cellular metabolism (especially energy metabolism), higher flux rates in catecholamine metabolism and permanent leukocyte activation. The purpose of this study was to investigate the role of oxidative stress associated anemic prevalence among Leprosy patients.
Methodology: The study involves fifty Leprosy patients who are the inmate of Government Rehabilitation Centre for Leprosy, Pudhupatti, Madurai. The samples were collected at the informed consent of the patients. Serum sample were collected and Reactive Oxygen Species (ROS) was quantified using Nitro Blue Tetrazollium (NBT) reduction assay. Result: Study Involves Gaussian distribution and statistical analysis confirms the existence of the extreme significance of oxidative stress among the study population ($p<0.0001$) one way variance analysis (ANOVA). Study document existence of Correlation between increased oxidative stress with anemic prevalence.
Conclusion: The excess production of ROS evidenced in leprosy patients leads to oxidative damage of the erythroid cells which plays a crucial role in hemolysis due to ineffective erythropoiesis in the bone marrow and short survival of red blood cells (RBC) in the circulation leads to anemia . Intervention with antioxidant supplementation like vitamin E prevents oxidative stress mediated through ROS and activates the net antioxidant status thereby minimizing the risk of anemic associated complication among leprosy patients

P4.05.04
Immunotherapeutic effects of recombinant adenovirus encoding granulocyte-macrophage stimulating-colony factor in experimental pulmonary tuberculosis

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BALB/c mice with pulmonary tuberculosis (TB) develop a T helper cell type 1 that temporarily controls bacterial growth. Bacterial proliferation increases accompanied by decreasing expression of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and inducible nitric oxide synthase (iNOS). Activation of dendritic cells (DCs) is delayed. Intratracheal administration of only one dose of recombinant adenoviruses encoding granulocyte-macrophage stimulating-colony factor (AdGM-CSF) one day before *M. tuberculosis* (Mtb) infection produced significant decrease of pulmonary bacterial loads, higher activated DCs and increased expression of TNF-α, IFN-γ, and iNOS. When AdGM-CSF was given in female mice B6D2F1 (C57BL/6J X DBA/2J) infected with low Mtb dose to induce chronic infection similar to latent infection and used corticosterone to induce reactivation, a very low bacilli burden in lungs was detected and the same effect was observed in healthy mice cohoused with mice infected with mild and highly virulent bacteria in a model of transmissibility. Thus, GM-CSF is a significant cytokine in the immune protection against Mtb and gene therapy with AdGM-CSF increased protective immunity when administered in a single dose one day before Mtb infection in a model of progressive disease, and when used to prevent reactivation of latent infection or transmission.

P4.05.05
Clinical isolates of Mycobacterium tuberculosis in Argentina differentially induces respiratory burst via TLR2 and dectin-1 in neutrophils as possible mechanism of immune escape

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Some years ago it was thought that both illness and latency of Tuberculosis (TB) were primary related by host factors. Nonetheless, it becomes evident that there are also factors from the bacteria which participate. The goal of this study was to characterize the immune response of multidrug-resistant clinical isolates representative of the most successful families in South America (LAM and Haarlem). We observed that Ra from LAM family, induce high levels of apoptosis, dependent on ROS generation and mediated by dectin-1 and TLR2, with subsequent activation of the Syk and p38 pathways. In this process, lipid rafts coalescence is a key step for assembly of the NADPH oxidase. We propose the α-glucan of the mycobacterial capsule as potential ligands *Mtb* dectin-1, triggering ROS generation, via Syk activation in PMN. Particularly, the clinical isolate M, does not induce apoptosis as a result of poor induction of ROS, associated with a deficient lipid rafts formation and no or faulty interaction with dectin-1. These observed differences among clinical isolates of *Mtb* may be associated with structural and / or spatial variations of the cell wall, which condition their interaction with the target cell. Thus, we postulate that differences in composition of the cell wall at the level of α-glucans and ligands for TLR2, allow some bacteria to hide from the immune system, acting as a potential evasion mechanism, permitting perpetuation.

P4.05.06
P2X7 receptor activation by ATP released from necrotic cells is crucial to severity of tuberculosis caused by hypervirulent mycobacteria

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Progressive Primary Tuberculosis (PPT) is an aggressive form of severe tuberculosis, which is characterized by lung necrosis, pneumonia and bacillus dissemination. To determine the major features of the bacillus-host interaction that are responsible for the development of severe tuberculosis, we infected C57BL/6 mice via intratracheal injection with ~100 bacilli from phylogenetically distant and highly virulent mycobacterial strains (*Mycobacterium tuberculosis* (Beijing 1471) and *Mycobacterium bovis* (MP287/03)). We observed extensive areas of pulmonary inflammation and necrosis that culminated in bacillus dissemination, at the 28 days post-infection, and death of mice groups around 40 days post-infection. We wondered whether the recognition of damage signals released from necrotic cells by innate immune cells could aggravate the disease. The purinergic P2X7 receptor (P2X7R) is a sensor of extracellular ATP, a damage-associated molecular pattern that is released from necrotic cells and induces pro-inflammatory cytokine production and cell death. Our data show that both hypervirulent mycobacterial strains grew rapidly inside macrophages and induced death by a P2X7R-dependent mechanism facilitating the spread of bacilli. Moreover, the disease caused by these strains was attenuated in mice that lacked the P2X7R, as determined by lung bacterial burdens, pneumonia and bacillus dissemination without evidence of necrotic lesions. ATP release triggers a vicious cycle in which extracellular ATP exacerbates the pulmonary recruitment of inflammatory cells and results in further cell destruction through the activation of the P2X7R. These findings suggest the use of drugs designed to inhibit the P2X7R as a new therapeutic approach to treat the aggressive forms of tuberculosis.

P4.05.07
Mixed DC /macrophage lineage phenotypes in activated lepromatous lesions during reverse reaction

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Reverse Reaction (RR) in leprosy is a phenomenon incited by the rearrangement of the immune response with new waves of cellular activation and the disruption of the tolerance established by immunosuppression mechanisms. We hypothesized that in the cutaneous environment, these waves of migratory cells might affect different cellular populations in diverse stages of maturation, such as macrophages and dendritic cells. Our aim is to characterize the populations constituting the RR skin lesion and to determine the set of molecules involved in the onset of the episode. The immunohistochemical (IH) analysis showed in BL/LL, as well as RR skin lesions, the presence of specific markers of macrophage and dendritic cell populations, indicating the higher concentration of CD123. Immunofluorescence analysis showed that the dermal $CD123^+$ cell also exhibited expression of CD68, CD163, CD11c, CD86, CD14, HLADR, CD1a, CD1b, BDCA2 and BDCA4, without notable difference between the groups. In the RR group the gene expression of M-CSF (p=0.0077), GM-CSF (p=0.0033), TNF (p=0.0041) and IFN-γ (p=0.0312) was increased in comparison with the non-reactional group. CD123 gene expression was augmented in the RR patients when compared with the BL/LL group (p=0.0381), corroborating the IH analysis. The same was observed for the gene expression of CD209 (p=0.0394) and defensin 2 (p=0.0317). The expression of IDO (p=0.3148) was detected in BL/LL and RR lesions and although it seemed higher in the RR group, there was no statistical significance. The expression of IL-23 (p=0.0317) and IL-17 (p=0.0120) were also higher in the RR group in comparison to BL/LL.

P4.05.08
In vitro TLR2 and CD14 gene silencing effect in the control of Mycobacterium tuberculosis infection

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Introduction: TLR2 and CD14 are considered as key receptors for the penetration of Mycobacterium tuberculosis in macrophages. The increased expression of TLR2 enhances bacterial survival, proliferation and produces a large activation in infected macrophages, but little is known about the absence of TLR2 and CD14 in human macrophages. This work studied TLR2 and CD14 silencing and its effect on death or survival of intracellular Mycobacterium tuberculosis. Methodology: We designed siRNA and transfected human macrophage cell line THP1 and the percentage of silencing was measured by RT-PCR and flow-cytometry. Once silenced receptors proceeded to infect them with M. tuberculosis strain H37Rv. Macrophage CD14 and TLR2 KD were infected with Mycobacterium tuberculosis and Mycobacterium bovis (BCG). At 48 hrs. after infection of macrophages part lysed and intracellular bacteria were cultured in Middlebrook 7H10 to evaluate the UFC. We analyzed the expression of mRNA of cytokines (IL-12, IFN-γ, TNF-α, TGF-β and IL-10), nitric oxide synthase (iNOS) as well as the quantification of cytokines by ELISA and concentration of nitrite with the Griess reagent. Results. TLR2 receptor silencing reflected an increase in expression of IL-12, TNF-α, IFN-γ as well as the iNOS mRNA, nitric oxide production. There was a decrease of 95% over the CFU(p <0.001) compared to control infection. Conclusion: These results suggest that the silencing of TLR2 and CD14 activates microbicidal mechanisms providing an environment able to kill bacteria, this is proposed as a new strategy of molecular treatments, silencing genes temporarily promoting an effective immune response to the M. tuberculosis infection.

P4.05.09
Pilot study: cytokine pattern in children under eight years with pulmonary tuberculosis

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The IFN-γ assays can help diagnose active tuberculosis in adults, but due to the immunological and clinical characteristics, it may not be as useful in children. Therefore, we decided to assess other cutoff points in the assay as well as other cytokines in patients under eight years, classified in three groups on the basis of culture result, clinical, epidemiological and radiological criteria: chronic lung disease (n=6), confirmed (n=3) and probable (n=9) pulmonary tuberculosis. IFN-γ, TNF-α, IL-10, IL-5, IL-4 and IL-2 were measured in the supernatant of PBMC stimulated with a mixture of early secretory antigenic target-6, culture filtrate protein-10 and tuberculosis 7.7 (QTF-TB Gold Tubes) by the flow cytometry bead array. When the IFN-γ values were interpreted according to Quantiferon-TB Gold, cero confirmed cases and three with probable tuberculosis were positive. No case of the chronic lung disease group responded. When a lower cut off was used, IFN-γ response was positive in one of the confirmed and seven of the probable cases, with no reaction among the control group. The response with other cytokines was heterogeneous in the confirmed and probable tuberculosis groups, although IL-5 and IL-2 were commonly positive. Low or null IL-4 response was observed in most tuberculosis cases. No relation between disease activity and TNF-α or IL-2 levels was found. It is concluded that lowering the Quantiferon-TB Gold assay cutoff point or testing other cytokines may improve immunodiagnostic of tuberculosis in children.

P4.05.10
Dynamics of the immune response to DosR and Rpf antigens of *Mycobacterium tuberculosis* in house hold contacts of tuberculosis patients

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Previously, we found a predominance of T $CD4^+$ response with a T_{CM} phenotype to selected DosR and Rpf antigens of *M. tuberculosis* (*Mtb*) in LTBI subjects (> 3 years), suggesting that this response may be important to maintain latent infection. To better understand the development of this response, we followed-up for one year the T CD4 and T CD8 expressing iIFNγ, the memory phenotype, and cytokine production in HHC of recently diagnosed TB patients. PBMCs from HHC were cultured for 7 days in presence/absence of DosR (Rv1737c, Rv2029c and Rv2628), Rpf (Rv0867c and Rv2389c), and control ESAT6-CFP10 (E6-C10) and PPD antigens. The frequency of T $CD4^+IFNγ^+$, T $CD8^+IFNγ^+$, and memory phenotype (CD45RO and CD27) was established by flow cytometry. The cytokine production (IL-2, IL-4, IL-5, IL-10, IL-13, IL-15, IL-17, IFNγ and MIP-1α) was determined by the Luminex technology. A significant decrease in the frequency of T $CD4^+IFNγ^+$ cells after 12 months in response to Rv1737c and Rv2029c, and E6-C10 was observed, concomitant with an increase in the frequency of $CD4^+IFNγ^+$ T cells with a T_{CM} phenotype. The levels of IFNγ, IL-10, IL-17, and TNFα in response to both DosR and Rpf antigens decreased significantly after 12 months of the follow-up. **Conclusions.** The decreased frequency of $CD4^+IFNγ^+$ T cells and cytokine production may be an indication of an attenuated inflammatory response to *Mtb* infection. The increased frequency of $CD4^+IFNγ^+$ with a T_{CM} phenotype could be crucial to the control of *Mtb* infection and TB reactivation.
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P4.05.11
Functional modulation by Cortisol and/or Dehydroepiandrosterone on THP1-derived macrophages infected with *Mycobacterium tuberculosis*

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Tuberculosis (TB) is an ancient disease caused by the intracellular pathogen *Mycobacterium tuberculosis* (Mtb). Partly because macrophages play a central role in the response against Mtb and our previous work indicating an unbalanced immune-endocrine response in TB patients, we have now studied whether cortisol and dehydroepiandrosterone (DHEA) were likely to modify the macrophage response upon infection with Mtb. Macrophages derived from THP-1 monocytic cell line were exposed to live Mtb strain H37Rv (MOI 5:1) during 3 hours in the presence or absence of cortisol (1 μM) and/or DHEA (1 and 0.1μM). Upon washing, cells were cultured for 1 (T0), 24 (T1) and 96 hours (T2) in the same media with or without hormones. End points included: phagocytic capacity (T0); cytokine levels in macrophage culture supernatants (TNF-α, IL-1β, IL-6, TGF-β and IL-10, T1), and number of CFU in agar-Middlebrook media exposed to lysed macrophages which had been cultured as mentioned (T0, T1 and T2).
Single cortisol-treated cultures had a decreased Mtb phagocytosis respect to untreated counterparts ($p<0.05$). Cortisol, alone or combined with DHEA, inhibited the Mtb-induced production of TNF-α, IL-1β and IL-10 ($p<0.05$). A time-related rise in CFU was seen in control cultures ($p<0.05$) whereas treatment with cortisol and DHEA resulted in an inverse trend ($p<0.05$). Combined treatment with cortisol and DHEA seems to favor the macrophage effector response, in the sense of bacillary growth control, apparently in no close correspondence with the profile of cytokines involved in such regard.

P4.05.12
Assessment the activity of blood phagocytes at tuberculosis of lungs

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Phagocitosis is a key element in protection of an organism against tuberculosis mikobakteriya. Studying of possibilities of a flowing cytofluorimetry in an assessment of activity of blood phagocytes of cages at tuberculosis of lungs became the purpose of work. Surveyed 39 people: 14-with an infiltrative form of tuberculosis, 15 - from tuberkuloma, 10 almost healthy people. In work used the CoulterEpicsXL device, Phagotest reagents (Orpegen Pharma), BurstTestKit (Glycotope Biotechnology) and monoclonal antibodies for definition of subpopulations of lymphocytes. Statistical processing is carried out with Statistica program use. It is established that the assessment activity of blood phagocytes is important criterion of determination of activity of tuberculosis of lungs at early stages of supervision and in the course of treatment, the flowing cytofluorimetry allows quickly, precisely and objectively to estimate activity of blood phagocytes. Tuberculosis of the lungs, being accompanied formation tuberkuloma, is characterized by considerable decrease in number and functional and metabolic activity of neutrophil phagocytes and the monocytes, the expressed reduction of number of T - and B - cells, increase in TNK cells. Infiltrative form of tuberculosis within one share of lungs is accompanied by considerable inflammatory reaction (leycocytosis), decrease in number of T - and B - cells, prevalence of cells with index early activation (CD25+), increase is functional - metabolic activity of neutrophil phagocytes. Markers of early activation of a cage can reflect activity of pathological process and be used for forecasting of a course of tuberculosis of lungs.

P4.05.13
Immunological assessment of inflammatory reaction at patients with different forms of tuberculosis of lungs

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Studying of biology of a mikobakteriya of tuberculosis in a separation from research of response of an organism on presence of a pathogen is wrong approach to a solution of the problem of treatment of patients. The susceptibility or resistance to this disease, and also dynamics of treatment in many respects is defined by macroorganism response. Inflammatory reaction which develops in the course of supervision becomes one of manifestations of protective reactions. Clarification of conditions of its formation and current is one of problems of the conducted research. 50 patients with various clinical forms of tuberculosis of lungs, and also group of healthy patients are surveyed and treated. Studied clinic - radiological research, the main biochemical indicators, and also definition of immunological indicators of existence of inflammatory reaction. Defined as soluble mediators of an inflammation (a complex interleukins, reagents Bender MedSystems eBioscience, GmbH, and estimated cellular reactions (phagocytosis micro and macro - phagocytes, used reagents of Phagotest reagents firm (Orpegen Pharma) and BurstTestKit (Glycotope Biotechnology). It is established that the indicators characterizing oxidizing explosion are sharply raised in neutrophils at infiltrative tuberculosis. In monocytes at all studied forms of tuberculosis they are a little lowered. Level of interleukin-4 and a factor of a necrosis of tumors in the majority of samples appeared below level of sensitivity of test system. But at patients tuberkuloma level of interleukins-4 made from 5,17 to 8,58 pg/ml. Concentration of TNF was in the range of 24,6 - 39,9 pg/ml of four serum samples.

P4.05.14
Mycobacterium tuberculosis antigen-specific immune responses among tuberculosis lymphadenitis patients

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The reporting rate of extra-pulmonary TB (EPTB) has increased in most high TB burden countries in recent years. Tuberculosis lymphadenitis (TBLN) is one of the most common forms of EPTB and is considered as a local manifestation of a systemic infection. Evaluating the immune responses of TBLN patients to *M. tuberculosis (*MTB*)* specific antigens will provide important inputs to efforts being made towards developing efficient TB diagnostic tools and vaccines. In this study, peripheral blood mononuclear cells (PBMCs), from non-pulmonary TB but clinically, cytologically and/or culture and PCR confirmed TBLN patients (n=48) and healthy controls (n=23) were isolated and frequencies of antigen-specific (ESAT-6, CFP-10, RV2031c (ACR), Ag85B, TB10.4 and PPD) IFN-γ producing T-cells (spot forming units/10^6 PBMCs) were measured using ELISpot assay. Considering PHA as negative and media alone as negative control, the median frequencies of CFP-10- and TB10.4-specific IFN-γ producing T-cells were significantly higher in TBLN patients compared to healthy controls ($p < 0.001$). Both groups showed poor responses to Ag85B. Strong responses to some of the selected TB antigens could demonstrate the role of the antigens in the identification of TBLN cases; however, more detailed analyses are warranted to satisfy the need for specific diagnosis of extra-pulmonary TB cases.

P4.05.15
Integrin alpha2 beta1 modulates MMP-1 expression in Tuberculosis

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In tuberculosis (TB) Matrix Metalloproteinase-1 (MMP-1), a collagenase, has a major role in tissue destruction and cavitation. MMP-1 secretion is amplified by networks such as those between monocytes and respiratory epithelial cells. Cell adhesion to the extracellular matrix is mediated by integrins, which can coordinate rapid responses to airway injuries. We hypothesised that adhesion to collagen I modulates MMP-1 secretion in primary Human Bronchial Epithelial cells (NHBEs) in TB.
NHBEs were stimulated with conditioned medium from Mtb-infected monocytes (CoMtb) or control medium, in the presence or absence of coated or free human collagen I. Integrin engagement was studied by neutralising anti-integrin alpha2 and alpha3 antibodies (mAbs), confocal microscopy and laser scanning cytometry. MMP-1 gene expression and secretion was measured by ELISA, Luminex, real-time PCR and functional DQ collagenase assay.

CoMtb stimulated NHBEs, adherent to 100µg/ml coated collagen decreased MMP-1 secretion from 1647±65pg/mL to 1127±91pg/mL (32%). In contrast, free collagen upregulated MMP-1 by 23%. Gene expression was consistent with secretion. Collagenolytic activity was 3-fold higher in absence of collagen than with coated collagen and 5-fold higher in the presence of free collagen ($p<0.05$). MMP-1 suppression with coated collagen was abolished by 2mM EDTA ($p>0.05$). Adhesion to coated anti-alpha2 mAbs reduced MMP-1 secretion by 30% ($p<0.01$). Confocal microscopy showed integrin alpha2 beta1 polarization on coated collagen while free collagen I caused integrin alpha2 beta1 microclustering.
In summary, MMP-1 activity, gene expression and secretion is regulated by collagen-cell interactions and integrins are potentially crucial in the control of inflammatory tissue destruction in TB.

P4.05.16
Degradation of cholesterol is essential for the pathogenicity of _Mycobacterium tuberculosis_

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Objective: *Mycobacterium tuberculosis* (Mtb) cholesterol oxidase (ChoD) and ketosteroid dehydrogenase (KstD) enzymes are involved in cholesterol degradation but its role in the infection of human macrophages is not preciously defined. Methods: Using the technique of gene replacement based on the process of homologous recombination we obtained MtbH37Rv mutants lacking functional copy of ChoD (Δ*choD)* or KstD (Δ*kstD*) as well as complemented strains obtained by insert intact *choD* or *kstD* genes. The ability of MtbH37Rv and its mutants to replicate in human resting macrophages and their impact on the macrophages functional response was evaluated. We also assessed the TLR2 involvement in the activation of bactericidal activity of macrophages. Results: Δ*choD* and Δ*kstD* mutants grew weaker in macrophages in comparison to wild-type and complemented strains. Blocking of TLR2-signalling pathway significantly increased the survival of both mutants in contrast to wild-type strain. Only mutants stimulated macrophages to NO production that was blocked in the presence of TLR2-signalling pathway inhibitor. In turn, ROS production was significantly less inhibited by mutants than by wild type and complemented strains. Wild-type and complemented strains contributed to significantly higher production of IL-10 by macrophages than Δ*choD* strain. Conclusion: We indicate a relationship between degradation of cholesterol by Mtb, Mtb intracellular survival and functional responses of macrophages. Cholesterol metabolism by Mtb may contribute to the production of specific virulence factors that affect NO/ROS production and disrupt the host cell TLR2-signalling pathway.
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P4.05.17
Granzyme A is an additional marker for tuberculosis (TB) to discriminate between patients with active disease and subjects with latent infection

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Tuberculosis is a major global health problem, with an estimated 8.7 million cases and 1.4 million deaths in 2011. Cytotoxic molecules such as granulysin, perforin and granzymes produced by cytolytic T cells directly contributes to immune defense against tuberculosis (TB). It is still difficult to discriminate active disease, latent infection (LTBI), past TB patients subjects based on Quantiferon TB-Gold in tube (QFT-IT) response; therefore the aim of this study was to evaluate if the responses to granzyme A-could help to discriminate between the different TB stages. We compared the release of granzyme A in whole blood cells stimulated with QFT-IT. We found significant higher level of this cytototoxic molecule in LTBI and past TB subjects compared to the level found in patients with active disease (at least $p<0.001$).
These results suggest that the evaluation of the level of cytotoxic molecules such as granzyme A could be considered as a biomarker of TB to better discriminate among LTBI, active disease, past TB.

P4.05.18
Mycobacterium tuberculosis Beijing induces a tolerogenic phenotype in dendritic cells

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Dendritic cells (DC) play a pivotal role during the immune response to Mycobacterium tuberculosis (Mtb). Different works have demonstrated that Mtb inhibits several functions of DC in order to delay and modify T cell responses. Furthermore, there is increasing evidence about the genetic diversity of Mtb strains and how these affect their interaction with the immune system. Beijing genotype has attracted attention because of their high prevalence and multi-drug resistance. Although it is known that this genotype is particularly hypervirulent and activates differentially macrophages when compared to other genotypes, little is known about its interaction with dendritic cells. In order to address this issue, bone marrow derived DC (BMDC) were stimulated with protein extracts from BCG, H37Rv, Canetti and Beijing genotypes. We observed that Beijing was unable to induce maturation of DC as assessed by MHC-II molecule expression, contrasting with other mycobacteria. Moreover DC stimulated with Beijing failed to produce IL-12 and TNF-α, but was the only mycobacteria able to induce IL-10 in BMDC. Interestingly, Beijing induced CCR7 and PDL-1 on BMDC, but was unsuccessful to induce CD86. These results points that Beijing is able to modulate DC functions that could be related to the pathogenesis induced by this genotype.

P4.05.19
Macrophage exposure to crystalline silica promotes growth of Mycobacterium tuberculosis by deregulating cell death pathways

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Background: Inhalation of crystalline silica (CS) particles increases the risk for pulmonary tuberculosis. The exact immune mechanism through which CS exposure is associated with *Mycobacterium tuberculosis* (Mtb) infection is unclear. The oxidative stress theory turns on cell death and interferes with the ability of phagocytic cells to eliminate pathogens, which might contribute to spread the infection. We speculate that deregulation of apoptosis *vs.* necrosis could explain the association between exposure to CS and tuberculosis. Methods: THP-macrophages were exposed to CS and infected with Mtb. Expression of surface markers was analyzed by flow cytometry. Cell death was assed by TUNEL. Intracellular expression of JNK1/2, ASK1, caspase 9, P-p38, Bcl-2 and Mcl-1 was analyzed by Western blott and cytokines by ELISA. Results: Pre-exposure to CS limits the control of Mtb growth. Reduced expression of TLR2, Bcl-2 and Mcl-1 and increased the expression of JNK1 and ASK1 molecules, was observed after macrophage exposure to CS. We identified that priming uninfected macrophages with CS increased DNA fragmentation but not in Mtb-infected macrophages. Finally, when pre-exposed macrophages were infected with Mtb the concentration of TNF-alpha, IL-1beta and caspase-9 expression increased. This pro-inflammatory profile of the macrophage unbalanced the apoptosis/necrosis pathway. Conclusions: CS exposure sensitizes macrophages to present cell death by inducing activation of the MAPK intracellular pathway. Secretion of TNF-alpha and IL-1beta by M.tb-infected macrophages promotes necrosis. Deregulation of cell death pathways may be detrimental to the host defenses because it favors the release of viable bacilli and; therefore of tuberculosis.

P4.05.20
Mycobacterium tuberculosis impairs autophagic flux in human dendritic cells by ESX-1

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The autophagic process has been investigated in human primary dendritic cells (DC) to optimize Mycobacterium tuberculosis (Mtb) vaccine strategies. Indeed, given the importance of autophagy in different facets of antigen presentation, we studied this process in DC, which represent key effectors of the immune response against Mtb.
We have observed that Mtb, but not the avirulent BCG, inhibits the late steps of autophagy and that a functional ESX-1 secretion system is required for this block. Infecting DC with recombinant BCG and H37Ra Mtb strains expressing the RD1 region, BCG::RD1, or the trascriptional factor phoP, (H37Ra::phoP) involved in the expression of the ESAT-6 molecule, we observed a restored capacity of these strains to induce autophagy blockage.
In addition, treatment with rapamycin (a pharmacological inducer of autophagy) restores autophagy in Mtb-infected DC and enables DC to prime a robust Th1 response. These findings underscore a previously unrecognized link between Mtb virulence and autophagy inhibition and, together with the findings on maturation and cytokine expression, indicate that Mtb may control DC immunoregulatory functions through ESX-1.

P4.05.21
Virulence, immunopathology and transmissibility of selected strains of M. tuberculosis from epidemiological studies in Colombia evaluated in a murine model

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Until recently the M. tuberculosis (Mtb) complex was considered genetically a highly conserved group of bacteria, however, over the last decade, a wide variety of genotypes have been discovered among Mtb strains. Also, epidemiological data has shown that differences in transmissibility and virulence could be related to the genetic background of the organism.
Six Mtb strains were selected based on relevant clinical/epidemiological patterns from pulmonary tuberculosis patients from Colombia and tested in a well-characterized BALB/c mouse model of progressive pulmonary tuberculosis to examine the course of infection in terms of strain virulence (mouse survival, lung bacillary loads, histopathology) and immunological response (cytokine expression). Also, a mouse model of transmissibility consisting of prolonged cohousing of infected and naïve animals was tested.
We observed that particular outbreak strains caused distinct disease patterns, regardless having the same genotype (LAM): 1) two of the strains showed high bacterial loads and more tissue damage including necrosis towards the 3rd week; 2) in contrast, the rest of the isolates showed lower bacterial loads and formation of granulomas and pneumonia around the 3rd week ; 3) all six strains could be transmitted to nïve animals.
According with epidemiological data, the two more virulent strains were isolated from females while the rest were obtained from men.
In conclusion, genetically different Mtb strains may affect the progression of the disease, and establishing the link between virulence and transmission is important to reach the goal for Tb control.

P4.05.22
Induction of Transcription Factors involved in T lymphocyte differentiation in BCG vaccinated subjects and pulmonary Tuberculosis patients with antituberculosis therapy

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Tuberculosis is still a leading cause of death in the world, by the intracellular pathogen Mycobacterium tuberculosis. One of the many strategies to evade the dissemination of the pathogen is through BCG vaccination. Acquired immune response mediated by subpopulations of T cells, like Th1 (TBET), Th2 (GATA3), Th17 (RORC2) and Treg (FOXP3) have been described. However, it remains unknown the precise role of Th cells after the antituberculosis therapy and after BCG vaccination. The aim is to evaluate the expression of transcription factors in peripheral blood mononuclear cells from patients with pulmonary tuberculosis after antituberculosis therapy and in subjects who received the BCG vaccine. Blood samples were obtained from healthy subjects and patients at times 0, 2 and 6 months after BCG vaccine or antituberculosis therapy. Mononuclear cells were cultivated for 48 hours in the presence of ESAT-6 10 µg/ml and CFP-10 10 µg/ml and the transcription factors were measured using Real-Time PCR. We found in BCG vaccinated subjects, a decrease in the expression of FOXP3 after 2 months of having received the vaccine, and an increase in the expression of RORC2 after 6 months. For tuberculosis patients, high levels of FOXP3 and low levels of RORC2 were observed. In contrast, after 2 months of therapy, the expression of TBET and FOXP3 were decreased and GATA and RORC2 were increased. In conclusion, the BCG vaccine induces Th17 cells and it may contribute to protected immune response and the 2 months-antituberculosis therapy induces Th17 and Th2 cells in tuberculosis patients.

P4.05.23
A partly normalization in the immune-endocrine circulating profile of tuberculosis patients undergoing specific treatment

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Tuberculosis (TB) continues to be a global health problem. Extending our earlier findings in which untreated TB patients revealed imbalanced immune-endocrine responses, we have now analyzed the immune-endocrine profile throughout the 6-month course of specific treatment and 3 month following its completion. Twenty healthy controls (HCo) and 19 age- and sex-matched TB -HIV negative-patients were bled at diagnosis (T0), two (T2), four (T4) and 6 months (T6) of treatment as well as 3 months later (T9). At diagnosis patients showed a low BMI ($p<0.001$) and lymphoproliferative response ($p<0.05$), without no major changes in clinical laboratory tests; respect to HCo. The two former ones increased during treatment. Lymphoproliferation reached values similar to HCo at T9, whereas BMI showed a partial recovery remaining below the values seen in HCo. Analysis of plasma endocrine compounds (Cortisol and dehydroepiandrosterone -DHEA), cytokines (IFN-γ, IL-6, TGF-β, IL-4, IL-17), C reactive protein and peripheral T regulatory cells were carried out at the same time-points. Pro-inflammatory mediators were increased at T0, as was the Cortisol/DHEA ratio ($p<0.01$ vs. HCo, all cases). Such an increase coexisted with a high percentage of T regulatory cells, augmenting even further at T2 ($p<0.05$); the time when inflammatory compounds decreased. Throughout treatment Cortisol remained a little but significantly increased, whereas DHEA levels reached the values seen in HCo, collectively resulting in a restored Cortisol/DHEA ratio. While the clinical improvement resulting from antituberculosis treatment coincided with a decreased inflammatory pattern, the activity of the hypothalamic-pituitary-adrenal axis remained somewhat disturbed.

P4.05.24
Mycobacterium bovis culture filtrate proteins induces expression of autocrine prolactin loop enhancing inflammatory response in monocytes THP1

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Prolactin (PRL) of pituitary origin is known to regulate many physiological functions such as proliferation, differentiation and cell survival. Also, its purpose is to act as an immunomodulator, which can interfere with lymphocyte activation and cytokine production. It has recently been associated with carcinogenesis and exacerbation of autoimmune diseases. The Tuberculosis in humans induces increased physiological levels of PRL, however, their involvement in disease progression has not been determined. The aim of this work was to investigate whether PRL autocrine modulates the proinflammatory response in THP1 monocytic cells incubated with Mycobacterium bovis culture filtrate proteins (CFP). The monocytes stimulated with CFP-M.bovis express autocrine Big PRL 60-80 kDa and short isoforms of PRLr 40 and 50 kDa. RT-PCR showed an increase mRNA of PRL and PRLr vs untreated cells $P<0.001$. For immunocytochemistry the PRLr and PRL were observed in medium, membrane and nucleus. As expected, M.bovis induced high concentrations of nitric oxide, IL1-β, (681.5 pg/mL) and IL-6 (600.4 pg/mL), and TNF-α (28.7 pg/mL). The levels were measured by the Greiss reaction and ELISA. However proinflammatory cytokines IL1-β, (100 pg/mL) and IL-6 (110.5 pg/mL), and TNF-α (16.4 pg/mL) significantly decrease secretion levels by inhibition of the PRLr. In summary, our results suggest the CFP-M.bovis induces overexpression of short isoforms of PRLr and autocrine synthesis of Big PRL in myeloid cells. This autocrine mechanism suggesting a primary pro-inflamatory role of this hormone and these results suggest the PRL autocrine might have a crucial role in resolving inflammation.

P4.05.25
Immunogenicity assessment of live TB vaccine candidates in human primary dendritic cells

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Vaccination strategies against Tuberculosis (TB) by engineering new mycobacterial strains are based on either on improvement of BCG (Bacillus Calmette-Guerin) vaccine through addition of relevant genes or attenuation of Mycobacterium tuberculosis (Mtb) through deletion of virulence genes.
Several live vaccine candidates have been tested for safety, immunogenicity and protection in pre-clinical studies in animal models. However, due to the different outcome of Mtb infection in animals versus man, it would be critical to test live vaccine in a human experimental setting composed by primary dendritic cells (DC), key effectors of the immune response against Mtb.
To this aim, we are investigating DC response to infection with BCG and Mtb strains mutated in ESAT-6 and CFP10 complex, whose virulence attenuation was previously characterized in mice. By a comparative analysis of DC phenotype, pro-inflammatory and regulatory cytokine production profile and the capacity to promote T cell response, differences were observed in immunogenic potential of the recombinant BCG and Mtb clones.
Collectively, our data indicate that a model based on human DC could be used to select the most immunogenic vaccine candidate among the available recombinant strains displaying a similar attenuated phenotype. In addition a deeper understanding of host-pathogen interaction will improve our knowledge on human vaccinology and for designing of vaccination strategies against Mtb.

P4.05.26
B cells are important to modulate the immune response against Mycobacterium tuberculosis (Mtb) in mice

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Tuberculosis is the second cause of death by infectious disease in the world. The unique vaccine certified nowadays is BCG. However, the role of B cells in BCG immunization and tuberculosis infection is poorly understood. We want to investigate the role of B cells in tuberculosis progression in mice. C57BL/6 and Bko mice were immunized with BCG and 30 days after immunization, mice were infected with Mtb H37Rv. After 10, 30 or 60 days post infection, the percentage of B cells and T memory cells were determined by flow citometry in lungs. In parallel the bacilli charge was determined in the lungs and the inflammatory infiltrate was shown by histological analyses using HE. Adittionally, mRNA of INF-γ, IL-6, IL-17 and TGF-β were quantified using RT PCR. Results shown that in animals only infected with Mtb, lack of B cells caused an exacerbation of lungs pathology by promote an increase of inflammation and migration of memory T cells, although didn't not affected the number of CFU. Even in lung of animals previously immunized, BCG couldn't be able to promote protection in Bko mice, because this immunization did not caused a significant reduction of inflammation and CFU as seen in WT mice. Additionally, lack of B cells induced a drastic decrease in expression of IFN-γ mRNA and increase expression of IL-6, TGF-β and IL-17 mRNA in lungs of Bko. B cells seem to be important to control tuberculosis immunopathology and bacterial growth, beyond to promote the optimal efficacy of BCG immunization.

P4.05.27
***Mycobacterium leprae* DNA and cytokine profile of household contacts resident in Governador Valadares, MG, Brazil.**

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Hansen's disease has a long incubation period and essentially clinical diagnosis. Household contacts, which live with patients, may be infected and asymptomatic. Therefore, the validation of a diagnostic method to identify infection earlier is required. We evaluated the qPCR as a tool to identify DNA of ML in samples of dermal scrapings of patients and their household contacts, and also the cytokine profile of those individuals to indicate the possibility of existing subclinical infection. We had 156 individuals, 43 index cases and 113 household contacts. The qPCR was performed to amplify 16S rRNA fragment, specific for ML and was positive in 48.8% of 43 patients with leprosy while the BAAR was positive in only 30.2% of the total. For household contacts 23.9% of 113 subjects showed bacterial DNA. The intracellular cytokine pattern was evaluated following antigen stimulation. Patients classified as PB showed high frequency of IL-10 high producer cells. However, it was observed a higher frequency of IFN-γ high producer cells in these individuals when compared to MB patients. On the other hand, MB patients showed a higher frequency of IL-4 and IL-10 high producer cells than PB. The cytokine profile of household contacts, showed a higher frequency of IFN-γ high producer cells among PB household contacts, in contrast, higher frequency of IL-4 high producer cells among MB household contacts. These data reinforce the relevance of monitoring household contacts that showed qPCR positive for ML and higher frequency of T cells producing IL-4 and IL-10 cytokines. Financial support: CNPQ/FAPEMIG/FIOCRUZ/UNIVALE/CREDEN-PES.

P4.05.28
Apoptotic body like liposomes carrying phosphatidic acid enhance antimicrobial and anti-inflammatory innate immune response

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We have generated novel asymmetric liposomes with phosphatidylserine (PS) distributed at the outer membrane surface, to resemble apoptotic bodies, and phosphatidic acid at the inner layer, to promote phagolysosome maturation, as a strategy to enhance innate anti-mycobacterial activity in phagocytes while limiting the inflammatory response. Our results show that the stimulation of human macrophages with apoptotic body-like liposomes carrying phosphatidic acid (ABL/PA) i) promote maturation of phagolysosomes containing *Mycobacterium tuberculosis* (MTB); ii) enhance intracellular MTB killing in infected type-1 and -2 macrophages; iii) induce intracellular killing of endogenous pathogens in bronchoalveolar lavage cells from 4 patients with active pulmonary tuberculosis and 1 patient with Klebsiella pneumonitis. Moreover, the intranasal administration of ABL/PA in MTB infected mice dramatically reduced pulmonary mycobacterial burden, with a concomitant 10 fold reduction of serum TNF-α, IL-1β, and IFN-γ in comparison with untreated mice. Furthermore, ABL/PA stimulated macrophages down-regulate Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-12, IL-1β, IL-18 and IL-23 and up-regulate Transforming Growth Factor (TGF)-β without altering IL-10, IL-27 and IL-6 mRNA expression. Finally, when ABL/PA stimulated macrophages were used as target cells and allogeneic purified naïve CD4+ T cells were used as responder cells, in a mixed lymphocyte reaction model, a dramatic inhibition of alloreactive CD4+ T cell proliferation was observed. Altogether, these results suggest that apoptotic body-like liposomes may be used as a Janus-faced immunotherapeutic platform to deliver polar secondary lipid messengers, such as PA, into phagocytes to improve and recover phagolysosome biogenesis and intracellular pathogen killing while limiting the potentially pathogenetic inflammatory response.

P4.05.29
Proteomics reveals dynamic phagosomal environment during *Mycobacterium* -HIV co-infection

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Mycobacterial infection is a major cause for increased mortality in HIV infected patients. In order to understand the coalition between Mycobacteria and HIV that makes these diseases more destructive when confronted together, we undertook a proteomics approach to identify differentially regulated proteins during intra-phagosomal phase of infection in macrophages under mycobacteria-HIV co-infection. A comparative protein expression pattern of host proteins during mycobacterial and mycobacteria-HIV co-infection of macrophages revealed 95 phagosomal and 70 mitochondrial proteins differentially regulated. An extreme cytoskeletal rearrangement was observed with categorical up-regulation of energy metabolism proteins during co-infection. Purinergic receptors, which are capable of adjusting cellular metabolic needs and also help HIV entry, were observed to be upregulated during co-infection. Interestingly, co-infected host cells mounted augmented inflammatory response, as evident from increased manganese superoxide dismutase and intracellular ROS levels. 94 mycobacterial proteins were differentially regulated upon co-infection, majority of which belonged to intermediary metabolism, respiration, cell wall synthesis and information pathways. During co-infection, Mycobacteria showed increased expression of proteins of ESX secretion systems that are instrumental in secretion of virulent proteins. An increased expression of cAMP dependent regulator (Rv2565), Rv1265 (regulated by cAMP

dependent regulator) and RecF (DNA repair protein) upon co-infection may protect mycobacteria from increased host ROS levels. Altered expression of toxin-antitoxin family of proteins during mono- and co-infection suggested regulated mycobacterial stress response that may influence programmed cell death. Conclusively, proteomics data suggested a dynamic host-pathogen environment during co-infection leading to identification of critical factors that explained the coalition between HIV and mycobacteria.

P4.05.30
Diagnosis of latent tuberculosis in immunosupressed patients with inflammatory bowel diseases

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Objective: This study was conducted in patients with inflammatory bowel diseases to evaluate the performance of Mycobacterium tuberculosis antigen-specific interferon-γ releasing assay (QuantiFERON®-TB Gold In-Tube) for the diagnosis of latent tuberculosis infection (LTBI) comparing to TST, to assess the impact of immunomodulator (IM) treatment in their performances and to analyze whether IGRA positivity is related to genetic susceptibility by screening for INFG+874T→A SNP.
Material and methods: TST by Mantoux method and QuantiFERON®-TB Gold In-Tube (QFT-GIT) in accordance with manufacturer's instructions, were prospectively performed in 100 consecutive IBD patients and 54 healthy individuals. The SNP genotyping was done by an ARMS-PCR technique.
Results: A better agreement was observed between test's results in controls (κ=0.40) than in patients (κ=0.16). Although QFT-GIT results were unaffected by IM therapy, the mean mitogen response was reduced in immunosuppressed patients (8.06 UI/ml) when compared to the rest of patients and controls (12.70 UI/ml). Similarly, lesser TST positivity was observed in those under IM (9.8%vs20.5%). Among the 12 QFT+ subjects, 10(88.3%) had the susceptibility allele (A). Additionally, 6 of them were homozygote for this allele (AA).
Conclusion: Although IM weakens the immunity strength, QFT-GIT seems to be,as previously described, more accurate for detecting LTBI's cases that would otherwise be missed using solely TST. In a large vaccinated population, QFT-GIT appears more reliable for excluding a false positive TST. Even though, the (INFG +874TA) SNP has been frequently associated with active tuberculosis, it seems that this SNP can also be associated with the latent form.

P4.05.31
CD4+ and CD8+ T cells producing IL-17 specific-ESAT-6 or CFP10 M.tuberculosis antigens correlate with therapy successful in MDR-TB patients

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Multidrug-resistant tuberculosis (MDR-TB) is a disease caused by Mycobacterium tuberculosis (*M. tuberculosis*) strains that are resistant at rifampicin and isoniazid the most effective anti-TB drugs, entails extended treatment and expensive and toxic regimens, higher rates of treatment failure and death. With the aim of assessing immunological biomarkers that can aid with MDR-TB treatment efficacy, we enrolled six MDR-TB patients and followed during drug therapy under program Directly Observed Therapy (DOT). Blood samples were obtained before and monthly trough 2 year of treatment. Then peripheral mononuclear cells (PBMC) were isolated and the frequency of IFN-g producing cells and CD4+ and CD8+ T cells producing IFN-γ, IL-2, IL-4 and IL17 in response to the *M. tuberculosis* antigens ESAT-6 and CFP10 or PPD were assessed by ELISpot and FACS assays respectively. The overall rate cure was of 100%. The successful of MDR-TB treatment was accompanied by a significant reduction of CD4+T and CD8+ T cells producing IL-17 in response to ESAT-6 and CFP-10 (P≥0.05). An inverse response was observed on CD8+ T cells producing IL-17 in response to PPD (P=0.05). We did not find correlation in the CD4+ or CD8+ T cells producing IFN-γ, IL-4, IL-2 or IFN-γ with successful therapy. These findings suggest a correlation of CD4+ and CD8+ specific T cell producing IL-17 with mycobacterial load, which also decrease during successful therapy and suggest that CD4 and CD8 T cell producing IL-17 could represent a potentially useful parameter in the treatment of MDR-TB.

P4.05.32
Attenuation of BCG Delays Cell-to-Cell Spread in the Lung and Promotes its Clearance by T cells

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To identify differences between persistent and resolving mycobacterial infection, we compared the course of infection with *M. bovis* BCG Pasteur or *M. tuberculosis* H37Rv in WT and TCRβ/δ$^{-/-}$ mice. *M. tb*-infected TCRβ/δ$^{-/-}$ mice die ~42 d post infection (p.i.), while BCG-infected TCRβ/δ$^{-/-}$ and WT mice both survived >150 days p.i. TCRβ/δ$^{-/-}$ mice infected with 3x10^3 BCG had 10^5 bacteria in lungs 100 days p.i., while WT mice had no detectable CFU at that time, indicating that T cells promote elimination of BCG. We then compared the distribution of bacteria in subsets of lung myeloid cells. 14 d p.i., only 12% of Rv-infected cells were AM, indicating that Rv spreads rapidly to other lung phagocytes. At the same time point, 40% and 82% of BCG-infected cells are AM in WT and TCR β/δ$^{-/-}$ mice, respectively, implying that BCG does not spread efficiently beyond AM, and that T cells prevent spread of BCG beyond AM. 150 days p.i. in TCR β/δ$^{-/-}$ mice, BCG spreads beyond AM and the cellular distribution resembles that of *M. tb*. We conclude that the attenuation of BCG delays cell-to-cell spread in the lung; T cells prevent the further spread of BCG and eliminate the infection.

P4.05.33
IFN-α adjuvant effect in BCG-vaccinated mice against mycobacterial infections

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Tuberculosis is still a global health problem. The current vaccine BCG, is the only prophylatic measure for *M. tuberculosis* (*MTb)*, however, it fails to protect adults from pulmonary Tb. Heterologous prime-boost protocols have been demonstrated to promote long-term memory responses. T cellular responses play a key role in the defense against *MTb*. Type I interferons (IFN-I) (α/β) are cytokines that exerts profounds effects in DCs function as well as in T cellular responses. There is a controversial issue on *in vivo* effects of type Í IFNs on mycobacterial infections. In a preliminary study, we have found that IFN-α exerts mostly a systemic immune protection (reduction of CFUs in spleen). However, the precise role of IFN-α in mediating an adjuvant effect able to improve BCG or other Ag-based vaccines *in vivo* has to be assessed. Thus, the objective of this study was to investigate at different intervals of IFN-α boosting of BCG primed BALB/c adult mice, the IFN-α role in augmenting BCG immunogenicity *in vivo*. Here, we found that intramuscular administration of IFN-α specifically associated to BCG vaccine, exerted a mostly protector effect when the BCG primed mice received a very small concentration of IFN-α at successive days (two weeks after BCG priming) than at intervals of two weeks. Preliminary results using electronic microscopy suggest that IFN-α might promote germinal centers in spleen. Overall our findings provide evidence of the positive role of IFN-α as a potential candidate to be used in BCG-based vaccines against mycobacterial infections.

P4.05.34
Analysis of polymorphisms in TLRs and FcgRs in susceptibility to tuberculosis in a Mexican population

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Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. In México, specifically in the state of Veracruz, there´s a relative high prevalence, so it´s important to identify factors which affect the susceptibility to TB in this population. To investigate the influence of genetic variants in TLRs and FcγRs in TB, we conducted a case-control study for the polymorphisms: *TLR1* (p.N248S), *TLR4* (p.D299G; p.T399I), *TLR6* (p.S249P), *TLR10* (p.A163S; p.V298A), *FCGRIIA* (p.R131H) and *FCGRIIIA* (p.V158F).
In a total of 125 cases and 272 controls, we found a protective association to the *TLR4* 299G and 399I variants (OR=0.268 and OR=0.179 respectively) when comparing between alleles. Although the association didn´t reach statistical significance (p=0.062 and p=0.063). A similar association (OR=0.299, p=0.236) was found when comparing genotypes 399TT versus 399TI.
The variant 131H of *FCGRIIA* was found associated with susceptibility to TB when comparing between alleles and homozygotes (OR=1.586, p=0.106 and OR=1.301, p=0.087 respectively), which were not statistically significant.
For *TLR4* SNPs, the haplotype G-I was more frequent in controls than in cases (2% and 0.4%, p=0.085) and the D-T haplotype was more frequent in cases than in controls (99.2% and 96.9%, p=0.049). The analysis of linkage disequilibrium (LD) indicated that *TLR4 (chr:9, r^2=0.6)* and *TLR10 (chr:4, r^2=0.85)* polymorphisms were in LD.
Our results so far indicate that none of the SNPs analyzed were statistically associated with TB in our population. However we found several correlations with other studies in different populations.

P4.05.35
Cathepsin S at the crossroads between innate and adaptive immunity to TB infection

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Although the TB vaccine (derived from the non-pathogenic mycobacterium M. bovis bacillus Calmette-Guérin, or BCG) is generally safe and rarely induces disease in human, it appears to mimic virulent M. tuberculosis (Mtb) strains in their capacity to inhibit macrophage (MØ) functions that initiate adaptive immune response. Our investigations have revealed that MØ infection with conventional BCG down-modulates surface expression of mature MHC class II molecules by mechanisms that are in part dependent on the inhibition of Cathepsin S (CatS) expression. Normal phagosome biogenesis, which is a prerequisite for optimal Ag presentation, is prevented by pathogenic Mtb, and also significantly blocked by BCG. Therefore these inhibitory effects of the current BCG vaccine on MØ functions may, at least partially, explain its failure to induce efficient immunity to TB.
CatS is a cysteine protease required for normal processing and maturation of MHC class II molecules in MØ. Therefore, we generated a recombinant BCG strain secreting human active CatS (BCG-CatS) and demonstrated that infection with BCG-CatS (i) restores normal levels of MØ CatS, (ii) induces phagosome-lysosome fusion and apoptosis and (iii) restores the expression of mature class II molecules as well as their capacity to present mycobacterial Ag to specific CD4 T cells. On the other hand, preliminary animal experiments showed a significant decrease in the number of virulent Mtb in the lung of mice vaccinated with BCG-CatS. Such findings were consistent with concomitant observations that CatS also stimulates the MHC class I pathway in cells infected with mycobacteria.

P4.05.36
Prime-boost Mycobacterium smegmatis recombinant vaccination improves protection in mice infected with Mycobacterium tuberculosis by enhancing humoral and cellular specific immune responses

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Tuberculosis (TB) presents one of the main causes of death by infectious agents. BCG, the available vaccine for TB, although safe, does not protect against TB in adults. Several proteins from M. tuberculosis (Mtb) were tested as subunit vaccines or in recombinant vectors and, the vaccines composed by more than one protein/antigen showed a good potential to proceed to clinical assays. A fusion protein construction (CMX) containing epitopes from Ag85C, MPT-51 and HSP-X from Mtb were immunogenic in both mice and in individuals with active TB, thus presenting a potential use as vaccine for TB. M. smegmatis (MC2) was used as a vector for a new CMX vaccine. The specific immune response to CMX was evaluated after CMX-MC2 vaccination and challenge with Mtb. IgG1 and IgG2a to CMX were induced, as well also specific CD4+IFN-γ and CD8+IFN-γ cells responses. It was observed higher induction of CMX specific TCD4+IL-17+ cells in the lungs. After Mtb infection, a substantial increase in the CD4+IFN-γ+ cells were observed in the spleen and lungs. The CMX-MC2 vaccine reduced at least to half of the amount of bacteria recovered from the lungs of infected animals. In conclusion a CMX-MC2 vaccine increased protection to mice infected with Mtb by inducing both humoral and cellular immune response to CMX.

P4.05.37
Blockade of PD-1 signal pathway causes exacerbation of Mycobacterium tuberculosis infection via excessive IFN-γ production by antigen-specific Th1 type CD4+ T cells

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$CD4^+$ T cell-mediated acquired immunity is essential for the control of *Mycobacterium tuberculosis* (Mtb) infection. However, the immune response is also responsible for the pathological outcome in lung. Therefore, appropriate regulation of antigen-specific $CD4^+$ T cell response is required to prevent bacterial replication and lung tissue damage. Recent studies of the programmed cell death 1 (PD-1) have revealed the important role in the regulation of T cell response in a wide rage of infections with viruses and bacteria. In this study, therefore, we infected wild type and PD-1 knockout (KO) mice with Mtb to clarify the role of PD-1 in Mtb infection. PD-1 KO mice were highly susceptible to Mtb infection. We observed higher bacterial burden and severe tissue damage in lungs of PD-1 KO mice in the early phase of infection. In addition, the production of inflammatory cytokines and chemokines were markedly increased in the lung. Adoptive transfer study showed that $CD4^+$ T cells of PD-1 KO mice could not confer protection on recipient RAG2 KO mice. Furthermore, administration of anti-IFN-gamma IgG ameliorated severe tissue damage and excessive inflammation in lungs of Mtb-infected PD-1 KO mice. These data indicate that Mtb-induced severe inflammation in lungs of PD-1 KO mice was mainly caused by an enormous amount of IFN-gamma produced by Th1 cells and PD-1 signal is essential to have control over a magnitude of Th1 response to Mtb in the early phase of infection.

P4.05.38
Anti-Interferon-Gamma Autoantibodies in Adults with Disseminated Nontuberculous Mycobacterial Infections are Associated with HLA-DRB1*16:02 and DQB1*05:02 and the Reactivation of Latent Varicella-Zoster Virus Infection

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Adult patients with disseminated nontuberculous mycobacterial (dNTM) infections usually have severe immune system defects. Recently, several reports have shown that anti-IFN- gamma

autoantibodies may play an important role in the pathogenicity of dNTM infections. A considerable proportion of reported cases of anti-IFN-gamma autoantibodies show either clinical or laboratory evidence of autoimmune disease. In this study, we identified 19 formerly healthy adults who later developed dNTM infections, and 17 were further investigated immunologically. High-titer anti-IFN-gamma autoantibodies capable of inhibiting IL-12 production in vitro were found in the plasma of all of these patients. In addition to dNTM infection, 35% and 71% of our patients also suffered from salmonellosis and herpes zoster, respectively. This observation suggests that IFN-gamma may be crucial in controlling salmonella infection and reactivating latent varicella-zoster virus (VZV) infection in humans. Two HLA alleles, DRB1*16:02 DQB1*05:02 (odds ratios 8.68, 95% CI: 3.47-21.90, P=1.1×10-6, Pc=3.08×10-5 and 7.16, 95% CI: 3.02-17.05, P=1×10-7, Pc=1.4×10-6, respectively), were found in 82% (14/17) of our patients. In conclusion, our data suggest that anti-IFN-gamma autoantibodies may play a critical role in the pathogenesis of dNTM infections and reactivation of latent VZV infection and are associated with HLA-DRB1*16:02 and DQB1*05:02.
This work is supported by the National Health Research Institutes (NHRI-EX10110028SC).

P4.05.39
Mycobacterium tuberculosis Dos Regulon Gene Rv0079 Encodes a Putative, 'Dormancy Associated Translation Inhibitor (DATIN)' and Induce Proinflammatory Responses

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Tuberculosis is a human disease which is caused by Mycobacterium tuberculosis. Mtb present in one third of the world population under dormant condition. DosR regulon is mainly responsible for dormancy of mycobacteria. At the time of dormancy Mycobacteria live in the environment which is unfavourable like fewer nutrients, less oxygen condition and more nitric oxide condition. In such unfavourable condition bacteria should slow down its metabolic activity to remain live. Here we discuss the systematic characterization of Rv0079 and shown that it is important in the persistent of Mycobacteria and maintenance of granuloma. Rv0079 is involved in stabilization of ribosome and growth arrest of Mycobacteria during dormancy. This protein is also having antigenic peptides which make it immunomodulatorry protein. Rv0079 interact with TLR2 and induces proinflammatory cytokines in macrophages which are important for granuloma maintenance during dormancy. All the experiments were done with recombinant protein (rRv0079) and Mycobacterium bovis BCG overexpressing Rv0079. TRL interaction and cytokines induction study was done in engineered HEK293, THP-1cell line and PBMCs isolated from human patients blood.

P4.05.40
Protection evaluation following vaccination with a Mycobacterium abscessus virulence factor in a pulmonary infection cftr-/- mice model

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Cystic fibrosis (CF) is a disease caused by a mutation in the CFTR gene encoding a transmembrane regulator functioning as a Cl channel within the epithelial membrane. The main mutation is linked to the loss by deletion of a Phe in position 508 (ΔF508). Among the CF pathogens, Mycobacterium abscessus (Mabs), a rapid-growing mycobacterium (RGM), is responsible for severe respiratory infections in CF patients.
The specific objective of our project was to evaluate the protection induced by a Mabs virulence factor, the phospholipase C (PLC) (MAB_0555), administered under the form of a nanovectorized recombinant DNA in a model of systemic infection (intravenously) and a model of pulmonary (aerosol route) infection using as mouse model the ΔF508 CFTR mice ("CF" mice) and their wild-type counterpart (FVB genetic background
Protection was evaluated by CFU counts in the spleen, liver and lungs of animals at day 1, 7, 14 and 21 for the aerosol challenge and day 1, 14 and 45 for the IV challenge.
We only observed a protective effect after the aerosol challenge. After the aerosol challenge of FVB wild-type mice, no protective provided by immunization with the nanovectorized PLC gene effect was observed. However, a protective effect was observed in homozygous ΔF508 CFTR mice. A difference was observed at D7, which was confirmed at D21 ($p = 0.038$).
In conclusion, the use of a nanovectorized gene coding for a virulence factor is able to provide local protection and accelerated clearance of M. abscessus in the lungs of "CF" mice.

P4.05.41
Association between DC-SIGN genetic variants and pulmonary tuberculosis in Chinese Uighurs

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DC-SIGN and TLR2 on Dentritic cells and macrophages involve in the recognition of *Mycobacterium tuberculosis (MTb)* and play essential roles in the initiation of the immune response against *MTb*. Genetic studies show that these two pathway genes, DC-SIGN, TLR2 and TIRAP, contribute susceptibility to pulmonary tuberculosis in some populations, while no strudies were perforned in Chniese Uighurs. In this study, 15 SNPs in three candidate genes were genotyped in 200 cases suffering active pulmonary tuberculosis and 154 matched controls of Chinese Uygurs by using SnaPshot, including 11 tagSNPs in promotor or 3`UTR of DC-SIGN,rs5743708 and rs121917864 in exon of TLR2, rs8177374 and rs7932766 in TIRAP. We also screened the promotor and 3` UTR of DC-SIGN in 32 control samples by direct sequencing of genomic DNA. The increased risk of variants of -871GG in promotor and *443AA in 3`UTR of DC-SIGN on the occurance of tuberculosis were confirmed, especially in populations never inoculated with BCG or contacted with patients closely, and the epidemiology analysis showed that age, sex, BCG, contact and smoking were all essential factors associated with tuberculosis. The further dual-luciferase assay showed -871GG variant can not promote the transcription of DC-SIGN *in vitro*, but a -336 varient reported before can. No variant was found in rs121917864 of TLR2, and other 3 SNPs in TLR2 and TIRAP did not show significant difference between cases and controls. The presented evidence suggests variants of -871GG and *443AA in DC-SIGN increased the pulmonary tuberculosis risk in Uighur population.

P4.05.42
Mycobacterium bovis culture filtrate proteins induces expression of autocrine prolactin loop enhancing inflammatory response in monocytes THP1

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Prolactin (PRL) of pituitary origin is known to regulate many physiological functions such as proliferation, differentiation and cell survival. Also, its purpose is to act as an immunomodulator, which can interfere with lymphocyte activation and cytokine production. It has recently been associated with carcinogenesis and exacerbation of autoimmune diseases. The Tuberculosis in humans induces increased physiological levels of PRL, however, their involvement in disease progression has not been determined. The aim of this work was to investigate whether PRL autocrine modulates the proinflammatory response in THP1 monocytic cells incubated with Mycobacterium bovis culture filtrate proteins (CFP). The monocytes stimulated with CFP-M.bovis express autocrine Big PRL 60-80 kDa

and short isoforms of PRLr 40 and 50 kDa. RT-PCR showed an increase mRNA of PRL and PRLr vs untreated cells P<0.001. For immunocytochemistry the PRLr and PRL were observed in medium, membrane and nucleus. As expected, M.bovis induced high concentrations of nitric oxide, IL1-β, (681.5 pg/mL) and IL-6 (600.4 pg/mL), and TNF-α (28.7 pg/mL). The levels were measured by the Greiss reaction and ELISA. However proinflammatory cytokines IL1-β, (100 pg/mL) and IL-6 (110.5 pg/mL), and TNF-α (16.4 pg/mL) significantly decrease secretion levels by inhibition of the PRLr. In summary, our results suggest the CFP-M.bovis induces overexpression of short isoforms of PRLr and autocrine synthesis of Big PRL in myeloid cells. This autocrine mechanism suggesting a primary pro-inflamatory role of this hormone and these results suggest the PRL autocrine might have a crucial role in resolving inflammation.

P4.05.43
Suppression of immune regulation in a murine model of progressive pulmonary tuberculosis

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Mycobacterium tuberculosis (Mtb) is the etiologic agent of tuberculosis (TB). Many aspects of TB are not completely elucidated, such as the complex process of immunoregulation mediated by T regulatory cells (Treg) and the enzymes hemoxygenase 1 (HO-1) and indoleamine 2,3-dioxygenase (IDO). When BALB/c mice are infected with Mtb strain H37Rv by the intratracheal route a rapid progressive disease is developed. This model has two phases, the early phase with an efficient control of bacilli growth mediated by activation of the innate and acquired (Th-1) immunity, and the progressive phase characterized by a high production of anti-inflammatory Th-2 cytokines. High bacilli loads and tissue damage causes death. We studied the kinetics of T-reg cells, HO-1 and IDO along the infection. In the lungs of tuberculous animals during the progressive phase there was high gene expression and production of HO-1 and IDO, and a highest amount of T-reg cells. In order to study this, T reg cells were eliminated during progressive disease by the administration of specific monoclonal cytolitic antibodies (clone PC61). In separate experiments the activity of HO-1 and IDO was inhibited using the specific inhibitors zinc protoporphyrin-9 (ZnPP9) to suppress HO-1 and 1-methyl-DL-tryptophan (1-MT) to block IDO. In comparison with control animals, mice treated with any of these molecules showed a significant decrease of bacilli loads improving survival but they developed higher lung inflammation. Thus, immune-regulation mediated by T-reg cells, HO-1 and IDO is important to control excessive inflammation, permitting bacilli proliferation and disease progression.
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P4.05.44
Reduced frequency of BCG-specific IFN-γ Producing T Cells in Ugandan infants when Bacillus Calmette-Guerin vaccination is delayed from birth to 6 Weeks Of Age

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Background: In Uganda, Bacillus Calmette-Guerin (BCG) is administered at birth within a health facility. However, homebirths are prevalent therefore some newborns receive BCG later. We compared BCG-specific $CD4^+$ and $CD8^+$ T cell response in infants who received BCG at birth or at 6 weeks of age. We hypothesised that infants vaccinated at birth would show lower frequencies of BCG-specific $CD4^+$ and $CD8^+$ T cells compared with infants vaccinated at 6 weeks of age.
Methods: Nine months-old infants who received BCG at birth or at 6 weeks of age were enrolled. Blood was drawn from each infant and left unstimulated or stimulated with BCG in a short-term whole blood assay. Plasma was harvested after 7 hours for soluble cytokine levels by luminex. After 12 hours, cells were fixed, harvested and cryopreserved for later intracellular cytokine staining and flow cytometry.
Results: We enrolled 50 infants vaccinated with BCG at birth while 42 were vaccinated at 6 weeks of age. All infants showed a robust BCG-specific IL-2, IFN-γ and TNF-α, IL-17 and perforin $CD4^+$ and $CD8^+$ T cells response. Birth vaccinated infants showed a higher frequency of $CD4^+$ and $CD8^+$ T cells producing IFN-γ and lower levels of BCG-specific soluble IL-10 than six weeks old BCG vaccinated infants.
Conclusions: The higher social economic levels in birth than delayed vaccinated infants may partly explain the observed differential specific T cell responses in the two groups. Our findings prompt follow up studies that will give more insight into clinical relevance of these findings.

P4.05.45
Effects of multi-drug therapy (MDT) on cellular immunity in patients with leprosy

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Leprosy is one of the most neglected infectious tropical diseases of the skin and the nerves caused by the intracellular pathogen *Mycobacterium leprae*. Despite eradication programs, the annual new case and prevalence rate remain consistent in affected countries, especially in India, here with a WHO estimate of 127,000 newly infected individuals per year. Multi drug therapy (MDT) has been established for different disease presentations, such as multibacillary (MB) and paucibacillary (PB) infections. However, the response to these treatment regimens remains diverse across individuals. Protection against intracellular pathogens such as *M. leprae* is critically dependent on the function of NK cells at early stages of the immune response.
Natural killer cells (NK) can interact with macrophages, the preferred host cell for mycobacterium, and this interaction will trigger an adaptive or antigenic-specific immune response. We investigated the transcriptome profile of NK cells in paucibacillary and multibacillary patients who responded to MDT, and in MDT non-responders. We observed a marked differential upregulation of gene-products active in inflammation and cellular defence via mass array studies.
Additionally, we also discovered specific genotypes within the TAP1, TAP2, NOD2, LRRK2, and RIPK2 genes that are associated to disease outcome. These differential signatures and knowledge on differential regulatory pathways during the disease onset and treatment will lead to important repercussions in understanding how NK cells and *Mycobacterium leprae* interact. This may guide us to a supportive treatment by activating innate immunity in general or NK cells in particular via a supplementary supportive treatment of leprosy.

P4.05.46
The role of CD137 ligand (CD137L) signaling pathway during M. tuberculosis infection

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The role of the CD137-CD137 ligand (CD137L) signaling pathway in T cell co-stimulation has been well established. Dysregulated CD137 or CD137L stimulation leads to pathological conditions such as inflammatory diseases and cancer. However, the contribution of the CD137-CD137L interactions in the control of infectious diseases has not been extensively studied, with the few available reports focusing mainly on viral infections. Here we investigated the role of the CD137-CD137L interactions during Mycobacterium tuberculosis infection. Using CD137L deficient mice, we found that the absence of CD137L resulted in impaired priming of T cells by dendritic cells in vitro and a delayed CD4+ T cell activation in vivo. We found increased cellular

infiltrates in the lungs of CD137L deficient mice and reduced production of key cytokines such as TNF-α and IL-12 but similar levels of IFN-γ in the broncho-alveoar lavage fluid. However, these disorders translated only into a modest transient increase in the bacterial loads and granulomatous lesions in the lungs of the CD137L deficient infected mice. Our data thus suggests that compensatory mechanisms exist in these mice.

P4.05.47

Prolactin modulate proinflammatory inmune response induced by culture filtrate protein from Mycobacterium bovis in THP1 cells through different signaling pathways.

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Prolactin (PRL) has been implicates as a modulator of immune function and some of its actions may be linked with nitric oxide (NO) and cytokines synthesis. Several reports show evidence of a protective role of PRL during different infectious diseases caused by bacteria, fungi and protozoa. Significant increase of prolactin plasma levels was observed in patients with advanced tuberculosis. Monocytes/macrophages play an essential role in innate defense against microbial infection. The aim of this work was determine if PRL module a proinflamatory immune response in THP-1 cell stimulated with *Mycobacterium bovis* culture filtrate proteins (CFP) and its signaling pathway associated. The THP-1 cells were stimulated by PRL, *M. bovis* CFP and both. The levels of IL1-β, IL-6, TNF-α, IL-10 and the oxidation products of NO in THP1 cells were measure by ELISA and the Griess reaction respectively. Activation of STAT3, ERK1/2 and Akt were evaluated by western blot. As expected, *M. bovis* CFP induced high concentrations of NO also IL1-β, IL-6, and TNF-α in THP-1 monocytes. PRL (20 ng/ml) treatment considerably decreases IL1-β, IL-6, TNF-α secretion and NO concentration in THP-1 cell stimulated by *M. bovis* CFP, however increased IL-10 production. The effect of PRL was corroborated by inhibition of their receptor binding. Significantly enhanced expression p-STAT-3 and p-ERK 1/2 with PRL, and with *M. bovis* CFP treatments was observed, however with both treatments was diminished. PRL could be mediating an anti-inflammatory response during a mycobacterial infection and may be, in turn, regulated by the JAK/STAT-3 pathway.

P4.05.48

Human memory T cells in latent *Mycobacterium tuberculosis* infection are directed against three antigenic islands and are found in a CXCR3$^+$CCR6$^+$ Th1 subset

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In this study we performed a systematic analysis of the specificity and functional properties of memory T cells in individuals with latent tuberculosis by combining a genome-wide library of *Mycobacterium tuberculosis* (MTB)-derived predicted HLA class II epitopes with a high throughput cellular screening method. We found that natural immunity to MTB is directed against multiple epitopes and antigens, with more than 80 antigens necessary to capture 80% of the response. As a result of this analysis, 34 novel antigens and 3 broadly immunodominant antigenic islands were defined. The majority of the novel antigens identified are associated (contained within or in close proximity of) with these antigenic islands, which all contain Esx protein pairs and PE/PPE proteins, and are part of a putative secretory system. We also found that the response to MTB is virtually confined to a CCR6$^+$CXCR3$^+$ memory Th1 cell subset, with a minority of MTB-specific cells displaying a CCR6$^+$CXCR3$^-$ Th1/Th17 phenotype. The origin and lineage relationship of CXCR3$^+$ and CXCR3$^-$ MTB-specific T cells remain to be defined: while it is possible that they represent separate lineages (Th1 and Th1/Th17, respectively), they may also represent two differentiation stages within the same lineage. TCR deep sequencing experiments are ongoing to determine the T cell repertoires in the two T cell subsets. This study demonstrates the power of genome-wide, high throughput cellular screenings to analyze the human immune response to complex pathogens.

P4.05.49

Plasma cytokines and chemokines differentiate between active disease and non-active tuberculosis infection

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To analyse cytokines and chemokines from unstimulated plasma samples for detection of active TB disease, latent TB, discriminating active TB cases from latently infected contacts and for monitoring anti TB treatment. We analysed ex vivo plasma samples from 33 TB patients (17 HIV negative and 16 HIV positive) and 30 healthy household contacts with Luminex. We found statistically significant differences ($p < 0.05$) in median plasma concentrations of EGF, fractalkine, IFN-γ, IL-4, MCP-3 and IP-10 between contacts and TB patients. Single cytokines or chemokines predict with an area under the Receiver Operating Characteristic (ROC) curve of 0.59 for VEGF to 0.98 for IP 10 while a combination of fractalkine, IFN-g, IL-4, IP-10 and TNF identified 96.87% of TB cases and 100% of household contacts. However, none of the cytokines were significantly different in QFT positive and QFT negative contacts ($p > 0.05$). HIV does not affect the median plasma level of any of the cytokines or chemokines and there was not significant difference between HIV positive and HIV negative TB patients ($p > 0.05$) in any of the cytokines or chemokines. The median plasma concentrations of IFN-γ, IL-4, MCP-3, MIP-1β and IP-10 were significantly different ($p < 0.05$) before treatment and after treatment. Plasma cytokines and chemokines could be used as immunological markers for diagnosing active TB disease and for monitoring effective antituberculosis therapy.

P4.05.50

Knockdown of suppressor of cytokine signalling 3 mediates killing of Mycobacterium tuberculosis in THP1 derived macrophages

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Mycobacterium tuberculosis (M.tb) is the etiological agent of tuberculosis (TB), which initiates with pulmonary manifestations. M.tb resides in alveolar macrophages of the host, the very cell type that has evolved to phagocytose and destroy microbes entering the lungs. M.tb inhibits phagosome maturation and phagolysosome fusion. It induces alternative activation of the host macrophage and perturbs cytokine signalling. Genome-wide transcription analysis in our lab has identified upregulation of the human suppressor of cytokine signalling 3 (SOCS3) as a consequence of colonization of macrophages by M.tb. SOCS-3 is a negative regulator of cytokine signalling working by inhibiting Janus kinase (JAK). SOCS3 thus inhibits cytokine signalling from γ-interferon (IFN-γ) which is a potent proinflamatory cytokine. This report describes knock-down of SOCS3 siRNA using morpholino oligonucleotides chemically conjugated to a dendrimer. Upregulation of SOCS3 RNA by 127-fold was observed in M.tb infected THP1 derived macrophages. Knockdown of of SOCS3 resulted in significant reduction of intracellular M.tb survival. The colony count of M.tb recovered from THP-1 cells infected with Mtb but treated with a scrambled morpholino-oligonucleotide sequence was 5.73 log/ml, while parallel wells subjected to SOCS3 knockdown with 1 and 0.1 µM of siRNA had 5.64 and 5.63 log units of surviving bacteria. It was concluded that restoration of cytokine signalling is sufficient to induce bactericidal activation of macrophages infected with M.tb.

P4.05.51

Association of SLC11A1 gene polymorphisms with susceptibility to Lepromatous Leprosy

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Leprosy is a debilitating infectious disease of human skin and nerves caused by Mycobacterium leprae. The range of clinical forms varies from tuberculoid (TT) to lepromatous leprosy (LL) based in immune response variations to M. leprae. Human SLC11A1 gene is a key candidate to host resistance and susceptibility to certain pathogens. The study aimed to evaluate the association of SLC11A1 gene polymorphism with resistance, susceptibility or clinical forms of leprosy in Mexican patients. Methods: We enrolled 80 LL, 8 TT patients and 148 healthy subjects (HS), which polymorphisms were studied simultaneously. By polymerase chain reaction amplification of genomic DNA followed by restriction fragment length polymorphism analysis, we evaluated the polymorphisms of SLC11A1 gene at INT4 (469 + 14G/C), D543N (codon 543, Asp to Asn) and 5´promoter (GT)n microsatellite. Results: C/C genotype and C allele frequencies in INT4, were significantly associated with susceptibility to LL (p=0.005, OR: 0.4211 [IC 95%, 0.16-1.11]; and p=0.002, OR: 0.33 [IC 95%, 0.52-0.80] respectively). However, variants analyses of D543 and (GT)n loci from LL and TT patients did not reveal any statistical significance versus controls. Conclusions: Our data support the association between the C/C genotype and C allele in INT4 of SCL11A1 gene, which increases the risk to develop LL in Mexican patients.

P4.05.52

***Mycobacterium tuberculosis P27* protein targets host cell mitochondria**

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Tuberculosis (TB) is a major health problem worldwide, the selection and spread of multidrug–resistant (MDR) *M. tuberculosis* strains worsens the scenario. A precise knowledge on mycobacteria–host cell interactions is required for successful control of TB. Mycobacterial infection affects mitochondrial function in host cells. However, the identity of the molecules responsible for such effect has not clearly defined. The AIM of this work was to identify M. tuberculosis proteins that target mitochondria and identify its protein targets within mitochondria. Material and Methods: *M. tuberculosis* protein sequences were analyzed by bioinformatic tools such as MitoProt II–v1.101. PSORT II. *M. tuberculosis* Rv1411c (p27) protein was selected as a candidate and experimentally tested on J774.A1 murine macrophages, cells were incubated in the presence of *M. tuberculosis* His-tagged p27 recombinant proteins (p27), or α–crystalline, as negative control, cells were then labelled with MitoTracker red CMXRos (mitochondria), and FITC–labeled anti–His antibody (recombinant protein) and observed by confocal microscope. Results: Bioinformatic analysis singled out 19 proteins as mitochondria-targeting candidates, p27 experimentally targeted mitochondria, and 3 mitochondrial proteins have been shown as likely targets for p27.
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P4.05.53

Relationship between host Mycobacterium tuberculosis-specific CD4 T cell responses and sputum bacillary clearance during early anti-tuberculosis treatment

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Tuberculosis treatment is associated with changes in host Mycobacterium tuberculosis (Mtb) antigen-specific T cell responses. It is unclear how these changes relate to treatment outcomes. We investigated the relationship between Mtb-specific immune response, quantitative bacteriology and clinical outcomes of treatment in sputum smear-positive adult TB patients receiving anti-TB treatment at Queen Elizabeth Central Hospital, Malawi. Peripheral blood and sputum samples for PBMC isolation and serial mycobacterial culture respectively were collected from 45 patients irrespective of their HIV status before and after 56 days of treatment. Mtb-specific immune responses were assessed by quantification of IFN-γ secreting T cells in response to CFP-10 and ESAT-6 using an ELISPOT assay. Mtb clearance from sputum was evaluated by mycobacteria growth indicator tube culture system. Results are given as median and differences were considered statistically significant if $p<0.05$. Preliminary results show a difference in the rate of bacillary clearance and host responses with respect to HIV. 71% of HIV-uninfected and 50% of HIV-infected patients were culture negative by 2 months of treatment. Similarly, the number of IFN-γ secreting T cells reduced during treatment in HIV-uninfected patients (median change -60 SFU/106 PBMC, IQR 4-204), while there was a smaller reduction in HIV-infected patients (median change -14 SFU/106 PBMC, IQR 0-51) (Mann-Whitney U-test p = 0.085). Preliminary findings suggest an association between magnitude of host Mtb-specific T cell responses and mycobacterial load. We plan to analyze the data further by doing regression modeling to investigate the relationship between immunological parameters, HIV status and bacillary clearance.

P4.05.54

The accumulation of highly differentiated Mtb-specific CD4 T cells is associated with lung tissue destruction during pulmonary tuberculosis

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Immunological methods are widely used for the diagnostics of *M. tuberculosis* (*Mtb*) infection. In this study we show that these methods can also be used as a means to evaluate and monitor the degree of lung tissue destruction caused by *Mtb*. Lung destruction is a characteristic trait of pulmonary tuberculosis (TB). It underlies disease severity, causes dissemination of the pathogen and needs to be carefully evaluated. Our earlier studies in mice have demonstrated that *Mtb* infection induces the differentiation of effector CD4 T cells into highly differentiated $CD4^+CD27^-$ lymphocytes and that this differentiation takes place in the lungs. We hypothesized therefore that the accumulation of $CD4^+CD27^-$ cells should mirror tuberculosis activity. In this study, we evaluated the percent of $CD27^-$ cells within the population of *Mtb*-reactive CD4 T cells ("Mtb^+CD27^-" cells) in the blood of TB patients. Compared to healthy donors, TB patients had significantly higher percent of Mtb^+CD27^- cells ($p<0.0001$). Within the group of TB patients, the percent of Mtb^+CD27^- cells varied. Among different characteristics of TB disease (i.e., pulmonary destruction, disease duration, et al.), high degree of pulmonary destruction was the main factor associated with high percent of Mtb^+CD27^- cells ($p<0.0001$). ROC-curve analysis revealed that evaluation of Mtb^+CD27^- cells allows assessing pulmonary destruction (sensitivity, 89%; specificity, 74%). During the treatment, a decrease in the percent of Mtb^+CD27^- cells was indicative on the repair of lung destruction ($p<0.01$).
The determination of the percent of Mtb^+CD27^- cells allows assessing pulmonary destruction and its repair following TB therapy.
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P4.05.55
Mycobacterium tuberculosis adapts its immune-evasion mechanisms in dependence of the antigen presenting cell and shifting to dormancy.

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Immune responses to Mycobacterium tuberculosis (Mtb) drive the bacteria into a dormant state, but rarely eliminate them. The latent state of Mtb is reversible and upon reactivation Mtb causes post-primary tuberculosis (TB). Dormant (D-) Mtb determines latent TB infection (LTBI), but it was also observed in sputa of patients with active TB. However, little is known on the capacity of the immune system to recognize D-Mtb.
We observed that human CD4+ T cell clones specific for mycobacterial antigens recognize more efficiently D- than replicating (R)-Mtb. The increased presentation of D-Mtb was observed using both professional, macrophage or DC, and non professional APC, such as fibroblasts. The difference was not dependent on a different capacity of the two forms of Mtb to infect APC or to induce different APC activation. In fact, APC were induced to secrete cytokines and to express MHC class I and II molecules and activation markers at the same levels. However, D- but not R-Mtb co-localized with mature phagolysosome markers Lamp-1 and vATPase in infected macrophages. In addition, R- but not D-Mtb increased the phagosome pH and inhibited the proteolytic efficiency of macrophages. Mice infected with D-Mtb developed a specific immune response earlier than mice infected with R-Mtb. Together, data indicate that D-Mtb does not block phagosome maturation and antigen processing capacity of APC.
Our results suggest that the capacity of Mtb to switch to dormancy may represent a mechanism of survival in LTBI, but also a strategy to modulate the host adaptive immune system in active TB.

P4.05.56
A novel therapeutic vaccine against tuberculosis using the cynomolgus monkey model and mice

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Purpose and Methods. Multi-drug resistant (MDR) Mycobacterium tuberculosis (TB) is a big problem in the world. We have developed novel TB vaccines [HVJ-Envelope/HSP65 DNA +IL-12 DNA vaccine, granulysin vaccine and killer specific secretory protein of 37kDa (Ksp37) vaccine] using Hemagglutinating virus of Japan (HVJ)-envelope. Induction of CTL by these vaccines was investigated. Results. HSP65+IL-12DNA vaccine provided remarkable protective efficacy in mouse and monkey models compared to BCG. This vaccine induced strong CTL differentiation against TB, while BCG vaccine induced little CTL. The vaccine also provided strong therapeutic efficacy against MDR-TB and XDR-TB in murine model. Furthermore, the synergistic therapeutic effect of this vaccine in the combination of first line chemotherapy Isoniazid (INH) was observed. The vaccine exerted therapeutic effect on TB-infected monkeys via the augmentation of IL-2 production. On the other hand, granulysin is an important defensive molecule expressed by human T cells. We found granulysin induced the differentiation of in vitro and in vivo CTL. Granulysin vaccine exerted therapeutic efficacy against TB in monkeys and mice. Ksp37 vaccine also augmented in vivo and in vitro differentiation of CTL. The combination of HSP65 vaccine and granulysin vaccine exerted synergistic therapeutic efficacy against TB. Recently, we first established granulysin transgenic mice and Ksp37 transgenic mice, showing strong in vivo anti-TB activity. Conclusions. These data indicate that additive therapy of HSP65-, granulysin- and Ksp37-vaccines to first line chemotherapy might provide very strong therapeutic strategy against MDR-TB. These also indicate that our vaccines provide efficacy against TB via the induction of CTL.

P4.05.57
Role of $CD8^+$ T cells in triggering reversal reaction in HIV/leprosy patients

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It has been reported that the initiation of HAART is associated with the development of reversal reaction (RR) in co-infected HIV / leprosy patients. Nevertheless, the impact of HIV and HAART therapy on the cellular immune response to *M. leprae* (ML) remains unknown. In the present study, we observed that *ex vivo* PBMCs of both RR and RR/HIV patients presented increased percentages of activated $CD4^+$T cells when compared with the HC group. The frequency of $CD8^+CD38^+$ cells increased in the PBMCs of RR / HIV but not in RR patients when compared with the HC group. RR and RR/HIV skin lesion cells presented similar percentages of activated $CD4^+$ cells, but the numbers of activated $CD8^+$ cells were higher in RR/HIV in comparison to the RR group. The frequency of IFN-γ-producing cells was high in response to ML regardless of HIV co-infection. In ML-stimulated cells, there was an increase in TCM $CD4^+$ T cell frequencies in the RR and RR/HIV groups, but an increase in TCM $CD8^+$ T cell frequency was only observed in the RR/HIV group. ML increased granzyme B^+ TEM $CD8^+$ T cell frequencies in the RR/HIV PBMCs, but not in the HC and RR groups. Our data suggest that the increased expression of TEM $CD8^+$ T cells together with greater perforin/granzyme B production could be an additional mechanism leading to the advent of RR in co-infected patients. Moreoever, this increased expression may explain the severity of RR occurring in these patients.

P4.05.58
Mycobacterial Immune response in tuberculous meningitis is influenced by HIV infection

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Tuberculous meningitis (TBM) is one of the common and serious forms of extrapulmonary tuberculosis prevlent in many developing countries. The laboratory diagnosis of the disease is always difficult and delay in accurate treatment leads to serious consequences.The mortality due to TBM in children is high and can leave serious sequalae.
The present study comprises 27 autopsy proven TBM cases with and without HIV infection. Paired samples of CSF (lumbar, ventric) and serum samples collected were subjected for anti-TB antibody response, western blotting, Immune complexes and cytokine analysis. Non-neurological samples (LP-CSF) were used as controls in the study.
The anti-mycobacterial antibody response in LP-CSF was up to 60% in TBM cases which was higher than in serum samples highlighting in-situ synthesis antibodies in the CSF compartment. Pro-and anti-inflammatory cytokines were quite varied in these cases. IL-4 and TNF signals were noticed to be weak whereas, the signals for IFN-gamma and IL-6 were exaggerated. The IFN response in HIV positive cases was relatively low. Further, wherever individuals were negative for acid fast bacilli in the CSF the gamma interferon response was poor. It thus appears that the HIV infection abrogates the immune response in TBM cases.

P4.05.59

M2-like polarization of Mycobacterium tuberculosis infected human macrophages by extracellular AMP

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Tuberculosis (TB) is a major infectious disease spread worldwide. Mycobacterium tuberculosis-containing droplets are inhaled into the pulmonary alveoli. Following encounter with the bacillus, alveolar macrophages (Mϕ) allow the recruitment and activation of immune cells, leading to the formation of granuloma. As the host fails to control mycobacterial infection, the center of granulomas exhibits necrosis as a result of the dying infected Mϕ and the release of cell content. How Mϕ will respond then to the M. tuberculosis infection in this new environment is poorly understood.
Here, we show that extracellular ATP, an endogenous danger signal released upon cell death modulates human Mϕ response to M. tuberculosis infection. Using a transcriptomic approach, we found that eATP dampens the inflammatory response of M. tuberculosis infected MΦ and contributes to tissue repair. This gene profile is in accordance with a polarization of the infected cells toward an M2-like phenotype. Alternative activation of MΦ was dependent of ATP degradation by the ectonucleotidase CD39. We provide strong evidence that adenosine monophosphate (AMP) mediated the observed effect through the stimulation of the adenosine A2A receptor. These data point to an unrecognized role of extracellular AMP in host response against M. tuberculosis limiting tissue damage but it may also favor bacterial immune escape.

P4.05.60

Differences in the levels of host markers detected in saliva and serum and their potential for diagnosing TB disease

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Despite the recent introduction of molecular beacon assays into clinical practice, the diagnosis of tuberculosis (TB) disease remains challenging in individuals with difficulty in providing good quality sputum. Host biosignatures of inflammatory markers could be valuable in such cases, especially if they are based on more easily obtainable samples such as saliva, and are developed into rapid, point-of-care tests. This study aimed to compare the levels of host markers detectable in the saliva and serum of patients with possible pulmonary TB, and to assess the diagnostic utility of these markers for TB disease.
Methods: Saliva and serum samples were collected from 38 TB suspects,recruited from a community health centre in Cape Town, 33 host markers were evaluated in the samples using the Luminex platform.
Results: ActiveTB disease was confirmed in 11(28.9%) by sputum culture. The levels of most markers were above the minimum detectable limit in both sample types.The levels of fractalkine, IL-17, IL-6, IL-9, MIP-1β, CRP, VEGF and IL-5 in saliva, and those of IL-6, IL-2, SAP and SAA in serum, were significantly higher in TB patients, in comparison to the levels obtained in those without active TB ($p<0.05$). The area under the ROC curve was ≥ 0.70 for most of these markers, thereby confirming their diagnostic potential for TB disease.
Conclusions: There were vast differences in the levels of host markers expressed in saliva in comparison to serum and some markers in both sample types have potential in the diagnosis of TB disease.

P4.05.61

Different immunosuppressive mechanisms in multi-drug-resistant tuberculosis and non-tuberculous mycobacteria patients

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Previous studies have demonstrated that cells from both multi-drug-resistant tuberculosis (MDR-TB) and non-tuberculous mycobacteria (NTM) patients respond poorly to mycobacterial antigens in vitro. In the present study, we compared the in vitro response of cells isolated from sensitive TB (NR-TB)-, MDR-TB- and NTM-infected patients. Analysis of T cell phenotype ex vivo revealed that both MDR-TB and NTM patients present an increased percentage of CD4+ CD25+ FoxP3+ and CD4+ CD25+ CD127- Treg cells when compared to NR-TB. Increased numbers of Treg cells and interleukin (IL)-10 serum levels were detected in MDR-TB, whereas elevated serum transforming growth factor (TGF)-β was found in the NTM group. Cells of MDR-TB patients stimulated with early secretory antigenic target (ESAT)-6, but not purified protein derivative (PPD), showed a lower frequency of CD4+ /interferon (IFN)-γ+ T cells and enhanced CD4+ CD25+ FoxP3+, CD4+ CD25+ CD127- and CD4+ CD25+ IL-10+ T cell population. In addition, increased IL-10 secretion was observed in cultured MDR-TB cells following ESAT-6 stimulation, but not in NR-TB or NTM patients. In vitro blockade of IL-10 or IL-10Rα decreased the CD4+ CD25+ FoxP3+ frequencies induced by ESAT-6 in MDR-TB, suggesting a role of IL-10 on impaired IFN-γ responses seen in MDR-TB. Depletion of CD4+ CD25+ T lymphocytes restored the capacity of MDR-TB T cells to respond to ESAT-6 in vitro, which suggests a potential role for Treg / T regulatory 1 cells in the pathogenesis of MDR-TB. Together, our results indicate that although the similarities in chronicity, NTM- and MDR-TB-impaired antigenic responses involve different mechanisms.

P4.05.62

Increase of Reactive Oxygen Species in Natural Killer cells from Pulmonary Tuberculosis patients

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Tuberculosis (TB) remains today a threat for public health and one third of the world population is infected. Although the role of Natural Killer (NK) cells in vivo was never fully understood, their capacity to lyse not only infected macrophages, but also directly M. tuberculosis demonstrates their possible role in the immune response in addition to their regulatory function over other immune cells and their presence inside granulomas. However, their number is decreased and with impaired function in TB patients. Reactive oxygen species (ROS) produced by tissue-specific cells, mainly alveolar macrophages can be responsible for their suppressive state as happens in other diseases. In this work, we intended to demonstrate the increase of ROS in NK cells of patients with pulmonary tuberculosis.
We analyzed by flow cytometry the levels of oxidative stress in cells from peripheral blood of 14 TB patients with main focus in NK cells.
In our work we demonstrated that not only phagocytic cells with active NADPH oxidase have an increase of ROS, but also NK cells and NKT cells. We did not find any difference in ROS-producing CD8+ T cells comparing with healthy controls. Beyond expected decrease of total NK cell number and mainly CD56dim CD16+ subset, we found that almost CD56dim are CD16- corresponding these cells to an increase of oxidative stress. We also demonstrated that contrary to granulocytes and monocytes, in multi-drug resistant (MDR) TB patients the levels of oxidative stress in NK cells are equally higher to TB.

P4.05.63

Fatty acid, cytokines and chemokines profile in exhaled breath condensates (EBCs) of patients with Tuberculosis

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Introduction. *Mycobacterium tuberculosis* (*Mtb*) affects host lipid metabolism; differences in the fatty acid profile and inflammatory products from mononuclear phagocytes from TB patients and controls have been reported. These mediators are differentially produced depending upon the clinical TB and should be useful to understand pathology of paucibacillary individuals. *Objective.* To analyze the profile of fatty acid derivatives, cytokines and chemokines in EBC of healthy, asmatic and tuberculosis children (6-12 years) and healthy, smear-positive and paucibacillary (12 years and older). *Methods.* EBCs were collected by capture R-tubes, condensed and some of them used to detect cytokines and chemokines by LUMINEX. Other EBCs were freeze-dried and used to detect fatty acid derivatives by gas chromatography. *Results.* Saturated fatty acids were lower in paucibacillary due to the higher relative amounts of oleate and linoleate and lower proportions of stearate and myristate compared with healthy adults and smear-positive patients. These fatty acids were also present in cells from bronchoalveolar lavages isolated from paucibacillary patients. After six months of treatment, EBC from paucibacillary patients did not differ from healthy controls. EBC from TB children presented higher relative amounts of palmitate, oleate and linoleate and lower amounts of stearate compared with non TB children. *Conclusion.* A combination of different EBCs fatty acids, cytokines and chemokines will allow distinguishing different groups of TB with diverse clinical manifestations, and these will provide insights to understand the mechanisms leading to better understand TB immunopathogenesis. *Funding resources* COLCIENCIAS 1115-4592-1439.

P4.05.64

Profiles of fatty acids released during the differentiation of mononuclear phagocytes in the presence of mycobacterium tuberculosis - stearic acid effect on Differentiation

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Introduction. Our previous results support the idea that fatty acid profile provides a valuable tool to correlate differentiation of mononuclear phagocytes with effector mechanisms. Differentiation into macrophages correlated with increased ratios of saturation mainly due decreased oleate. Laurate correlated with the presence of mycobacteria and arachidonate and linolenate with necrosis in less differentiated cells. Objective. Evaluate which fatty acids are released into the extracellular medium and its possible effect on the differentiation of mononuclear phagocytes. Methods. Human monocytes were isolated from PBMC from healthy donors; cells were infected or not with M. tuberculosis or treated with PPD, and differentiated 120 hours. Supernatants were esterified and analyzed by gas chromatography. Differentiation was also studied in the presence of stearic acid, the highest derivative found in supernatants from cells differentiated in the presence of M. tuberculosis; differentiation markers, cytokines and mycobacterial control were studied. Results. A stearic acid derivative was the only one significantly higher in supernatants from cells differentiated in the presence M. tuberculosis and PPD, compared with untreated cells. Monocyte differentiation in the presence of stearic into macrophages improved effector mechanisms against mycobacteria. Stearic acid increased HLA-DR, CD64, cytokines such as IL-12, TNF-alpha and IL-6 and chemokines as MIP-1 alpha and beta, and this treatment also reduced the bacterial burdens. Conclusion. Our data suggest that during the differentiation process that the cells release a derivative of stearate which favors the control of mycobacteria, the relevance of this finding still requires additional evidence. Funding resources COLCIENCIAS 1115-4592-1439.

P4.05.65

The combined use of M. tuberculosis-specific CD4 and CD8 T-cell responses is a powerful diagnostic tool of active tuberculosis

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Background: Diagnosis of active tuberculosis disease (TB) from latent Mycobacterium tuberculosis (Mtb) infection (LTBI) remains complex and requires several clinical, radiological, histo-pathological, bacteriological and molecular parameters. However, we recently showed that the dominant cytokine profile (i.e. TNFα+/IFNγ-/IL2-) of Mtb-specific CD4 T-cells from TB patients could be used as a diagnosis tool. Furthermore, Mtb-specific CD8 T-cell responses were more frequently detected in TB patients than in LTBI subjects.
Methods: We combined the detection of Mtb-specific CD8 T-cell responses to the analysis of the cytokine profile of Mtb-specific CD4 T-cells in 178 TB and LTBI subjects and determined the performances of the combination of both parameters as diagnosis assay (called SCORE).
Results: The performances of the combined diagnosis assay merging both parameters (cytokine profile of CD4 T cells and detection of CD8 T cells) were significantly (AUC: 0.94; P<0.008) improved as compared to each single parameter. On the basis of the logistic regression analysis, an optimal cutoff of SCORE of 4 was determined (OR=58; specificity=83.5%; sensitivity=92%; PPV=60% and NPV=97.5).
Conclusions: The activity of Mtb infection (i.e. latent versus active) is associated to distinct profiles of Mtb-specific CD4 and CD8 T-cell responses. These parameters can be combined within the same flow-cytometry assay and can be accurately used for the timely diagnosis of TB.

P4.05.66

Functional and phenotypic characterization of Mycobacterium tuberculosis specific CD4+T cells in peripheral blood and at the site of infection

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Tuberculosis (TB) is a worldwide public health problem. *Mycobacterium tuberculosis* (Mtb) specific CD4+T cells have an efficient role in the immune response against TB since more than 90% of subject infected with *Mtb* do not developing active TB (ATB). However, during ATB, these cells seem have crucial role close to the site of infection. Characterization of these cells could provide biomarker candidates that still represent a real challenge in diagnostic and vaccine research on TB.
In this study, we used surface and intracellular staining flow cytometry methods to determine the memory phenotypes and the functional profiles of *Mtb*-specific CD4+T cells within 12 patients with pleural TB and 16 healthy subjects with latent Mtb infection (LTBI).
Our results showed that the rate of *Mtb*-specific CD4+ T cells simultaneously producing IFN-γ, TNF-α and IL2 cytokines is significantly higher in the peripheral blood (PB) within LTBI subjects compared to patients with pleural TB. Furthermore, these multifunctional cells are highly represented in the pleural fluid compared to the autologous PB within patients.

Analysis of the memory phenotype of Mtb-specific CD4+ T cells showed that the percentage of effector memory (EM) sub-population (CD45RA-/CCR7-) is also increased in the PB of LTBI subjects and in the pleural fluid of patients. Nevertheless, we showed that this repartition involve EM CD4+T cells obligatory producing IFN-γ.
These findings indicate that the characterization of the EM and polyfunctional Mtb-specific CD4+T cells in the PB and in the site of infection may help as a correlate of protection.

P4.05.67
NOD2 triggers an IL-32 dependent human dendritic cell program in leprosy

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It is unclear whether the ability of the innate immune system to recognize distinct ligands from a single microbial pathogen via multiple pattern recognition receptors (PRRs) triggers common pathways or differentially triggers specific host responses. In the human mycobacterial infection leprosy, we found that activation of monocytes via NOD2, by its ligand muramyl dipeptide, vs. TLR2/1, by triacylated lipopeptide, preferentially induced differentiation into dendritic cells (DC), dependent upon a novel IL-32-dependent mechanism. IL-32 was sufficient to induce monocytes to rapidly differentiate into DC. Although IL-32 or GM-CSF derived DC efficiently process and present antigen via MHC class II to CD4+ T cells, IL-32 derived DC were more efficient in presenting antigen to MHC class I-restricted CD8+ T cells. Importantly, IL-32 induced DC showed the capacity to cross-present antigen, allowing MHC class I molecules to present peptides derived from exogenous antigens taken up by the endocytic pathway. Our finding that the NOD2 IL-32 dependent DC pathway of cross-presentation is present at the site of disease in tuberculoid leprosy suggests the hypothesis that IL-32 induction of cross-presentation of mLEP antigens results in activation of CD8+ T cells and is important for host defense.

P4.05.68
The role of follicular helper T cells in mycobacterial infection

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Immunological protection against Mycobacterial infection requires the activation of the cellular arm of the immune response. However, the role of humoral immunity against Mycobacterial infection is not well understood until now. Follicular helper T (Tfh) cells are one of CD4+ helper T cell subsets that regulate humoral immunity through B cell mediated antibody secretion. IL-21 secreted by Tfh cells plays an important role of induction of the humoral immunity. It is however less clear whether Tfh cells participate cellular immunity. To investigate the role of Tfh cells in mycobacterial infection, we focused on the induction and differentiation of Tfh cells. We used Mycobacterium bovis BCG (Bacille de Calmette et Guérin) that is a popular vaccine against Mycobacterial tuberculosis. Using our recently reported IL-21-GFP reporter mouse, we have compared the frequency, phenotype and capacity to produce IL-21 from Tfh cells in both lymphoid and non-lymphoid organs following BCG and influenza virus infection.

P4.05.69
Specific TCD8+IL-10+ and TCD8+TGF-beta+ cells in active tuberculosis patients

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Tuberculosis (TB) is responsible for causing over 1.5 million deaths each year. TCD4+ together with TCD8+ cells and their cytokines are important for protective immune response to TB. Although TCD8+ cells have been studied in TB, several aspects are not yet clarified. In the absence of TCD4+ cells, TCD8+ cells increase their cytotoxic function and production of different cytokines in order to respond to Mycobacterium tuberculosis (Mtb) infection. To understand the role of TCD8+ cells as regulatory cytokine producers in TB, peripheral blood TCD8+ cells were characterized and compared their profile with healthy controls (TST-). PBMC from 15 patients with active pulmonary TB (mean age = 40.5) and 15 healthy controls TST-, matched by sex and age to TB patients, were cultivated for 96 hours with Mtb recombinant antigen (rMPT-51). TCD8+ cell subsets were analyzed by flow cytometry. TB patients presented higher levels of TCD8+IL-10+ (medium=1.2±0.9; PHA=12.8±1.5; rMPT-51=5.0±1.4) and TCD8+TGF-beta+ (medium=1.6±1.3; PHA=14.1±1.7; rMPT-51=8.0±1.1) in response to rMPT-51 than TST- individuals (TCD8+IL-10+: medium=1.3±1.1; PHA=11.9±1.7; rMPT-51=3.0±1.5); (TCD8+TGF-beta+: medium=2.4±0.8; PHA=13.6±2.3; rMPT-51=2.8±1.2). The cytotoxic markers of TCD8+ cell such as Perforin and Granzime-B are independent of the rMPT-51 stimulus, and TB patients had lower percentage of triple positive cells than the healthy controls (TST-=21.7±2.8; TB=9.1±1.3). Conclusions: Patients with TB presented Mtb specific TCD8+ cells positive for IL-10 and TGF-beta, whereas presented lower cytotoxic markers. These results suggest that TCD8+ cells can contribute to the regulatory immune response in active tuberculosis.

P4.05.70
Deciphering Dendritic Cell Function during Mycobacterium tuberculosis Infection by RNA Interference

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With rising incidence of acquired drug resistance amongst life-threatening pathogens, alternative approaches to improve therapy and vaccination have taken centre stage. To this end genome wide and pathway specific siRNA libraries are being increasingly employed to identify genes that regulate immune responses against a number of pathogens. In this study using calcium and cysteine protease pathway specific siRNA libraries we identified genes that play critical roles in modulating diverse functions of dendritic cells (DCs) during Mycobacterium tuberculosis (M. tb) infection. Knockdown of many of these genes in the two pathways resulted in reduced bacterial burden within DCs. These included genes that regulated activation of transcription factors, ubiquitin specific peptidases, and genes that are involved in autophagy and neddylation. Knockdown of certain genes increased the expression of IL-12p40 and surface densities of costimulatory molecules in an antigen and receptor specific manner. Increased IL-12p40 and costimulatory molecules on DCs also promoted the development of Th1 responses from a Th2 inducing antigen. Further, modulation of autophagy and oxidative burst appeared to be one of the mechanisms by which these genes regulated survival of M. tb within DCs. While some genes regulated specific responses, others regulated multiple responses that included IL-12 production, T cell priming as well as intracellular survival of M. tb. Further dissection of the mechanisms such as neddylation, by which these genes regulate immune responses, would improve our understanding of host parameters that are modulated during M. tb infection.

P4.05.71
Clinical isolates of Mycobacterium tuberculosis exacerbate pulmonary inflammation in mice

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***Background:** Mycobacterium tuberculosis (Mtb)* is a virulent intracellular pathogen that infects and persists in host macrophages, resulting in granuloma formation and collagen deposition in the lung. The mechanisms that confer resistance to *Mtb* or result in establishment of disease are poorly understood. Data from the

literature suggest that differences in *Mtb* virulence contribute to the development of disease. ***Objective:*** Our purpose was to investigate lung inflammatory reactions in mice induced by three isolates of *Mtb.* These isolates were recovered from patients with non-cavitary (SV009), cavitary (SV038) and extra-pulmonary (SV068) active tuberculosis and compared to H37Ra (avirulent) and H37Rv (virulent) *Mtb* strains. ***Methods:*** Female Balb/c mice were inoculated intratracheally (i.t) with $1x10^5$ CFU of *Mtb* or 100 µL PBS. Neutrophil and mononuclear cell recruitment to the lung was assessed by bronchoalveolar lavage (BALF). Mouse survival, colony formatting units (CFU), lung histology, and cytokines were evaluated. ***Results:*** Mice infected with SV009, SV038 and SV068 presented with more robust cellular recruitment into BALF at 30 and 60 days post infection (p.i.) and survival was reduced compared to H37Rv/a. Spleen CFU recovered 90 days p.i. was higher in mice infected with SV009 strain. Lung histology demonstrated that SV068 infected mice had greater numbers of foamy macrophages containing *Mtb* and intense perivascular and perialveolar infiltration of neutrophils, mainly at 60 days p.i. Cytokine production was variable among the groups, especially IFN-γ, TNF-α, IL-6 and IL-10. ***Preliminary Conclusion:*** Our findings suggest that SV009, SV038 and SV068 are more virulent than H37Rv/a *Mtb* strains.

P4.05.72
Role of Slc11a1 gene in macrophage activation during BCG infection

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The *Slc11a1* gene regulates resistance against *S. enterica* Typhimurium, *L. donovani* and *M. tuberculosis*. AIRmax and AIRmin mouse sublines, homozygous for *Slc11a1* R and S alleles ($AIRmax^{RR}$, $AIRmax^{SS}$, $AIRmin^{RR}$ and $AIRmin^{SS}$) were produced in our Lab to study the effect of this gene in the inflammation background. The aim of this work is to evaluate the effect of this gene in peritoneal macrophage (MΦ) activation during *M. bovis* BCG infection. Mice were inoculated ip with BCG (14 days) or PBS (control). The peritoneal cells were collected and placed in culture, the adherent MΦ stimulated or not with LPS were used in all experiments. Spleens were homogenized and cultured in MB7H10 medium and the bacterial colonies were counted. Production of NO, H_2O_2 and cytokine were determined in culture supernatants. In BCG infection only $AIRmax^{RR}$ mice were capable of controlling bacterial proliferation, which was accompanied with high levels of IL-1β, IL-12, TNF alfa and IL-6 produced by activated macrophages. On the other hand, susceptible $AIRmin^{SS}$ mice produced higher amounts of NO, H_2O_2 and IL-10 suggesting that both inflammatory background and *Slc11a1* alleles interfere on resistance to BCG infection. Thus, we conclude that the high inflammatory response associated to *Slc11a1* R alleles (in $AIRmax^{RR}$ mice) are mechanisms for efficient bacterial killing, through production of cytokines by MΦ which activates the immune response.
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P4.05.73
Development of a new point-of-care test for leprosy

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Leprosy is a dermato-neurological disease that remains an important public health problem in many endemic countries. Leprosy presents as a spectrum and no laboratory test is commercially available for its diagnosis/prognosis. Here we report the development of a new point-of-care (POC) test for leprosy using LID-1 fusion-protein and phenolic glycolipid-I (PGL-I) (NDO-LID,Orangelife®/Brazil). A new rapid-test reader platform integrated in a cell phone was also used (Smart Reader application-SR®). NDO-LID® tests were evaluated among newly diagnosed untreated multibacillary (MB=108) and paucibacillary (PB=104) leprosy patients, (Ridley & Jopling classification); leprosy household contacts (HHC=75); pulmonary tuberculosis patients (sputum positive/HIV negative;TB=53) and healthy endemic controls (EC=101) recruited in central-western Brazil. NDO-LID® anti IgM/IgG reactivity (SR®:cut-off=10) was compared to anti PGL-I IgM ELISA (cut-off:optical density=0.250). Across the spectrum of leprosy forms (from lepromatous/LL to tuberculoid/TT pole) a gradual decrease in the seropositivity to NDO-LID® test was observed: 97.4% for LL, 83.7% for borderline lepromatous/BL, 76.9% for borderline borderline/BB patients; 26.2% for borderline tuberculoid/BT and 14% for TT patients. Seropositivity among HHC was 5.3%, 5.7% for TB and 3% among EC. The estimated sensitivity of NDO-LID® test was 87% and the specificity was 96.1%. The new NDO-LID® POC test represents a useful tool for MB leprosy detection/diagnosis and treatment and can also contribute to monitor treatment by leprosy controls programs. MB patient are important *Mycobacterium leprae* disseminators and are at increased risk of complications such as reactional episodes. The digital and automated rapid test SR®-app also represents an improvement towards more accurate results.
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P4.05.74
The involvement of proteins of DNA double-strand breaks repair systems of *Mycobacterium tuberculosis* in the infection of human macrophages

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Objectives: Double-strand breaks repair systems as non-homologous end-joining (NHEJ) and homologous recombination (HR) are important for the *Mycobacterium tuberculosis* (Mtb) survival *in vitro*. We determine the involvement of proteins Ku, ligase D (crucial for NHEJ system) and RecA (major for HR) in the Mtb intracellular survival. Methods: MtbH37Rv mutants with inactivated genes: *ku, ligD* (Δ*ku/ligD*), *recA* (Δ*recA*) or *ku, ligD, recA* (Δ*ku/ligD/recA*) were obtained using the technique of gene replacement based on the process of homologous recombination. Intracellular growth of bacteria and antimicrobial activities of macrophages: nitric oxide (NO) and reactive oxygen species (ROS) production were examined. The involvement of EKR1/2 signalling pathway in the response of macrophages to Mtb infection was assessed. Results: We found that Δ*ku/ligD/recA* mutant grew significantly weaker inside the macrophages than wild-type and the rest of mutants. Inhibition of ERK1/2 pathway in macrophages caused the increased intracellular growth of Δ*ku/ligD/recA*. Δ*ku/ligD/recA* and Δ*recA* mutants, but not wild-type or Δ*ku/ligD*, stimulated NO and ROS production. Nevertheless, inhibition of ERK1/2 signalling pathway blocked NO and ROS production by macrophages infected with Δ*ku/ligD/recA* or Δ*recA*. Only the triple mutant did not disturb the phosphorylation of ERK1/2 proteins induced by phorbol ester. Conclusion: Both NHEJ and HR systems are important for the survival of Mtb inside human resting macrophages. The RecA protein affect the bactericidal activity of macrophages. We suggest that HR repairing pathway can be one of the virulence factor of Mtb.
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P4.05.75
The role of Mycobacterium tuberculosis secreted protein in the induction of Th1 immune response

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Peptide-25 is the major T cell epitope for Ag85B of Mycobacterium tuberculosis and induces Th1 differentiation in vivo. Using transgenic

mice expressing a T cell receptor specific for Peptide-25, we demonstrated that Peptide-25 induced transient expression of T-bet, and as a result induced Th1 differentiation in the absence of IFN-γ and IL-12. In this study, we examined the role of T-bet in Peptide-25 induced Th1 differentiation. Using T-bet-/- P25 TCR-Tg mice, we demonstrated that Peptide-25 induced Th1 differentiation even in the absence of T-bet, indicating that Peptide-25 induces the expression of transcription factors that induce the chromatin remodeling of ifn-γ gene in a T-bet independent manner. To identify the transcription factors, we analyzed gene expression profile in CD4+ T cells after Peptide-25 stimulation by using Affymetrix GeneChip. Our criterion for the selection of genes induced by Peptide-25 stimulation was that their expressions are more than two-fold changed compared to unstimulated cells and they possess the transcription activity. As a result, we identified 53 genes as a transcription factor induced by Peptide-25 stimulation in the CD4+ T cells from both T-bet-/- and WT P25 TCR-Tg mice.
We are now confirming whether these candidate genes can induce the chromatin remodeling of ifn-γ gene by using retroviral expression system.

P4.05.76
Anti-tuberculosis chemotherapy reveals differences in host immunity to *Mycobacterium africanum* and *Mycobacterium tuberculosis* infection

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Mycobacterium tuberculosis (*Mtb*) and *Mycobacterium africanum* (*Maf*) are the two main strains causing tuberculosis (TB) in The Gambia. *Maf* is more likely to affect immune-compromised individuals, implying that it is less virulent than *Mtb*. This study aimed to evaluate differences between the host immune response to both pathogens.
HIV-negative TB patients were classified as *Maf* (n=26) or *Mtb* (n=49) infected based on spoligotyping of sputum isolates. Clinical and immunological evaluation was performed before, and after 2 and 6 months treatment. Undiluted whole blood was stimulated overnight with mycobacterial antigens and controls, multiple cytokines in culture supernatants were measured using the Bio-Plex assay and mRNA expression levels of 85 innate and adaptive immune markers were assessed by dual-colour RT-MLPA assay.
Before treatment, IL-9 secretion and expression was significantly higher in *Maf* than *Mtb*-infected patients in response to PPD and live-*Maf* respectively. Following treatment, IFN-γ, IL-4, IL-13, IL-9 and CCL11 secretion as well as CCL4, Granzyme B and autoimmune regulator (AIRE) expression significantly increased and were higher in *Mtb* than *Maf*-infected patients after treatment in response to ESAT-6/CFP-10, live-*Mtb* and live-*Maf*. There was greater improvement in body mass index and chest X-ray presentation in *Mtb* than *Maf*-infected patients after treatment.
Mtb may have the ability to suppress an immune-competent host response, which is restored following treatment. *Maf*-infected patients may have an intrinsic weaker immunity with high IL-9 production or may require a modified treatment regimen. These data provide an important addition to our understanding of pathogenesis of TB with public health implications.

P4.05.77
The interaction of CD43 with Mycobacterium tuberculosis Cpn60.2 modulates cytokines production

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The pathology of tuberculosis is considered to begin following the first interactions between *Mycobacterium tuberculosis* (Mtb) and alveolar macrophages through the production of IFN-gamma and TNF-alpha, two essential cytokines for the eradication of the bacteria. Additionally, IL-10 has been found to play an important role in infection and to block Mtb-phagosome maturation. Several molecules such as complement, Fc-gamma and mannose receptors, Scavenger receptors, Sp-A receptors, CD14, TLRs and CD43 participate in these cytokines production. Particularly, CD43KO mice fail to produce TNF-alpha in response to mycobacteria exposure. Given that Cpn60.2 and DnaK, two highly conserved chaperones among mycobacteria (including *Mycobacterium bovis* (BCG)), are presumed ligands for CD43, our aim was to evaluate the role of CD43 in TNF-alpha and IL-10 production in macrophages stimulated with BCG or Cpn60.2 and to elucidate the signaling pathway for the production of these cytokines. We generated clones of THP-1 cells stably transfected with a plasmid that down-regulates the expression of CD43; alternatively we derived BMMs from CD43KO and wild type mice; all cells were stimulated with BCG or Cpn60.2. We found that the lack of expression of CD43 correlated with a down modulation in TNF-alpha production, but surprisingly in enhanced IL-10 secretion. We also found that the CD43-mediated signaling pathway leading to TNF-alpha production depends on PI3K, PLC-gamma and members of the PKC family. All together these data highlights a role for CD43 in regulating the pathology of tuberculosis.
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P4.05.78
Combined detection of IFNγ, TGFα and IL-6 can discriminate between active TB disease and latent infection

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Interferon gamma release assays are used for diagnosing Mycobacterium tuberculosis (M.tb.) infection. However these tests fail to differentiate active TB from latent TB infection (LTBI).
Aim: Selection of informative biomarkers for diagnosing M.tb. infection and for discriminating between latent TB infection (LTBI) and active TB disease.
Materials and Methods: We recruited 54 TB patients, 47 TB contacts and 43 healthy donors and performed the “QuantiFERON-TB Gold In-Tube” (“Cellestis”, Australia). Using XMap technology levels of 10 cytokines (EGF, MIP-1β, VEGF, IL-2, IL-4, IL-6, IL-1α, IFN-α2, TGFα, TNFα) as well as sIL-2Rα and sCD40L were evaluated in unstimulated (NIL) and Mycobacterium tuberculosis specific antigen (AG) stimulated plasma. We also determined levels of IP-10 in 48 patients by ELISA.
Results: Six out of 13 biomarkers distinguished active TB from LTBI . As a result of “decision tree” in JMP 9.0 software, we choose three the most informative cytokines. The combination between IFNγ, TGFα and IL-6 may allow to distinguish active TB from latent infection with sensitivity 96,3% and specifity 80,7% (AUC=0,9). We also observed very high levels of IP-10 and IL-2, which correlated with IFNγ levels (r=0,71 and 0,79 respectively).
Conclusion: IL-2 or IP-10 as well as IFNγ, detection could be used as a first step to diagnose (M.tb.) infection. A second step of the test could be performed if positive IL-2 / IP-10 / IFNγ results are obtained to measure three-marker combination of IFNγ, TGFα and IL-6 to differentiate individuals with active TB from LTBI.

P4.05.79
Modelling human granuloma to study the mechanisms by which isoniazid inhibits Mycobacterium tuberculosis growth.

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Tuberculosis (TB), caused by the intracellular pathogen *Mycobacterium tuberculosis* (Mtb), is one of the most devastating infectious diseases worldwide. One of the main anti-TB antibiotic used is isoniazid (INH), which inhibits mycolic acid synthesis, an important bacteria's cell wall component. *In vitro* studies have suggested that INH-dependent bactericidal efficiency requires both a prolonged time of drug exposure as well as elevated concentrations in infected macrophages. In contrast, clinical studies have shown that INH promotes an initial killing of 95% of bacteria during the first 2 days of treatment, pointing to an indirect effect of this drug, which is found at low nM-range concentrations within the granuloma. To investigate the mechanisms by which INH inhibits Mtb survival during cellular infection, we have utilized an *in vitro* model in which these human leukocytes acquire granuloma conformation upon bacteria infection. Interestingly, treatment with INH inhibited Mtb growth in granulomas from PPD negative, but not in PPD positive donnors. Levels of TNF, IL-1beta and IL-6 showed no major diferences in PPD+ versus PPD- granulomas, except for IL-8, which was found to be highly decreased in INH-treated granulomas from PPD+ donnors. These results suggest that INH direct modulates adaptive lymphocyte responses within the granuloma. Experiments to address this hypothesis are in progress.
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P4.05.80
Aptamer against an immunosuppressive epitope-ManLAM inhibits virulent *Mycobacterium tuberculosis* infection in mice and rhesus monkeys

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The major surface lipoglycan of *Mycobacterium tuberculosis* (*M. tb*), mannose-capped lipoarabinomannan (ManLAM) is an immunosuppressive epitope of *M. tb*. We used Systematic Evolution of Ligands by EXponential enrichment (SELEX) to generate an aptamer (ZXL1) that specifically bound to ManLAM from the virulent *M. tb* H37Rv strain. Aptamer ZXL1 had the highest binding affinity (equilibrium dissociation constant-[KD] of 8.91 X 10^{-8} M) and competed with the mannose receptor for binding to ManLAM and *M. tb* H37Rv. ZXL1 significantly inhibited the ManLAM-induced immunosuppression of $CD11c^{+}$ dendritic cells (DCs) and enhanced the *M. tb*-antigen-presenting activity of DCs for naïve Th1 $CD4^{+}$ cell activation. More importantly, we demonstrated that a single injection of aptamer ZXL1 significantly reduced the progression of *M. tb* H37Rv infections, bactieral loads in lung and prolonged the survival rates in mice or rhesus monkeys. These results suggest that aptamer ZXL1 is a new potential antimycobacterial agent and TB vaccine immune-adjuvant.

P4.06 Immunity to fungal infection

P4.06.01
Anti-aspergillus fumigatus IgY antibodies and their protective efficacy in immunocompromised BALB/c mice

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Earlier the protective and therapeutic effect of immune IgY on normal mice challenged with A. fumigates was established and reported. In the present study immunocompromised, instead of normal mice were used.
Egg laying hens were immunized with a heat-killed suspension of A. fumigatus. IgY- rich extracts were obtained from the yolk of eggs laid post-immunization and pooled. The same was done with eggs laid pre-immunization. The preparations were dialyzed and then lyophilized. The presence of IgY anti-A. fumigatus antibodies in the post-immunization and its absence in pre-immunization extracts was confirmed by ELISA. Pre and post immunization IgY extracts were compared by polyacrylamide gel electrophoresis (PAGE). Groups of BALB/c mice were treated with cyclosporin A for 10 days prior to challenge with viable A. fumigatus, and were given IgY extract either 24hrs before, during, or 24hrs after the A. fumigatus challenge. Survivals were monitored daily and compared to control groups for a period of two weeks.
The post-immunization, but not the pre-immunization extract, protected mice against lethal challenge with A. fumigatus. Protection was observed when the extract was given 24 hours before, simultaneously with, or 24 hours after challenge.
It appears that antibodies produced in egg laying-hens directed against A. fumigatus are protective and therapeutic in the immunocompromised mice when given in proximity of a lethal dose of the fungus. A clinical trial to test their prophylactic/ therapeutic potential in immunocompromised patients might be considered.

P4.06.02
HeNe laser increases the pro-inflammatory cytokines production and the in vitro phagocytosis of P. brasiliensis by macrophages

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Cutaneous lesions caused by the fungus *Paracoccidioides brasiliensis* (Pb) lead to painful ulcerative wounds, which are difficult to be cured by conventional treatment with anti-fungal drugs due to their adverse effects and the multidrug resistance that some fungal isolates present. We have previously showed that Helium-Neon (HeNe) laser irradiation of paracoccidioidomycotic lesions in mice accelerates the wound healing as well as the fungal clearance, which is very probably linked to the death of the fungus by macrophages. In this study we evaluated, *in vitro*, whether HeNe laser irradiation was capable of modulating the phenotypic and functional profile of human and murine macrophages. Peritoneal macrophages from BALB/c mice were irradiated with different dosages of HeNe laser (0.836; 1.5 and 3J/cm^2) and cultivated with Pb yeast cells (1:4, macrophage:fungi) for 6 hours. Flow cytometry analysis of macrophages showed that laser irradiation promoted an increase in the expression of TLR-4, which are molecules involved in yeast recognition and internalization, but did not alter other markers, like CD11b, F4/80, MHC-II, for example. Also, co-cultures of irradiated macrophages and Pb showed a higher phagocytic index and a higher pro-inflammatory (IFN-γ, IL-6, TNF-α) cytokines production. Similar results were obtained with a human macrophage cell line (THP1) even under the lowest incidence energy of the laser. Collectively, our results indicate that the accelerated wound healing process, observed after the treatment of paracoccidioidomycotic lesions with the HeNe laser, must be associated with a direct action of irradiation on macrophages.
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P4.06.03
Cryptococcus gattii infection inhibits the induction of Th1 immune responses

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Cryptococcal infections are caused mainly by two Cryptococcus species; C. neoformans and C. gattii. Unlike C. neoformans that infects primarily the immunocompromised hosts, C. gattii can cause life threating diseases in healthy individual. Accumulating studies suggest that C. gattii infection evades host immune response by preventing leukocyte recruitments into the lungs and inhibiting immune response. However, the underlying mechanisms that immunocompetent host fails to mount effective immune response against C. gattii infection remain unidentified. In this study, we report that C. gattii infection fail to elicit effective Th1 responses. Compared

with C. neoformans-infected mice, mice infected with the high virulent C. gattii strain R265 exhibited reduced recruitments of inflammatory leukocytes and CD4+ T cells in the lungs and developed much fewer antigen-specific IFN-γ-producing T cells. Unexpectedly, this high virulent strain R265 did not impair the development and recruitments of Th17 cells. The failure to mount effective Th1 response in mice infected with C. gattii was further confirmed by the significant reduction of Ifng, but not Il13 or Foxp3 transcripts in their lung compared to in those of C. neoformans-infected mice. Furthermore, we showed that the loss of Th1 response correlated positively with reduced Il12p35 mRNA expression in the lung of mice infected with C. gattii, compared to that of C. neoformans-infected mice. Our data thus suggest that C. gattii is capable of not only inhibiting the recruitments of inflammatory cells, but also dampening protective Th1, but not Th17 responses, possibly by downregulating their Th1-promoting cytokine IL-12 expression in the lung.

P4.06.04
Disentangling immunity and tolerance in candidiasis

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The ability to tolerate *Candida albicans*, a component of human microbiota, implicates that host defense mechanisms of resistance and tolerance cooperate to limit fungal burden and inflammation at the different body sites. We have disentangled resistance and tolerance components of murine and human *C. albicans* mucosal infection and have introduced the challenging notion of a disease due to a defective tolerance mechanism. While some degree of inflammation is required for protection at mucosal tissues during the transitional response occurring between the rapid innate and slower adaptive responses, progressive inflammation worsens disease and ultimately prevents pathogen Resistance and tolerance mechanisms were both activated in murine intestinal and vaginal candidiasis through the contribution of innate and adaptive immune responses, involving distinct modules of immunity, IL-22 and Th1/Th17 cells for resistance and IL-10-producing regulatory T cells for tolerance., with a major contribution by the enzyme indoleamine 2,3-dioxygenase 1 (IDO1). IDO1 was responsible for the production of tolerogenic kynurenines, such that replacement therapy with kynurenines restored immunoprotection to murine vaginal candidiasis. In humans, two functional genetic variants in *IL22* and *IDO1* genes were found to be associated with heightened resistance to recurrent vulvovaginal candidiasis, a disease of unknown origin, and they correlated with increased local expression of IL-22, IDO1 and kynurenines. Thus, IL-22 and IDO1 are crucial in balancing resistance with tolerance to *Candida*, their deficiencies are risk factors for mucosal infection resulting from transition from symbiont to pathobiont.

P4.06.05
Paracoccidioides brasiliensis* induces neutrophil extracellular traps *in vitro

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Paracoccidioides brasiliensis is a dimorphic fungus that is the causative agent of paracoccidioidomycosis, a chronic, subacute or acute mycosis, with visceral and cutaneous involvement. Recently, studies have focused on the role of neutrophils that are involved in primary response to the fungus. In addition to their ability to eliminate pathogens by phagocytosis and antimicrobial secretions, it has been shown recently that neutrophils can trap and kill microorganisms by release of extracellular structures composed by DNA and antimicrobial proteins, called neutrophil extracellular traps (NETs).The aim of this study was to demonstrate whether *P. brasiliensis* induces NETs release by human neutrophils activated or not with cytokines that promote fungicidal activity of these cells. Human neutrophils were isolated from peripheral blood of healthy donors and incubated with *P. brasiliensis* for 60, 90, 120 and 150 minutes. Some of these cultures were pré-treated with DNAse (to degrade NETs) or PMA (positive control of NETs release). Samples analyzed by scanning electron microscopy and photomicrographs demonstrated that *P. brasiliensis* induces NETs 60, 90, 120 and 150 minutes after interaction with neutrophils. DNAse treatment degraded these structures, whereas PMA induced their formation. Moreover, by using confocal imunofluorescence microscopy the following NET constituents were identified in activated cells with TNF-α, IFN-γ and GM-CSF: DNA (stained with DAPI, elastase (stained with FITC) and histones (stained with TEXAS RED). Thus, we demonstrated that *Paracoccidioides brasiliensis* is able to induce NETs. FAPESP 2010/17405-5; FAPESP 2010/18957-1.

P4.06.06
Characterization of Regulatory T Cells in Pulmonary Paracoccidioidomycosis Using Foxp3-GFP Transgenic Mice

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The involvement of Foxp3-expressing regulatory T (Treg) cells in suppressing immunity against paracoccidioidomycosis, the most prevalent deep mycosis in Latin America, has been previously demonstrated. However, more direct approaches using Foxp3-GFP mice have never been used. In the present study, Foxp3-GFP mice were intratracheally infected with 10^6 yeast cells and examined 2 and 10 weeks post-infection. Flow cytometric analyses showed the expansion of $Foxp3^+$ $CD4^+$ T cells at the site of infection and the up-regulation of diverse cell surface markers, including those associated with the “natural Treg” phenotype at both post-infection periods. In addition, increased expression of Treg-related effector molecules were observed at the chronic phase of *Paracoccidioides brasiliensis* infection. Treg cells were then depleted by administration of anti-CD25 mAb (PC61) at an early phase of infection. Treg ablation led to reduced fungal burden in the lungs as well as virtually no dissemination to liver and spleen both in early and late stages of infection, as shown by a colony forming units assay. Furthermore, PC61-treated mice displayed an increased number of effector T cells and diminished tissue pathology, as revealed by flow cytometry and histopathologic analysis of lung sections, respectively. These data support the deleterious effects exerted by Treg cells on PCM and help to elucidate some of the immunoregulatory phenomena involved in this systemic mycosis.

P4.06.07
Deciphering the class and specificity of the T cell response to C. albicans

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From the immune system point of view, microbes (pathogens or commensals) are complex antigens that occupy distinct niches and consequently trigger different types of immune responses. The complexity of the microbial proteome, in particular that of bacteria and fungi, represents a considerable challenge to our capacity to analyze the human T cell response. We are using complementary approaches in order to study the human T cell response to Candida albicans and to identify immunodominant and protective antigens. On the one hand, we are performing a wide screening of HLA-binding peptides, identified through bioinformatic analysis, from 80 fungal proteins belonging to different classes for their capacity to be recognized by different memory T cell subsets. On the other hand, we isolate and identify proteins contained in cell wall extracts that are recognized by human IL-17-producing memory T cells (Th17) and induce strong immune responses when used as vaccines in mice. These studies are expected to improve our understanding of the immune response to complex pathogens, to define the correlation between class of antigens and type of T cell response elicited, and, finally, to provide useful information for the design of subunit vaccines against C. albicans.

P4.06.08
Variability in autologous/heterologous binder serum activity anti-Candida albicans and relationship with oral presence of the yeast, in patients with risk factors

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Candida albicans is an opportunistic yeast that often causes candidiasis in immunocompromised individuals. Variability in binder serum activity (IgM) autologous/heterologous anti-Candida albicans and oral presence of yeast were determined, in patients with risk (steroids, chemotherapy, HIV + and hemodialysis), without yeast (n = 140). The yeast was isolated and identified by culture in the medium YMAB and Chrom-Agar). The IgM was measured by agglutination plates. The prevalences obtained were 37.14% -13/35, -13/30 43.33%, 40% and 13.33% -12/30 -4/30, in the respective low risk groups. The autologous binder reactivity (self-isolated) was 46.15% (6/13-esteroides), 61.53% (8/13- chemotherapy), 58.33% (7/12-VIH+) and 25% (1/4-hemodialysis), with titers between 1:1-1:32. Frequencies of Heterologous Serum Agglutination (FHSA) most representative corresponded to steroids and hemodialysis: 41.66% and 66.66% of heterologous sera, agglutinated to 91.66% and 100 - 11/12 % -3/3 of heterologous strains respectively. The FHSA were low in chemotherapy and HIV+: only 16.66% and 18.18% of heterologous sera agglutinated to 66.66% or 50% and 100% of the heterologous strains, respectively, indicating low variability in the response. They showed high levels of Heterologous Representativeness Index (HRI) in few strains: 1.0 in steroid therapy (5 strains), 0.92 in chemotherapy, 0.73 and 1.0 in HIV+ in hemodialysis. The prevalence of yeast in the first three groups is higher than that reported in healthy individuals. Autologous serum reactivity tends to be lower, enhancing the risk and requiring prevention. There were no differences (p 0.05) between the prevalence or autologous reactivity and sex. Also among agglutination titers in different Groups.

P4.06.09
Granulomas developed in paracoccidioidomycosis in oral lesions of patients and in experimentally infected mice present similar patterns

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Severe paracoccidioidomycosis is characterized by the presence of numerous loose granulomas, and mild forms by few compact granulomas. We characterized oral lesions in paracoccidioidomycotic patients, and compared with previously obtained data from experimentally infected susceptible and resistant mice.
We did qualitative microscopical analysis of HE and Grocott-stained slides in 38 paracoccidioidomycosis cases and compared to similar preparations from mice, analyzing architecture of the lesions, presence of morphologically preserved or destroyed fungi and composition of the cellular infiltrate.
In patients (mostly 40-50 years old male adults,) lesions were mainly found in gengiva, hard and soft palate and oral mucosa. Microscopy revealed predominance of loose granulomas with multinucleated giant cells (MGC) of the Langhans type and intense, diffuse inflammatory infiltrate, constituted by lymphocytes and plasmocytes, Fungi were frequently found dispersed in connective tissue and inside MGC. Susceptible mice showed similar pattern, with disseminated diffuse granulomas presenting numerous fungi and MGC and influx of neutrophils, lymphocytes, and plasmocytes.
The overall aspect of the lesions of a patient treated for 6 months with remission of the symptoms was similar to that observed in resistant mice, showing few, compact lesions.
Based on these results, we can conclude that paracoccidioidomycosis is a condition affecting mainly male adult patients in south of Minas Gerais state. The lesions present predominance of an intense mononuclear inflammatory infiltrate, diffusely distributed in the tissues. The small numbers of compact granulomas can indicate a severe depression of cellular immunity in these patients, analogous to that described in susceptible mice.

P4.06.10
Cutaneous candidiasis in BALB/c mice

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Individuals with diabetes mellitus are predisposed to cutaneous candidiasis. BALB/c mice were chosen as model of chronic cutaneous candidiasis because in further studies, this mycosis will be studied in diabetic BALB/c. *C. albicans* pseudohyphae ($5X10^6$) were injected in the deep dermis of the hindpaw and PBS in the contralateral paw. After 0, 1, 4, 7,14 and 21 days of infection the mice were euthanized and tissues injured by infection and the popliteal lymph nodes collected for histopathological and cytokines analysis. The extent of the inflammatory area was evaluated daily with a paquimeter. The peak of inflammatory area occurred at day 6 of infection (2.64 ± 0.57 mm), persisted until day 9 (2.70 ± 0.54 mm), reducing significantly in size until day 15 as compared to day 9. *C. albicans* caused significant thickening of the epidermis and induced migration of inflammatory cells to the lesion site. IFN-γ, IL-6, TNF-α and IL-10 were found in low levels at the infection sites, whereas the immune response at the popliteal lymph nodes was intense: the levels of IFN-γ (994±54 pg) and TNF-α (437±31pg) were high until day 4 of infection; those of IL-6 were high (520±8pg) until day 4 and those of IL-10 remained low in the very initial phase and increased from day 4 on. These cytokines at these levels could play important roles in this model of cutaneous candidiasis: IFN-γ and TNF-α in *Candida* antigens processing and presentation and therefore fungal clearance, IL-6 in neutrophils migration and IL-10 in lesion repair.

P4.06.11
Immune response investigation in a murine model of sporotrichosis

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Major constituents of Sporothrix schenckii cell wall are peptido-polysaccharide complexes containing rhamnose, mannose and galactose. These complexes are organized in two distinct layers in the yeast's cell wall and those forming the inner one are called cell wall peptido-polysaccharide (PPC), which is being used as antigen in this study along with the heat-killed S. schenckii yeast (HKSS). Antigens released by the fungus directly participate in evasion of the immune system, also serving as targets for fungus elimination by binding of antibodies or innate immune cells like macrophages. Our results showed that PPC induces inflammatory cytokines in a more pronounced way when compared to HKSS, which induces higher levels of IL-10. We also suggest that, in the animal model used, IL-10 doesn't act as a suppressor of splenocytes proliferative response, and that IL-4 plays a role at the resolution phase of the infection, but not as a means of immunologic escape by the fungus. Also, we've shown for the first time that the assayed antigens are capable of inducing IL-17 secretion in splenocytes cultures, in a pattern close to that of the Th1 cytokines evaluated. In conclusion, the inflammatory response is slightly better triggered by the PPC, while IL-10 seems specially more responsive to HKSS. However, both antigens have been capable of inducing IL-17A liberation, suggesting, for the first time, Th17 response involvement in sporotrichosis. Financial support: FAPESP and CNPq.

P4.06.12
Identification of NETs (neutrophil extracellular traps) in tegumentary lesions of patients with paracoccidioidomycosis

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Paracoccidiodomycosis is a systemic mycosis caused by Paracoccidioides brasiliensis (Pb), which is endemic in Latin America. In recent years, works in paracoccidioidomycosis have focused the role of neutrophils (PMNs), since the literature has demonstrated the dynamic involvement of these cells in host defense against various microorganisms. Recent studies have shown that neutrophils may use a third strategy to destroy microorganisms called NETosis, a type of neutrophil death, reported to be distinct from apoptosis, phagocytosis-induced cell death, and necrosis. This cell death pathway involves the release of extracellular traps by activated neutrophils, known as neutrophil extracellular traps (NETs). The NETs are composed by fibers containing decondensed chromatin, histones and several other granular proteins such as elastase, which in addition to ensnare and/or kill microorganisms, increase the inflammatory response. It has been proposed that NETs destroy microorganisms that were not phagocytosed by neutrophils, and this could be proposed for the Paracoccidioides brasiliensis, since the fungus yeast can present various sizes and morphologies. In this context, the objective of this study was identify the presence of NETs in vivo, analyzing tegumentary lesions of patients with paracoccidioidomycosis. Tissue section from formalin-fixed biopsies were stained with DAPI, anti-elastase (FITC) and anti-histone (Texas-Red); and analyzed by confocal imunofluorescence microscopy. The images revealed the presence of NETs constituents, nuclear and extracellular localization of DNA stained with DAPI, co-localization of elastase and histone, which was confirmed by the overlay of these 3 stains. Thus, we identified the mechanism of NETosis in paracoccidioidomycosis patient lesions. Financial Support: FAPESP/CNPq/Fundunesp

P4.06.13
High-throughput miRNA sequencing of human monocyte-derived dendritic cells confronted with Aspergillus fumigatus

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Aspergillus fumigatus is an ubiquitous mold which normally lives in soil and decaying biomass. While in healthy individuals its reproductive spores, called conidia, are recognized and eliminated by the innate immune system, they can germinate in the lung of immunocompromised patients and enter the blood vessels. MicroRNAs (miRNAs) specifically bind to mRNA targets thereby leading to translational repression or target degradation and gene silencing. The role of miRNAs and their influence on innate immune cells during A. fumigatus infections are not well understood.
Isolated monocytes from healthy donors were differentiated to monocyte-derived dendritic cells (moDCs) and co-cultivated either with A. fumigatus conidia, germlings (MOI 1) or LPS (1µg/ml). Following total RNA isolation, miRNA sequencing (Illumina HighSeq® System, FDR < 0.05) and mRNA expression arrays (Affymetrix HGU 219) were performed.
Germlings induced n=16 miRNAs to be regulated after 6 h with a time dependent increase of 20 miRNAs after 12h. In contrast, conidia only induced miRNA regulation after 6h (11), but not after 12h. LPS as positive control had the strongest influence on the miRNA profile, with 22/38 regulated miRNAs after 6 and 12 h of stimulation, respectively. Interestingly, miR-155-5p was regulated by germlings and LPS at both time points, while miR-132-3p/5p, miR-212-5p and miR-222-5p were characteristic for the stimulation with germlings. In parallel, mRNA levels showed significant changes in chemokine/cytokine expression. In summary, we were able to describe new significantly regulated miRNAs by A. fumigatus and their influence on mRNA levels in moDCs.

P4.06.14
Involvment of Notch-TLR signaling in experimental paracoccidioidomycosis

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Introduction: Paracoccidioidomycosis is a systemic mycosis of deep nature and granulomatosis that preferentially affects the lung tissue caused by *Paracoccidioides brasiliensis*, a thermal dimorphic fungus. It interacts with macrophages, cells that play an important role in regulation of immune response. Recently it has been demonstrated the importance of Notch receptors in regulating the activity of macrophages. Given that Notch signaling may be involved in modulating macrophage function, we evaluated the ability of the fungus modulate the activation of this pathway. **Methods and Results:** J774 macrophages, pre-stimulated with LPS or not, were interacted with yeast fungus, followed by analysis of real-time-PCR, phagocytosis-index and ELISA. The transcriptional analysis of Notch1 revealed an increased level of transcription in 24 hours, while there is a reduction of its ligand Delta4. However, we found that the fungus alone is not able to induce transcription of NF-kB, but when we stimulated with LPS together inhibition of Notch pathway, an increased level of transcripts was observed, suggesting that this factor is activated in the absence of Notch. In the same context, we observed that phagocytosis of yeasts by macrophages became more efficient. It was possible to verify that the fungus has the ability to promote the production of IL-6 via TLR-Notch, making us assume that this cytokine is important to the establishment of the disease. We also observed that the increased level of IL-6 is associated with decrease of TNF-α. In resume ours results suggest that *P.brasiliensis* uses the Notch signaling pathway as an escape mechanism.
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P4.06.15
Thymic alterations during Paracoccidioides brasiliensis infection

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Thymus is a lymphoid organ responsible for the development and maturation of T lymphocytes. To exercise this function, the thymic microenvironment should be preserved by a fine control of chemokines and growth factors released by stromal components. Such soluble thymus components directly influence the migration of T lymphocyte precursors from perivascular space to the region cortical and subsequent migration to the medullar region. Despite its importance, the thymus has been reported as a target organ in several pathologic conditions, e.g. infections. Studies from our laboratory have shown that experimental infection with Paracoccidioides brasiliensis (Pb), the causative agent of the most prevalent systemic mycosis in South America, is able to invade the thymus and to induce thymic microenvironmental changes. Herein, thymuses from mice infected with the P. brasiliensis virulent isolate (Pb18) in its yeast phase were analyzed for thymic index, changes in thymic architecture, accumulation of adipose tissue, and expression of matrix metalloproteinases (MMPs) as well as their tissue inhibitors (TIMPs). Our results show severe thymic atrophy, which is accompanied by loss of its architecture and replacement of stromal cells by adipose tissue. Besides, an enhanced expression and widespread distribution of MMP-2, MMP-9, TIMP-1 and TIMP-2 were observed in thymus from Pb-infected animals. We assume that these thymic alterations are related to the high death of thymocytes and premature egress of immature thymocytes to the peripheral lymphoid organs. Our results reinforce the idea that thymic compartment disturbances may be involved in the immunossupressive phenomenon frequently associated to Pb infection.
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P4.06.16

Anti-DEC-205 receptor carrying P10 induces protective response in experimental Paracoccidioidomycosis

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Paracoccidioidomycosis is a systemic mycosis caused by *Paracoccidioides brasiliensis*, a thermally dimorphic fungus. It occurs in Latin America and is the most frequent endemic systemic mycosis in many countries of South America, where almost 10 million people are believed to be infected. In Brazil, it is ranked as the tenth cause of death among chronic infectious and parasitic diseases. It is likely that vaccines will need to elicit strong T cell-mediated immunity composed of IFNγ secreting $CD4^+$ helper and $CD8^+$ cytolytic T lymphocytes. To induce such responses, it would be valuable to harness the dendritic cell (DC) system of antigen-presenting cells. To assess the potential of targeting P10, a peptide derived from gp43 secreted by the fungus, directly to DCs, we cloned its sequence in fusion with a monoclonal antibody to the DEC-205 receptor, an endocytic receptor abundant on these cells in lymphoid tissues. We verified that αDEC/P10 antibody response induced large IFNγ-producing cells after single dose administration in relation to P10, even being administered at a lower concentration. However, this response was not maintained after the second dose of the antibody. After challenge of animals immunized with two doses of the antibody, we detected high levels IFNγ and IL-4 in the lung tissue was statistically higher in αDEC/P10 and ISO/P10 compared to administration of P10 and control Poly I:C. The results obtained indicate that targeting of P10 through αDEC/P10, in the presence of Poly I:C, is a promising strategy for vaccination against *P. brasiliensis*.
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P4.06.17

Concanavalin-A induces IL-17 production during the course of *Candida albicans* infection

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In a previous study, our group verified that 100% of mice survived to a lethal dose of *Candida albicans* following pretreatment with concanavalin-A (Con-A) for 3 days. This work proposed to investigate whether treatment could mediate an adaptative immune response involving TH17 cells. A significant increase in IL-17 levels at 6 h postinfection was observed and was maintained up to 18 h in the Con-A group, whereas in control mice, a reduction in this cytokine was verified. In addition, TH17 cells develop in the presence of TGF-beta, IL-1 beta, and IL-6 that were increased significantly 2 h postinfection in Con-A-treated mice. Macrophages were involved in the process, engulfing greater numbers of yeast cells, and were activated through TNF-alpha and interferon-c produced at significant levels at 2 h postinfection. A significant increase in IL-12 levels was also observed at 2 h postinfection. Thus, activated macrophages were probably more capable of killing and processing *Candida* antigens, signalizing an adaptative immune response. Macrophages from controls did not prevent yeast-to-hyphae transition and were partially destroyed, as shown in scanning microscopy. These results suggest that treatment with Con-A facilitated the triggering of TH17 and TH1 responses via IL-17 and IFN-gamma production, leading to the resolution of *C. albicans* infection.

P4.06.18

Inhibition of PGE_2 and LTB_4 production by dendritic cells induced by *Paracoccidioides brasiliensis* is associated with the non maturation of these cells

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The fungus *Paracoccidioides brasiliensis* is the etiological agent of paracoccidioidomycosis, a systemic mycosis endemic in Latin America. Dendritic cells (DCs) play a crucial role in the detection of pathogens, triggering an initial response from the host as well as the instruction of the adaptive immune response. Mediators released by DCs, by an autocrine way, modulate their functions. Among these, we highlight the prostaglandins and leukotrienes. We therefore assessed whether human DCs produce PGE_2 and LTB_4 in response to fungus, the participation of recognition receptors of molecular patterns (PRRS) in this production, as well as the modulating role of these eicosanoids on phenotypic maturation of these cells. Human immature DCs ($CD14^-$/ $CD1a^{high}$/$CD83^{low}$) derived from the differentiation of monocytes cultured with GM-CSF and IL-4 (7 days) released substantial concentrations of PGE_2 and LTB_4 which increased significantly in the case of PGE_2, with incubation with LPS for 1h, 2h, 4h, 8h, 12h, 18h, 24h or 48h. However, the levels of PGE_2 and LTB_4 were significantly inhibited after challenge with two different strains of the fungus. Assays using monoclonal antibodies to block PRRS showed the involvement of mannose receptor in PGE_2 inhibition by the fungus. Additionally, phenotyping assays showed that after challenge with the fungus, DCs did not change their phenotype of immature cells to mature ones. Accordingly, we detected a clear association between inhibition of the production of PGE_2 and LTB_4 fungus and inability to induce maturation of DCs. Assays using exogenous PGE_2 and LTB_4 confirmed this association.
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P4.06.19

Therapy with scFv transfected-dendritic cells induce a decrease levels of specific IgG2b upon Paracoccidioides brasiliesnsis infection

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Paracoccidioidomycosis is a systemic mycosis and the etiologic agent of disease is the *Paracoccidioides brasiliensis (Pb)*. A gp43 is the major antigen of *Pb*. It has been shown that mice immunized with anti-gp43 monoclonal antibodies (Ab1), induce the idiotypic cascade, which produced of anti-Id antibodies (Ab2). Ab2 inhibited the binding of gp43 to Ab1, suggesting that the Ab2 binds to the idiotope, thus fulfilling the internal image criteria. To better characterization of Ab2 we developed antiidiotypic antibody single chain variable fragments (scFv) and we showed that dendritic cells (DCs) transfected with scFv from Ab2 mimicking original antigen gp43 induces protection against experimental paracoccidioidomycosis. Since DCs are not typically found in normal muscle tissue, the objective of this study was analyze the capacity of DCs in migrate to the site of DNA inoculation and analyze if DCs may then either take up the plasmid DNA directly or cross-present expressed antigen to initiate immune responses. Mice were immunized with scFv and we observed an increased of DCs into regional lympho nodes with a decrease level of IL-4. These DCs expressed high levels of CD40, CD8, DEC205 and MHCII suggesting the capacity of presenting antigen and a decreased of regulatory T cells also was observed. Besides, therapy with scFv transfected-DCs induced increased levels of IgG2b upon *Pb* infection. These studies describe that scFV can be utilized to recruit DCs into regional lymph nodes and initiate the immune responses of T lymphocytes, besides to induce an efficiently immune response with a protector antibody production.

P4.06.20

In pulmonary paracoccidioidomycosis, IDO exerts a protective effect to susceptible mice but a deleterious effect to resistant mice

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In pulmonary paracoccidioidomycosis, the regulatory mechanisms mediated by innate and cellular immunity are still unclear. It is known that indoleamine 2,3-dioxygenase (IDO), an IFN-ɣ-induced enzyme which catalyzes the tryptophan metabolism, can control pathogen growth due to tryptophan starvation and inflammation by its immunosuppressive effects on innate and adaptive immunity. Our previous studies demonstrated that P. brasiliensis infected mice showed increased IDO expression, controlling fungal growth but suppressing T cell responses of resistant (A/J) and susceptible (B10.A) mice to P. brasiliensis infection. The aim of this study was to further characterize the role of IDO in the behavior of pulmonary dendritic cells and the expansion of T cell subpopulations. B10.A and A/J mice were treated with 1-methyl-DL-tryptophan (1MT, an IDO inhibitor) or left untreated, and intratracheally infected with yeasts. Our data demonstrated that in both mouse strains IDO activity reduced the migration of IL-6+ DCs, but increased the numbers of TGF-B+ and IL-12+ DCs in A/J and B10.A mice, respectively. IDO expression also reduced the migration of IL-17+ CD4+ and CD8+ T cells concomitantly with an increased influx of Treg cells to the lungs of B10.A and A/J mice. Furthermore, mortality and histopathological studies revealed that IDO exerted a protective effect on B10.A mice due to its inhibitory activity on the pro-inflammatory response associated with their susceptible pattern, but a deleterious effect on A/J mice due to its enhancing effect on the tolerogenic activity of DCs and the expansion of Treg cells, which delayed the expression of protective T cell immunity.

P4.06.21

Evaluation of infiltration with important acquired immune cells in kidney and spleen of mouse model in groups affected by systemic candidiasis, treated by etanolic extract of Zataria multiflora Boiss. in comparison to control groups

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Systemic candidiasis is a disease mainly caused by Candida albicans. This disease has increased dramatically in recent years and therefore use of effective fungalcidal drugs such as fluconazole turned with the emergence of resistance to these drugs in human societies. So now, one of the major challenges in relation to pharmaceutical researches, manufacturing of antimicrobial drugs from natural resources such as *Zataria multiflora* Boiss. Because Candida albicans is a stimulator of cellular immunity, evaluation of increase or decrease in different subgroups of T cells in involved tissues, which can image a desirable mechanism in terms of extract impact on the immune system. In this study, *Zataria multiflora* Boiss extract and selective antifungal drugs were injected in patient groups including balb/c mice over IP (intra peritoneal). After 7 days of treatment, spleen and kidney mononuclear cells (SMNCs & KMNCS) of them were cultured in the presence of heat killed Candida albicans (HKC) and anti-CD28 and the cell precipitate was assessed by flow cytometric techniques. Briefly, the percentage of Th1 cells and Th17 in SMNCs & KMNCS of group received extract were significantly higher than other groups ($P < 0.05$).In total, regarding to doses of this extract and other pharmaceutical drugs, including fluconazole, thymol and carvacrol were used in this study and useful effect of the extract in reducing fungal burden and strengthen the immune system, we can hope to application of the extract and its obtained fraction in the systemic candidiasis, especially in patients with acuired immunodeficiency syndrome(AIDS).

P4.06.22

Dectin-2 mediated adaptive immunity to Candida glabrata

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The C-type lectin receptor dectin-2 *(Clec4n)* is mostly expressed by dendritic cells, macrophages and neutrophils. It recognizes mannan structures and plays an important role in host defense against fungi by preferentially inducing Th17 cell differentiation. Little is known about dectin-2 recognition of *Candida glabrata*, the second most important pathogenic *Candida* species. In order to investigate the role of dectin-2 in fungal infections, we modified genetically mice to lack *Dectin-2* (*Dectin-2*$^{-/-}$ mice) and assessed the susceptibility of these mice to disseminated *C. glabrata* infection at three time points following the infection. *Dectin-2*$^{-/-}$ mice were significantly more susceptible to *C. glabrata*, as shown by a delayed elimination of the fungus from the kidneys on day 7 and 14 of infection. This increased susceptibility to infection was accompanied by 30 to 50% lower production of Th-derived cytokines by spenocytes of *Dectin-2*$^{-/-}$ mice, while the macrophage-derived cytokines were less affected. In an *ex-vivo* phagocytosis and killing assay, Clec4-/- activated peritoneal macrophages had a 15-20% lower capacity to kill *C. glabrata*. Dectin-2 $^{-/-}$ neutrophils also exhibited a significantly decreased phagocytosis and killing of live *C. glabrata*, which correlates with up to 25% less ability of deficient neutrophils to secrete reactive oxygen species (ROS) upon recognition of both *C. glabrata* and *C. albicans*. Understanding the nature of activation of the innate immune cells by Dectin-2 represents an important step towards elucidating the host defense mechanisms against this important fungal pathogen, which may lead to the design of novel immunotherapeutic approaches.

P4.06.23

Differential immune reactivity of Paracoccidioides brasiliensis phylogenetic species S1, PS2 and P. lutzii antigens by paracoccidioidomycosis patients` sera

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Paracoccidioidomycosis (PCM) is a systemic mycosis and recent researches show that its agent *Paracoccidioides brasiliensis* can be divided into phylogenetic species S1, PS2, PS3 and *P. lutzii,* which have distinct geographical distributions. The aim of this work was to evaluate the serum levels of IgG to soluble antigens of *P. brasiliensis* phylogenetic species S1, PS2 and *P. lutzii* in chronic PCM. Patients' sera (n= 35) from the northern and west regions of Paraná, Brazil were evaluated by ELISA. *P. brasiliensis* B339 (S1), IFM 54649 (PS2) and *P. lutzii* (*Pb01-like*; IFM 54648) were used to obtain cell free antigens (CFA) and 43 kDa (gp43) antigens from each strain. ELISA results, expressed as O.D. at 492 nm, showed higher reactivity of sera with CFA from *P. brasiliensis* S1 than PS2 and *P. lutzii* ($p < 0.05$) and also reactivity was higher with gp43 from *P. brasiliensis* S1 than PS2 ($p<0.05$) and PS2 higher than *P. lutzii* ($p<0.05$). In conclusion, chronic PCM patients' sera may distinctly recognize antigens from *P. brasiliensis* phylogenetic species S1, PS2 and *P. lutzii* and this difference is possibly related to geographical distribution of these agents.

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P4.06.24
FIBCD1 binds to Aspergillus fumigatus PAMPs and modulates the inflammatory response

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We have recently identified and characterised fibrinogen C domain-containing protein 1 (FIBCD1) as a homotetrameric type II transmembrane protein expressed by epithelial cells. FIBCD1 is an endocytic receptor that binds crab chitin and other acetylated compounds calcium- and acetate-dependently through the C-terminal fibrinogen-related domain (FReD).
In the present study, we investigate the role of FIBCD1 in the immune reaction against Aspergillus fumigatus (Afu), a pathogenic fungus involved in the development of asthma and allergy in western society.
We show that FIBCD1 binds the Afu cell wall alkaline-insoluble fraction (AIF), which is primarily composed of chitin, beta-glucan, and galactomannan, in a calcium- and acetate-independent manner. We demonstrate that FIBCD1 recognises chitin-rich zones in the fungal cell wall in different cellular stages of Afu and that FIBCD1 is expressed in ciliated airway epithelial cells from patients with aspergillosis.
We show that conidia, AIF, chitin, and beta-glucan induce IL-8 secretion from A549 lung epithelial cells in a time- and dose-dependent manner. Furthermore, our results indicate that FIBCD1-transfected A549 cells display reduced IL-8 secretion compared to sham-transfected A549 cells when stimulated with any of these compounds and that this reduction is significantly increased when the compound is a FIBCD1 ligand.
Collectively, these results indicate that FIBCD1 is present in human airway epithelial cells, binds Afu cell wall PAMPs, and influences the inflammatory response in the lung.

P4.06.25
Protein-based Mycosis Vaccines: Mechanism and Prospects for Future Human Use

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Invasive aspergillosis (IA) has a persistently high mortality rate and is the main one obstacle for the treatment of lymphoma, leukemia and other hematological malignancies by hematopoietic stem cell transplantation (HCT). Between 5.8 to 8.1% of all HCT recipients contract IA or similar fungal infections, and a staggering 57.8% of HCT recipients with IA die. Here we present our research on the development of an aspergillosis/mycosis vaccine to prevent IA and other mycoses in HCT recipients.
We previously developed a protein vaccine that protects mice from IA. Is was based on vaccinations with recombinant Asp f3, and induced both antibody and T cell responses. While antibodies were not protective, Asp f3-specific $CD4^+$ T cells mediate protection in cortisone acetate immunosuppressed mice. Neutrophils, although present in high numbers at the site of a pulmonary *A. fumigatus* infection, were dispensable for vaccine protection. Apart from mice, the rAsp f3 vaccine was also used to safely immunize South African penguins.
To broaden the spectrum of protection to fungal pathogens other than *A. fumigatus*, and to enhance the potency of the vaccine, we have conducted a quantitative analysis of fungal cell wall proteomes. Promising vaccine candidates were proteins with high abundance, high degrees of sequence homology between fungi, and little homology to human or mouse. We produced several of the most promising candidates as recombinant multivalent multi-epitope protein vaccines, and are presenting data on their protective effect. We will discuss the prospects of our strategy to implement a mycosis vaccine for human use.

P4.06.26
Preliminary evaluation of antigens from Paracoccidioides brasiliensis responsible for protective immune response induced in P. brasiliensis-infected mice by treatment with complete Freund's adjuvant

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Paracoccidioidomycosis (PCM) is an infectious disease caused by the dimorphic fungus Paracoccidioides brasiliensis. Among the deep mycoses, PCM is the most prevalent in Latin America and represents a major public health problem in countries where there is a higher incidence of the disease. In Brazil, PCM corresponds to the eighth leading cause of death among chronic or recurrent infections and parasitic diseases. Previous results from our group have shown that complete Freund's adjuvant (CFA) has a therapeutic effect in P. brasiliensis-infected mice. In the current study, we propose to isolate P. brasiliensis antigens associated with the protective immune response induced by CFA in infected mice. Exoantigens (ExoAg) and somatic antigens (SoAg) were isolated from yeast cells of virulent Pb18 strain of P. brasiliensis, and electrophoresis of the preparations showed high complexity of proteins. Both antigenic preparations were able to induce delayed-type hypersensitive (DTH) reactions in BALB/c mice previously infected with the fungus, though more prominent and significant DTH reactions were elicited when the mice were treated with CFA. Moreover, these preparations induced a proliferation in CFSE-labeled CD3+ spleen cells from mice infected with P. brasiliensis and treated with CFA significantly higher than those from only infected mice. These results suggest that ExoAg and SoAg contain antigens that are potential target to immunotherapeutic intervention in PCM. Others assays in vitro and in vivo are being conducted to isolate antigens from P. brasiliensis capable of inducing and/or enhancing the development of a protective cellular immune response.
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P4.06.27
CD5 modulates the inflammatory response to fungal cell wall components

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The lymphocyte surface receptor CD5 is a member of the Scavenger Receptor Cysteine-Rich superfamily, expressed in all T cells and certain B cell types (B1a, B10). CD5 is physically associated with the TCR and BCR, modulating their intracytoplasmic signals during lymphocyte activation and differentiation. CD5 can also bind and signal the presence of β-glucans, fungal PAMPs present on saprophytic and pathogenic species. Accordingly, the infusion of a recombinant soluble form of CD5 (rshCD5) in a septic shock-like model induced by zymosan, a fungal cell wall derivative from Saccharomyces cerevisiae, significantly improved the survival of CD1 mice. To gain further insight into the putative role of CD5 in host response to fungal structures, the same model was evaluated in wild-type (WT) and CD5-deficient (CD5KO) mice of C57BL/6 background. Ex vivo experiments showed that 15 µg/mL rshCD5 significantly reduced the zymosan-induced activation of purified spleen CD4+ and CD8+ T cells from WT mice as measured by the expression levels of CD69 surface marker. In vivo experiments showed that WT mice exhibited an increased response to zymosan challenge as reflected by a worse clinical condition, a more robust inflammatory response (higher plasma levels of pro- and anti-inflammatory cytokines as well as circulating monocytes in the blood) and higher early mortality compared to CD5KO. Infusion of 25µg rshCD5 either 1h before or after zymosan challenge decreased the peritoneal cytokine levels without improving mice survival. These results highlight the immunomodulating role of CD5 (both soluble and membrane-bound) in the inflammatory response induced by fungal structures.

P4.06.28
Host parasite interaction in Drosophila melanogaster

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Drosophila melanogaster is a model organism to study innate immunity in invertebrates. Temperate and tropical D. melanogaster populations are exposed to different environments and have possibly to face different parasites and parasite pressures. This could result in differences in immune investment. In order to test this hypothesis we have to assess the immune ability of D. melanogaster populations and the response they unfold upon infection. Here we infected two tropical and two temperate D. melanogaster out-cross populations with two genotypes of the fungal entomopathogen Beauveria bassiana. Mortality rate is recorded for all populations and early transcriptional response is assessed both by full genome microarray and RNA sequencing. Genes that are similarly induced among populations and genes induced privately in each population are identified. Genetic variability is assessed for candidates from both categories in a panel of D. melanogaster sequenced lines from all continents in order to assess pattern of selection at the promoter region of these genes.

P4.06.29
Inhibition of neutrophil extracellular traps (NETs) by Cryptococcus neoformans: involvement of the capsular polysaccharide Glucuronoxylomannan (GXM)

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We characterized the production *in vitro* of human NETs (neutrophil extracellular traps) induced by the opportunistic fungus *Cryptococcus neoformans*, evaluating participation of capsular polysaccharides GXM and GalXM in this phenomenon. The strain CAP67 (mutant non-capsulated) and the capsular polysaccharide GalXM were capable of inducing the production NETs. In contrast, we observed that the wild-type strain and the polysaccharide GXM were not capable of inducing the release of NETs. We described that wild-type *C. neoformans* and GXM inhibited NETs production by PMA. In parallel, we found that the NETs induced by the fungus CAP67 had microbicidal action on the wild-type strain and that the neutrophil elastase, myeloperoxidase, collagenase and histone were key killing components of NETs. We investigated the signaling pathways associated with the NETs induction, focusing on the role of reactive oxygen species (ROS), we concluded that both strains were able to induce the production of ROS. Finally we evaluated whether the GXM and GalXM modulate signaling pathways that lead to the production of ROS by neutrophils; and we observed that alone, both capsular polysaccharides do not induced the production of ROS; however in neutrophils activated with PMA, both capsular polysaccharides were capable of inhibiting ROS production. Therefore we described that the *C. neoformans* and its capsular polysaccharide GXM inhibited NETs production, and this processes could be considered a description of a new virulence mechanism of this pathogen.

P4.06.30
The resistance to infection by Paracoccidioides brasiliensis requires production of IL-6, IL-17 and IL-23

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Paracoccidioides brasiliensis (Pb), a thermally dimorphic fungus, is the causative agent of one of the most frequent systemic mycosis that affects the rural population in Latin America. Th17 cells are an arm of the immune system that enhances host protection against several infections, including mycosis. To better understand the mechanisms which are involved in resistance to P. brasiliensis infection we evaluated the role of IL-6, IL-17 and IL-23 during the PCM experimental. We showed that Pb triggers the secretion of IL-6, IL-17 and IL-23 by splenocytes. Intravenous infection of C57BL/6 (WT) mice with 1x106 yeast forms of Pb18, a highly virulent Pb strain, also induced an increased production of these cytokines compared with uninfected mice. We observed that the absence of IL-6, IL-17R and IL-23 impaired the control of the fungal replication at lung, liver and spleen from knockout mice in relation to WT group. Histopathological analysis showed that IL-6 and IL-17 contribute to compact granulomas formation due to adequate production of reticulin fibers. The deficiency of these cytokines was accompanied of disorganized CD4+ T cell infiltration at lung. The absence of IL-6, IL-17R or IL-23 resulted in lower production of IFN-γ and IL-10 compared with WT lung. Additionally, the frequency of Th17 cells, as well as IL-17 production, was decreased in IL-6-/- or IL-23-/- mice. This was associated with an impaired neutrophils and macrophages recruitment. Taken together, these results demonstrate that IL-6, IL-17 and IL-23 contribute to control of experimental Pb-infection through an efficient granulomatous organization.

P4.06.31
***In situ* expression of regulatory cytokines in oral paracoccidioidomycosis lesions**

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Paracoccidioidomycosis (PCM) is the most prevalent systemic mycosis in Latin America. The disease is caused by the dimorphic fungus *Paracoccidioides brasiliensis*. In humans, the disease is characterized by a broad spectrum of clinical manifestations ranging from localized mucocutaneous lesions to widespread manifestations. Immune response mediated by CD4 + T cells is critical for the outcome of infection. The role of different effector and regulatory cytokines needs to be established on PCM oral lesions. Biopsies from 16 patients with oral lesions (PCM) and health controls were analyzed by immunohistochemistry.
It was observed a significantly lower number of mast cells in patients with PCM compared with the control group. Conversely, fibrosis was significantly higher in PCM patients. The expression of IL-10, IL-4, IL-17 and FoxP3 were significantly higher in patients with oral PCM compared with the control group. Moreover, when PCM biopsies were categorized by level of organization, the expression of IL-10 and IL-4 were significantly higher in patients with biopsy of no organized granuloma when compared with organized granulomas. These cytokines appear to play an important role in the control of infection and the development and maintenance of lesions in the oral PCM. The better understanding of the mechanisms involved in immunoregulation of PCM oral lesions has applicability on therapeutical approaches and differential diagnosis with other oral pathologies.
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P4.06.32
Interferon-gamma production by human neutrophils upon challenge with *Paracoccidioides brasiliensis*

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Paracoccidiodomycosis is a systemic mycosis caused by the fungus *Paracoccidioides brasiliensis* (Pb), endemic in Latin America. Our laboratory has studied the relationship between human neutrophils (PMNs) / Pb focusing the effector mechanisms of these cells against the Pb. Studies have shown that PMNs can modulate the immune response with production and release cytokines. We have evaluated whether PMNs stimulated with Pb can modulate the immune response to a Th1 phenotype through the production of IFN-γ and the role of pathogen recognition receptors (PRRs) TLR2, TLR4 and Dectin-1. Furthermore, we have determined if cell activation by IL-12, IL-15 and IL-18 could result in increased levels of these cytokines.

Peripheral blood PMNs obtained from 20 healthy donors were nonactivated or activated with IL-12, IL-15 or IL-18 in different concentrations and challenged with Pb (18) strain for 24h and 48h and evaluated for IFN-γ production, by ELISA. In other experiments, PMNs were treated with monoclonal antibodies anti-TLR2, TLR4 and Dectin-1, challenged with Pb and evaluated for IFN-γ production. We found that Pb induces human PMNs to produce IFN-γ, probably by binding TLR4 and Dectin-1 receptors expressed by these cells. Moreover, cytokine levels were significantly increased when cells were activated with each of the tested cytokines or a combination of two of them, the association IL-12 plus IL-15 the most effective. The results support our hypothesis that during infection by Pb, human PMNs via the production of IFN-γ modulate the adaptive immune response to a Th1 response pattern. Support: FAPESP 2010/17405-5.

P4.06.33

Interaction between monocyte-derived dendritic cells from active and cured paracoccidioidomycosis patients and *Paracoccidioides brasiliensis* antigens: differences on phenotype, cytokines release and lymphoproliferation

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Cellular immune response is the most effective expression of defense in human paracoccidioidomycosis (PCM), an endemic mycosis in Latin America; however, the role of dendritic cells (DCs) in human PCM has not yet been explored. We investigated monocyte-derived DCs from patients with treated (TP) and active PCM (AP) compared to healthy non-PCM donors (CO). DCs from the TP group stimulated with either 43kDa glycoprotein (gp43) or cell-free antigen (CFA) from *Paracoccidioides brasiliensis* showed higher expression of HLA-DR and CD86 and higher levels of IL-12p40 compared to CO, whereas AP showed similar expression to CO. Production of IL-10 was up-regulated by gp43 and TNF-α only on the TP group when compared to DCs treated with TNF-α alone. CFA induced a stronger autologous lymphocyte proliferation and higher levels of IFN-γ and TNF-α on TP and AP groups when compared to CO while gp43 showed little or no effect over the proliferation. CFA may be a suitable antigen to induce cellular response through DCs antigen presentation even on active PCM patients. Higher expression of surface molecules with increased IL-12p40 may indicate a better activation of DCs after treatment of PCM. Our findings suggest that DCs may be crucial in the protective response to P. brasiliensis and that in vitro-generated DCs might be useful in enhancing antifungal immunity, especially during active PCM.
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P4.06.34

Frequencies of Heterologous Serum Agglutination (FHSA) Heterologous Representativeness Index (HRI) and Autologous Reactivity as indicators of variability in binder activity anti-Candida albicans - Evaluation in age groups of healthy individuals carriers of the yeast in oral cavity

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Serological diagnosis of candidiasis has highlighted problems of specificity and sensitivity. Considering the importance of protecting agglutinating antibodies were evaluated: a) FHSA: amount of heterologous sera (HS-for isolate not own) causing agglutination of a variable number of heterologous strains (HS), b) HRI: extent to which each strain agglutinated by a variable number of heterologous serum (HS), c) Autologous Reactivity (for isolate itself), as indicators of variability in binding activity (IgM) anti-Candida albicans. Was isolated and identified yeast (YMAB and Crhom-Agar) from mouth of age groups of healthy subjects (n = 129). The IgM was measured by agglutination plates. Autologous reactivity was> 75% in almost every age group and 57% for 4-9 years, with titles from 1:1-1:64. The most representative FHSA corresponded to 0-3, 10-18, 19-45 and 56-64 years: 100%, 87.5%, 69.2% and 66.7% (> 50%) of HS respective were pelleted by more than 40% of HS (minimum value selected as representative serum). The FHSA were low at 4-9, 46-55 and 65-85: <40% of HS agglutinated more than 50% of HS (minimum value selected as representative for strains), indicating low variability in the response. HRI (HS agglutinating HRIstrainX = # x / # total HS) revealed that few strains are recognized by more than 90% of HS, despite of being the same species. Evaluated parameters can be used as indicators of response variability.

P4.06.35

IL-18 does not modulate dectin-1 expression on human monocytes challenged with Paracoccidioides brasiliensis

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IL-18 is a proinflammatory cytokine and its role in paracoccidioidomycosis, a deep mycosis caused by Paracoccidioides brasiliensis (Pb) is not fully understood. Recently, we demonstrated that this cytokine, by modulating TLR4 and MR expression, play an important role on human monocytes response to P. brasiliensis. The aim of this study was first to evaluate the involvement of dectin-1 on IL-18, TNF-α and IL-10 production by human monocytes in response to P. brasiliensis. Moreover, we asked whether IL-18 can modulate the production of these cytokines by altering the expression of this receptor. Monocytes were stimulated with IL-18 and treated with specific neutralizing antibody anti-dectin-1 before challenge with more virulent (Pb18) and less virulent strain (Pb 265) of the fungus . After, dectin-1 expression was analyzed by flow cytometry and IL-18, IL-10 and TNF-α production by ELISA. Results showed that higher levels of IL-18 and IL-10 were detected after challenge with Pb 18, in comparison to Pb 265. By other hand, Pb 265 strain induced higher levels of TNF-α. However, only TNF-α induced by the strains were decreased after blocking dectin-1 receptor, showing the involvement of this receptor only in the production of this cytokine. Moreover, cells preincubation with IL-18 did not affect cytokines production by monocytes in response to P. brasiliensis, as well as dectin-1 expression. We can hypothesize that at least in relation to dectin-1 receptor expression, lack of modulation by IL-18 could contribute to susceptibility of the response against the fungus. Financial Support: FAPESP 2012/01836-2.

P4.06.36

Dectin-1 plays a prominent role in the immune response against the dermatophyte "Trichophyton rubrum" in murine model

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Introduction: *Trichophyton rubrum* is the main agent of dermatophytosis in humans. However, little is known about the immunological mechanisms underlying these mycoses.
Dectin-1 is an immune receptor involved in responses to fungi. It can induce production of cytokines and shape the immune response, like IL-1β, a key cytokine for the induction of T_H17 responses.
The aim of this study was to elucidate the role of Dectin-1 in the immune response against *T.rubrum*.

Materials and Methods: Bone-marrow derived macrophages (BMMs) from C57BL/6 mice (wild-type and dectin-1$^{-/-}$) were primed with LPS and incubated with *T.rubrum* conidia for 4, 6, 8 and 10 hours. The interaction was evaluated by optical microscopy and IL-1β levels were measured by ELISA.
Groups of 3 C57BL/6 mice (wild-type and dectin-1$^{-/-}$) were infected with *T.rubrum* conidia intraperitoneally. Animals were maintained for 7 and 14 days before being euthanized. Liver and spleen were collected for determination of fungal burden and cytokine measurements (IL-1β, IFN-γ, IL-4).
Results: Wild-type BMMs phagocytosed conidia and secreted IL-1β in response to the pathogen, but were destroyed by the fungal growth. Dectin-1$^{-/-}$ counterparts, however, showed reduced phagocytose and cytokine production, but conidia were unable to become hyphae.
Dectin-1$^{-/-}$ mice could not control the fungal burden and showed higher production of IFN-γ and IL-4, but lower levels of IL-1β, than their wild-type correlates.
Conclusions: Results showed that Dectin-1 is important for phagocytose of *T.rubrum* conidia and production of IL-1β. *In vivo* results suggest that dectin-1 is necessary for infection control by induction of IL-1β.
Supporting: FAPESP

P4.06.37
The role of IL-6 and IL-8 in vulvovaginal candidiasis in adolescent Girls

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Vulvovaginal candidiasis (VVC) - one of the diseases of modern civilization. Currently, this infection ranks second among of all infections of the vulva and vagina and is one of the most common reasons for treatment of female medical help. According to modern ideas, the imbalance of cytokine production, and in particular IL-6 and IL-8 plays a key role in the pathogenesis of many diseases. The aim of this study was to establish the degree of descriptiveness pathogenic cytokine production of serum IL-6 and IL-8 in patients with VVC to optimize the diagnostic and therapeutic and preventive measures. We examined 44 adolescent girls age from 12 to 15 suffering from VVC . Verification of the diagnosis VVC was conducted according to the WHO classification (ICD-X column in 37.3 and N 77.1). IL-6 and IL-8 serum levels were determined by ELISA ("Vector-Best", Russia). IL-6 and IL-8 levels can provide additional criteria to evaluate the inflammatory response in patients with various forms candid infection vagina (mostly asymptomatic candid carriers, acute and chronic relapsing forms). Changes in the parameters studied could reflect the state of the immune system and the inflammatory response characteristic of each VVC form.

P4.07 Immunity to helminth infection

P4.07.01
Involvement of Treg and alternatively activated macrophages in evasion strategies during hydatidosis

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Human hydatidosis is caused by the parasite *Echinococcus granulosus* (*E.g.*). It is one of the world's major zoonotic infections. It usually manifests as unilocular cyst(s) mainly located in the liver. We have previously highlighted an evident role of laminated layer (LL, accelular layer of hydatic cyst) in parasite survival by impairment of Th1 protective response.
The purpose of this study was first, to investigate the effect of LL extract (LLs) on IL-10 production by human mononuclear cells (PBMC) *ex vivo*. Second, the effect of LLs on macrophages phenotype and expression of some M1/M2 markers was also investigated. In this way, NOS2 and Arginase activities were assessed in PBMC cultures induced by LLs. Moreover, implication of mannose receptor (MR) and TGF-β on Arginase activity were evaluated using mannose (MR antagonist) and Anti-TGFβ. Activity of NADPH oxidase was also evaluated by chemiluminescence. Finally, TLR2, CD14 and CD23 expression was measured by flow cytometric immunoassay.
Interestingly, we showed that LLs enhanced IL-10 production. Moreover, while NOS2 and NADPH oxidase activities are inhibited, Arginase activity is activated. MR and TGF-β are involved in the Arginase induction by LLs. Furthermore, LLs increases TLR2 and CD14 expression and decreases CD23 expression in monocytes.
Collectively, ours finding suggest that *E. g.* laminated layer induced M2 phenotype. These macrophages induce Treg cells and impair M1/Th1/Th17 protective responses allowing parasite survival. Inhibition of these mechanisms constitutes an important issue to address an anti-hydatic treatment design.

P4.07.02
Galectin-like molecule in parasite inhibits remission of experimental autoimmune encephalomyelitis by enhancing auto-reactive B cells

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Although the expression and function of host galectins have been extensively appreciated, there is still scarce information on the repertoire of 'galectin-like molecules' expressed by pathogens and associated to their virulence. In a recent study, researchers reported that rTl-gal isolated from an adult worm of the gastrointestinal nematode parasite Toxascaris leonina attenuated clinical symptoms of inflammatory bowel disease in mice treated with dextran sulfate sodium (DSS). Noting that the role of rTl-gal in the inflammation disease, we tried to elucidate the effect of parasite via its galectin-like molecules (rTl-gal) on experimental autoimmune encephalomyelitis (EAE), a mouse inflammatory and demyelinating autoimmune disease model of human multiple sclerosis (MS). When we administered rTl-gal to EAE mice, no significant difference in clinical disease severity was observed between rTl-gal-treated and control group at peak stage. At remission stage, interestingly, rTl-gal-treated mice not only failed to recover after the peak of disease but also showed increased EAE severity, leading to autoreactive inflammation cell activation and persistent CNS damages such as neuroinflammation, demyelination, gliosis and axonal damages. We further verified the cell population whose infiltration was altered by rTl-gal treatment and observed that rTl-gal-treated EAE mice markedly increased the infiltration of CD45R/B220+ B cells. Consistent with these, cell population of CD45R/B220+ B cell and autoantibody production in periphery were significantly up-regulated. Our results suggest that galectin-like molecule isolated from a gastrointestinal parasite can deliver a harmful effect to EAE via strengthening autoreactive B cell function contrary to its beneficial effect on inflammatory bowel disease.

P4.07.03
Anisakis simplex allergens remain biologically active after canning and simulated gastric fluid treatment of parasitized fish

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Background: The ingestion of parasitized fish with *Anisakis simplex* live larvae L3 can result in the development of anisakiasis and sometimes can induce IgE-mediated reactions. Allergic episodes can be elicited by infection or exposure to allergen remaining in food containing dead larvae. It is believed that is due to heat stable and pepsin resistant allergens.
Objective: The aim of this work was to detect the presence of biologically *A. simplex* allergens after canning and simulated gastric fluid treatment of parasitized fish muscle.
Material and methods: Tuna fish fillets (*Thunnus thynnus*) were artificially parasitized with *A. simplex* and subjected to autoclaving during 40 minutes at 121ºC as an equivalent method to industrial

canning. Fish extracts were obtained by grinding followed by sonication and centrifugation. Then, extracts were incubated with simulated gastric fluid (SGF) for 30 and 60 minutes. The extracts were assayed by IgE-immunoblotting and a flow cytometric basophil activation test on *A. simplex*-sensitized patients (n=5).
Results: *A. simplex* allergens in parasitized fish after autoclaving and SGF treatment were able to activate basophils from sensitized patients. The capacity to induce basophil activation seemed to be patient-dependent since it ranged from 74% to 2% after 60 minutes of SGF incubation. Apparently there was not any relationship between patients' anti-*A. simplex* IgE values and percentage of activated basophils. IgE-immunoblotting analyses showed the presence of low-molecular weight allergens (about 20 and 18kDa) after both treatments.
Conclusion: *A. simplex*-sensitized patients can be exposed to biologically active parasite allergens when eating canned fishery products

P4.07.04
Immunomodulatory effect of Toll-like receptor 7/8-agonist (imidazoquinoline Resiquimod) in experimental secondary hydatidosis infection

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A variety of avoidance strategies are believed to be involved in cystic echinococcosis (CE) metacestode infection. Clinical evidence has demonstrated Th2 bias in chronic infection, functional to parasite survival, while a Th1 cytokine profile appears to typically dominate in effective chemotherapy treatments, indicating a role in the process of cyst degeneration.
Experimental secondary infection, by intra-peritoneum infection of mice with protoscoleces (PSC) of *Echinococcus granulosus*, constitutes the laboratory model for this study.
BALB/C mice have been experimentally infected with 100 or 3000 PSC and treated p.i with the Toll like receptor 7/8 agonist Resiquimod, an Imidazoquinolinamine with immune response modifying properties via IL-12 and IFN production and IL-4 and IL-5 inhibition.
A substantial reduction in the number of recovered cysts, together with a significant decrease in parasite load is observable in mice treated after infection with 100 PSC with respect to controls. Treatment of 3000 PSC-infected mice reduces the number of recovered cysts by more than 40%, comparable to Albendazole treatment. Parasite load was also found to be lower than controls, but less so in benzimidazole-treated animals, where most cysts are completely collapsed and have thickened walls.
Overall results indicate Th0/Th1 polarization during early developmental stages of secondary experimental hydatidosis, with a subsequent Th2 bias, functional to parasite survival. Imidazoquinolinamine has demonstrated potency in reducing cyst development, highlighting that Th1 response correlates with protective immunity, opening the way to new immunomodulatory strategies potentially useful also for natural infection.

P4.07.05
Immunomodulation by TLR-7-agonist, IL-27 or IL-25 in Hymenolepis diminuta infection in rats

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During infection with the intestinal Cestode *Hymenolepis diminuta,* a Th-2 bias is reported both in mice (unnatural host) and rats. Expulsion occurs only in severe infections, as higher levels of IL-13 increase smooth muscle contraction, goblet cell hyperplasia and increased mucin release, leading in turn to increased mucus entrapment of the worms progressively expelled.
To gain greater insight into the complex mechanism of dose-dependent expulsion and the role of Th1/Th2 polarization, Wistar rats have been infected with 3 or 50 Hd cysts before or after treatment with Resiquimod (imidazoquinolamine, agonist of TLR7/8 inducing Th-1-mediated immune response) or with regulatory cytokines IL-25 (regulator of Th2-cell response) or IL-27 (pivotal to driving naive cells into Th1 subset and with immunosuppressive property to Th17). Resiquimod is able to inhibit worm loss during expulsion in severe infection, both in pre- and post-infection treatment, with long lasting inhibition of at least up to 42 days p.i.. Vice versa no differences are observable respect to controls during mild infection irrespective of sacrifice time. Accordingly, experiments carried out using IL-25 showed accelerated destrobilation and loss of worms as early as 14 days p.i. Vice versa IL-27 appears to be able to prevent worm expulsion up to 35 days p.i. with a pattern similar to TLR7/8 agonist. Overall results clearly indicate different Hd expulsion features in Th1 or Th2-biased environments confirming a role for helminths as innovative approach to immunological distraction or regulation in autoimmune or inflammatory diseases.

P4.07.06
An essential role of galectin-1 in driving Th2-polarized responses during helminth infection

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Schistosomiasis, a disease caused by Schistosoma mansoni, affects approximately 238 million people in tropical and sub-tropical areas. Despite considerable efforts in elucidating the mechanisms underlying immune protection and evasion during S. mansoni infection, the endogenous mediators responsible of driving Th2-polarized reactions remain uncertain. Here we identified a crucial role for galectin-1 (Gal-1) an endogenous glycan-binding protein, in driving Th2 responses during S. mansoni infection. Schistosome Egg Antigen (SEA) induced a dose-dependent up-regulation of Gal-1 in bone marrow-derived dendritic cells (DCs), as shown by western blot, real time qPCR and ELISA ($p<0.05$). Analysis of the cytokine profile of wild type (WT) and Gal-1-deficient (Lgals1-/-) DCs exposed to SEA (DCSEA) revealed no significant differences in IL-23, IL-27, IL-10 and IL-12p70 secretion. In contrast, Lgals1-/- DCs showed lower IL-5 and IL-10 ($p<0.05$) and higher production of IFN-γ ($p<0.05$) than WT DCSEA in allogeneic co-cultures using Balb/c splenocytes. An in vivo infection model confirmed our findings showing that, compared to their WT counterpart, splenic DCs isolated from Lgals1-/- infected mice induced allogeneic T cells to produce lower amounts of IL-5 and IL-10 ($p<0.01$) and higher IFN-γ ($p<0.01$). This effect correlated with an increased Th2 response observed in T cells isolated from WT lymph nodes (LN) and spleen ($p<0.05$). Likewise Lgals1-/- infected mice displayed smaller size of granulomas and WT granulomas expressed higher amounts of Gal-1 as shown by qRT-PCR an IHC. These results show a pivotal role for Gal-1 in driving Th2 responses during helminths infection.

P4.07.07
The change of CD4+CD25+ regulatory T cells and related cytokines in mice infected with *Echinococcus multilocular*

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Echinococcus multilocularis infection is considered one of the most deadly diseases due to worm infection. In order to clarify the role of variations in CD4+CD25+ regulatory T cells and related cytokines IL-10 and TGF-β in E. multilocularis persistent infection and to explore the relationship between immune evasion mechanisms and the immunosuppressive effects of CD4+CD25+ regulatory T cells. During the early stages of E. multilocularis persistent infection, the ratios of CD4+CD25+ regulatory T cells in peripheral blood mononuclear cells (PBMC), splenocytes and lymph node lymphocytes were found to be low or significantly decreased compared to the control. As the infection progressed, the number and size of E. multilocularis cysts in

mice gradually increased, and the ratio of CD4+CD25+ regulatory T cells significantly increased. Especially in PBMC the ratio substantially increased in the middle stages of E. multilocularis infection. Furthermore, the related cytokines IL-10 and TGF-β were also augmented by infection.Therefore, CD4+CD25+ regulatory T cells may be a key part of the strategies employed by E. multilocularis for long-term survival in the host.

P4.07.08
***Heligmosomoides polygyrus* L4 stage from inflammatory *milieu* effects on dendritic precursor JAWSII cells**

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Infection with intestinal L4 stage *Heligmosomoides polygyrus* reduces inflammation in an experimental model of *colitis*. The protective effect of prior larvae which inhabit the small intestine on *colitis* is associated with macrophages and neutrophils infiltration into the small intestine and high concentration of pro-inflammatory cytokines IL-1β, IL-6, TNF-α and opioids in the small intestine and inhibition of those in the colon (Parasite Immunology, 2012, 34: 536–546). Interestingly, the active inflammatory reaction in the small intestine promotes development of the *H. polygyrus* L4. Enhanced number and length of L4 and different larvae location on the small intestine was detected. The weaker recognition and better adaptation of the nematode larvae in the small intestine was associated with different composition and activity of antigen presenting cells APC; dendritic cells, macrophages and B-cells of Payer's patches.
In the present study, we detected the effect of somatic, excretory-secretory antigen and live L4 stage of *H. polygyrus* from control infection and from mice with DSS-induced colitis on dendritic precursor JAWSII cells. L4 from mice with *colitis* induced higher expression of MHC class II, CD80/CD86 co-stimulatory molecules on JAWSII cells than control L4 *H. polygyrus* and higher production of IL-6, MCP-1, IL-22 with inhibition of IL-17A and no effect on regulatory cytokines IL-10 and TGF-β. Our studies show that the L4 larvae from the inflammatory *milieu* better induce phenotypic and functional maturation of dendritic precursor JAWSII cells than larvae of control infection.
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P4.07.09
Chitosan stimulates lymphocyte proliferation during muscle phase of *Trichinella spiralis* infection in mice

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Chitosan is intensively studied as a potential vaccine adjuvant that can combine high efficacy with low toxicity. Yet mixed information is available, how the polysaccharide affects immunity. It was observed that antibody titers were raised when antigen was administered with chitosan but it is not clear what mechanisms are involved in the phenomenon. As parasitic infection down regulates immune response, modification of suppressed reactions may be promising for the anti-parasitic protection.
Aim of the study was to evaluate the effect of chitosan administration on lymphocyte proliferative response in mice infected with *T. spiralis*.
C57Bl6 mice were intra-peritonealy injected with 500 µg of chitosan (> 375 kDa) dissolved in adipic acid, every day 5 days before till 9 days after infection with 400 L1 *T. spiralis*. Mesenteric lymph node cells were cultured without or with TCR stimulation (anti-CD3 and -CD28 antibodies); levels of proliferation and cytokines were measured.
Chitosan treatment restored cell responsiveness; they proliferated intensively and were again sensible to TCR stimulation. Also cytokine secretion was altered. Nonetheless the cells regained reactivity, the level of infection was intensively raised after chitosan treatment; over two times more muscle larvae were recovered from mice that obtained polysaccharide injections.
Although chitosan stimulated lymphocyte response, the effect of treatment was not protective.
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P4.07.10
The role of TLR3 in the development of a protective immune response against Plasmodium

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The understanding of mechanisms involved in the initiation of a protective immune response during *Plasmodium (P.)* infection is essential for developing tools and strategies to control the disease. An early innate immune response involving NK and NKT cells is implicated in the control of the primary *P. yoelii* infection in C57Bl/6 (B6) mice. Pattern Recognition Receptors such as Toll Like Receptors (TLR), which are expressed by innate immune cells, could be involved in the recognition of parasitic molecules during Plasmodium infection. Herein, we studied the role of TLR molecules in the clearance of *P.yoelii* in B6 mice. Our results showed that TLR3 deficient mice developed lower parasitemia compared to B6 mice. In this context, a phenotypique analysis of lymphocyte populations was conducted and showed an increase of the number of B cells correlated to immunoglobulins level in TLR3 KO mice when compared to wild type mice. We also observed a positive correlation between parasitic clearance and the B cell response. Further studies will be focused on the study of the antibody repertoire implicated in the elimination of *Plasmodium*.

P4.07.11
Oral Fluids: utility in parasitic infections diagnosis

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Current diagnostic methods of various parasitic diseases require invasive sampling procedures e.g visceral aspiration and/or blood drawing. Thus, the development of diagnostic tests using oral fluid, easier to collect would be more simple and practical especially under field conditions. In this regard, oral fluid samples were screened for the presence of anti-*Leishmania* and anti-*Echinococcus granulosus* in visceral leishmaniasis (VL) and hydatid cysts' patients respectively.
Oral fluid and blood samples of 37 VL cases and 40 healthy controls were analyzed by rK39 ELISA to detect specific *Leishmania* antibodies in oral fluids and sera. Moreover, anti-hydatid fluid antigens (HFA) IgG antibodies were screened by ELISA in oral fluids and sera of hydatid cysts' patients (n=37) as well as in healthy controls (n=30).
Leishmania antibodies detection in saliva had a sensitivity of 100% and a specificity of 97.5%. Antibody levels measured in sera and saliva showed a significant positive correlation (rho=0.655 and p=0.01). Salivary anti-HFA IgG showed a sensitivity of 86.5% and a specificity of 80%. A positive correlation was observed between anti-HFA IgG in saliva and in serum (rho=0.364 and p=0.02).
The detection of specific anti-parasite IgG promises to be interesting in the diagnosis of parasitic diseases.

P4.07.12
Schistosomiasis vaccine discovery using high throughput schistosome tegument proteins microarray recognition

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Schistosomiasis is a neglected tropical disease that affects more than 207 million people worldwide, and 700 million people are at risk in 74 endemic countries. Immunoepidemiological investigations have

shown a very small percentage of exposed populations in Brazil are naturally resistant to schistosomiasis despite never being drug-treated (praziquantel). We propose to evaluate the humoral response of endemic area individuals to schistosome tegument antigens spotted onto a protein microarray to understand the mechanisms involved and the antigens targeted by these individuals. To identify antigens that are uniquely/preferentially recognized by resistant compared with susceptible individuals, we constructed a protein microarray with S. mansoni and S. japonicum proteins. We used sera from individuals from Minas Gerais, Brazil, who were putatively resistant or chronically infected with low, moderate and heavy eggs counts per gram of feces or unexposed controls. We evaluated IgG subclasses and IgE responses comparing their recognition profiles. Resistant and infected groups recognised many of the proteins on the array. In general, we observed proteins with multiple antibody subtypes response and others had a unique subtype response. High throughput protein array is an excellent tool for protein discovery in helminthiasis. In schistosomiasis, it proved to identify the immune response in resistant compared to infected individuals. The search for pre-existing IgE response is crucial and should be excluded in further investigations. This report describes the construction and screening of a schistosome protein array with human sera, and the results presented will be valuable in the selection of antigens for human vaccine for schistosomiasis.

P4.07.13
Schistosomes: Potential of Dynein Light Chains as Vaccine

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Schistosomes are blood flukes that reside in human mesenteric veins and cause a chronic debilitating disease, Schistosomiasis. Millions of people in the tropical third world are affected by this diseases. The syncytial tegument of these parasites represents a dynamic interface that regulates nutritional and immunological interactions with the host. Dyneins are motor proteins that are important in the biogenesis and maintenance of Schistosome's tegument. Studies have identified dynein light chains (dlc) fragment (Sm10) as a possible vaccine candidate and immunolocalized it in the tegument. Other motor proteins that have been tested as Schistosomes vaccines include paramyosin and myosin-v and the EF-motif dyneins. In this study we have analyzed the three components of dlc: L8 (Sm10), L7 (roadblock) and Tctex in Schistosomes. We characterized these genes in the different life stages of schistosomes and their processing to cDNA. The transcripts are present in *S. mansoni*, *S. haematobium* and *S. japonicam,* the three species that infect human. These peptides can make conjugate peptide for vaccine evaluation.

P4.07.14
Differential expression of ovine TLR2 and TLR4 in peripheral blood mononuclear cells cultured with hydatid cyst derived antigens

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Recognition of helminth derived pathogen associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), including toll like receptors (TLRs) is the first step towards initiating anti-helminth immune responses. Understanding the molecular basis of innate immune recognition of different stages of parasite antigens will provide a better understanding of disease pathogenicity as well as the potential immunostimulatory and/or immunoregulatory roles of these antigens.
Using hydatid cyst derived antigens of Echinococcus granulosus, causative agent of cystic echinococcosis, the expression of ovine TLR2 and TLR4 in peripheral blood mononuclear cells (PBMCs) was analysed by real-time quatitative reverse-transcription polymerase chain reaction (qRT-PCR). Somatic and exceretory/secretory antigens of protoscoleces (PSC), germainal layer (GL) and hydatid cyst fluid (HCF) antigens were prepared to stimulate ovine PBMCs. A higher expression of TLR2 and TLR4 was observed in PBMCs cultured with somatic antigens of PSC, whereas only TLR2 was up-regulated by ES antigens of PSC. Similarly, we detected an increased level of TLR2 and TLR4 expression in PBMCs cultured with GL antigens. In contrast, the expression of TLR2 and TLR4 in PBMCs was down-regulated by HCF antigens. The results presented in this study suggest that, PSC (Somatic and excretory/secretory antigen) and GL antigens have immunostimulatory effects whereas HCF antigens exhibit an immunoregulatory effect on peripheral immune cells. In conclusion, this study provide for the first time evidence of induction of TLRs in ovine PBMCs by PSC, GL and HCF antigens of Echinococcus.

P4.07.15
Induction of regulatory T cells by Opisthorchis viverrini

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Opisthorchis viverrini is a liver fluke that prevalence in Thailand and neighboring countries. Our previous studies demonstrated that this helminthic infection could induce Th2 and regulatory T cell (T reg)-like cytokines, such as TGF-β, IL-4 and IL-10 in infected hamsters. This study aims to investigate CD4+ T cell responses against O. viverrini infection in hamsters. Hamsters were infected with 50 Mc for 10 months. The mononuclear cells of spleen and mesenteric lymph node (MLN) from infected or control groups were stimulated with 10 µg/ml of crude somatic antigen (CSAg) for 24 hrs and stained for CD4, CD25, IL-4, IL-10, IFN-γ and TGF-β, detected by flow cytometer. The IL-4+ and TGF-β+ CD4+ T cells in MLN were found significantly higher than control whereas IL-4+ and IL-10+ CD4+ T cells were increased in infected spleens. In contrast CD4+IFN-γ+ T cells in both spleen and MLNs were not significantly different between infected and control groups. Furthermore significant numbers of TGF-β+ CD4+ T cells in O.viverrini infected group in MLN were found to be CD25+. The result indicated the induction of IL-4+CD4+ T cells in systemic immune responses whereas TGF-β+CD4+ T cells are predominated in mucosal immune responses. To confirm the induction of regulatory T cells by parasite, Balb/c mice were immunized with CSAg, antigen-specific T cell clones were established and found to be TGF-β producing T reg cells. Taken together, our study demonstrated that O.viverrini could stimulate the T reg lead to down regulation of the host immune system making parasite survival.

P4.07.16
Down regulation of NKG2D and Fc gamma receptor I in peripheral blood mononuclear cells induced by excretory-secretory antigens from the third stage Gnathostoma spinigerum larvae (L3)

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Natural killer (NK) cells and monocytes play roles in protective immunity to helminth infection. The understanding in survival and evasion of Gnathostoma spinigerum (G. spinigerum) L3 from human cellular immunity for months or years is still obscure. This study aimed to demonstrate the excretory secretory antigens from G. spinigerum L3 (G. spinigerum ES) could interfere in human monocyte and NK cell functions via down regulating their receptor expression.

Cultures of normal peripheral blood mononuclear cells (PBMC) were used as immune cells within the circulation. Firstly, PBMCs co-cultured with the G. spinigerum ES for 18 hours were screened for the gene profiling correlated with the immune response by DNA microarray analysis. The profound gene groups associated with innate and cytotoxic immunity, particularly NK cell receptor; lectin like family (NKR) and Fc gamma Receptor-I (FcγRI) significantly down regulated. To verify the transcriptional regulation by the G. spinigerum ES, we determined the expression of NKG2D on NK cells and FcγRI on monocytes in PBMC cultured with the ES by flow cytometry, and quantitative real time PCR. The results revealed that NK cells co-cultured with the ES markedly decreased expression of NKG2D and FcγRI during 72 hours. These findings were consistent with the related mRNA expression during 90 min. Taken together, the down regulation of NK cell receptor and FcγRI expressions were markedly observed in PBMC co-cultured with low dose of the G. spinigerum ES. Our findings gain the understanding in a mechanism of the immune-evasive strategy of the G. spinigerum L3.

P4.07.17
Swiprosin-1/EFhd2 limits autoantibody and IgE levels by restricting co-stimulation dependent B cell activation

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Peripheral checkpoints account for appropriate selection of activated B cells by regulating their apoptosis and survival. Failure of this process can result in the occurrence of auto-antibodies. Furthermore, dysregulation of immunoglobulin subtypes, such as IgE, can contribute to hypersensitivity reactions. The Ca2+ binding adaptor protein EFhd2 (also named Swiprosin-1) is able to promote apoptosis in activated B cells. We therefore hypothesized that EFhd2 is involved in selection of activated B cells, and established EFhd2-/- mice. B and T cell development and basal immunoglobulin levels were normal but EFhd2-/- mice developed anti-nuclear antibodies spontaneously. Moreover, EFhd2-/- mice developed enhanced germinal center reactions. Upon immunization with trinitrophenol-lipopolysacharide or nitrophenol-keyhole limpet hemocyanin elicited higher antigen-specific IgM or IgG1 antibody titers EFhd2-/- but we did not observe enhanced affinity maturation towards the hapten nitrophenol. Iinfection with the hookworm Nippostrongylus brasiliensis induced strongly exaggerated IgE responses in EFhd2-/- mice and led to enhanced occurrence of IgG1+ and IgE+ plasma cells in a B cell intrinsic manner. This propensity to hyperactivation resulted in pronounced IL-6 production and induction of two NF-kappa B target genes, nfkbia and irf4, after combined BCR and CD40 stimulation of EFhd2-/- B cells. We conclude that EFhd2 constrains selection of activated B cells activation, with implications for autoimmunity, parasite biology and type I hypersensitivity reactions.

P4.07.18
Regulation of intestinal immune response by selective removal of the anterior, posterior, or entire pituitary gland in Trichinella spiralis infected golden hamsters

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The influence of anterior pituitary hormones on the gastrointestinal tract of humans and animals has been previously reported. Hypophysectomy (HYPOX) in the rat causes atrophy of the intestinal mucosa, and reduction of gastric secretion and intestinal absorption, as well as increased susceptibility to bacterial and viral infections. However, to our knowledge, no findings have been published concerning the immune response following HYPOX during worm infection, particularly that caused by the nematode *Trichinella spiralis*. The aim of this work was to analyze the effects of total or partial HYPOX on colonization of *T. spiralis* in the intestinal lumen, together with duodenal and splenic cytokine expression. Our results indicate that 5 days post infection, only neurointermediate pituitary lobectomy (NIL) reduces the number of intestinally recovered *T. spiralis* larvae. We observed that the mean intensity of all tested Th1 cytokines was markedly diminished, even in the duodenum of infected controls. In contrast, a high level of expression of these cytokines was noted in the NIL infected hamsters. Likewise, a significant decrease in the fluorescence intensity of Th2 cytokines (with the exception of IL-4) was apparent in the duodenum of control and sham infected hamsters, compared to animals with NIL surgeries, which showed an increase in the expression of IL-5 and IL-13. NIL hamsters showed an exacerbated inflammatory infiltrate located along the lamina propria in the duodenal mucosa. We conclude that hormones from each pituitary lobe affect the gastrointestinal immune responses to *T. spiralis* through various mechanisms.

P4.07.19
The effects of astaxanthin in improving age associated declines of Th2 immune responses

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Objective. Astaxanthin is one of the natural antioxidant carotenoid mainly derived from crab,salmon and trout. This study aims to determine whether dietary astaxanthincould modulate mucosal immunity in the gut in aged mice.
Method. Aged (18 months old) Balb/c female mice were orally administered with6mg/kg body weight of astaxanthin or saline for 4 weeks. After 3 weeks, micewere infected with *Nippostrongylus brasiliensis(Nb)*, which is gastroinfectional nematode in rodents. Tissues were taken from the small intestine, and total RNA was extracted and analyzed by real time PCR for IL-4, IL-13, IL-10, IFNγ and NOS2. For lipid peroxidation measurements in the liver, the thiobarbituric acid (TBA) assay was also performed.
Results. The lipoperoxide level in the 18M mice were significantly higher compared with that in the 3 months old mice. However, it significantly decreased after astaxanthin administration. Aged mice failed worm expulsion with declining Th2 immune responses, while Th2 cytokine gene expression was recovered and worm expulsion progressed in astaxanthin-fed aged mice. INFγ didn't show any change. NOS2 was down-regulated and IL-10 increased after infection in astaxanthin fed-mice. FACS analysis revealed that CD4+CD25+Foxp3- T cells increased in aged mice, but these cells disappeared after astaxanthin administration.
Conclusions. These data showed that astaxanthin exhibited the effect of inhibition of oxidative stress and improved Th2 immune responses which declined during aging.

P4.07.20
Dendritic cell capture and transfer of HIV-1 to CD4$^+$ T lymphocytes is inhibited by helminth parasite antigens

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Areas endemic for parasitic infections often have a high HIV-1 prevalence. Helminths are known for their immuno-modulatory properties however, their impact on HIV-1 susceptibility is unknown. We have studied the effects of soluble egg antigen (SEA) of *Schistosoma mansoni* and whole *Brugia malayi* adult (BMA) worm antigen extract for their capacity to interfere with HIV-1 infection. These antigens bind C type lectin receptors (CLRs) which could influence direct and indirect HIV-1 infection of CD4$^+$ T cells. Additionally, CLR signaling on DCs can modulate the induction of Th cell populations, potentially affecting their susceptibility for HIV-1 infection and replication. Direct infection, as determined by viral outgrowth in CD4$^+$ T lymphocytes pre-incubated with SEA or BMA, was unchanged for both CCR5 and CXCR4 using HIV-1 isolates. Contrary, utilizing a DC-SIGN-Fc/gp120 binding ELISA we observed that both SEA and BMA potently inhibited HIV-1 Env from binding DC-SIGN. Furthermore, this was confirmed by showing that both SEA and BMA could prevent Raji-DC-SIGN cells and the more physiologically relevant moDCs from capturing HIV-1 and supporting *trans*-infection of CD4$^+$ lymphocytes. Currently, we are testing the HIV-1 susceptibility of Th cells induced by moDCs matured in the presence of either SEA or BMA, since the cytokine/ chemokine profile of CD4$^+$ T lymphocytes can influence HIV-1 infection and replication. We have demonstrated that helminth parasite antigens can block HIV-1 from interacting with DC-SIGN thereby disrupting a pathway important for HIV-1 transmission and subsequent propagation.

P4.07.21
Helminth infection alters mood and short-term memory as well as neurotransmitter and cytokine levels in the mouse hippocampus

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Helminthic infections are important causes of morbidity and mortality in many developing countries, where children bear the greatest health burden. The ability of parasites to cause behavioral changes in the host has been observed in a variety of host-parasite systems, including the *Taenia crassiceps*-mouse model. In murine cysticercosis, mice exhibit a disruption in sexual, aggressive and avoidance predator behaviors. The present study was undertaken to characterize short-term memory, depression-like behavior as well as neurotransmitters and cytokine levels in the hippocampus of cysticercotic male and female mice. Chronic cysticercotic infection induced a decrease in short-term memory in male and female mice, being the effect more pronounced in females. Interestingly, infected females showed a significant increase in forced swimming tests with a decrease in immobility. In contrast, male mice showed an increment in total activity and ambulation tests. Serotonin levels decreased by 30% in the hippocampus of infected females whereas noradrenaline levels significantly increased in infected males. The expression of IL-6, IFN-γ in the hippocampus was markedly increased in infected male and female mice. The expression of IL-4 increased in infected female mice, but decreased in infected male mice. Our study suggests that intraperitoneal chronic infection with cysticercus leads to persistent deficits in tasks dependent on mice hippocampal function. Our findings are a first approach to elucidate the role of the neuroimmune network in controlling short-term memory and mood of *T. crassiceps* infected mice.

P4.07.22
CD28 is required for protection against Nippostrongylus brasiliensis secondary infection and recall of memory responses in inducible CD28 deleting mice

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IL-13 driven Th2 immunity is indispensable for host protection against infection with the gastrointestinal nematode *Nippostronglus brasiliensis*. Disruption of CD28 mediated costimulation impairs development of adequate Th2 immunity, showing an importance for CD28 during the initiation of an immune response against this pathogen. However, any requirement for CD28 during recall immunity to secondary infection with pathogens has remained largely controversial. In this study, we used global CD28$^{-/-}$ mice and a novel mouse model that allows for inducible deletion of the *cd28* gene by oral administration of tamoxifen (CD28$^{-/lox}$Cre$^{+/-}$+TM) to address these questions. Following primary infection with *N. brasiliensis*, CD28$^{-/-}$ mice failed to expel adult worms in the small intestine in contrast to wild-type C57BL/6 mice. Impaired expulsion was associated with reduced production of Th2 cytokines, particularly IL-13, reduced humoral immunity and failure to develop CXCR5$^+$ T_{FH} cells. Interestingly, interference with CD28 expression in CD28$^{-/lox}$Cre$^{+/-}$ mice by oral administration of tamoxifen prior to secondary infection with *N. brasiliensis* resulted in impaired worm expulsion. This correlated with reduced production of Th2 cytokines IL-13 and IL-4, diminished serum titres of antigen specific IgG1 and total IgE and reduced CXCR5$^+$ T_{FH} cell populations. Importantly, abrogation of CD28 during secondary infection inhibited the recruitment of central and effector memory CD4$^+$ T cells and follicular B cells to the draining lymph node. Therefore, it can be concluded that CD28 is essential for the development of protective memory Th2 immunity against *N. brasiliensis* infection and for expansion of CXCR5$^+$ T_{FH} cells and follicular B cells.

P4.07.23
Analysis of Schistosoma mansoni candidate antigens as diagnostic targets for schistosomiasis

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Demonstration of schistosome eggs in stool/urine is the definitive clinical test for clinical examination of schistosomiasis but this test has low sensitivity. Antibody based assays cannot distinguish between past and active infections thus unsuitable for follow up after drug administration while molecular techniques are expensive and unavailable at the point of care centers. Bioinformatics and Proteomics can be used to characterize schistosome proteins from different life cycle stages that include worm gut, worm tegument, egg secretions or released products of dead eggs that are released into the bloodstream and/or urine forming good diagnostic targets. We identified three schistosome proteins; Cathepsin B (Sm31), Asaparaginyl endopeptidase (Sm32) and Sm200 tegument protein using these approaches. Peptide sequences from these proteins synthesized as multiple antigenic peptides (MAPs) were used to immunize rats. Serum from immunized rats were used to test the suitability of these targets using Enzyme Assays, Blot Assays and Immunocytochemistry with worm sections. Results show that antibodies to MAPs raised in rats have good specificity for the peptides and native schistosome antigens which identifies them as possible diagnostic targets that can be developed further to assess

their sensitivity aiming at developing an assay capable of detecting the lowest number of worms in the host.

P4.07.24
Leukocyte adhesion to mesenteric endothelial cells mediated by $P2Y_1$ receptor activation during schistosomiasis

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Introduction: Previous work showed a downregulation of endothelial purinergic P2X7 receptor during chronic inflammation associated with schistosomiasis (Oliveira et al., 2013, Purinergic Signal. 1:81). We also observed an increased ectonucleotidase enzymatic activity, which produces ADP, an agonist of $P2Y_1$ receptors ($P2Y_1R$) that stimulates leukocyte adhesion to endothelial cells. Present work evaluated $P2Y_1R$ signaling in mesenteric endothelial cells (MECs) during schistosomiasis.
Methods: Cultured MECs and mononuclear leukocytes (control and infected mice) were obtained as previously described (Oliveira et al., 2011, PloS One 6:e23547). Western blotting assays investigated MECs $P2Y_1R$ expression and fluorimetric assays evaluated intracellular Ca^{2+}. We used the agonist 2-MeSATP in the absence or presence of the selective antagonist MRS2179 (0.3 µM).
Results: In the control group, 2-MeSATP (30 and 60 µM) increased MECs intracellular Ca^{2+} and leukocyte adhesion from 9.4 ± 1.3 to 24.5 ± 1.6 and 28.7 ± 1.7 cells/field, respectively (n=26-36 replicates, $P<0.01$). MRS2179 prevented cell adhesion (9.2 ± 1.2 cells/field, n=36). However, in the inflammation model, there was an increased spontaneous adhesion (22 ± 2.0 cells/field, n=33), and the agonist had no significant effect. No alteration of $P2Y_1R$ expression was observed, but, the antagonist MRS2179 reduced basal adhesion (9.6 ± 1.0 cells/field, n=33), *i.e.*, in the absence of 2-MeSATP. One possible explanation is that $P2Y_1R$ were being continuously activated by locally produced ADP.
Conclusion: Schistosomiasis primes MECs *in vivo* which keep the acquired phenotype *in vitro*. These data indicate that schistosomiasis activates MECs $P2Y_1R$ signaling and consequently leukocyte adhesion.

P4.07.25
Purinergic signaling in mesenteric endothelial cells and peritoneal macrophages in chronic inflammation

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Introduction Schistosomiasis causes a chronic inflammation related with physiological vascular alterations. Our group evaluated the immunomodulation of the purinergic signaling in mesenteric endothelial cells (MECs) and macrophages from *S. mansoni*-infected mice. Methods: The P2X7 receptor (P2X7R) function was evaluated by ATP-inducing membrane permeabilization to ethidium bromide (BE) measured by flow cytometry in MECs and macrophages. P2X7R expression was accessed by Immunocytochemistry and Western blotting. P2X7R-mediated nitric oxide (NO) production and ectonucleotidase activity were evaluated in MECs. Results: Schistosomiasis reduced the P2X7R expression in MECs and macrophages. We observed that 3 mM ATP was less efficient in inducing BE-uptake in MECs from infected (25 ± 2%; n=8) than uninfected mice (43 ± 1%, n=10). The selective P2X7R agonist BzATP induced a smaller NO synthesis in MECs from infected than control mice (5 ± 2% and 22 ± 2%, n=15, respectively). On the other hand, the ectonucleotidase activity was higher in MECs from infected than control mice (17 ± 3 and 7 ± 1 pmol Pi/µg of protein, n=16-14, respectively). The infection reduced the BE-uptake induced by 1 mM ATP in macrophages (95 ± 2% and 54 ± 6%, infected and uninfected mice respectively, n=12). Preliminary results also showed a reduced BzATP-mediated Ca^{2+} mobilization and increase of anti-inflammatory cytokine TGF-β in macrophages from infected-mice. Conclusion: Altogether, our data indicate that schistosomiasis modulates P2X7-receptor signaling in two cells types important to immune response. This could limit vascular inflammation and morbidity. Financial support: CNPq, FAPERJ-PRONEX, FAPERJ, INCT-INPeTAm/CNPq/MCT

P4.07.26
Atopic responses among pre-school children in a schistosomiasis endermic community in Zimbabwe

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The study was conducted in the Mashonaland East Province of Zimbabwe where *Schistosoma haematobium* is endemic. A longitudinal, community-based study survey was conducted at Magaya and Chitate schools. Parasitology collection, questionnaires and treatment of the study population was done. A skin prick allergen reaction test on mother and child only were carried out. Blood samples were obtained and used to determine immunological profiles. Magaya area had high infection prevalence greater than 50% and Chitate area had moderate infection (10> prevalence<50%). All infected participants were treated with praziquantel tablets. The overall prevalence of skin prick reactivity was 19% and the most prevalent allergic response was directed against the house dust mite with 12% of the population reacting against it. Anti-schistosome treatment with praziquantel did not result in an increase in atopic responses 6 months after treatment in children aged 6 months -5 years of age. Most infected individuals did not have any marked allergenic responses to the testing antigens. Treatment resulted in significant increase in protective IgE which has been associated with protection against re-infection. IgE and IgG4 antibodies against Derp-1 increased after treatment but the ratio IgE: IgG4 significantly decreased in the high transmission area suggesting that removal of the parasites by PZQ treatment decreases allergic responses, contrasting with the hygiene hypothesis.
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P4.07.27
Impaired CXCR4/CXCL12 chemokine receptor/ligand axis limits filarial infection

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Filariases are chronic diseases affecting 160 million people worldwide. Despite considerable effort to reduce disease burden, particularly through mass drug administration programs, filarial infections remain a major public health problem requiring new therapeutic approaches. In our study, we used *Litomosoides sigmodontis* as a well-established murine model of filarial infections. Previous studies have shown that the CXCL12 chemokine and its receptor CXCR4 participate to the mice resistance mechanism to the filarial infection, suggesting CXCR4 and CXCL12 as potential therapeutic targets. To decipher their role on the infection progression, we used a newly developed murine model of a rare combined human immunodeficiency disorder (WHIM: Warts, Hypogammaglobulinemia, recurrent Infections and Myelokathexis) caused by a gain of CXCR4 function and also characterised by a profound lympho-neutropenia. WHIM mice reproduce this leucopenia, associated with defective thymopoiesis and B-cell development and lymph node disorganised architecture. Our results on filarial infection in those mice showed that filarial parasitic success was drastically decreased by 70% in WHIM mice compared to control wild-type mice. In addition, a significant neutrophilia became noticeable from 15 days

post-infection, normalising the circulating neutrophils to the control levels although the lymphopenia remained in the WHIM mice throughout the infection. Moreover, 6 hours post filarial infection, cell recruitment in the skin was more important in those WHIM mice, suggesting a stronger local immune response to *L. sigmodontis*. Further analyses are currently conducted in order to elucidate the mechanisms behind this immune response in respect to the role of the CXCL12/CXCR4 axis in filarial infection.

P4.07.28
Opisthorchis viverrini induces Th17 cytokine responses in hamsters

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Opisthorchiasis, a major helminthic disease in many parts of Southeast Asia, is caused by the liver fluke Opisthorchis viverrini (OV). Disease ranges from asymptomatic to diarrhea, epigastric pain, fever and jaundice. Long term chronic inflammation may lead to cholangiocarcinoma. Since the discovery of Th17 cells, our current knowledge of T helper (Th) cell responses and their roles in OV infection is incomplete. In particular the production of cytokines from Th1, Th2, Th17 and Treg cells requires careful examination. Although the hamster is the best animal model, immunological studies in the hamster have been limited due to the lack of available reagents. In this study, we infected hamsters with different doses (5, 50 and 100 metacercariae) and examined cytokine levels at different times (2, 8 and 24 weeks) after infection. Th17-related cytokines including TGF-β, IL-6, IL-23, IL-17A and IL-21 were assayed in the spleens, livers and mesenteric lymph nodes (MLNs) using RT-qPCR. Hamster sera Th1/Th2/Th17/Treg cytokine levels were quantified using a bead-based Luminex technology. The results showed that Th17 cytokines were detectable together with Th1, Th2 and Treg cytokines. Generally TGF-β was expressed, correlated with dose and increased with time up until at least 6 months in all organs. In contrast IL-17A and IL-21 were only expressed in spleen. Th17 cytokine transcripts were higher in MLNs than spleen and liver where the pathology occurs. This study provides important insights in immunity to opisthorchiasis and suggests possible roles for Th17 cytokines in immunoprotection and/or immunopathology.

P4.07.29
Study of nitric oxide metabolites(NOx) production during human hydatidosis: Relationship with cyst fertility

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Nitric oxide (NO) and its stable metabolites nitrite and nitrate (NO2−+ NO3−) have been identified as major effector molecules during the majority of parasitic infections. Production of NO has been shown to be induced by interferon gamma (IFN-γ) during human hydatidosis suggesting the relevant role of NO in the host defense. This parasitic infection constitutes a major health problem in Algeria. It is characterized by a prolonged coexistence of Echinococcus granulosus and its host without effective rejection of the parasite. In this study, we investigated in vivo production of NO products NOx (NO2−+ NO3−) in sera of Algerian patients carrying different cyst locations. NOx levels were evaluated by the Griess method. Our results indicated that the levels of NOx were significantly higher in the sera of hydatic patients than those of healthy controls supporting the possible involvement of NO in antihydatic action. The levels of NOx in sera of the patients with hepatic hydatidosis were significantly higher than those with pulmonary infection. The lower serum NOx levels were observed in the relapsing cases. In the current study, our results show high levels of NOx in fertile liver and lung fluids compared to infertile fluids. Our results suggest that the presence of NO products in hydatic fluids seems to be related to the location and the fertility of hydatic cysts. In addition, the presence of NO products in the cyst fluid suggests a possible local NO production by the larvae.

P4.07.30
Evaluation of CCA urine test for diagnosis of *Schistosoma mansoni* infection in Brazil

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The circulating cathodic antigen (CCA) immune-chromatographic dipsticks was developed as an alternative diagnostic test, which indicate the presence of alive worms by detecting excreted schistosome antigens in host urine. A fresh urine sample was tested with a commercially available circulating cathodic antigen (CCA) assay (Rapid Medical Diagnostics, Pretoria, South Africa). In the present study we compared the accuracy of CCA testing to Kato-Katz (duplicate thick smears from tree stool samples collected in consecutive days). Feces and urine sampling took place in August and September 2012 in tree rural districts in Governador Valadares, Minas Gerais, Brazil. A total of 106 individuals aged 7-89 years old participated of this study. For the Kato-Katz test, 45 individuals were egg negative and 61individuals were egg positive, showing a range of 4 to 2288 eggs/g of feces. Results from the CCA test were recorded according to the visual intensity of the CCA reaction band: trace, weak (+), medium (++) and strong (+++). Kato-Katz examination and CCA testing were in broad agreement, revealing mean prevalence of 58% and 53%, respectively. Following urine testing with CCA dipsticks, we observed that 13%(14/106) of results were categorized as trace, 16% (17/106) as single positive, 10% (11/106) as double positive and 13% (14/106) as triple positive. The sensitivity (SS) was found to be 82% and specificity (SP) 85%, when trace was considered as positive. Our results suggest that urine-based assays for CCA may be valuable in screening for *S. mansoni* infections. Financial support: BWF/UCSF and UNIVALE

P4.07.31
High IL-4 production related to granuloma development in high-tolerogenic mice infected with *Schistosoma mansoni*

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The *Schistosoma mansoni* infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. TR strain present good inflammatory responses and a non-tolerogenic profile while TS are non-inflammatory but high-tolerogenic, with high percentages of CD4+CD25+Foxp3+ T regulatory cells, and able to produce high levels of inhibitory citokynes such as IL-10. The aim of this study is to correlate the cytokines production to the pathology caused by infection. TS strain have higher weakness, apathy, prostration and mortality due to hepatosplenomegaly more intense due to the larger size of hepatic granulomas and extensive fibrosis of these granulomas. Both strains produced IFN-γ, but TS produced IL4 and IL-10 in a larger quantity, however IL-10 was not able to regulate the growth of hepatic granulomas exacerbated this lineage. High levels of IL-4 in TS strain are consistent with the exacerbation of granulomas, since IL-4, as well as IL-13, induces collagen synthesis and is related to the development of fibrosis in schistosomal granuloma. Additional studies are needed to confirm our proposals and to understand the mechanisms underlying the difference in immune response of these strains in the schistosoma-host relationship.

P4.07.32
Reduction of B lymphocytes production in mice genetically selected for humoral response and infected with *Schistosoma mansoni*

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The *Schistosoma mansoni* infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. TR strain present good inflammatory responses and a non-tolerogenic profile while TS mice are non-inflammatory but high-tolerogenic profile. The aim of this work is the study of the production of B lymphocytes in mice selected for humoral response to oral tolerance infected with *S. mansoni*. TS strain has higher weakness, apathy, prostration and mortality due to anemia and hepatosplenomegaly more intense due to the larger size of hepatic granulomas. We observed a reduction of percentage of T CD4+ lymphocytes in the liver of infected mice in both strains and a reduction in all subpopulations of B lymphocytes in bone marrow (precursors, immature, mature and plasma cells) more pronounced on TS strain than TR strain, possibly due to extensive mobilization of immature B cells induced by inflammation and hematopoiesis deviation for synthesis of granulocytes in TS mice. TR strain didn´t show changes in their subpopulations of B lymphocytes. Additional studies are needed to confirm our proposals and to understand the mechanisms underlying the difference in immune response of these strains in the schistosomal-host relationship.

P4.07.33
Ultrastructural alterations in adult *Schistosoma mansoni* harbored in low-inflammatory mice

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The *Schistosoma mansoni* infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. The objective was to analyze by Transmission Electron Microscopy the influence of the host immune regulatory profile on the worm morphology. Parasites recovered from TR mice showed no morphological changes. However, specimens collected from TS mice, exhibited tubercle swelling with blunted and shortened spines in lower density. These tegument alterations were similar to those described with artemether or praziquantel treatment, supporting observations that the host immune system influences the tegument development and function of worms harbored in non anti-helminthic treated TS mice. The ileum oogram from TS mice showed a higher percentage of dead eggs and a lower percentage of immature eggs than TR mice, but had similar quantities of collected eggs. This suggests that in TS mice the alterations in adult worm tegument prevented egg development, as a consequence of decreased glycogen granules and extensive lysis of internal structures, but not egg production reduction. These results corroborate our previous Scanning Electron Microscopy study indicating the influence of the host immune regulatory profile on the development and function of the worm reproductive system and tegument.

P4.07.34
Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in *Strongyloides venezuelensis*-infected mice

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When animals are infected with helminthic parasites, resistant hosts show Th2 immune responses to expel worms and develop eosinophilic inflammation in their lungs. But the mechanism for this lung eosinophila is not clear. Here we show the relevant roles of endogenous IL-33 for *Strongyloides venezuelensis* (*S. venezuelensis*) infection-induced lung eosinophilic inflammation by using *Il33*$^{-/-}$ mice. We found alveolar epithelial type II cells (ATII) but not macrophages express IL-33 in their nucleus, and infection with *S. venezuelensis* increases in the number of ATII cells and in the level of *Il33* mRNA and IL-33 protein. Recently, newly identified type II innate lymphoid cells (ILC2) are shown to express ST2 and produce IL-5 and IL-13 when stimulated with IL-33. *S. venezuelensis* infection induces pulmonary accumulation of ILC2 in IL-33 dependent manner, and these cells produce IL-5 and IL-13 ex vivo. Furthermore, *Rag2*$^{-/-}$ mice increase the number of ILC2 and eosinophils in their lungs after infection, while *common*$\gamma^{-/-}$*/Rag2*$^{-/-}$ mice, in which ILC2 are absent, do not show these responses. Finally, IL-33-stimulated ILC2 produce IL-5 and IL-13, which in turn induce lung eosinophilic inflammation, and might aid to expel infected worms in the lungs.

P4.08 Immunity to protozoan parasite infection

P4.08.01
CCL3, CCL4 and CXCL8 chemokines mRNA expression in human neutrophils in response to *Leishmania infantum*

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Zoonotic visceral leishmaniasis caused by *Leishmania infantum (Li.)* is potentially fatal; however, subclinical forms are relatively common. Initial cytokine response at the early stage of infection is important in the outcome of infection. Polymorphonuclears (PMN) are the first cells that recruited to sites of *Leishmania* infection, where they can influence the recruitment of other immune cells by producing chemokines. Little is known about chemokines produced by human PMN in response to *Leishmania*. In this study, we investigated CCL3, CCL4 and CXCL8 mRNA expressions from PMN treated with or without *Li.* promastigotes. The mRNA expression of chemokines was quantified by qRT-PCR. The mean dCT (CT number of chemokine gene - CT number of reference gene (β-actin)) for treatment and the mean dCT for control for each chemokine were compared by t-test. The results showed that PMN express significantly higher CXCL8 after stimulation with *Li.* ($p=0.007$). However, CCL3 and CCL4 expression were suppressed in 65% and 62%, and remained unchanged in 12% and 8% of individuals, respectively. There was a significant correlation ($p<0.0001$) in mRNA expression between CCL3 and CCL4. Our Findings suggest that induced CCL8 and suppressed CCL3 and CCL4 expression in *Li.*-stimulated PMN, induce PMN and suppress early macrophages recruitment. Since early influx of PMN has been demonstrated to be beneficial for *Leishmania* survival and early arriving macrophages does not seem to be a suitable host for free extracellular promastigotes this selective release of chemokines will favor parasite survival and establishment.

P4.08.02

Trypanosoma cruzi oral/intragastric infection in murine model: infection and immune response features of the host

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Nowadays Trypanosoma cruzi oral transmission through ingestion of contamined food promotes acute Chagas' disease outbreaks in several Brazilian states and other Latin America countries. Few reports address carditis and immune response on this model, so our study has evaluated parasitologic and immunologic features, and also target organs after T. cruzi oral/intragastric infection. In this purpose we have infected BALB/c mice with $5x10^4$ trypomastigotes (Tulahuen strain) through intraperitoneal (IP), intragastric (IG) or oral (IO) route. IP group have presented higher parasitemia and mortality than IG and IO (0%, 45% e 20% respectively remain alive after 31 dpi), and also IO group have presented higher values than IG. Initially evaluating target organs histopathology, hearts have presented microinfiltrates that have enlarged through time and from 9, 12 and 15 dpi, amastigotes nests in IP, IG and IO groups, respectively. Besides, liver has showed mild/moderate lesions in IG group that were smaller and less than IP. Concerning to host immune response, we have observed that serum cytokine profile in IP group was mainly T_H1 and in IG, T_H2. Therefore we have analyzed cellularity and lymphocyte phenotyping in subcutaneous (SCL) and mesenteric (ML) lymph nodes, spleen and Peyer patches (PP). IP group presented hyperplasia in SCL (increased $CD8^+/CD19^+$ population) and spleen (increased $CD4^+/CD8^+/CD19^+$ population) earlier than IG. PP hypoplasia (decreased $CD19^+$ population) was observed in IP and IG groups. Our results suggest that IO/IG infection affects parasitemia, mortality and target organs commitment, triggering alterations in serum cytokines and secondary lymphoid organs compared with IP.

P4.08.03

Congenital infection with *Trypanosoma cruzi* primes cord blood Vδ2 T cells to proliferate in response to isopentenyl pyrophosphate

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Trypanosoma cruzi is the protozoa agent of Chagas' disease in South America. Our team has previously demonstrated that newborns congenitally-infected with *T. cruzi* develop a parasite-specific CD8 αβ T cell immune response *in utero*. In the present study, we have investigated whether cord blood TCRVδ2 T cells were also involved in such response, by analysing their phenotype and their capacity to proliferate in response to isopentenyl pyrophosphate (IPP) in both uninfected and congenitally-infected newborns. We found that Vδ2 T cells can be expanded following congenital infection with *T. cruzi*. Cord blood Vδ2 T cells from congenitally-infected newborns expressed inhibitory NK cell receptors such as NKG2A and killer inhibitory receptors. Moreover, they vigorously expand in response to IPP combined with IL-2. Altogether these results demonstrate that Vδ2 T cells can be activated *in utero* and that these cells can be primed to respond to phosphoantigens.In conclusion.In conclusion, this work demonstrates that congenital infection with *T. cruzi* induces significant activation of fetal γδ T cells *in utero*, and supports the notion that it's possible to boost the γδ T cell immune response in early life

P4.08.04

Phenotypic and functional analysis of CD4+FoxP3+ regulatory T cells (TREG) in the early phase of murine infection with Trypanosoma cruzi

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Introduction: We previously observed that in T. cruzi-infected mice (Y strain) depletion of TREGs by anti-CD25 mAb treatment does not affect acute parasitemia, suggesting that TREGs do not regulate the early anti-T. cruzi response. Using FoxP3+GFP+ mice, we now studied the phenotypic changes in spleen TREGs along the early infection with SylvioX10/4 T. cruzi parasites and evaluated their suppressive activity.
Results: Peak spleen cellularity and CD4+ cell number occurred on the 11th day post-infection (d.p.i.). No major changes in TREG number were observed from the 4-18 d.p.i., despite an increase in large TREG cells. Regarding expression (MFI) of different markers by TREGs, we observed a slight increase in FoxP3 at 7-14 d.p.i., small increases in Fas at 11 and 18 d.p.i., strong increases in CD25 that peaked at 14 d.p.i. and late increases in GITR and FasL. No significant changes in OX40 or CD127 were observed. Of note was the appearance of TREG subsets with higher expression of CTLA-4 (at 7 d.p.i.), CD69 (from 7-14 d.p.i.) and ICOS (from 7-18 d.p.i.) that peaked at 7 d.p.i.. According to their suppressive activity upon the anti-CD3-induced proliferation of CD4+FoxP3- splenocytes and upon IFN-γ production by total splenocytes, there were no major differences between TREGs cells from control and 7d-infected mice. Moreover, responding 7d-CD4+FoxP3- splenocytes showed similar susceptibility to suppression by control and 7d-TREGs than control CD4+FoxP3- cells.
Conclusion: We demonstrate that during the early infection by T. cruzi TREGs maintain their suppressive activity and responding CD4+cells do not become resistant to suppression.

P4.08.05

Phagocyte-derived from cells B1 (B1CDP) facilitate Leishmania major infection in vitro via IL-10 and PGE-2 secretion

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B1 cells are a small fraction of the population of B lymphocytes of the spleen and they represent the major population of B lymphocytes in the pleural and peritoneal cavity. It has been demonstrated that B1 cells secrete IL-10 and thereby they modulate the phagocytic activity of macrophages. Furthermore, they described a new role for B-1 cells as part of mechanisms that suppress the immune system via production of IL-10. Reports over the past years have shown evidence that both lymphoid and myeloid lineage unexpectedly run a close relationship. This information is based on studies showing that, in mice and human, B1 cells can generate offspring phagocytic, exhibiting similar characteristics to those of macrophages. The B1 cells in primary cultures proliferate and become a mononuclear phagocyte. These cells were called phagocyte-derived from cells B1 (B1CDP). This information motivated us to study the actions of B1CDP cells in infectious and inflammatory processes and for this propose we will use the model of experimental infection with *Leishmania major*. Our data demonstrated that B1CDP are more susceptible to infection by *L. major* in vitro and this effect is blocked when added doses of neutralizing anti-IL-10 but not TGF-beta. Also, we observed the presence of a large number of lipid bodies in B1CDP and PGE-2 production. *B1CDP cells are defective in IL-10 production in response to COX-2 inhibitors. These data suggest that B1CDP cells are cellular component that might compromise host immune responses to infection diseases.*

P4.08.06
Analysis of Metalloproteinases and their Inhbitors in Chronic Chagas'Cardiomyopathy

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Trypanosoma cruzi infection affects 10 million persons in Latin America. Around 30% of those develop chronic Chagas disease cardiomyopathy (CCC), an inflammatory dilated cardiomyopathy characterized by an intense cardiac remodeling, hypertrophy, fibrosis and a myocarditis rich in T cells and macrophages. The survival of patients with is shorter than that of patients with dilated cardiomyopathies of non-inflammatory etiology. Pathological cardiac remodeling includes the restructuring of the extracellular matrix (ECM) which is mediated largely by matrix metalloproteinases (MMPs) and they specific inhibitors (TIMPs). 25 MMPs have been described in humans where modification of their expression and/or activity correlates to various pathological conditions including cardiovascular diseases. We have assessed the expression of major MMPs and TIMPs by Real Time PCR and immunoblotting in myocardial tissue samples of explants from patients subjected to heart transplantation of end-stage CCC, idiopathic dilated cardiomyopathy (DCM), and from donor hearts not used from transplantation (CONT). We observed upregulation of gene expression of MMP2 and TIMP2, an inhibitor of MMP2, in DCM vs. CCC. We also observed by immunoblotting an increased expression of MMP2 and TIMP2 in CCC and DCM as compared to CONT. These results suggest that of MMP2 might be involved in cardiac remodeling in CCC as well as DCM.

P4.08.07
Role of IL-9 in the intestinal inflammation induced after Toxoplasma gondii infection

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IL-9 is a pleiotropic cytokine and functions as both a positive and negative regulator of immune responses. IL-9 has long been thought to be a Th2 cytokine, as it promotes allergic inflammation and is associated with various Th2 responses. Recent studies revealed that the IL-9 is produced mainly by T cells generated in the presence of the cytokines TGF-β and IL-4 termed Th9. However, whether IL-9 and Th9 cells are involved in infection immunity by intracellular pathogen has not been investigated. In this work, we evaluated role of IL-9 and Th9 cells in model the inflammation intestinal induced by T. gondii. Susceptible (C57BL/6) and resistant (BALB/c) mice were orally infected with 100 cysts of the ME49 strain of T. gondii and after 7 days post infection, we evaluated the expression of IL-9 by qPCR and the frequency of Th9 (CD3+CD4+IL-9+) cells in spleen and mesenteric lymph nodes by FACS. Our results shown that infection by T. gondii induce the expression of IL-9 in gut on both the lineages of mice. In additon, we found an increase the Th9 cells during disease progression. At day 7 of infection, an intense intestinal inflammation is observed in C57BL/6 but not BALB/c mice. However, the C57BL/6 mice exhibited a lower frequency of Th9 cells compared with BALB/C mice that have an increase these cells in the spleen and mesenteric lymph nodes. Taken together, these findings suggest that IL-9 plays an important role in immune response regulation favoring resistance to T. gondii infection.

P4.08.08
Splenic phagocytes shape the immunity to Plasmodium infection in distinct but complementary ways

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During experimental malaria, several changes in structure and cell numbers occur in spleen. The effects of these modifications on phagocytosis by spleen dendritic cells (DCs) and macrophage subsets are so far unknown, and the relative importance of this activity is not well understood. By using different in vivo and ex vivo approaches, we described some aspects of parasite phagocytosis and the implications to immune responses to infection. In naïve mice, DCs and F4/80+ red pulp macrophages (RpMOs) promptly recognized and internalized parasites, and enhanced the ability of making stable contacts with T and B cells inside spleen, respectively. The importance of DCs and RpMOs to activation of respectively T and B cells, as well as for survival after infection, was confirmed by depletion strategies. During the parasitemia peak, migrating inflammatory monocytes (MMs) were also able to phagocytize parasites, thus helping the parasitemia elimination observed in this model. However, these MMs were also crucial for controlling excessive CD4+ T cell activation by production of nitric oxide (NO); this control was necessary for full CD4+ T cell memory responses to parasite challenge. Thus, DCs, RpMOs and MMs contribute in distinct ways for parasite elimination and the induction of a massive, yet controlled, immune response to blood stage malaria.

P4.08.09
Neutrophil migration during placental malaria

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Neutrophils play an important role in the innate immune response against invading pathogens. The role of neutrophils, especially during placental Plasmodium falciparum (P. falciparum) infections, has been poorly investigated. The aim of this study was to investigate the role of neutrophils during placental malaria. For this purpose an in vitro model system was used to assess immunological changes induced in a placental cell line (BeWo) and in neutrophils by P. falciparum parasites. BeWo cells were stimulated with either purified infected red blood cells (iRBC) or uninfected red blood cells (uRBC) and the supernatants were collected and analyzed for cytokines, chemokines and angiogenetic proteins by cytometric bead arrays and proteome profile arrays. The results show that BeWo cells are secreting a lot of immunoregulatory proteins and cytokines upon stimulations with P. falciparum parasites. One dominant factor produced was IL-8 which is a known chemoattractant for neutrophils. To test if P. falciparum parasites could induce neutrophil recruitment by BeWo stimulated cell-culture supernatants, a chemotaxis assay was set up. The results show that neutrophil migration towards iRBC supernatants is enhanced compared to migration towards supernatants from uRBC stimulations. By performing neutralization assays we hope to further define the factor(s) of importance for this increased migration. These findings show that trophoblast cells are immunologically active during placental malaria and secrete factors that are capable of influencing and/or contributing to the local maternal environment, in this case by recruiting neutrophils to the infected placenta. In addition, we will also focus on phenotyping the recruited neutrophils.

P4.08.10
Histopathological study in intestinal wall of dogs naturally infected with Leishmania in the intestines

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Canine Visceral Leishmaniasis (CVL) is caused by a protozoa parasite of the specie *Leishmania* (*L.*) *infantum,* endemic for humans and dogs in many regions of Brazil. The purpose of the present study was to evaluate the histopathological alterations in intestinal tissues naturally infected with *Leishmania* amastigotes in a group of polisymptomatic CVL dogs. Intestinal tissue samples, including small and large intestines were harvested from 11 dogs euthanized by Zoonotic Disease Control Center. By histo and immunohistochemical techniques, *Leishmania* amastigotes as well as inflammatory cells were quantified in the intestinal wall layers of dogs. *Leishmania* amastigotes were detected in lamina propria of mucosa submucosa and muscular layer from small intestines and in mucosa and submucosa from colon in 05/11 (45.5%) dogs. Amastigote forms were detected inside hypertrophic macrophages abundant in the mucosa at the tip of villi in small intestines, and surrounding crypts and submucosa in the colon. The inflammatory reaction was characterized by a chronic infiltrate of mononuclear cells; macrophages, lymphocytes and plasma cells in those animals intensely infected with the parasite. Mast cells quantified in these animals indicated less concentration of these cells in the mucosa where amastigotes were detected in higher numbers. Neutrophils and esosinophils were detected in higher numbers in dogs with low parasitic burden. In conclusion, positive dogs by intestinal leishmaniasis with high parasitic load had a chronic mononuclear inflammation, while dogs with low intestinal parasitic burden had an acute inflammatory reaction characterized by hemorrhagic enteritis, mast cell and polymorphonuclear hyperplasia.

P4.08.11
Comparative evaluation of rK39 and soluble antigen ELISA, IFAT and parasitological tests for Canine Visceral Leishmaniasis diagnosis

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Canine Visceral Leishmaniasis (CVL) is a chronic parasitic infection caused by Leishmania (L.) infantum that infect dogs and humans in rural and urban areas. The aim of the present work were to compare the serological methods by ELISA using two antigens (recombinant-rK39 and soluble extract-SE antigens), indirect fluorescence antibody test (IFAT) and parasitological exams (PA) in order to evaluate a better and safer method for CVL diagnosis. For this study, serum and lymph node aspirate samples of 70 dogs were used for serological and parasitological exams. The statistical comparative analysis was done using Kappa (k) index and the interpretation of the data was according to Nigel Paneth classification, which k > 0.80 was considered excellent; k= 0.60 - 0.80 was good; k= 0.40 - 0.60 was regular and k< 0.40 was bad concordance. According to the results there was good agreement among the diagnostic methods for CVL in 34.3% (24/70) dogs. 100% of positive dogs by PE were also good by ELISA-SE in 68.7%, k=0.4217. Similar results were observed between PA x IFAT with 71.6% of concordance and k= 0.4654. In contrast, the agreement between ELISA-SE x ELISA-rk39 was 58.6%, k=0.0330 (41/70) and IFAT x ELISA-rk39 was 52.8%, k=0.0069 (37/70), bad agreement. ELISA-SE x IFAT were the methods that presented excellent and the highest agreement in 82.8%, k=0.7237 (60/70). The results of positivity indexes of the dogs revealed that parasitological exams in association with ELISA-SE and IFAT was more appropriated for CVL diagnosis.

P4.08.12
Role of L-arginine during acute phase of Chagas disease in pregnant Wistar rats infected with the Y strain of *Trypanosoma cruzi*

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Chagas disease induces a strong immune response during acute phase of infection, mobilizing different compartments of the immune system. L-arginine is an essential amino acid that plays an important role in homeostasis of the immune system. During pregnancy, the immune and endocrine systems underwent important changes in order to establish a protective role in the feto-maternal relationship, avoiding fetus rejection and at the same time keeping adequate maternal host defense mechanisms to fight infection. The aims of this study were to evaluate parasitemia, corticosterone levels, peritoneal macrophage counts, production of nitric oxide (NO) and histopathology of heart and placenta. Twenty pregnant Wistar rats (180-220g) were grouped in: pregnant control (PC), pregnant control and L-arginine supplied (PCA), pregnant infected (PI), pregnant infected and L-arginine supplied (PIA). Females were infected with $1x10^5$ tripomastigotes of the Y strain (3rd day of pregnancy). Animals were supplied with 21 mg of L-arginine/Kg/day (gavage) during 14 days. PIA showed significant decreased levels of corticosterone and parasitemia. For control groups, L-arginine supplementation did not show any alteration in NO production by peritoneal cells; for PIA, enhanced nitrite concentrations were observed as compared to PI, despite similar levels of macrophages. A reduction in the amastigote burdens in cardiac tissue was observed with L-arginine therapy but similar placental parasitism was found when comparing PIA and PI. Based in these results, the administration of L-arginine can be considered a co-adjuvant alternative therapy during acute phase of Chagas disease, enhancing the immune response. Financial support: Fapesp and Capes.

P4.08.13
Zinc intake improves immune response during pregnancy in wistar rats infected with Y strain of *Trypanosoma cruzi*

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Several changes in the levels of various hormones and serum factors occur during pregnancy. Occurrence of infectious diseases such as Chagas disease during pregnancy has significant effects on maternal health and can lead to adverse pregnancy outcomes. It is known that zinc is an essential element important for the normal function of the immune system. The objective of this work was to examine the potential role of zinc supplementation during the acute phase of Chagas disease in pregnant Wistar rats. Twenty pregnant Wistar rats (180-220g) were grouped in: pregnant control (PC), pregnant control supplied with zinc (PCZ), pregnant infected (PI) and pregnant infected supplied with zinc (PIZ). Females were i.p. infected with $1x10^5$ tripomastigotes of Y strain of *T. cruzi* on the 3rd day of pregnancy. Rats were orally supplied (gavage) with zinc sulphate (20 mg/kg) once a day during the experiment. On day 18 of pregnancy (15º day post infection) some parameters were evaluated such as parasitemia, IFN-γ and TNF-α concentrations, peritoneal macrophages counts and histological analysis of heart, placenta and fetuses stained with haematoxylin and eosin. PIZ group showed a significant reduction of parasitemia levels, enhanced concentrations of IFN-γ and significant reduction of amastigotes nests in the placentas and hearts. Absence of parasites in fetal tissues was observed. Upon this scenario, zinc intake during the early pregnancy period plays a vital protective role, improving immune response and consequently preventing adverse outcomes. Supported by Fapesp and Capes.

P4.08.14
T cell immunoglobulin- and mucin-domain-containing molecule-3 secretion by patients with active cutaneous leishmaniasis

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Defective IFN-gamma and increased IL-10 production favors the occurrence of ulcerative lesions and therapeutic failure in human cutaneous leishmaniasis (CL). Recently, it has been demonstrated that T-cell immunoglobulin, mucin domain-3 (Tim-3) is a regulatory membrane protein expressed on mature IFN-gamma secreting Th1 cells. Soluble sTIM-3 isoform was detected in mice only. Here, fifteen patients with active lesions (aCL), 12 patients with healed lesions (hCL) and 13 asymptomatic without clinical record of leishmanial lesion (rCL) were enrolled. Peripheral blood was collected and PBMC separated by centrifugation gradient. Cells were cultured in the absence or presence of 5ug/mL PHA or L. braziliensis antigens (AgLb) alone or with 10ug/mL recombinant human IFN-gamma (rhIFN-gamma). Levels of TIM-3 were measured by ELISA in supernatants using commercial mAbs. Patients with active CL lesions secreted higher amounts of sTim-3 in response to AgLb and AgLb+rhIFN-gamma stimulation than cells from hCL or rCL subjects. However, when the capacity of PBMC to respond to these stimuli was evaluated compared to baseline production of sTIM-3, results varied among groups. In the presence of AgLb alone, higher detection of sTIM-3 was observed in cells from rCL subjects whereas stimulation of cells with AgLb+rhIFN-gamma improved sTim-3 secretion by cells from aCL and hCL patients but not from rCL subjects in comparison to PBMC cultured in medium alone. This is the first detection of a soluble TIM-3 isoform in human samples. New perspectives in understanding the events regulating the immune system that control human parasitic diseases are expected.

P4.08.15
Trypanosomiasis-associated modulation of B cell lymphopoiesis and immune function

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African Trypanosomiasis is a parasitic disease affecting both humans and livestock in Sub-Saharan Africa. The disease in cattle approximately causes an annual loss of 4,5 billion dollars and has therefore a major impact on the socio-economic development of the affected rural areas. Polyclonal B cell activation has long been described as a hallmark of African trypanosomiasis. Recently, we have shown that murine T. b. brucei infection induces depletion of B cells in developmental, immature and mature stages and induces loss of vaccine-induced memory B cell responses, even against non-related antigens. In order to understand the extent of this problem, we have so far taken two parallel approaches. First, we studied trypanosomiasis-associated destruction of various B-cell compartments in a Hen Egg Lysozyme specific B cell transgenic mouse model (MD4). In this model depletion occurs with the same kinetics as in infected WT mice, indicating that the detrimental infection-associated effects occur independent of B cell receptor specificity. Second, we have addressed the possibility of B-cell compartment restoration following drug cure of trypanosomiasis. Infected mice were treated with berenil (diminazene aceturate), a widely used chemotherapeutic agent for animal Trypanosomiasis. B-cell recovery was shown to take place over a period of three weeks following treatment. Future work using other anti-trypanosome drug compounds as well as drug-resistant parasite models will provide further insights in the mechanisms that govern both B-cell destruction and recovery.

P4.08.16
Towards an immunosense vaccine to prevent toxoplasmosis

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The ideal vaccine to protect against toxoplasmosis in humans would include antigens that elicit a protective T helper cell type 1 immune response, and generate long-lived IFN-γproducing CD8+ T cells. Herein, we utilized bioinformatic algorithms to identify novel, T. gondii-derived, CD8 + T cell epitopes restricted by the HLA-A02, -A03, and -B07 supertypes, which collectively provide broad coverage 90% of the human population worldwide. A total of 19 surface and secreted proteins from the type II T. gondii strain, ME49, were selected for bioinformatic analysis. Peripheral blood mononuclear cells from seropositive T.gondii donors were tested for response to these peptides by using IFN-γElispot assays. 15 peptides elicited production of IFN-γfrom PBMC of HLA-A02, -A03, and -B07 supertype persons seropositive for T. gondii infection. These peptides displayed high affinity binding to HLA-A02, -A03, and -B07 supertype. Immunization of HLA-A*0201, -A*1101, -B*0702 transgenic mice with these pooled peptides, with a universal CD4+ epitope peptide called PADRE, formulated with adjuvant GLA-SE, induced high CD8+ T cell IFN-γproduction and protected against parasite challenge. Peptides identified in this study provide candidates for inclusion in immunosense epitope-based vaccines.

P4.08.17
Effect of congenital toxoplasmosis on the uNKs cells population and their products in a mouse model

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Uterine natural killer (uNK) cells reside in the placenta at early gestational stages. They mainly produce VEGF-A, IFN-γ and IL-18 which induce an inflammatory environment which protects the fetus from infection. Nevertheless, *Toxoplasma gondii* infection might exacerbate the Th1 immune response locally, which could lead to abortion. In order to evaluate the effect of *T. gondii* on fertility, uNKs, inflammation and fetal damage, female Balb/c mice were infected with different doses of ME49 strain tachyzoites at 10th days of gestation. Samples from maternal spleen and maternal-fetal interface (MFI) were taken 72 hours later to assess damage and parasite load by histopathology and qPCR. The number of fetuses reabsorbed was significantly higher in the cases inoculated with 10 million tachyzoites than in other groups. From the 5 million-dose on, parasites were found in the placenta and the fetuses, but the former presented around 100 times the burden than fetuses. MFI presented discrete focal coagulative necrosis, more intense at the maternal side, as well as congestive changes especially in the decidua and the chorionic labyrinth. The number of uNK VEGF-A+-cells was similar among groups, consistent with no changes in the number of blood vessels. The number of local IFN-γ+ uNKs increased in relation to parasite dose as it did the magnitude of inflammation and variety of inflammatory cells. In conclusion, *T. gondii* alters the local structure at the MFI, increasing the IFN-γ+-uNKs which augment the Th1 inflammatory environment, and consequently triggers fetal death.

P4.08.18
Metalloproteinases 2 and 9 are differentially expressed in patients with indeterminate and cardiac clinical forms of Chagas disease

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Dilated chronic cardiomyopathy (DCC) from Chagas disease is associated with myocardial remodeling and interstitial fibrosis, resulting in extracellular matrix (ECM) changes. In this study, we characterized for the first time, the serum MMPs 2 and 9 levels, as well as their main cell sources in peripheral blood from patients presenting the indeterminate (IND) or cardiac (CARD) clinical forms of Chagas disease. Our results showed that serum levels of MMP-9 are associated with the severity of Chagas disease. The analysis of MMPs production by T lymphocytes showed that CD8+ T cells are the main source of both MMP-2 and MMP-9 molecules. Using a new 3-dimensional model of fibrosis we observed that serum from patients with Chagas disease induced an increase in the extracellular matrix components in cardiac spheroids. Furthermore, MMP-2 and MMP-9 showed different correlation with matrix proteins and inflammatory cytokines in patients with Chagas disease. Our results suggest that MMP-2 and MMP-9 show distinct activities in Chagas disease pathogenesis. While MMP-9 seems to be involved with the inflammation and cardiac remodeling of Chagas disease, MMP-2 does not correlate with inflammatory molecules.

P4.08.19
Kinetics of the IgG antibodies acquisition in Plasmodium vivax infected individuals living in a low malaria transmission area in Brazil

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In malaria, antigens from asexual stage of the Plasmodium life cycle are key driving-forces towards host acquired immunity. Among these antigens, the Merozoite Surface Protein 1 (MSP1) and Apical Membrane Antigen (AMA-1) are the most studied and well characterized. The main goal of the study was to assess the kinetics of IgG antibodies to P. vivax and P. falciparum MSP1 and AMA-1 antigens. Serological samples from 285 malaria-infected individuals were collected at days 0, 7, 14, and 30. Individuals were divided into three groups, according to their data collection profile; group 1 (days 0 and 7), group 2 (days 0 and 14) and group 3 (days 0, 7 and 30). Antibody levels were assessed and determined using ELISA. At day 0, the sero-prevalence across all groups was 86.7% (PvHis6MSP119), 70.5% (PvGSTMSP119), 51.9% (PvAMA1), 11.2% (PfMSP119) and 9.8% (PfAMA1). We observed that IgG antibodies response against PvMSP1 was higher than PvAMA-1. In group 1, the positivity to PvHis6MSP119 was 86.0% and 99.0% at days 0 and 7, respectively. In group 2, it was 80.5% (day 0) to 95.1% (day 14). In group 3, it was 86.5%, 94.2%, and 98.08% at days 0, 7 and 30, respectively. For PvAMA-1, we observed similar qualitative behavior up to 30 days. For P. falciparum antigen, seropositivity to each antigen individually was low. In P. vivax infection, antibody positivity against MSP1 and AMA-1 antigens seems to remain stable, or even increased, up to 30 days after treatment thus not showing a significant decay during this timeframe.

P4.08.20
Molecular basis of CD8+T cell's protective response to Toxoplasma infection

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Toxoplasma gondii infects up to a third of the world's population. Recent studies showed *Toxoplasma* Infection may link to schizophrenia, suicide. In immune-compromised patients, reactivation of latent disease can cause life-threatening encephalitis. Infection acquired during pregnancy may also cause severe damage to the fetus. An immunodominant and protective decapeptide HF10 confers protection against toxoplasmosis, which is mediated by GRA6 reactive CD8+ T cells. GRA6 is a polymorphic protein secreted in the parasitophorous vacuole. We have determined the crystal structure of GRA6 TCR bound to HF10 presented by H-2Ld MHC class I(MHCI). Surprisingly, the GRA6 TCR recognizes the MHCI and peptide (pMHC) in an unconventional orientation. GRA6 TCR docks on pMHC almost parallel to the HF10 peptide. The GRA6 TCR CDR2 and CDR3 loops of both α and β chains interact extensively with Ld α2 helix. However, there are almost no contacts between the TCR and Ld α1 helix. The decapeptide does not bulge out the binding groove. Instead, HF10 peptide forms a helical structure in the peptdie binding groove, and interacts with both CDR3s loops. The GRA6 TCR CDRα3 undergoes major conformational change to better the binding to HF10 peptide, compared with unbound GRA6 TCR structure. The current structural analysis provides an alternative docking framework that enables the TCR interaction with infectious pathogens and shed light on how HF10 elicits protective immunity.

P4.08.21
NOD2-dependent Th1 response impairs host survival during N. caninum infection

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Neospora caninum is an intracellular parasite that infects a wide range of warm-blooded hosts worldwide. NLRs represent an important family of microbial sensor proteins that have been identified as key host molecules in innate immune recognition and the inflammatory response to microbial products. However, the role of NLR in the induction of adaptative immune response against intracellular protozoan infection is unclear. In this study, we evaluated the role of NLRs in host response to *N. caninum* infection. For that purpose, $Nod2^{-/-}$ and WT mice were infected with *N. caninum* tachyzoites to evaluation of acute phase parasitism, inflammatory cell migration and cytokine production. $Nod2^{-/-}$ mice exhibited higher parasite burden to WT mice. Inflammatory cell migration was impaired in both compartments, as $Nod2^{-/-}$ mice presented decreased migration of DCs, B and T lymphocytes to the peritoneal cavity. Mononuclear cell infiltrates were also significantly reduced in the lungs of $Nod2^{-/-}$ mice, as compared to WT. Additionally, we observed that dendritic cells and macrophages from Nod2-/- mice presented lower MHCII expression, fact that was associated to lower IFN-γ production in spleen cell antigenic recall, and in lung and brain homogenates. However, no difference in IL-10 production was observed. Surprisingly, Nod2-/- mice demonstrated increased survival than WT mice. Based on the results herein presented we proposed that Nod2 has an important role in T_H1 programming initial immune responses to *N. caninum.* This additional activation of T_H1 response appears to be important to parasite clearance, but could contribute to pathogenesis and mortality during *N. caninum* infection.

P4.08.22
Glucocorticoid mediated caspase-8 and caspase-9 activation promotes thymocyte apoptosis and thymus atrophy in Trypanosoma cruzi infection

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Trypanosoma cruzi acute infection leads to thymic atrophy, largely as a result of death of immature CD4+CD8+ (DP) T cells. In a second vein, the glucocorticoid hormone imbalance promotes DP T cell apoptosis in infected mice. Herein, we assessed the involvement of glucocorticoid and caspase signaling in thymocyte death during T. cruzi acute infection. BALB/c mice were infected with 100 trypomastigote forms of T. cruzi and analyzed from 7 to 19 dpi. Thymocyte apoptosis was observed in early stages of infection, increasing along with time postinfection. Immature CD4-CD8- (DN) and DP as well as CD4+ and CD8+ thymocytes from infected mice showed increased activation of caspase-8, -9, and -3. In vitro treatment of thymocytes from infected mice with a general caspase inhibitor or the combination of caspase-8- and caspase-9-specific inhibitors increased the number of living thymocytes. Intrathymic injection of the general caspase inhibitor, but not caspase-8 or -9 inhibitors individually, prevented thymic atrophy and thymocyte depletion in infected mice. Moreover, blockade of glucocorticoid receptor activity with RU486 prevented DP thymocyte apoptosis, together with caspase-8 and -9 activation. These findings indicate that DP T cell apoptosis following experimental T. cruzi acute infection is dependent on glucocorticoid stimulation, promoting caspase-8 and -9 activation.

P4.08.23
Melatonin and lycopene role on immune system against Chagas'disease.

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Chagas'disease is an infectious illness caused by the protozoa *Trypanosoma cruzi*, affects over 15 million people being responsible for the high indices of morbidity/ mortality. Melatonin is a pleiotropic hormone secreted by the pinealocytes displays several functions such as to regulate other hormones, keep the body's circadian rhythm, antioxidant effects and immunomodulating actions. Lycopene, a potent antioxidant and immunomodulatory carotenoid is synthesized by some commonly consumed plants such as tomatoes, but not synthesized by animals. This work aims to evaluate the influence of oral melatonin and lycopene treatment in i.p. *T. cruzi* infected male *Wistar* rats, (1 x 105 blood trypomastigotes of the Y strain). Animals were treated with melatonin (5 mg/kg) and lycopene (10mg/kg) once a day over the course of the experiment. Phenotypic and functional macrophage analysis, expression of CD11b/c marker on spleen cells and blood parasitaemia were performed. Animals were grouped in: control, infected, melatonin infected and lycopene infected. Studies were performed on 7 days after infection. Peritoneal cells were adjusted to 2×106 cells/ml, being phenotyped by means of cytofluorometric analysis. Parasites were counted by Brener's method. Melatonin and lycopene treated animals displayed a significantly reduced number of parasites when compared to untreated counterparts. Infected and treated groups showed enhanced significant number of macrophages, as well as on its functional analyze (RT1B+) between infected treated and untreated groups. There was an increase on CD11b/c (APC marker) for treated infected groups. It seems that melatonin and lycopene contribute to reduce parasite replication and exert an immune regulating role.

P4.08.24
Impact of the absence of galectin-3 on the course of experimental *Trypanosoma cruzi* infection

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Galectin-3, a β-galactoside binding lectin, is expressed in cells of the immune system such as macrophages and lymphocytes, modulating activation and cytokine production. Chagas' disease, caused by the protozoan *Trypanosoma cruzi,* is a public health problem, without effective vaccines and therapeutics. Here we evaluated the involvement of galectin-3 in experimental *T. cruzi* infection. Wild-type (WT) and galectin-3 deficient (Gal-3$^{-/-}$) mice were infected with the Tulahuén strain. Cytokine levels were determined by ELISA in culture supernatants of activated T cells obtained from subcutaneous lymph nodes (LSC) and spleen. Quantification of cell number and presence of CD4, CD8, CD19, CD25 and FoxP3 were analyzed by flow cytometry. To evaluate macrophage susceptibility to infection, Gal-3$^{-/-}$ and WT peritoneal macrophages were infected *in vitro* and intracellular amastigote numbers were counted. Our results showed that Gal-3$^{-/-}$ mice are more susceptible to infection than WT, with higher mortality rate and increased blood parasitemia. In addition, infected Gal-3$^{-/-}$ mice had lower cell expansion in LSC and spleen, with a decrease in CD4^{+}, CD8^{+} and CD19^{+} cell numbers in LSC and CD19^{+} cell number in spleen, compared to infected WT mice. Analysis of the supernatants from infected Gal-3$^{-/-}$ mice showed a decrease in IL-4, IL-5 and an increase in IFN-γ secretion. Preliminary data showed that infected Gal-3$^{-/-}$ macrophages are less effective in eliminating intracellular parasites than those from WT animals. Our results suggest that galectin-3 is important in modulating the host response against *T. cruzi*, with implications for susceptibility to infection.
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P4.08.25
Casein diets prevent experimental cerebral malaria by enhancing host pro-inflammatory immune responses during early stage

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Cerebral malaria (CM) is still a severe complication threatening children under five in malaria endemic areas. Malaria infection reduces the nutritional status of children, and malnutrition aggravates malaria mortality. Casein hydrolysate can regulate host immune responses. Here, we investigate the related immune mechanisms in casein diet preventing experimental cerebral malaria (ECM). C57BL/6 mice were fed 0% (NC group), 5%, 20% and 35% casein diet for one month before infection with Pb ANKA, respectively. Casein diets can reduce parasitemia and prolong survival of ECM mice. On d3 p.i., casein diets increased splenic Th1 cells and macrophages ($P<0.05$), and a similar trend was observed in IFN-γ, TNF-α and NO levels ($P<0.05$). However, on d5 p.i., Th1 cells and macrophages and related IFN-γ, TNF-α and NO levels were significantly lower than NC group ($P <0.05$), and CD4+, CD8+ T cells, neutrophils and IFN-γ, TNF-α, ICAM-1, VCAM-1 mRNA levels in the brain, and ICAM-1 and VCAM-1 expression levels in the brain vascular endotheliocyte were significantly down-regulated in casein diet groups (compared with NC group, $P <0.05$). Casein diet does not affect the number of Treg and IL-10 levels. These data present the mechanism of casein diets in preventig ECM by improving host pro-inflammatory immune responses to control parasitemia during the early stage of infection, thereby reducing inappropriate pro-inflammatory immune responses to maintain blood-brain barrier integrity, which offers a new theoretical basis for the prevention of children CM.

P4.08.26
Association of IL-1β in the severity of diffuse cutaneous leishmaniasis

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Leishmania mexicana can cause two types of the disease localized (LCL) and diffuse (DCL) cutaneous leishmaniasis. Yet, little is known about factors regulating disease severity in affected patients. We present our findings of a case-control study about severity association to SNPs in IL-1β -511, CXCL8 -251, IL-1RA +2018, IL-10 (-819, -592, -1082), IL-6 -174, TNF-α (-308, -238), CD14 260, TLR-4 299 and IRAK-1 532 in a cohort of Mexican mestizo patients with LCL and DCL. Results show a significant difference in the distribution of IL-1β (-511 C/T) genotypes between patients and controls (heterozygous OR), with respect to the reference group CC. This suggests that this SNP in IL-1β represents a variable influencing the risk to develop the disease in these patients. Additionally, the *in vitro* production of IL-1β shows an increase in monocytes. Expression of this cytokine in sera correlates with the severity of the disease being significantly higher in DCL patients heavily infected with *L. mexicana*. Also, the distribution of IL-1β in lesions varied according to the number of parasites harbored in the tissues. Among heavily infected LCL patients and in all DCL patients, the cytokine was scattered diffusely throughout the lesion. In contrast, in LCL patients with lower numbers of parasites in the lesions, IL-1β was confined to the cells. These data suggest that IL-1β plays a key role determining the severity of the disease in DCL patients. The analysis for all others SNPs showed no significant differences.

P4.08.27
Oxidized ATP action on macrophage during infection with Leishmania amazonensis

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Leishmaniasis is a disease caused by parasites of the genus Leishmania. By affecting 12 millions of people around the world have been investigated for the discovery of new drugs. Previously we have described that macrophage treatment with ATP impairs intracellular growth of Leishmania amazonensis through triggering of P2X7 receptor. In the present study, we evaluated the directly effect of periodate-oxidized ATP (oATP), a P2X7 antagonist, in parasite control in leishmania-infected macrophages.
We found that oATP impaired the attachment / entrance of L. amazonensis promastigotes to C57BL/6 mouse macrophages in a P2X7 receptor-independent manner, as macrophages from P2X7-/- mice were similarly affected. Although oATP directly inhibited the growth of axenic promastigotes in culture, promoted rapid ultrastructural alterations, and impaired their internalization by macrophages, it did not affect intracellular parasite multiplication. Upon infection, phagosomal acidification was diminished in oATP-treated macrophages, and this was accompanied by reduced endosomal proteolysis. Likewise, MHC-II expression and ectoATPase activity was decreased by oATP added to macrophages at the time of parasite infection. These inhibitory effects were not due to a cytotoxic effect, as no additional release of lactate dehydrogenase was detected in culture supernatants. Moreover, macrophage capacity to produce NO and ROS was not affected in the presence of oATP during the infection.
We conclude that oATP directly affects extracellular parasite integrity and macrophage functioning.

P4.08.28
Purinergic receptor functionality controls murine infection by L. amazonensis

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P2X7 is a purinergic receptor associated to control of parasite infection, such as caused by Toxoplasma gondii and macrophage infection by Leishmania amazonensis. Leishmania is the causative agent of leishmaniasis, a disease that affects millions of people around the world. On this ground, we decided to perform in vivo experiments to study its importance on leishmaniasis control.
P2X7 knockout (KO) and wild type (WT) mice were inoculated in footpad and the lesion was accompanied during the infection. We observed the lesion developed earlier in P2X7 KO than WT mice. Furthermore, P2X7 KO mice had more parasitic load and cellularity in footpad in PID 47, as evaluated through limiting dilution assay and count of viable cells after macerating of the footpad. We found that peritoneal macrophages of P2X7 KO mice produced less oxide nitric and reactive oxygen species upon stimulation by LPS and Zymosan in vitro, when compared with macrophages from WT mice. The analysis of popliteal lymph node has shown a larger cellularity in P2X7 KO than WT mice, despite P2X7 KO mice exhibit less cells in this lymphoid organ. The proliferation of T lymphocytes was larger in P2X7 KO after in vitro incubation with anti-CD3, evaluated by CFSE staining. Finally we verified that P2X7 KO mice have had a decreased ectonucleotidase activity in cells from popliteal lymph node, when compared with WT mice, using a colorimetric assay of Pi generation.
Our findings reveal that the P2X7 receptor is important to control the in vivo infection by L. amazonensis.

P4.08.29
A non-regulated Th1/Th2 inflammatory immune response is linked to clinical problems in congenital toxoplasmosis

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Toxoplasma gondii is a ubiquitous intracellular parasite with worldwide distribution which may be vertically transmitted and cause clinical problems to the fetus. In adults a Th1 polarized immune response is protective if regulated by IL-10 or TGF-β. An early Th2 profile does not inhibit parasite replication and thus is non-protective. Paradoxical observations about immune response in congenital toxoplasmosis in animal models have been reported: a but it may induce sterile abortion because of inflammatory damage at the placenta if not regulated or may favor parasite passage to thefetus. The aim of this work was to analyze the cellular/humoral pattern in *T. gondii* infected pregnant women and their newborns and to relate it with perinatal and neonatal problems. IgE and IgG1 to IgG4 specific antibodies were determined by ELISA. Specific PBMCs proliferation and cytokine production were measured by flow cytometry. Specific type/ subtype antibodies correlated with cellular immune response profile. T-CD4+ cell proliferation was observed in all cases, including newborns, while CD19+ and CD8+ specific responses were found in some of them. A Th1/Th2 inflammatory non-regulated immune response was found in adults. In newborns, a non- regulated Th2-polarized response prevailed, which correlated with systemic clinical problems observed in these neonates. Finally a lower immune response was observed in the cases of newborns in comparison with adults, as described in the literature. These preliminary results support the hypothesis that an inflammatory Th1/Th2 non-regulated response is responsible for clinical problems in congenital toxoplasmosis in humans.
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P4.08.30

Sero-immunoproteomics of Leishmania infantum: a reappraisal of the role of antibodies in protection to the development of kala-azar and antigen discovery for the design of bio-markers and vaccines

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The majority of infections with the etiological agent of visceral leishmaniasis in Brazil, Leishmania infantum, do not result in disease. The scientific consensus is that antibodies do not have a role in this outcome and may contribute to susceptibility to visceral leishmaniasis; nevertheless recent studies challenge this view. We hypothesized that individuals presenting asymptomatic infections with L. infantum produce antibodies with functional properties that differ from those produced by individuals who develop visceral leishmaniasis. Sera were collected from 208 kala-azar patients, 197 from individuals with asymptomatic infection and 138 healthy donors living in endemic areas. ELISAs were performed in order to screen IgG antibodies against soluble antigens (SA), membrane antigens (MA) and secreted/excreted antigens (SEA) of L. infantum. We show that kala-azar patients present higher absolute OD of IgG (~10 fold) against L. infantum SA, MA and SEA than asymptomatic individuals. Despite this data, a statistically significant difference between asymptomatic individuals and healthy controls was observed (p<0,001), which indicates that specific IgG properties (such as their subclasses) should be evaluated. Furthermore MA was recognized by 58% of asymptomatic individuals, contrasting with SA, recognized by 48% and suggests that MA may be more suitable for the identification of asymptomatic infection by immunoassays. MA and SEA were separated by 2D polyacrilamide gel, and western blots were performed with sera from kala-azar patients or infected asymptomatically. Indeed, the pattern of recognition of sera from asymptomatic individuals recognizes a set of antigens that differs both by specificity and intensity compared to kala-azar patients.

P4.08.31

Myeloid-derived suppressor cells are key players to balance the exacerbated inflammatory response during acute Trypanosoma cruzi infection

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Myeloid-derived suppressor cells (MDSC), CD11b+Gr1+, are main players of the immune suppressive network. Previously, we reported that during acute infection with the causative agent of Chagas disease, *Trypanosoma cruzi*, BALB/c mice showed a lesser inflammatory response and improved survival than B6 mice which developed severe inflammation and pathology. Recent studies have demonstrated the MDSC presence in this scenario though the suppressor mechanisms have not been fully explored. In this study we demonstrated a higher increase of MDSC in spleen and liver of infected BALB/c compared to B6 mice. The phenotypic analysis of two major MDSC subsets revealed a greater number of granulocytic cells ($CD11b^{+}LY6G^{+}LY6C^{low}$) in spleen from BALB/c respect to B6 mice whereas monocytic population ($CD11b^{+}LY6G^{-}LY6C^{high}$) was the predominant subset in the liver of both mouse strains. Notably, splenic MDSC purified from infected BALB/c mice inhibited the ConA induced splenocyte proliferative response. Mechanistic studies demonstrated that inhibitors of reactive oxygen species (NAC) and nitric oxide synthase (L-NMMA) restored the proliferation. In addition, an up-regulation of NADPH oxidase p47 phox subunit and p-STAT3 occurred in MDSC. A higher number of infected CD8+ T-cells suffered surface-nitration compared to uninfected controls. Infected IL-6 deficient mice showed less recruitment of MDSC and impaired survival. Remarkably, the in vivo depletion of MDSC by 5FU led to an increased production of IL-6, IFNγ and Th17 response with increased parasitemia and mortality. In summary, our data show for the first time the mechanisms of MDSC as crucial regulators of inflammation during *T. cruzi* infection.

P4.08.32

Evaluation of immunological responses against cocktail DNA vaccine of *Leishmania major* genes in BALB/c mice

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Leishmaniasis is caused by *Leishmania* species which in the infected host is obligate intracellular parasite. LACK and TSA are immuno-dominant antigens of *Leishmania major*. In the present study, we constructed a DNA cocktail, containing plasmids encoding LACK and TSA genes of *Leishmania major* and evaluated the immune response and survival rate in comparison with control groups in BALB/c mice. IgG and IFN-γ values were markedly increased in the group immunized with DNA cocktail vaccine, which were significantly higher than control groups (p<0.05) following the immunization and after challenging with *Leishmania major*. IL-4 values significantly decreased in the immunized group compared to controls (p>0.05**)**. The mice immunized with the cocktail DNA vaccine presented a significant reduction in lesion diameter compared to control mice (p<0.05). The survival time of mice immunized with the DNA cocktail vaccine was significantly higher, after challenge with *Leishmania major*, than controls (p<0.05). The mice immunized with DNA cocktail vaccine had significantly lower parasite load compared to control mice (p<0.05). These findings indicate that the cocktail DNA vaccine increases the cellular response and survival rate and induces partial protection against infection with *Leishmania major* in BALB/c mice.

P4.08.33

Role of arachidonic acid metabolites in *Trypanosoma cruzi* infection

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Cardiac inflammation plays an important role in the pathophysiology of Chagas disease, caused by *Trypanosoma cruzi*. Lipid mediators derived from arachidonic acid (AA), like prostaglandins and leukotrienes, are considered regulators of homeostasis and inflammation. These molecules are produced by a biosynthetic pathway controlled by enzymes as cyclooxygenases and lipoxygenases. The role of cyclooxygenase-2 (COX-2) in immunosuppression during the acute phase of *T. cruzi* infection has been described using non-steroidal anti-inflammatory drugs, which are inhibitors of this enzyme. In this study, we first investigated the expression of enzymes involved in AA metabolism during *T. cruzi* infection. Susceptible and non-susceptible mouse models of infection were used to further analyze the role of lipid mediators in *T. cruzi* infection. Our results confirm the expression of several of these enzymes in *T. cruzi*-infected heart, in particular COX-2. $CD68^{+}$ heart-infiltrating macrophages were the major cell type expressing COX-2. $CD11b^{+}$ heart-infiltrating myeloid cells were purified and they were able to produce prostaglandins PGE_2 and $PGF_2\alpha$. Studies using gene-deficient mouse models indicated that regulation of COX-2 and PGE2, through PGE2 receptor 2 signaling, was in part responsible of cardiac inflammation in *T. cruzi* infection. The key role of lipid mediators in Chagas disease inflammation and the availability of drugs that inhibit their synthesis and their receptors could be useful for the treatment of this neglected disease.

P4.08.34
Role of B-1 cells in experimental Leshmania amazonensis infection

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Leishmaniasis belong to a group of diseases caused by protozoan parasites from *Leishmania* genus. *L. amazonensis* can lead to various clinical manifestations of leishmaniasis. Experimental evidence suggests that B cells and/or antibodies are potentially involved in the development of infection caused by *L. amazonensis*. B-1 cells are a subtype of B lymphocytes whose role in the physiology of the immune system as well as in the pathogenesis of various diseases is still poorly understood. These cells have ability to engulf pathogens, to migrate to inflammatory focus and to modulate immune response in several experimental models, such as paracoccidioidomycosis and murine melanoma. In addition, B-1 cells produce large amounts of IL-10 cytokine, which plays a key role in immunosuppression in several diseases, such as leishmaniasis. However, the importance of these cells in leishmaniasis has not been clarified. The objective of this study was investigate the role of B-1 cells in experimental infection of *L. amazonensis*. Preliminary results demonstrated that Balb/c mice subcutaneously infected at the right hind-foot with $1x10^7$ promastigotes of *L. amazonensis* for 70 days showed significant reduction in peritoneal B-1 cells population (28,46% ± 5,43), as compared to non-infected group (45,30% ± 5,05; p<0,01). These preliminary data suggest that B-1 cells population can be modulated in response to *L. amazonensis* infection. Further studies have been conducted to better understand the biological significance of these findings. The role of B-1 cells in the development of cutaneous leishmaniasis is also under investigation in our lab.

P4.08.35
Identification of *Toxoplasma gondii* sequences of potential diagnostic value via cDNA library immunoscreening

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Laboratory diagnosis plays a major role in diagnosis of toxoplasma infection and research on improvements of the diagnosis of the infection is still ongoing. This includes identification of antigens that can be used as diagnostic markers. Thus in this study, *T. gondii* cDNA library was immunoscreened with selected set of serum samples in order to identify cDNA sequences of potential diagnostic value.
First, pooled positive serum samples from Group I and polyclonal anti-human IgM-HRP were used as primary and secondary antibodies respectively. Screening of *T. gondii* cDNA library was also performed using pooled positive sera (from Group I) and probed with anti-human IgG-HRP. Finally three clones were found to show the best differentiation between negative and positive serum samples, namely clones,5ii 7aG and 11aG, and tested with the 45 individual sera samples. The use of a combination of clones 7aG and 11aG produced sensitivity of 88% and specificity of 80% for IgG detection of sera from toxoplasmosis patients. The three clones (clones 5II, 7aG and 11aG) were then sent for sequencing, followed by database search using Toxo DB website. The DNA sequencing revealed that clone 5ii coded for dense granule protein while both 7aG and 11aG coded for hypothetical proteins. In conclusion, this study has identified three *T. gondii* cDNA sequences of potential diagnostic value. The results of this study are potentially useful for development of IgM and IgG detection tests for toxoplasmosis.

P4.08.36
Effect of Phlebotomus papatasi saliva extracts on humoral and cellular immune response in individuals living in an endemic area for leishmania major infection

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Cell proliferation, IFNg and IL10 responses were analyzed after stimulation by salivary gland extracts (SGE), alone or in the presence of anti-IL10 antibody, in 378 individuals subdivided according to the leishmanin skin test response and the presence or absence of scars. We demonstrated significant proliferative and IFNg only in naive individuals. However, the use of anti-IL10 antibody was associated with an increase in proliferative and IFNg responses in healed and asymptomatic groups. No IL10 production was observed after SGE stimulation. Evaluation of humoral responses (mean relative OD: mROD) to SGE in 763 individuals showed a positive response in all groups. A multivariate analysis performed on 29 new CL cases observed after one year transmission showed that the main risk factor to develop CL was the IgG anti-SGE. Indeed, individuals with mROD≥4 had a risk factor of 2,65 (p=0.023) to develop infection. Interestingly, no new CL cases were observed among the naïve individuals, with positive SGE proliferative responses unlike those with negative proliferative responses (6 new cases). These results suggest that cellular responses developed against SGE in the absence of parasites may induce a protection against Leishmania infection. We used Luminex technology to measure up to 30 cytokines and chemokines levels in 5 Healed individuals showing positives IFNg anti-SGE and 5 naïve individuals with negative IFNg responses to SGE. We observed significant IL-6 and IL-2R levels in the first group, after SGE stimulation. Analysis of these profiles may allow to better understand the mechanisms of immune response against leishmaniasis in humans.

P4.08.37
Cytokine responses & parasite genotypes associated with pathogenesis of human African trypanosomiasis trypanosomiasis in North-eastern Uganda

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Human African trypanosomiasis (HAT) or sleeping sickness is caused by protozoan parasites of the genus trypanosoma. The disease is a major cause of human morbidity and mortality in 36 countries in sub-Saharan Africa. Mouse models have indicated that a balance between pro- and counter-inflammatory cytokines is central to the outcome of the disease. Pro-inflammatory cytokines like TNF-α have been shown to control parasitaemia by direct trypanocidal effect. However, over production of these pro-inflammatory cytokines (TNF-α & IFN-γ) has been liked with exacerbated pathology and also favor parasite growth. In a study of HAT in Uganda, early and late stage infections were characterized by elevated levels of IFN-γ, TNF-α and IL-10. CSF of both T. b. gambiense and T. b. rhodesiense patients has been characterized by increased levels of IL-10 and IL-6. In this study we aim to determine the role of the host inflammatory cytokines (Th1 and Th2) and parasite genotype in the pathogenesis of HAT. HAT diagnosis will be done by microscopic detection of trypanosomes in wet blood films or giemsa stained thick blood films prepared from finger prick blood. After admission, a detailed clinical history and a physical examination is performed on all HAT patients. Both neurological and non-neurological signs of HAT will be recorded. Neurological involvement will be assessed using the GCS. Ten milliliters of blood is obtained before commencement of treatment using EDTA vacutainers. Plasma and CSF concentrations of IL-1β, IFN-γ, TNF-α, IL-6, TGF-β and IL-10 is quantified using solid phase sandwich ELISA.

P4.08.38
Induction of protective immune responses against cutaneous leishmaniasis in BALB/c mice by a vaccine containing a new low pathogenic strain of Leishmania major along with imiquimod

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Despite decades of research in countries around the world, an effective vaccine against leishmania infections has not yet been established. Selection of *Leishmania major* strains with low pathogenicity might be a straight way to protect the susceptible mice against cutaneous leishmaniasis for vaccine studies.
Here we used a new low pathogenic strain of *L. major* (MHOM/IR/03/IPI-DA39), isolated from an endemic area of cutaneous leishmaniasis located in north of Iran (Damghan) for vaccination compared to a high pathogenic reference strain of Iran (MRHO/IR/75/ER) which has been used previously in some clinical trials. Whole killed parasites of both strains were inoculated in the hind footpad along with imiquimod as a Th1 adjuvant. BALB/c mice immunized in three doses at two weeks intervals and challenged with reference strain parasites, two weeks after the last immunization. Parasite burden by limiting dilution assay and IFNγ, IL4 mRNA expression by qRT-PCR were performed four weeks post challenge. Lesion size measurements were followed for twelve weeks.
The group vaccinated with DA39+imiquimod, revealed significant decrease in the parasite burden compared to the reference strain group ($3.41\times10^8\pm2.19\times10^8$ and $3.21\times10^9\pm1.2\times10^9$ live parasites/lymph node, respectively; p=0.05). In DA39+imiquimod group, smaller lesion size in the footpad was observed from week five to week twelve (p<0.001). Mean fold increase of IFNγ mRNA also exhibited a dominant Th1 response in DA39+imiquimod compared to reference strain group (350.80±10.55 and 198.61±45.43, respectively; p=0.01).
The results indicated that using a low pathogenic strain of *L. major* could lead the immune responses toward partial protection against cutaneous leishmaniasis.

P4.08.39
Antiparasitic effects of Foeniculum vulgare extracts against Toxoplasma gondii

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Toxoplasma gondii is a common parasite of animals and humans and can cause serious opportunistic infections. However, the majority of infections are asymptomatic, possibly because the organism has co-evolved with its many vertebrate hosts and has developed multiple strategies to persist asymptomatically for the lifetime of the host. Fennel (Foeniculum vulgare) is a plant species in the genus Foeniculum, has been used in folk medicine as a medicinal plant, as well as a spice and food in many countries. This research was carried out to evaluate the antiparasitic effect of Fennel (Foeniculum vulgare) extracts (aqueous - ethanol - acetone) against Toxoplasma gondii (T.gondii) in vivo.
The effect of these extracs against the proliferation of T. gondii was evaluated by animal testing.
T. gondii in a dose-dependent manner compared with sulfadiazine. After T. gondii invasion, the viability of T. gondii-infected mice treated with Foeniculum Vulgare extracts (aqueous - ethanol - acetone). 15 groups of BALB/c mice selected to during the period of the experiment. The animals were infected with T. gondii RH strain and received single dose from each extracts (100-200-300-400-500 µg/ml) intraperitonally, with survival and the immune response monitored.
These results demonstrate Foeniculum Vulgare extracts (aqueous - ethanol - acetone) not only induced anti-T. gondii effects causing the reduction of T. gondii tachyzoites in the spleen of infected host but also has antiparasitic properties, with lower tissue parasitism observed in infected animals.

P4.08.40
Serum nitric oxide levels in subjects with high bilirubin value in comparison of normal healthy control subjects

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Introduction: Bilirubin is excreted in bile and urine, and elevated levels may indicate certain diseases. Serum bilirubin are inversely related to risk of certain heart diseases. Since nitric oxide (NO) is produced by three types of Nitric Oxide Synthases (NOSs), rapid changes in stable oxidized metabolites (nitrite and nitrate) in the tissues and blood should be represented by the amount of stable forms in serum and may reflect changes in the body. Objective: The aim of this study was to evaluate the correlation between nitric oxide (nitrite & nitrate) production and bilirubin levels in serum. Methodology: The serum samples were collected from individuals with high levels of bilirubin and normal range controls. Nitrite was measured by a Griess reaction while nitrate was measured using the enzymatic one step assay with nitrate reductase. Results: The total 36 samples (18 normal range (N) and 18 high bilirubin values (H)) were evaluated for the NO levels. The age group varies from 4-70 & 5-65 for normal & high levels of bilirubin, respectively. The levels of bilirubin in the normal range & high values varies from 0.81-0.98 (mean=0.88±0.01) & 1.12-20.18 (mean=5.96±2.07), respectively. When the nitrite (14.48±1.05 µM versus 13.42±0.96 µM, P>0.05) and nitrate (25.94±2.04 µM versus 24.69±1.53 µM, P>0.05) levels were compared between these groups no significant differences were observed. Conclusion: There is no correlation between nitric oxide production and the levels of bilirubin.

P4.08.41
CD4-CD8- T cells, B cells and complement are critical for resolution of Babesia microti infection in the absence of CD4

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Babesiosis caused by the protozoan parasite Babesia microti is an emerging infectious disease in the United States. In immunocompromised patients, particularly in those treated with the B cell depleting drug rituximab, babesiosis is severe and persistent. To understand how the immunocompromised host clears B. microti, we developed a mouse model. Intense, persistent parasitemia was observed in athymic mice and in mice lacking MHC class II. Surprisingly, in cd4-/- mice, parasitemia was intense but resolved. In their spleens, CD4-CD8- (DN) T cells, CD8+ T cells and B cells expanded. Parasitemia resolved in cd4-/- cd8-/- mice, but persisted in cd4-/- igh6-/- mice. In cd4-/- mice, the rise in circulating Babesia specific IgGs was delayed but concomitant with resolution of parasitemia. All subisotypes were produced. Fcγ receptors were dispensable for resolution of parasitemia, but C3 was required. DN T cells producing both IL-21 and IFN-γ expanded, but not as fast as CD4+ T cells producing both cytokines in wild-type mice. In contrast, DN T cells producing IFNγ alone expanded as fast as their CD4+ T cell counterparts. DN T cells failed to produce IL-21 alone whereas CD4+ T cells producing IL-21 alone quickly expanded. Parasitemia resolved in cd4-/- mice treated with a neutralizing antibody against IFN-γ. IL-21, however, appeared critical. Our studies indicate that DN T cells and B cells are key for resolution of B. microti infection in the absence of CD4. Despite the production of IgGs, resolution of infection requires complement activation, but not Fc receptors.

P4.08.42
Alteration of s-Fas and s-FasL ligand expression during canine visceral leishmaniasis

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Apoptosis has been implicated in canine visceral leishmaniasis (VL). A major regulator of apoptosis is the Fas/Fas ligand (FasL) system involved in down-regulation of immune reactions and in T cell-mediated cytotoxicity. Fas (CD95 or APO-1) is a member of the TNF/NGF receptor superfamily which can be expressed in transmembrane or soluble forms. The role of sFas is not understood fully, but is thought to inhibit Fas-mediated apoptosis through neutralizing its ligand, FasL. sFasL is produced by cleavage of the membrane-bound FasL by unidentified metalloproteases. In this study, we measured the levels of sFas and sFasL in extract from spleen dogs with VL and control. Twenty two dogs presenting clinical symptoms compatible with leishmaniasis and diagnosis confirmed by the detection of anti-Leishmania antibodies were studied and eight healthy dogs, from Araçatuba City, Brazil, were used as control. Samples from spleen tissue (300 mg) for each dog were homogenized with an Ultraturrax T50 IKA (Labortechnik, Staufen, Germany) apparatus for 5 min at 4°C. Homogenized tissue was centrifuged at 10,000 × g for 15 min, and the supernatant stored at -20ºC. sFas and sFasL concentration in spleen homogenized were assayed in duplicate by ELISA (Enzyme Linked Immuno Sorbent Assay Kit, Life Science, Houston, USA). sFas levels were lower in dogs with VL when compared to in healthy controls, while sFasL was increased in dogs with VL when compared to healthy controls. These findings suggest that the molecules sFas and sFasL are involved in immunopathogenesis of canine visceral leishmaniasis.

P4.08.43
Phagocytosis of promastigotes of Leishmania amazonensis by B-1 cells

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Leishmaniasis are caused by Leishmania parasites which mainly infect macrophages. B-1 cells are a subpopulation of B cells resident in the peritoneal and pleural cavities of mice. These cells are able to differentiate in vitro into mononuclear phagocyte-like cells with phagocytic properties. Nevertheless, the role of B-1 cells in phagocytosis of Leishmania has not yet been clarified. This study aimed to investigate the phagocytosis of L. amazonensis by mononuclear phagocytes derived from B-1 lymphocytes in vitro and in vivo. Phagocytes derived from B-1 cells were able to internalize in vitro promastigotes of L. amazonensis. Our results showed that phagocytic index was higher in phagocytes derived from B-1 (2885 ± 264.46), compared to peritoneal macrophages (1236.33 ± 355.92) and bone-marrow-derived macrophages (421.5 ± 6 36; p <0.001). L. amazonensis stimulated TNF-α production by B-1 cells. TNF-α amounts were higher in B-1 cells that phagocytosed L. amazonensis, as compared with B-1 control (cells non-stimulated with parasites). The in vivo phagocytic ability of B-1 cells was also evaluated. A flow cytometric method was performed to evaluate the peritoneal B-1 cells-L. amazonensis interaction. Parasites were labeled with stable intra-cytoplasmic fluorocrome, 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) and subsequently inoculated in peritoneal cavities of mice. After 8, 16 or 24 h peritoneal cells were collected and labeled with anti-CD19 and anti-CD23. We found that many CD19+ CD23- cells were positive for FITC, which we attributed to phagocytosis of the parasite by B-1 cells. This is the first report that B-1 cells can internalize Leishmania species.

P4.08.44
A Mechanistic Insight Into Arabinosylated Lipoarabinomannan (Ara-LAM) Mediated Modulation Of IFN-γ signaling and Different Transcription Factors During Experimental Visceral Leishmaniasis

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Visceral Leishmaniasis (VL), commonly known as kala-azar is one of the most widespread infectious diseases affecting the third world countries. The hallmark of VL, caused by *Leishmania donovani*, is a strong suppression of the host-protective anti-leishmanial responses in a susceptible host. Arabinosylated Lippoarabinomannan or Ara-LAM isolated from an avirulent strain *Mycobacterium smegmatis* is a TLR2 ligand and has potent immunomodulatory properties such as up-regulation of pro-inflammatory cytokines, NO production and induction of TLR-2 mediated signaling in macrophages. In the present study, we have demonstrated that Ara-LAM confers protection against leishmanial pathogenesis via the modulation of IFN-γReceptor expression as well as its downstream signaling. By inhibiting the ceramide generation, Ara-LAM maintained the cell membrane integrity of the parasitized macrophages and increased the MHC-II expression. Along with this, Ara-LAM, significantly upregulated the phosphorylation level of JAK1 and JAK2 following IFN-γ-stimulation in infected macrophages, which ultimately induced the nuclear translocation of STAT1. Not only that, Ara-LAM was also found to differentially regulate Interferon Reulatory Factors (IRFs), among which IRF8 is directly involved in IFN-γ signaling . Therefore, our study demonstrates that by modulating IFN-γ responsiveness in *L. donovani* infected macrophages, Ara-LAM helps in the improved antigen presentation as well as better parasite clearance.

P4.08.45
Leishmania-induced biphasic ceramide generation in macrophages is crucial for uptake and survival of the parasite

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The initial macrophage-Leishmania donovani interaction results in the formation of membrane platforms, termed lipid rafts that help in the entry of the parasite. Therefore, it is imperative that the parasite designs a strategy to modulate its uptake and survival within the macrophages. Herein, we report Leishmania-triggered biphasic ceramide generation. In the first phase, L. donovani promastigotes induce activation of acid sphingomyelinase (ASMase), which catalyzes the formation of ceramide from sphingomyelin. Inhibition of ASMase resulted in reduced uptake and infection with the parasite. In the second phase, de novo synthesis generates ceramide that reduces the cellular cholesterol level and displaces the cholesterol from the membrane, leading to enhanced membrane fluidity, disruption of rafts, and impaired antigen-presentation to the T cells. The results reveal a novel role for ceramide in the perspective of L. donovani infection and help formulate an antileishmanial strategy that can possibly be applied to other intracellular infections as well.

P4.08.46
Leishmania infantum excreted/secreted proteins impairs differentiation and modulates cytokine production in human dendritic cells and monocytes

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Several data have shown that Leishmania (L.) parasites as well as some bioactive compounds they secrete are able to modulate innate immune responses through effects on dendritic cells (DCs) and macrophages. We previously demonstrated that Leishmania promastigotes interfere with differentiation and cytokine production in

human DCs and monocytes. In this study, we examined the immunomodulatory effects of 4 Leishmania excreted/secreted (ES) proteins that have been identified and characterized in our laboratory: Elongation Factor 1-alpha (EF-1a), AAA-ATPase and proteins with unknown functions P15 and P23. We analyzed the capacity of each ES protein to modulate IL-12p70, IL-10 and TNFa production in human monocytes and DCs and to interfere with DCs differentiation. DCs were able to produce significant levels of IL-12p70 when stimulated by ES proteins, unlike monocytes. In presence of IFNg, ES proteins similarly induced a significant enhance of IL-12p70 production by DCs as well as monocytes. ES proteins were also able to induce significant IL-10 and TNFa production by DCs and monocytes, with higher levels observed with P15 and AAA-ATPase. Interestingly, the presence of ES proteins during DCs differentiation led to a significant decrease in CD1a. P15 and AAA-ATPase proteins were able to inhibit up to 80% of CD1a expression. We showed that ES proteins induce IL-12p70 production by DCs, suggesting that they could be potential candidate vaccines. Furthermore, ES proteins and particularly P15 and AAA-ATPase strongly interfere with DCs differentiation suggesting a possible involvement of these proteins in mechanisms established by the parasite for its survival.

P4.08.47

Leishmania infantum excreted/secreted proteins impairs human dendritic cells differentiation and modulates cytokines production by human dendritic cells and monocytes

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Several data have shown that Leishmania (L.) parasites as well as some bioactive compounds they secrete are able to modulate innate immune responses through effects on dendritic cells (DCs) and macrophages. We previously demonstrated that Leishmania promastigotes interfere with differentiation and cytokine production in human DCs and monocytes. In this study, we examined the immunomodulatory effects of 4 Leishmania excreted/secreted (ES) proteins that have been identified and characterized in our laboratory: Elongation Factor 1-alpha (EF-1a), AAA-ATPase and proteins with unknown functions P15 and P23. We analyzed the capacity of each ES protein to modulate IL-12p70, IL-10 and TNFα production in human monocytes and DCs and to interfere with DCs differentiation. DCs were able to produce significant levels of IL-12p70 when stimulated by ES proteins, unlike monocytes. In presence of IFNγ, ES proteins similarly induced a significant enhance of IL-12p70 production by DCs as well as monocytes. ES proteins were also able to induce significant IL-10 and TNFα production by DCs and monocytes, with higher levels observed with P15 and AAA-ATPase. Interestingly, the presence of ES proteins during DCs differentiation led to a significant decrease in CD1a. P15 and AAA-ATPase proteins were able to inhibit up to 80% of CD1a expression. These results showed that ES proteins induce IL-12p70 production by DCs, suggesting that they could be potential candidate vaccines. Furthermore, ES proteins and particularly P15 and AAA-ATPase strongly interfere with DC differentiation, suggesting a possible involvement of these proteins in mechanisms established by the parasite for its survival.

P4.08.48

Role of neutrophils in parasite persistence and in host immunity during the initial phase of *L. chagasi* experimental infection

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Leishmania chagasi is the aetiological agent of visceral leishmaniasis (VL). In the last years the role of neutrophils or polymorphonuclear cells (PMN) during *Leishmania spp*. infection has been the subject of great interest from the scientific community. This immune cell population is described to play protective functions but also with responsibility in disease progression.
The main purpose of this work was to evaluate the role of PMN during the initial phase of experimental *L. chagasi* infection, by immunophenotyping different leukocyte subsets recruited to the site of parasite inoculation, quantification of chemokine and Toll-like receptors expression on the infection site and quantification of cytokine expression in internal organs after PMN depletion in C57BL/6 mice.
It was observed that the absence of PMN did not interfere with chemokine and TLR-4 expression and leukocyte recruitment to the site of infection, although it seems to be a delay in parasite elimination. Nevertheless, *Leishmania* parasites spread to cervical lymph nodes. In these organs, PMN depletion influences cytokine profile induced by the parasite in the beginning of infection (3 hours), leading to expression of pro and anti-inflammatory cytokines (IL1-β, TNF-α,TGF-β and IL-17). 144 hours post infection, serine protease granzyme B production along with IFN-γ inhibition appear to be a reflection of the initial deficiency of PMN.
With this work we provided evidence that PMN have a major role on cellular immunity during the early phases of *L. chagasi* infection that could be important for the development of alternative strategies for the control of VL.

P4.08.49

Salivary antigen SP32 is the immunodominant target of the antibody response to Phlebotomus papatasi bites in humans

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Introduction: Zoonotic cutaneous leishmaniasis (ZCL) due to *Leishmania major* is highly prevalent in Tunisia and is transmitted by a hematophagous vector, *Phlebotomus papatasi (P. papatasi)*. While probing for a blood meal, the sand fly injects saliva into the host's skin, which contains a variety of highly immunogenic compounds. We recently showed that the presence of anti-saliva antibodies was associated with an enhanced risk for leishmaniasis and identified the immunodominant salivary protein of *P. papatasi* as a protein of approximately 30kDa. **Material and methods:** We cloned and expressed in mammalian cells two salivary proteins from *P. papatasi*, PpSP30 and PpSP32, with predicted molecular weights close to 30kDa. We tested both recombinant proteins in serological experiments using sera from individuals living in endemic areas for ZCL and naturally exposed to *P. papatasi* bites. While recombinant PpSP30 (rPpSP30) was poorly recognized by sera, rPpSP32 was strongly recognized by all the tested samples. The binding of IgG antibodies to native PpSP32 was inhibited by the addition of rPpSP32. Consistently, experiments in mice showed that PpSP32 induced the highest levels of antibodies compared to other *P. papatasi* salivary molecules while PpSP30 did not induce any detectable antibodies. **Conclusion:** We demonstrate that PpSP32 is the immunodominant target of the antibody response to *P. papatasi* saliva. We also show that the recombinant form of PpSP32 is similar to the native one and represents a good candidate for large scale testing of human exposure to *P. papatasi* bites and perhaps for assessing the risk of contracting the disease.

P4.08.50

Role of P2X7 receptor in the response of phagocytes to Plasmodium chabaudi infection

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Malaria is characterized by intense activation of the immune system that seems to contribute to protection and pathogenesis. ATP recognition by P2X7R in immune cells is important for cell activation and death. In this study, we evaluated the effects of P2X7R-mediated signaling in phagocytes during infection with *Plasmodium chabaudi*. C57BL/6 and P2X7R$^{-/-}$ mice were infected with *P. chabaudi* infected red blood cells (iRBC) and clinical parameters were measured.

Kinetics of phagocytic cells and cell death in the spleen of infected animals was determined by flow cytometry. *In vitro* assay was performed with bone marrow derived macrophages (BMDM) from both mice strains in presence of iRBC and ATP. Between days 9 and 17 post-infection, 80% of $P2X7R^{-/-}$ mice died, while the C57BL/6 group presented no death. Clinical manifestations and their improvement were delayed in knockout mice in comparison to wild-type. $P2X7R^{-/-}$ mice showed a higher number of $CD11b^{+}$ cells at day 7 post-infection compared to the wild-type mice. The percentage of cell death found in spleen in the same day was lower in knockout group. Moreover, BMDM from C57BL/6 mice became significantly more sensitive to ATP when exposed previously to iRBC in a P2X7-dependent way when compared to that without iRBC. Thus, mice lacking the P2X7R seem to be more susceptible to *P. chabaudi* infection, presenting more severe clinical manifestations. Furthermore, the elevated number of phagocytes in spleen from $P2X7R^{-/-}$ mice when compared to C57BL/6 mice could be due to the reduced rate of cell death found in those cells.

P4.08.51
Relationship between parasite load and renal lesion in acute experimental Chagas disease

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Introduction: The acute phase of Chagas` disease is documented as being multifactorial. Besides the heart another affected organ is the kidney. Unfortunately, the pathophysiology and mechanisms of renal dysfunction associated with acute Chagas disease remains unclear. Thus, we evaluate the Influence of Parasite Load on Renal Function in Mice Acutely Infected with *Trypanosoma Cruzi*.
Methodology/results: Low, medium and high parasite loads were generated by infecting C57BL/6 mice with $3x10^2$, $3x10^3$ or $3x10^4$ "Y" strain trypomastigotes. By analyzing different parameters related to kidney injury, we demonstrated that mice infected with *T. cruzi* trypomastigotes show increased renal injury in a parasite load-dependent manner. The infections with low, medium and high doses of trypomastigotes resulted in reduced urinary excretion and creatinine clearance. We also observed a marked elevation in the ratio of urine volume to kidney and body weight, blood urea nitrogen, chloride ion, nitric oxide, pro- and anti-inflammatory cytokines as well as the number of leukocytes in the blood and/or renal tissues of infected mice. In addition we showed the presence of the parasite in the cortical/medullary and peri-renal region, an increase in the inflammatory infiltrate and in the vascular permeability of the kidney.
Conclusion: These data support the idea that *T. cruzi* impairs kidney function in a parasite load-dependent manner. Moreover, the data allow us to suggest that in addition to the cardiovascular effects, renal injury may be an important indicator of the magnitude of the infection and the severity of the illness.
Key words: kidney, *Trypanosoma cruzi*, parasite load

P4.08.52
Temporal transcriptomic dynamics of human macrophage response to Leishmania major infection

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Leishmania are pathogenic protozoan, obligate intracellular pathogens that develop mainly in macrophages. These cells act as a safe shelter for the pathogens and also as effector cells responsible for their killing. Macrophages are able to secrete a diverse set of regulators known to influence the physiological functions and differentiation of neighboring cells to trigger an adaptive immune response of the protective Th1-type cells, whereas parasites have developed a wide range of mechanisms to circumvent the host's immune responses. Most of our understanding of this host-parasite conflict has been gleaned from studies investigating the macrophage infection at unique time points after infection.
Here we have analyzed the transciptomic profile of monocyte-derived human macrophages at different time points during the first 24h upon in vitro infection, using a high-throughput microarray platform.
The gene expression profile of 17,838 genes showed high expression variability among human donors at different time points post-infection. To circumvent this, only genes that underwent common modulation in the three donors were analyzed. Cross comparison allowed the identification of a common set of expressed genes coding for inflammatory and chemotactic molecules, transcription factors, apoptosis inhibition such as members of the NR4A sub-family, glucose synthesis and heme metabolism such as HMOX-1.
Our findings suggest that transcriptome dynamics of macrophages early during the first 24h post infection enables the identification of the different key pathways deregulated at early time points upon L. major invasion.

P4.08.53
MAP kinases activation in macrophages during infection with Neospora caninum

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Due to the high prevalence and economic importance of neosporosis by N. caninum, the development of safe and effective vaccines against this parasite preventing abortions and vertical transmission in cattle has been a priority in the field and it is crucial to limit infection in natural hosts. The major aim of the present study was to approach the signaling mechanisms in bone marrow-derived macrophages (BMDMs) under N. caninum infection to understand the mechanisms of immune response to the development of an effective method. It was evaluate the immune response from BMDMs infected with N. caninum and stimulated with its soluble antigen (NLA), and the role of MAPKs. The immune response in BMDMs was determined from IL-12 and IL-10 production in BMDMs culture supernatants treated with inhibitor of MAPKs and infected by N. caninum. Phenotyping of BMDMs was determined by expression of costimulatory molecules and MHC. According to these results, the p38 inhibitor (SB203580) was the inhibitor with the most relevant results. Thus, in vivo assay was performed with immunization of NLA, NLA plus SB203580 inhibitor in C57Bl/6 mice. These experiments demonstrated that the SB203580 is more important in the immune response by N. caninum, as a modulation of cytokines production, expression of the costimulatory molecules. These results clearly showed that N. caninum can use mainly the p38 via to regulate the host immune response and to develop an infection. Therefore, this piece of information can be useful to develop a cellular vaccine against neosporosis.

P4.08.54
Seroepidemiology of toxoplasmosis in childbearing women of eastern azarbaijan - iran

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Background: *Toxoplasma gondi* (*T. gondi*) is cause to the most common parasitic infection in the world. Congenital transmission, prenatal mortality and abortion are major problems of T. gondi. Prevalence of toxoplasmosis is high in Iran. Current literature reviewed in this paper reveals results pertaining to various regions of Iran. The present study was designed to evaluate the seroprevalence of toxoplasmosis in childbearing women of Azerbaijan.

Methods: In this cross-sectional e-study, we evaluated 1659 women in childbearing age from different cities of Eastern Azerbaijan (Tabriz, Maragheh, Ahar, Marand, Sarab, Miane) from July 2009 to August 2010. Women aged between 20 to 40 years (childbearing period) and seeking prenatal care were enrolled in the study. Subjects' sera were examined with indirect fluorescent antibody (IFA).
Results: A total of 1659 subjects were examined. Titers ranged 1:100-1:800. 889 (54.13%) subjects were seropositive. The highest frequency of seropositivity was shown in 1:200 dilution (36.08%) and in subjects from Maragheh (84% of 211 subjects). There was a direct linear relationship between seropositivity and age ($p<0.001$). Also, seroprevalance of toxoplasmosis was higher in subjects with primary school/lower educational level ($p<0.001$) and subjects living in rural regions ($p<0.001$).
Conclusions: More than 50% of women in childbearing age were at risk of toxoplasmosis in Eastern Azerbaijan. Increasing seroprevalance of toxoplasmosis with age was a predictable result because of longer time of exposure to the bacterium. The relationship between increasing seroprevalence and lower educational level as well as living in rural region is in line with latest epidemiological findings which also show such relationships due to lower socioeconomic status.

P4.08.55
A successful safety trial of gentamicin-attenuated Leishmania infantum vaccine in an endemic area in Southeast of Iran

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Leishmania infantum is an obligatory intracellular protozoan and causative of visceral leishmaniasis in human and dogs. Vaccine development in the reservoir host, the domestic dog, is a current research priority. The aim of this study was to conduct safety trial of gentamicin-attenuated L. infantum (L. infantum H-line) in dogs in the endemic area of Southeast Iran.
The household dogs were vaccinated subcutaneously with L. infantum H-line. The prospective incidence of natural infection by this vaccine was assessed during a two-year follow up period. The number of animals vaccinated and non-treated control groups was 70 and 50 respectively. The safety of the vaccine on the interruption of the endemic area transmission of the disease was assessed by monitoring the clinical signs and measuring the serum specific anti-Leishmania IgG. No clinical signs of disease including anorexia, apathy, local swelling on injection site, vomiting and diarrhea were developed or observed after the vaccine injection. Therefore, our gentamicin-attenuated L. infantum vaccine induced a significant and strong protective effect against canine leishmaniasis and the consequent transmission to human in the field.

P4.08.56
Excreted/secreted proteins of Leishmania major induce production of IFN-g and granzyme B by CD4+T cells

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Previous reports have shown that the excreted/secreted proteins released by Leishmania in the phagolysosomal compartment are strongly immunogenic and protective in mice and dogs. The protective mechanisms involve both Th1 and cytotoxic T cells. The potential immunogenicity of Leishmania major excreted/secreted proteins (LmES) was evaluated in humans using peripheral blood mononuclear cells (PBMCs) from donors whith a previous contact with L. major parasite and from appropriate healthy controls. PBMCs from all immune individuals proliferate and produce high levels of IFN-γ. Interestingly, granzyme B (GrB), used as a marker of activated cytotoxic T cells, was also highly produced by PBMCs of all immune individuals when stimulated with LmES. Total leishmania antigens also induce both IFN-γ and GrB by PBMCs from immune individuals. ell depletion experiments showed, unexpectedly, that both IFN- γ and GrB were mainly produced by CD4+ T cells after stimulation with LmES or SLA antigens. In attempt to identify the sub-population of CD4+T cells involved in IFN-γ and GrB production, CD4+CD25+ and CD4+CD25- T cell subsets were purified from the peripheral blood of a representative set of immune individuals. Our results showed that both populations produced IFN-γ and GrB when stimulated with LmES. Further experiments showed that CD4+CD25+CD127dim/- regulatory T cells were not involved in such production. Collectively, our data indicate that excreted/secreted proteins from L. major are highly immunogenic in humans constituting a potential candidate of vaccine. Our results also emphasize the involvement of potentially cytotoxic CD4+ T cells in immune response and protection against Leishmania.

P4.08.57
Polymorphisms of cytokines genes associated with ocular toxoplasmosis

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Toxoplasmic retinochoroiditis (TR) is a consequence of the congenital or acquired infection at birth with T gondii parasite. The genetic functional polymorphisms in the cytokine genes can interfere with the expression of such molecules and they play an essential role in the genetic regulation of the inflammatory response and the infectious diseases resistance or susceptibility. To consider the associated risk to polymorphisms in cytokines genes with ocular toxoplasmosis. To determine the polymorphisms prevalence in the IL-1α, IL-1β, IFN-ɣ, TNF-α, IL-10, IL-12 and IL-17R cytokines genes and P2RX7 and TLR9 receptors in general population and patients with ocular toxoplasmosis. The minisequencing technique or "ddNTP first extension" will be used Previous extraction of the genomic DNA of obtained of peripheral blood cells, the amplification of the regions that contain the SNPs to be studied will be amplified. The product will be analyzed by capillary electrophoresis by using the ABI prism 3100-Avant analyzer. The multiplex standardization for the polymorphisms evaluation in the cytokines genes and receptors already mentioned was made by defining the experimental conditions that allowed to detect several polymorphisms in a same amplification reaction with 15 samples of TR patients and 15 healthy controls. The preliminary results show that the studied polymorphisms are found in our population. There is a tendency for the $IL\text{-}10^{-1082G/A}$ polymorphism that indicates a possible association of this polymorphism to (TR) resistance and a tendency to the $TNF\text{-}\alpha^{-857C/T}$ polymorphism to be TR susceptibility associated.

P4.08.58
Immunomodulation of Leishmania infection by murine mesenchymal stromal cells in vitro

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Leishmania are protozoa that cause leishmaniasis disease in animals and humans. Despite the identification of several molecular targets, there is no vaccine for leishmaniasis or new drugs anti-leishmania. Mesenchymal stromal cells (MSCs) have been used in therapy for some disorders mediated by the immune system, but little is known about the effects of MSCs on protozoa. We evaluated the influence of MSCs on the production of NO and leishmanicidal activity of macrophages. We also investigated the interaction between MSCs from adipose tissue of C57BL/6 mice and *L. amazonensis*. NO production by activated MSCs, and by macrophages stimulated with LPS/IFN-gamma in the presence of MSCs' conditioned medium (CM) and/or aminoguanidine, was assessed by Griess. The influence of MSCs on the leishmanicidal capacity of macrophages was determined by quantifying the number of alive promastigotes after their growth *in vitro*. After 3 and 6 hours of infection, we observed the intracellular presence of amastigote forms of *L. amazonensis* in MSCs. Activation of MSCs with LPS significantly increased NO production (3 to 8-fold) suggesting that MSCs may exert microbicidal activity dependent on NO. It was also found that the CM of MSCs

inhibited NO production by macrophages by up to 95%, and increased the survival of pathogens by up to 61.6%. These results suggest that MSCs may serve as reservoirs for *Leishmania*, as well as contribute to the establishment of infection in macrophages by reducing their leishmanicidal activity.

P4.08.59
Effect of *Entamoeba histolytica* calreticulin in the immune response of patients with acute amoebic liver abscess

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Entamoeba histolytica is a human intestinal parasite that causes amoebiasis, which in Mexico is considerate as an endemic disease. Some molecules are involved in pathogenicity and virulence of *E. histolytica*. It was previously reported that *E. histolytica* calreticulin (*Eh*CRT) is a highly immunogenic protein in patients with amoebiasis. The aims of this work were to characterize the isotype of antibody response in serum samples of patients during the acute phase of amoebic liver abscess (ALA), to measure the ability of *Eh*CRT to bind human C1q protein and to define the stimulation properties of *Eh*CRT upon peripheral mononuclear blood cells (PMBC). We compared individuals with ALA and healthy subjects, which were characterized by coproparasitoscopic and ELISA assays and took a peripheral blood sample to obtain serum and mononuclear cells. PMBC were isolated and cultured in an *in vitro* system and stimulated with recombinant r*Eh*CRT. The stimulation index was calculated. Results show that the major isotypes of serum antibodies against *Eh*CRT were IgM and IgG class. Levels of IgA in serum were low and levels of IgE isotype of antibodies were extremely low. Besides, r*Eh*CRT-C1q caused the inhibition of classical complement pathway *in vitro*. PMBC of patients with ALA display a comparatively higher stimulation index to control PMBC. These preliminary results suggest that *Eh*CRT can be a useful antigen and have a modulating role to the adaptive immune response in invasive amoebiasis. Work performed with support PAPIIT/UNAM: IN226511 and CONACYT 210CO1-140990.

P4.08.60
Polymorphism of *lcr1* gene in different *Leishmania* species

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Introduction: *Leishmania* parasites are the causative agent of a wide range of diseases in human and other mammals. There is no efficient preventive measure for these diseases and their therapy remains difficult with many side effects. Hence, search for a vaccine for this disease is important. *Lcr1* is an immunogenic gene discovered in *Leishmania infantum chagasi* that causes immunologic response in human, so its molecular identification will be very important. The aim of this research is to study the DNA sequence of *lcr1* gene by molecular methods in some species of *Leishmaina* parasites.
Materials and Methods: *Two Leishmania infantum* isolates (strains MHOM.IR.04.IPI-UN10 and MCAN/IR/2010/Meshkinshahr) and one *Leishmania tropica* isolate (strain MHOM.AF.88.KK27) were cultured in NNN media and genomic DNA were extracted. Parasite species were verified as by sequencing of ITS1 gene. *Lcr1* gene was amplified by *lcr1* specific primers in all the strains. The PCR products were electrophoresed and their restriction fragment patterns were defined. All PCR products were sequenced at least twice by forward and reverse primers specific for *lcr1.* The DNA sequences were analyzed by softwares and the correct sequence were determined.
Conclusion: Our results show that *lcr1* gene is completely conserved between the two strains of *Leishmania infantum* and it is nearly conserved in *Leishmania tropica*. Conservation of this immunogenic gene (*lcr1)* adds to its value as an immunogenic molecule with potential in prevention, diagnosis, and treatment of leishmaniasis.

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P4.08.61
Analysis of the cardiac disorder caused by the intracellular parasite *Toxoplasma gondii*

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Toxoplasma gondii is known to cause serious heart disease. The detailed mechanism, however, still remains to be investigated. In this study we described experimental heart diseases caused by *T. gondii* in wild type (WT) and interferon gamma knockout (GKO) mice as a model of immunocompetent and immunocompromised hosts, respectively. C57BL/6 WT mice and GKO mice with C57BL/6 background were infected perorally with Fukaya strain of *T. gondii*. Parasite load, mRNA expression levels of cytokines, chemokines, chemokine receptors, and adhesion molecules, types of infiltrating cells, and pathologic findings were analyzed. Parasite load in WT mouse hearts reached a peak 14 days after infection, and decreased thereafter, whereas that in GKO mouse hearts kept increasing. In WT mouse heart the expression levels of IFN-γ, CCL2, CCL3, CCL4, CCL5, CXCL2, CXCL10, CCR2, CCR5, CCR7, CXCR2, CXCR3, and ICAM-1 increased, reaching peaks 14-28 days after infection. On the other hand, in GKO mice these molecules, except IFN-γ, CXCL10, CXCR3, and ICAM-1, increased slightly. The expression levels of CXCR4 and CXCR5 were absent in both mice during the course of infection. While $CD4^+$, $CD8^+$, monocyte, and neutrophil cell numbers increased significantly in the infected hearts of WT mice, the neutrophils in the infected hearts of GKO mice increased dramatically. In the heart of WT mice increased cell types controlled the infection, although increased neutrophils in GKO mice could not. Thus, IFN-γ plays a pivotal role in the pathogenesis of heart disease caused by *T. gondii*.

P4.08.62
Adoptive transfer of Treg ($CD4^+Foxp3^+$) cells to B6 mice during *Toxoplasma gondii* infection induces down-modulation of exacerbated inflammatory immune response but does not generate protection

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Toxoplasmosis is an infection disease affecting one third of the world population and is caused by the protozoan parasite *Toxoplasma gondii*. T_H1 immune response is necessary for protection, IFN-γ being the major mediator of resistance. An exacerbated T_H1 immune response however, is detrimental. Regulatory T (Treg) are $CD4^+Foxp3^+$ cells that actively suppress pathological and physiological immune responses, thereby contributing to the maintenance of immune homeostasis. It has been reported that during acute toxoplasmosis in B6 mice, Treg cell number decreases. In order to analyze if Treg cells are involved during *T. gondii* infection, we carried out adoptive transfer of Treg cells to infected mice. We observed a decreased mortality when Treg cells were adoptively transferred but an increased number of cysts in brain was detected. A decreased pathology in the small intestine, a decreased production of IFN-γ in serum, a decreased activation in $CD4^+$ cells, and a decreased production of IFN-γ by $CD4^+$ cells was also observed in the same mice. IL-2 levels in sera however, remain unchanged. All these results suggest that adoptive transfer of Treg cells during *T. gondii* infection down-modulate the exacerbated immune response, but it does not generate protection in B6 mice.

P4.08.63
Natural isotypic response against potential vaccine candidate *Plasmodium falciparum* Glutamate-Rich Protein (GLURP) in individuals living in Brazilian malaria-endemic area

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The *Plasmodium falciparum* Glutamate-Rich Protein (GLURP) is a malaria vaccine candidate undergoing clinical trials. Studies have shown association between high levels of anti-GLURP antibodies with low parasitemia and protection against clinical malaria. The aim of this study was to evaluate the antibody response profile induced by GLURP in naturally exposed individuals from a Brazilian endemic area. The study was carried out in the farming area of Colina (CL) and the riverside fishing community of Ribeirinha (RB), Rondonia state. The population of CL consists of migrants from non-endemic areas, and the population of RB consists of natives from the Amazon Basin. The antibody response against immunodominant regions of the GLURP (R0 and R2) and synthetic peptides (*R0*: P3, P4, P5, P8, P9, P10, P11, S3; *R2*: S4) was evaluated by ELISA. The results showed a high prevalence of individuals with antibodies against R0 (72% and 92% in CL and RB groups, respectively) and R2 regions (79% and 97% in CL and RB groups, respectively). The R0-induced antibodies were predominantly IgG1 and R2-induced antibodies were predominantly of cytophilic subclasses. S4 and P11 epitopes were identified as immunodominant B-cell epitopes. However, in RB group, P3 and S3 epitopes induced higher levels of cytophilic antibodies. Our results show that GLURP is immunogenic in natural conditions of exposure and the seropositivity to GLURP increases with exposure. Also, GLURP is able to induce cytophilic antibodies that can participate of protective acquisition immunity. In conclusion, our results highlight the importance of GLURP like a malaria vaccine candidate.

P4.08.64
Th2-skewed response depends on Insulin-like growth factor I in Leishmania (Leishmania) major-infection

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In Leishmania infection extrinsic insulin-like growth factor-I (IGF-I) favors the parasite proliferation and the infection development. To study the role of constitutively expressed macrophage intrinsic IGF-I in Leishmania infection, we evaluated the parasitism under optical microscopy and IGF-I mRNA expression by qRT-PCR and confocal microscopy in Leishmania (Leishmania) major-infected RAW 264.7 macrophage cell line upon IGF-I mRNA silencing with 150µM small interfering RNA (siRNA) and Th2 cytokine IL-4 (2ng/ml) and IL-13 (5ng/mL) stimuli. siRNA treatment of cells resulted in 70% reduction of IGF-I mRNA expression. The parasitism in the control without IGF-I siRNA was 149 (median) parasites per 100 cells. With siRNA treatment it decreased to 93 ($p<0.05$). Cells under IL-4 plus IL-13 stimuli had the parasitism increased to 160. In siRNA-treated cells with IL-4 and IL-13 stimuli instead of an increase we observed a decrease to 102. Using individual cytokine stimulus, IL-4 stimulus increased the parasitism to 171 but in siRNA-treated cells IL-4 stimulus did not induced an increase but a decrease to 87. Similarly IL-13 stimulus increased the parasitism to 157 but in siRNA-treated cells IL-13 stimulus induced a decrease to 76. Expression of IGF-I mRNA expression accompanied the increase or decrease of parasitism. These results suggest that IGF-I is directly related to parasitism and that even with the cytokine stimuli the presence of IGF-I is required to promote susceptibility, strongly suggesting the dependence of the presence of IGF-I for the effect of Th2 cytokines in Leishmania infection.
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P4.08.65
Modulation of T helper immune response by photodynamic therapy in experimental leishmaniasis

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Cutaneous Leishmaniasis is a parasitic disease caused by protozoan flagellates that belong to the *leishmania* genus. *Leishmanias* develop inside the macrophage and Th1 cytokines are essential in protecting against all *Leishmania* infections due to NO production. Conversely, the Th2 cytokines are associated with susceptibility. Photodynamic Therapy (PDT) utilizes an otherwise nontoxic chemical to create oxidative damage in cells when exposed to light. In this study, we analyzed the local and systemic expression of Th1 and Th2 cytokines in BALB/c mice experimentally infected with *L. braziliensis* after PDT-Aminolevulinic acid. Skin foot pad and spleens from infected animal, submitted or not to 1, 2 or 3 PDT sections were homogenized, centrifuges and supernatants were used for cytokines titration by CBA. Parasite burden were evaluated in skin imprints'. We observed that IFN-gamma and IL-4 levels were higher in skin lesions than in spleens whereas levels of TNF-alpha, IL-2 and IL-5 were similar. Also, after a single PDT an increase of IL-4 levels and a decrease of IFN levels were observed only on skin samples. After the second and the third sections, levels of these cytokines were restored. Levels of TNF-alpha, IL-2 and IL-5 were not affected by PDT in any therapeutical approach used in this study. Moreover the parasites number decreased after a first PDT section and maintaining reduced during consecutive sections (about 4 times). This data suggested that a single PDT section affect Th1/Th2 balance, probably due to a direct effect on T lymphocyte or an indirect effect on *Leishmania* lyses.

P4.08.66
Th17 and Tc17 cells on pregnant women seroposite and seronegative for toxoplasmosis

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The immune response is an important mechanism to control infection by *Toxoplasma gondii*. Is clearly established the major role of Th1 cytokines, however, e role of IL-17 is not clearly established, especially during pregnancy. Cells that produce IL-17 (Th17) are responsible for the recruitment of neutrophils, important early in the control of parasite infection. The objective was to evaluate the synthesis of cytokines (Th1 and Th2) by mononuclear cells after culture with live tachyzoites of *T. gondii* and to characterize the role of Tc17 and Th17 cells in pregnant and non-pregnant seronegative and seropositive women for toxoplasmosis. CBA's method was used to measure cytokine (IL-2, TNF-α, IFN-γ, IL-4, IL-5 and IL-10) and immunophenotyping method for characterizing and CD8+ and CD4+ cells expressing IL-17 (Tc17 and Th17). The addition of live tachyzoites in cultures induced a significant increase on the synthesis of IL-5, IL-10 and TNF-α by seronegative pregnant women. In cell cultures stimulated with live *T gondii*. the expression of IL-17 was significantly higher on CD4+ T cells in relation to the CD8+. Furthermore, the addition of *T. gondii* promotes a significant increase of IL-17 on CD4+ cells only on seropositive non pregnant women. We conclude that both, CD4+ and CD8+ T cells are able to produce IL-17 and that there is a repertoire of Th17 cell specific against *T. gondii*, on seropositive non-pregnant women, suggesting that these cells contribute on eliminating the parasite.
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P4.08.67
Inflammatory response of a prostate stromal cell induced by live *Trichomonas vaginalis*

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Trichomonas vaginalis cause urogenital infectious diseases in men as well as women. More than 50% of men is reported to be suffered by prostatitis in their life. *T. vaginalis* has been accounted for about 10% of pathogenic organisms causing prostatitis. The objective of this study is to identify whether *T. vaginalis* is able to induce inflammatory response in prostate stroma. To address this goal human prostate stromal cell line, WPMY-1, and *T. vaginalis,* were used. When WPMY-1 was stimulated with live trichomonads, various inflammatory mediators including IL-8, MCP-1, showed increased production. Increased expression of TLR4 mRNA on WPMY-1 was suggested that this receptor may involved in upstream pathways of signaling on stimulation of *T. vaginalis* to the cell. Signalling molecules such as ROS, MAPK and NF-κB were also increased when WPMY-1 cocultured with *T. vaginalis*. Agents inhibiting of ROS, MAPKs, NF-κB activities significantly reduced IL-8 and MCP-1 production compared to control. These findings suggested that ROS, MAPKs and NF-κB may be involved in IL-8 and MCP-1 production. On the other hand, the culture supernatant of WPMY-1 coincubated with *T. vaginalis* (=WPMY-1 conditioned medium) including IL-8 and MCP-1 induced increased migration of human neutrophil and monocyte (THP-1) compared with those of culture supernatant of WPMY-1 without trichomonads. In conclusion, it is suggested that *T. vaginalis* is able to induce inflammatory responses in prostate stroma via production of IL-8 and MCP-1.

P4.08.68
Do different T cell receptor sequences dictate the phenotype of memory CD8 T cells in the Toxoplasma gondii-infected brain?

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The protozoan parasite *Toxoplasma gondii* is the most common parasitic infection in man. In immunocompetent hosts the acute phase of infection is generally asymptomatic and proceeds to the chronic phase, which is incurable and defined by tissue cyst formation preferably in the brain. The parasite poses a serious health threat to immunocompromised individuals, especially AIDS patients. IFNγ and CD8 T cells are essential for the control of chronic *Toxoplasma* infection.
We are defining how affinity of TCR-MHC interaction shapes the phenotype of memory CD8 T cells in the chronically *Toxoplasma*-infected brain. I am using CD8 T cells from three lines of transnuclear mice that harbour in its endogenous locus a different T cell receptor expressed on CD8 T cells specific for the same *Toxoplasma* antigenic epitope Rop7. Our preliminary data show that these three CD8 T cell clones have a distinct and fixed hierarchy in effector function e.g. in *ex vivo* cytokine production, proliferation capacity and ability to control acute phase *Toxoplasma* load in the peritoneum. With the unique tool, we will be able to show if trafficking, maintenance and effectiveness of CD8 T cells against *Toxoplasma* is dependent on their varying T cell receptor affinities. This research will advance the knowledge of control of *Toxoplasma* by CD8 T cells specifically and memory CD8 T cell in brain in general.

P4.08.69
Chagas'disease: evaluation of melatonin and lycopene influence on cytokines'profile during acute phase

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Inflammatory mediators and cytokines play important roles in the pathogenesis of a vast number of human illnesses, including Chagas disease. The antigens of *Trypanosoma cruzi* have the ability to induce an inhibitory/regulatory action over Th1 profile, as a strategy to maintain immune homeostasis. Lycopene, a carotenoid that is mainly present in tomato and its products, is an antioxidant, anticarcinogenic and antithrombotic activities, besides exerting an immunomodulatory effect reflected by enhanced levels of IL-2 and IL-4, T helper cell populations in healthy individuals. Melatonin is synthesized and secreted by pineal gland and is able to regulate the imbalance of cytokine network in some diseases. The modulation of cytokines, like CityIFN-γ, StateIL-10, IL-4, placeplaceCityTNF-α, placeIL-12 and IL-6, by melatonin in different models *in vivo* suggests a correlation between melatonin and the immune response. To characterize the immuno-modulatory effects of lycopene and melatonin, this research investigated intracellular cytokines production in $CD4^+$ and $CD8^+$ T lymphocytes. Animals were grouped: control, infected, melatonin infected and lycopene infected. Rats were i.p. infected with 1×10^5 blood trypomastigotes (Y strain) of *T. cruzi* and treated with melatonin (5 mg/kg) or lycopene (10mg/kg) daily, until 7^{th} day after infection and intracellular cytokines (IFN-γ, IL-10, IL-4, TNF-α) production were detected by flow cytometry. Melatonin treatment showed significant differences on IL-10 production and lycopene on TNF-α concentrations of $CD4^+$ lymphocytes, otherwise, $CD8^+$ T lymphocytes produced enhanced concentrations of IFN-γ. We conclude that melatonin and lycopene displayed immuno-enhancing effects in the intracellular cytokines production of distinct immune cell types.

P4.08.70
Increased levels of interleukin-4 (IL-4), interleukin-10 (IL-10) and IFN-γ in Iranian Visceral Leishmaniasis (VL) Children

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Leishmaniasis is a disease caused by obligate intracellular parasites. Visceral leishmaniasis (VL) as a prevalent type of it is a public health threatening in developing world. Functional immunity to VL depends on cytokine profile. IFN-γ, major cytokine of TH_1, plays a crucial role in immune responses against leishmaniasis in the contrast with IL-4 and IL-10 activity. All active cases in the endemic area were children under 12 years old. Sera from VL patients of north-west area of Iran were analyzed for the concentration of IFN-γ (patients=23, controls=9), IL-4 (patients=26, control=9) and IL-10 (patients=26, control=9) by ELISA method and were studied TH_1/TH_2 paradigm via their associated signature cytokines. We detected circulating levels of IFN-γ in 23 patients (mean=40.57pg/ml) higher than control group (mean=7.18pg/ml) and also the results show increased concentrations of IL-4 in the sera of 26 patients (mean=72.92pg/ml) in comparison with control group (mean=33.28pg/ml).Results achieved from measurement of IL-10 serum levels showed 4 cases with very high IL-10 levels (case1=1512pg/ml, case2=2255pg/ml, case3=1713pg/ml, case4=2031pg/ml) and the mean IL-10 level in 22 patients (34.78pg/ml) was higher than control group (mean=9.37pg/ml, n=9). Accumulating evidence points toward an antagonism between TH_1 and TH_2 and also their associated cytokines in leishmaniasis. In the acute phase of VL, TH_2 responses are dominant and TH_1 cytokines are protective. Our findings suggest that the cytokine pattern in VL patients is not polarized and the profile of these cytokines is not characterized by the TH_2 phenotype as in mice. Although both TH_1 and TH_2 cells appear to have proliferated and be involved in host immune responses.

P4.08.71
Infection with Leishmania major induces a cellular stress response in tissue resident macrophages

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We investigated early cellular responses induced in C57BL/6 (B6) macrophages by infection with *Leishmania major*. Infection activated the c-Jun NH2 -terminal kinase (JNK)/c-Jun/FasL cellular stress pathway in resident tissue macrophages, but not in inflammatory macrophages. In addition, infection triggered the secretion of a defined array of cytokines and chemokines, including IL-1ra, IL-6, TNF-α, TIMP-1, KC, MCP-1, MIP-1α, MIP-1β and MIP-2. Infection also increased secretion of metalloproteinases and soluble FasL. In spite of increased Fas and FasL expression, infected macrophages remained viable, as measured by a Lyzozyme release assay. As assessed in FasL deficient *gld* mutant B6 mice, neutrophil recruitment induced by infection did not depend on FasL expression. Moreover, inhibition of JNK, but not ERK, p38 or FasL, reversed *L. major* induction of KC by macrophages and also diminish parasite burden. Altogether, these data argue for a role of JNK in parasite survival and inflammatory responses following infection of resident macrophages.

P4.08.72
Identification of proteins in *Leishmania amazonensis* extract that may enhance the parasite infection

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Leishmania parasites secrete molecules that modulate the host immune system. The aim of this study was to purify, characterize and identify molecules with immunomodulatory activity in extracts of *Leishmania amazonensis* amastigotes (*La*E). The extract was subjected to electrophoresis in 12% polyacrylamide gel. Fractions with molecules with apparent molecular weights of 68, 45, 36 and 28 kDa were purified, based on previous results. BALB/c mice were injected intradermally with saline, *LaE* or fractions. After one week they were infected in the footpad with *L. braziliensis* promastigotes. The evolution of the lesions was monitored weekly, until six weeks after infection. The fractions of 68 and 28 kDa increased significantly the lesion. Parasite loads in the footpads were estimated by limiting dilution, and injection of the fractions of 28, 36 and 68 kDa produced results significantly different from the injection of saline. No statistically significant differences were observed between IL-4 and IL-10 concentrations in the supernatants from cultures of anti-CD3 - stimulated draining lymph nodes cells of fraction-injected and saline-injected animals. The proteins from these bands were digested in gel and the generated peptides were extracted and analyzed by mass spectrometry. Sixteen, 18, 18 and six proteins were identified in the fractions of 28, 36, 45 and 68 kDa, respectively. One of the identified proteins in the 36 kDa fraction was LACK, which has been shown to induce an infection-enhancing immune response in an elegant experiment using LACK-tolerant mice. No protein with reported immunomodulatory or infection-enhancing activities was identified in the other fractions.

P4.08.73
Theileria parva: Genotypic and antigenic diversity in buffalo and buffalo associated- cattle

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Theileria parva causes East Coast fever and Corridor disease, diseases of economic importance in Africa. T. parva is an intracellular protozoal parasite transmitted via the tick vector, Rhipicephalus appendiculatus from buffalo to cattle and between cattle. However, most buffalo-derived infections in cattle are not transmissible by ticks. Current evidence suggests that buffalo-derived T. parva are genotypically more diverse than those maintained in cattle. Further analysis of genotypic and antigenic diversity of T. parva populations is required to develop a more complete understanding of immunity, potentially contributing to improvements on the current vaccine. Cattle that recover from clinical infection with T. parva exhibit a strong but strain-specific immunity, believed to be mediated predominantly by CD8 T lymphocytes. Ten T. parva CD8 T lymphocyte target antigens (Tp1 - Tp10) have been identified in immune cattle. The antigenic diversity of parasites was assessed in parasitized buffalo cell lines isolated from 32 naturally infected buffalo in Kenya, and buffalo and cattle in the Laikipia area of Kenya. Tp1 through Tp10 were analysed in the 32 buffalo. Results showed defined polymorphisms within Tp1 andTp2 epitope regions, but little to no variation within the Tp4, Tp5 or Tp8 epitope regions. Mini-, micro-satellite and 454 sequencing are being conducted on the field samples. Additionally, a vaccine trial using the infection and treatment method (ITM) of vaccination is being undertaken to determine its efficacy against buffalo-derived parasites. ITM vaccination involves inoculation of susceptible cattle with a cocktail three T. parva isolates, and simultaneous administration of long-acting oxytetracycline.

P4.08.74
Plasma cytokine as biomarker of cardiac morbidity in Chagas disease

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The present study was designed to determine if the expression of immunological markers in patients with different forms of Chagas disease is associated with determining factors of cardiac morbidity in this disease. Individuals infected with *Trypanosoma cruzi* were grouped as indeterminate (IND) and cardiac (CARD) patients ranging from 23 to 69 years of age (mean of 45.6 ±11.25). The IND group included 82 asymptomatic individuals with no significant alterations in electrocardiography, chest X-ray, and echocardiogram. The CARD group included 94 patients presenting dilated cardiomyopathy, characterized by dilated left ventricle with impaired ventricular systolic function. A total of 24 healthy individuals, ranging from 29 to 55 years of age (mean of 42.6 ±8.8), from a non-endemic area for Chagas disease and showing negative serological tests for the infection were included as a control group (NI). Data analysis demonstrated that IND patients have a higher intensity of IL-10 expression when compared with individuals in the other groups. By contrast, the forms of inflammatory cytokine expression, such as IFN-γ, TNF-α, IL-6, and IL-1β, proved to be the highest in the CARD group. Correlated analysis showed that high IL-10 expression was associated with better cardiac function, as determined by left ventricular ejection fraction and left ventricular diastolic diameter values. Altogether, these findings indicate that a fine balance between regulatory and inflammatory cytokines represents a key element in the establishment of distinct forms of Chagas disease. Furthermore, this study helped to shed light

on the complex cytokine network underlying the immunopathogenesis of chronic chagasic cardiopathy.

P4.08.75
A new highly sensible method for division of patients with different forms of Chagas disease according to cytokine production

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The aim of this work was to employ an alternative strategy in an attempt to establish the cut-off edge for a division of patients with different forms of Chagas disease according to cytokine production. A total of 176 patients with positive specific serology for Trypanosoma cruzi were enrolled in this study. In addition, 24 healthy individuals were also included within the control group. Detailed analysis of individual levels of IL-10, IFN-γ, TNF-α and IL-6 expression was obtained for the NI, IND, and CARD groups by Cytometric Bead Array immunoassay kit (BD Biosciences, USA). Data were acquired in a FACScalibur flow cytometer and the analyses were performed using BD CBA software. Sixty-nine percent of CARD patients were defined as high IFN-γ producers, whereas only 4.9% of IND patients presented this profile of IFN-γ expression ($p<0.001$). The CARD group showed 71.3% of high TNF-α producers, as compared to the IND group which was confined to the range established by cut-off points for low and medium TNF-α producers ($p<0.001$). Similar results were identified when analyzing the IL-6 levels in the evaluated groups, although to a lesser extent. A small proportion of IND patients presented high levels of IFN-γ, whereas the most of these patients 76% expressed high levels of IL-10. It is also possible that these patients with high levels of IFN-γ tend to be prone to developing cardiomyopathy in the near future. Furthermore, it is likely that immunoregulatory mechanisms are present in the disease that can strongly influence its clinical evolution.

P4.08.76
High IL-17 expression is correlated with better cardiac function in human Chagas disease

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This study was designed to determine whether IL-17 expression can be correlated with patients' cardiac function. Individuals infected with Trypanosoma cruzi were grouped as indeterminate (IND) and cardiac (CARD) patients ranging from 23 to 69 years of age (mean of 45.6 ±11.25). The IND group included 82 asymptomatic individuals, with no significant alterations in electrocardiography, chest X-ray and echocardiogram. The CARD group included 94 patients presenting dilated cardiomyopathy. A total of 24 healthy individuals, ranging from 29 to 55 years of age, from a non-endemic area for Chagas disease and showing negative serological tests for the infection were included as a control group (NI). A Cytometric Bead Array (CBA) immunoassay kit - Human Th1/Th2/Th17 (BD Biosciences, USA) was used to measure the plasma levels of IL-17 as recommended by the manufacturer and described previously. Data were acquired in a FACScalibur flow cytometer and the analyses were performed using BD CBA software. The results revealed that IND group present a significantly higher plasma levels of IL-17, median of 12.8 ± 8.21, as compared to the CARD group, median of 5.715 ± 2.81 and the NI group, median of 5.44 ± 3.16 ($p<0.0001$). Correlative analysis showed that high IL-17 expression could be associated with better cardiac function, as determined by left ventricular ejection fraction and left ventricular diastolic diameter values ($r=0.878$ and $r=-0.8101$, $p<0.0001$, respectively). Taken together, the results of the present work corroborate with the protective role of IL-17 against cardiac damage in human Chagas disease.

P4.08.77
The effects of recombinant protein based on Trypanosoma cruzi P21-His6 treatment in infected mice with Leishmania amazonensis

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The cutaneous form of leishmaniasis is caused by the protozoan Leishmania amazonensis. This form of disease cause a lesion in the dermis characterized by a large inflammatory infiltrates composed, among others cell types, by macrophages infected with amastigotes forms inside a parasitophorous vacuole. P21 is a protein expressed in all forms of Trypanosoma cruzi. The recombinant protein based on the T. cruzi P21 (P21-His6) enhances phagocytosis on inflammatory macrophages. In this study we evaluate the effects of the treatment with P21-His6 on the site of lesion in BALB/c mice infected with L. amazonensis, and remove some aspects of the immune response of the hostage. The animals were divided in three groups: (1) Infected with L. amazonensis and not treated; (2) Infected with L. amazonensis and treated with 40µg of P21-His6 each 72 hours; and (3) Not infected and treated with 40µg of P21-His6 each 72 hours. The previous results showed a development of an inflammatory infiltrates in the group (3), composed by different cell types. The group (2) showed an increase of the inflammatory infiltrates and a large number of vacuoles and parasites internalized in comparison of group (1). The assays to measure the cytokines production, and the cell population residing in spleen tissue are being performed, to evaluate some aspects of the immune response against L. amazonensis in the presence of the P21-His6

P4.08.78
Recombinant human thrombopoietin inhibits the development of murine experimental cerebral malaria by decreasing the inflammatory response

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Cerebral malaria (CM), a deadly complication of malaria, remains to be a very thorny problem for its relatively high mortality and long-term neurological sequelae for patients. Thrombopoietin (TPO), the primary hematopoietic growth factor responsible for platelet production, has been shown to mediate various extra- hematopoietic functions. In this study, for the first time we demonstrate that recombinant human TPO (rhTPO) prolongs the survival of mice in experimental cerebral malaria (ECM). rhTPO administered on day one post-infection maintained blood-brain barrier integrity and significantly prevented the activation of brain vascular endotheliocyte via the down-regulation of ICAM-1 and VCAM-1 expression. More importantly, we found that rhTPO inhibited the development of murine ECM through decreasing the inflammatory response both in the brain and spleen. In the brain, leukocyte accumulation, levels of $CD4^+$ and $CD8^+$ T cells, as well as inflammatory cytokines (TNF-α and IFN-γ) and chemokines (CXCL9 and CXCL10) mRNA levels were down-regulated by rhTPO. In the spleen, rhTPO treatment reduced the Th1-type immune response, concomitant with decreased mDC proportion as well as DC maturation and activation, but conversely the proportion of Tregs increased. However, these effects were not accompanied by elevation of parasitemia, platelet and erythrocyte counts. These data provide the first evidence of protection against

murine ECM by rhTPO and hint to the mechanisms involved, offering a new theoretical basis for the treatment of CM patients.

P4.08.79
Patients with visceral leishmaniasis present an increased expression of TLR2 and TLR4 in lymphocytes and lipid and protein changes

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In order to have the development of adaptive immune response in visceral leishmaniasis is necessary that cellular receptors such as TLR2 and TLR4 recognize and play the parasite effector mechanisms. Moreover, the nutritional profile also has impact on immune response. Our objective was to evaluate the expression of TLR2 and TLR4 in CD14 and CD3 cells and correlate with nutritional status in patients with visceral leishmaniasis, pre and post-treatment. Were evaluated 8 patients pre-treatment, 3 post-treatment and 8 healthy subjects. Analysis of the expression of TLR2 and TLR4 receptors on CD3 and CD14 cells was performed by flow cytometry and the nutritional profile was performed by bioelectrical impedance and biochemical tests. In CD3 cells, expression of TLR2 was higher in patients pre-treatment than those post-treatment ($p < 0.05$) and controls ($p < 0.05$); TLR4 expression was higher in pre-treatment compared to controls ($p < 0.05$). In CD14 cells, TLR2 expression was lower in pre-treatment compared to controls ($p < 0.05$) and TLR4 expression did not differ between groups. Levels of HDL and albumin were lower in pre-treatment compared to post-treatment ($p < 0.05$) and controls ($p < 0.05$), phase angle was lower in pre-treatment than those post-treatment ($p < 0.05$) and triglycerides were higher in pre-treatment compared to controls ($p < 0.05$). Our results suggest that patients with LV activates expressed primarily TLR2 and TLR4 in lymphocytes, suggesting a possible involvement of these receptors in the development of the immune response. Patients also showed nutritional changes that could be interfering with the immune response against the parasite.

P4.08.80
Xanthine oxidase and inducible NOSynthase stress failed to efficiently suppress Leishmania major in BALB/c bone marrow dendritic cells

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Dendritic cells play a critical role in the initiation of immune response against Leishmania parasites. Moreover, phagocytes-dependent reactive oxygen species (ROS) and nitric oxide (NO) are potent leishmanicidal agents.
This study was performed with BALB/c mice bone marrow dendritic cells (BMDC) differentiated by 20 ng/ml GM-CSF for 8 days. BMDC were infected (1/10: cell/parasite) for 6 h and 24 h with a local strain of Leishmania major LIPA35 promastigotes (MHOM/DZ/06/LIPA35, Institut Pasteur Alger). The parasite load (percentage and index of DCs infection: Parasites number/DCs) was assayed on cytospin slides stained with May Grunwald Giemsa. Nitric oxide levels were determined in cultures supernatants, using Griess reaction. The cells were collected and used for the determination of xanthine oxidase (XO) by the uric acid assay.
Our results showed that Leishmania major enhanced XO activity by 76% ($P < 0.05$) and 235% ($P < 0.001$), respectively at hours 6 and 24, compared to control. Leishmania infection increased the nitrites levels by 52% ($P < 0.01$) only at 24h post infection compared to control DCs. In parallel, Leishmania major infected DCs by 50.33 ±3.05 at 6h vs. 47.66± 4.16 at 24h with an index of infection 2.39 ±0.33 vs. 2.47± 0.16, respectively.
In conclusion, these results suggested that oxidative stress via increasing xanthine oxidase activity and iNOS up regulation impaired Leishmania major killing by BMDC.

P4.08.81
Leishmania-reactive CD3+CD4-CD8- (Double negative) T cells display functional characteristics of memory T cells and protect against secondary challenge

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Although it is generally believed that $CD4^+$ T cells play important role in anti-*Leishmania* immunity, some studies suggest that $CD4^+$ T cells may be dispensable and MHC II-restricted $CD4^-CD8^-$ T cells may be more important in regulating anti-*Leishmania* immunity. Indeed, several studies have reported the expansion of $CD3^+CD4^-CD8^-$ (double negative, DN) cells in the blood of *Leishmania*-infected patients and dogs, and in spleens of *Leishmania*-infected mice. Although these cells have been proposed to contribute anti-*Leishmania* immunity, concrete evidence demonstrating this function has not yet been documented. Here, we report that DN cells are strongly activated, proliferate and produce IFN-gamma in the draining lymph nodes and spleens of mice following primary *L. major* challenge. Furthermore, we demonstrate that DN cells from mice that healed their primary *L. major* infection display functional characteristics of protective anti-*Leishmania* memory-like cells: they rapidly and extensively proliferate and produce IFN-gamma in response to *L. major* challenge *in vitro* and *in vivo*. These cells express predominantly alpha-beta T cell receptor (TCR) and are restricted by MHC class II molecules. Moreover, DN cells from healed mice, (but not from naïve mice), control parasite growth in infected macrophages *in vitro*. Using *in vivo* depletion and adoptive transfer studies, we show that DN cells from mice previously infected with *L. major* contribute to optimal secondary anti-*Leishmania* immunity in mice. Collectively, these results provide concrete evidence that $CD3^+CD4^-CD8^-$ cells play a role in both primary and secondary (memory) anti-*Leishmania* immunity.

P4.08.82
Molecular characterization T. gondii atypical strains isolated from pregnant women and their neonates in Southern Brazil

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Toxoplasma gondii is a protozoan parasite that shows ability to modulate the immune system in diferent ways. Toxoplasmic infection are of clinical importance in immunocompromised patients and pregnant women. There are few described clonal genotypes (I, II and III) seem to be responsible for the majority of toxoplasmic infections. Surface antigen 2 gene (SAG2) has been vastly used to determine genotyping T. gondii isolates. In northern hemisphere countries, human disease causing isolates are mainly type II or III, whereas T. gondii isolates from Brazil are mainly type-I, specially related to congenital toxoplasmosis. Information about the prevalent T. gondii genotypes in Brazil (southern regions) and other South-American countries mostly refers to animals. In this study we report genetic characterization of atypical T. gondii isolates obtained from clinical samples of pregnant women and their neonates. All the samples analyzed corresponded to SAG2 type I isolates, differing clinically from other T. gondii strains isolated in northern hemisphere countries. Our results indicate that further studies to define the prevalence of T. gondii are of outmost importance to better comprehend clinical causes of congenital toxoplasmosis in Brazil and neighboring countries.

P4.08.83
Unusual T. gondii strains associated to congenital toxoplasmosis

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Congenital toxoplasmosis may lead to several risk factors to pregnant women; as well as to the foetus. Parasitological direct methods and Toxoplasma gondii isolation in vivo and in vitro, constitute an option

to diagnostic determination of this disease. All biological samples were obtained from pregnant women (n= 24) selected through their clinical and immunological status. The analytic methods chosen were: direct observation of amniotic fluid samples, intraperitoneal inoculation of mice, cell culture inoculation and histopathological analysis of placenta, genotype definition through RFLP-PCR. Tachyzoites were observed both in free and intracellular forms during direct parasitological method, in all amniotic fluid and placenta samples. Parasites were also observed in peritoneal exsudate from inoculated mice since the 10th intraperitoneal passage, with a mantainance period of 55 passages. Cerebral cysts were seen in one animal that showed neurological distresss. All T. gondii strains were characterized as genotype-I (atypical strains). During in vitro isolation an average concentration of tachyzoites (222 tachyzoites/mL) in a mean time of 16,7 days was obtained, and during mantainance growth period the average was 1X106 parasites/mL (5 - 6 months). The growth (multiplication) period occurred between 5 -12 months, with a initial average concentration of 6,15 1X107 parasites/mL. Significative histopathological alterations were seen in placental samples. The results obtained in this study revealed that isolated T. gondii strains were atypical both clinically as well as in cell culture and in vivo models.

P4.08.84
Serological prevalence of Toxoplasma gondii antibodies in pregnant women from Southern Brazil

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The assessment of gestational toxoplasmosis is complex and requires interdisciplinary teams working toward better clinical follow-up, laboratory diagnosis, and treatment. It is essential to observe the clinical and laboratory parameters of pregnant women from the prenatal period to the postnatal period. To determine which diagnostic method(s) is safer; this must constitute the basis of an algorithm for clinical and laboratory-based decision making in monitoring pregnant women. The aims of the present work were to determine the seroprevalence of anti- T. gondii antibodies among pregnant women served by the Health Units of the public service system in the city of Curitiba, Brazil and evaluate the serological profile of that population. 20,389 women showing positive pregnancy tests were included in a serological evaluation of toxoplasmosis prevalence using automated immunoenzymatic assays. Serum samples were tested for the presence of IgG and/or IgM antibodies. Overall, 53.03% of the women were positive for IgG and 3.26% were positive for IgM; the analysis used a chi-square adherence test and significance level of 0.05 (χ2=14,720.35; p=0.00). To discriminate between recent and past infection, IgG avidity tests (n=166) were carried out, of which 28.3% (n=47)presented low avidity. The seroconversion index observed in this study was 0.44%. Seroprevalence results obtained were similar to other regions of Brazil. These data demonstrate the importance of continuous regional and national seroepidemiological inquiries to define public health strategies that can revert and reduce serological prevalence, as described in other countries where toxoplasmosis monitoring is mandatory.

P4.08.85
Congenital Toxoplasmosis: Current Brazilian public health policy

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Toxoplasmosis is caused by the protozoan parasite, Toxoplasma gondii. In pregnant women, the prenatal diagnosis should be made as early as possible, so that therapy may be applied to reduce parasitemia and avoid transplacental infection. Despite the high prevalence of infected individuals around the world (20-90%). In Brazil, seroprevalence may vary between 40% and > 80%, reflecting a significant disparity between the public health policies and resources applied in the different regions of the country and the human development index (HDI) of each of them. The lack of consistent and periodical data on seroprevalence per region makes it difficult to understand the significance of this infection and to plan specific public health policies and strategies. Regarding basic and clinical research, toxoplasmosis is not a priority if compared to the allocation of government funds and incentives to other tropical illnesses, such as: Chagas disease, Leishmaniasis, Dengue fever. There are also few study groups in Brazil that focus in this basic area, for genomics and proteomics studies of T. gondii strains-for the design of new methods of diagnosis, antiparasitic drugs and a vaccine model for humans and animals. This study highlights taxonomic and epidemiologic aspects, diagnosis methods, treatment and technical-scientific aspects of Toxoplasma gondii and toxoplasmosis in the Brazilian context.

P4.08.86
Effect of *Leishmania mexicana* amastigotes in the regulation of L-arginine metabolism in classically and alternatively activated macrophages

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Macrophages are decisive effector cells that either kill or host intracellular amastigotes of *Leishmania* depending on the balance of two inducible enzymes, nitric oxide synthase 2 (iNOS) and arginase 1 (Arg-1). These two enzymes share a common substrate, L-arginine, and are competitively regulated by Th1 or Th2 cytokines during macrophage activation. In classically activated macrophages (CAMφ), Th1 cytokines induce the expression and function of iNOS, which oxidizes L-arginine into the leishmaniacidal metabolite nitric oxide (NO). In alternatively activated macrophages (AAMφ), Th2 cytokines induce the expression and function of Arg-1, which hydrolyzes L-arginine into L-ornithine, an amino acid that is a basic source for polyamine synthesis, being these vital for *Leishmania* intracellular development. In the present work, we analyzed the effect of *Leishmania mexicana* amastigotes in the regulation of L-arginine metabolism in murine CAMφ and AAMφ. Bone marrow macrophages were differentiated with M-CSF, infected with *L. mexicana* amastigotes, and classically or alternatively activated with TNF-α+IFN-γ or IL-4, respectively. The enzymatic activities and changes in protein synthesis of iNOS and Arg-1, as well as parasite burdens were determined in both types of macrophages. In CAMφ there were a high iNOS activity and protein synthesis, which increased as a result of the infection with *L. mexicana* amastigotes. On the other hand, in AAMφ a high Arg-1 activity and protein synthesis were detected, which increased with the infection with *L. mexicana* amastigotes. Regarding to parasite burdens, classical activation of macrophages resulted in significant control of *L. mexicana* amastigotes growth, while alternative activation exacerbated it.

P4.08.87
B-1 cells increase susceptibility to murine viscetal leishmaniasis

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B-1 cells are a subtype of B lymphocytes whose biological role in immunity is not completely elucidated. In this study we investigated the role of B-1 cells in the pathogenesis of visceral leishmaniasis. Experiments were performed with BALB/c, BALB/Xid or BALB/Xid mice that receive adoptively peritoneal B-1 lymphocytes. These groups were infected or uninfected with *Leishmania (Leishmania) chagasi*. Our results showed that BALB/Xid mice, a mouse strain deprived of B-1 cells, infected for 45 days with *L. (L.) chagasi* had a significant reduction in parasite load in the spleen ($0.63 \times 10^7 \pm 0.4$ parasites/mg tissue) when compared to control animals BALB/c ($1.87 \times 10^7 \pm 0.6$ parasites/mg of tissue) or BALB/Xid that received adoptive transfer of B-1 cells ($1.44 \times 10^7 \pm 0.5$ parasites/mg tissue). Flow cytometry analysis demonstrated changes in peritoneal B-1 cell population. Infected BALB/c mice showed a significant increase in the percentage of peritoneal B-1 cells (70.0% ± 1.50) compared to control (63.3% ± 1.88). On the other hand, BALB/Xid that received adoptive transfer of B-1 cells and were infected with *L. (L.) chagasi* showed a significant decrease in the percentage of peritoneal B-1 cells (6.14 ±

3.01%) when compared to uninfected group (25.48 ± 7.60%). The cytokines production was evaluated in supernatants from spleen homogenates of uninfected and infected mice. BALB/Xid that received adoptive transfer of B-1 cells and were infected with parasite had significant higher levels of IL-10 and IFN-γ when compared with unifected mice.

P4.09 Malaria pathogenesis and vaccines

P4.09.01
Comparison of population structure of Plasmodium vivax currently circulating in Sri Lanka and of that a decade ago based on diversity of four selected antigen encoding loci

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As Sri Lanka achieved malaria pre-elimination status in 2008, understanding population genetic structure of current and previous local Plasmodium vivax isolates is important to (i) examine the degree of genetic isolation of these populations, and (ii) ascertain whether subsequent outbreaks would be due to residual transmission or due to introduction of new parasite strains to the parasite population.
Sequences of four polymorphic antigen encoding loci of P. vivax isolates collected a decade ago were retrieved from the Genebank (pvmsp3α: N=17; pvdbp, pvmsp1 and pvcsp:N=30). PCR amplification and sequencing of these four loci were carried out using 16 isolates collected recently (2011-2012). DNasp 5.1 software was used to examine the expected heterozygocity (He) and the genetic differentiation (Fst), to draw comparison of current and previous population genetic structures.
Low mean He in the current P. vivax population (He=0.76) compared with previous population (He=0.92) was observed for all four genes. Genetic differentiation amongst the two test populations was highest in pvmsp3α (Fst=0.20719), moderate in pvdbp (Fst=0.06785) and was absent in pvmsp1 and pvcsp (Fst= - 0.02876, Fst= - 0.01103). Though a low degree of overlap of amino acid haplotypes of these four proteins was observed between current and previous populations, most current haplotypes were novel. Thus these results suggest the possibility that new P. vivax variants have been introduced to the island; however, residual transmission of alleles that were not previously detected cannot be ruled out.
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P4.09.02
Targeting a *Plasmodium vivax* merozoite surface protein 1 fragment to the DEC205$^+$ dendritic cell population elicits strong antibody and T cell responses.

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Dendritic cells (DCs) are critical in the interaction between the innate and adaptive immune systems, as they are able to process and present antigens to T and B cells. In the last decade, studies from several groups have shown that it is possible to target antigens directly to different DC populations using monoclonal antibodies (mAbs) to receptors present on the DC surface fused with the antigen of interest. An anti-DEC205 mAb has been used successfully to target antigens to the DEC205$^+$CD8$^+$ DC population. The administration of low doses of the fusion mAb together with DC maturation stimuli is able to activate antigen-specific T cells and induce production of high antibody titers. Here we genetically fused the DEC205 mAb with the 42kDa fragment derived from the *Plasmodium vivax* merozoite surface protein 1 (MSP1), a candidate vaccine for malaria. During *Plasmodium* invasion into the red blood cell, the 42kDa fragment is further cleaved into 33 and 19 kDa fragments. The 19kDa fragment is normally target for antibody response while the T cell epitopes are restricted to the 33kDa portion of the molecule. The administration of two doses of the DEC-MSP1$_{42}$ fusion mAb in the presence of the TLR3 agonist poly I:C to either C57BL/6 or C57B10.A mice induced high anti-MSP1$_{19}$ antibody titers in the immunized mice. We also detected strong T cell immunity against a peptide present in the MSP1$_{33}$ sequence. Overall, our results indicate that targeting a *Plasmodium vivax* antigen to the DEC205$^+$CD8$^+$ DC population improves B and T cell responses.

P4.09.03
T Regulatory cell function in Lethal Plasmodium berghei ANKA and Non-Lethal Plasmodium yoelii infection: Role of Th17

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The outcome of malaria infection is determined, in part, by the balance of pro-inflammatory and regulatory immune responses. Host immune responses in disease including malaria are finely regulated by the opposing effects of Th17 and T regulatory (Treg) cells. Here we have examined the role of Treg cells and Th17 cells during malaria infection and find that low levels of Treg cells influence the outcome of infections with the lethal strain of Plasmodium berghei ANKA (PbA). In contrast, we observed that possibly high level of Treg cells influencing the outcome of non lethal Plasmodium yoelii (P. yoelii) infections. We observed decreased expressions of TGF-β, CD4IL-2 and IL-10 during PbA infection, whereas expression remains high during P. yoelii infection. On the other hand TNF-α, IL-6, IFN-γ and IL-23 expression is high during PbA infection and lower during P. yoelii infection. In combination with functional studies, we posit that Treg may convert to Th17 during PbA infection whereas; Th17 initially high during P. yoelii infection possibly converts to Treg cells. Thus, results from this study suggest that the critical balance between Treg and Th17 might have a key role on host pathogenesis during malaria infection.

P4.09.04
Systems immunology approach to identify the molecular profile of T cells during *Plasmodium* mono and mixed infections

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Despite intense research for many decades, the mechanisms of protective immunity against malaria are poorly understood and there is no immune correlate of protection. Recent technological developments provide an opportunity to characterize, at the molecular level, how the *Plasmodium sp.* parasite modulates the host immune response to infection or exert cross-species regulatory effects. Accordingly, we are applying cutting-edge systems immunology approaches to a unique resource of experimental infections in humans to define T-cell molecular profiles associated with *P. falciparum* or *P. vivax* blood stage mono or mixed infection. We are assessing the host-parasite interaction at transcriptional, translational and post-translational levels by investigating messenger and micro RNA expression, cytokine secretion and phenotype of key T cell subpopulations, as well as phosphorylation status of transcription factors involved in immune regulation using PCR arrays, multiparameter and phospho-specific flow cytometry. We hypothesize that the combination of these datasets will identify molecular signatures associated with species-specific immune protection and signatures of cross-species regulation occurring during mixed infections. To date we have identified a specific set of microRNAs expressed in the peripheral blood early post-infection with *P. falciparum,* a species-specific signature for phenotype and phosphorylation status of STATs in T cells during acute infection, and a high degree of polyfunctionality and increased frequency of *P. falciparum* circulating antigen-specific T cells during convalescence. Ultimately, it is anticipated that our studies will shed light on the mechanisms of protective immunity, define robust correlates of T-cell mediated protection against malaria and facilitate the development of a universal malaria vaccine.

P4.09.05
Immunogenicity and efficacy of Plasmodium falciparum MSP3-based immunogens in Saimiri sciureus monkeys

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Three recombinant protein constructions derived from the merozoite surface protein-3 (MSP3) from Plasmodium falciparum were evaluated for immunogenicity and efficacy against a blood stage challenge in Saimiri sciureus, a WHO-recommended non-human primate model for malaria. Each recombinant protein was formulated in Montanide ISA720 adjuvant and 15µg of the protein injected subcutaneously. Animals received three shots at 4-week intervals. Blood was sampled three weeks after each immunization and antigen-specific antibody titres were measured by ELISA and immunoblot. The non-splenectomized monkeys were challenged intravenously with $2x10^8$ P. falciparum (FUP strain) parasitized red blood cells five weeks after the third immunization. One of the recombinant proteins tested induced a consistent antibody response, with high titers being achieved especially after the third shot. Although all the challenged animals became parasitized, the immunized monkeys showed lower parasitemias compared to monkeys receiving the adjuvant only. All animals cured the infection spontaneously by the third week post-inoculation. The infection induced a potent boost in the anti-MSP3 antibody response in the immunized animals. The results of the experiments with the other two immunogens are currently being analyzed.

P4.09.06
The response of P2X7 deficient and competent immune cells to Plasmodium chabaudi AS infection in chimeric mice

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The immune response against the malaria has to be relatedwithboth, protection against infection and pathologic damage to the host. P2X7 receptor is responsible for ATP recognition by immune cells, which promotes inflammassome activation and induces proinflammatory cytokine production and cell death.Our group showed that P2X7 deficient mice were more susceptible than C57BL/6 mice to Plasmodium chabaudi AS infection. Then, ATP released by infected erytrocytes could be an important mechanism to activate the innate immune response. To evaluate the effects of P2X7 in the immune cells specifically and intrinsicly in response to uniform levels of parasitemia we used chimeric mice, ruling out any influence of P2X7 expressed on non-immune cells, what could change the millieu and modify the immune responses. For chimera construction, bone marrow from P2X7-/- donor mice was harvested and injected i.v. into non-lethally irradiated C57BL/6 mice. Blood and splenic monocytes and T-cells are reconstituted as expected (40-45%), while donor cells replaced most B-cells (80%). The basal activation of phagocytes and T-cells is lower on donor compared with recipient cells. P. chabaudi AS infection induced a higher activation on phagocytes from recipient then donor mice at day 8 post-infection. In contrast, CD8-T-cells from donor mice showed the higher activation profile, suggesting a delay on their activation process. Infection also induced an enhancement in 7AAD+ donor cell frequencies, but not in the recipient counterparts. These data suggest that the expression of P2X7 on immune cells is important for their activation during P.chabaudi infection.

P4.09.07
$CD4^+$ T cell promote B cell polyclonal activation in an ICOS-dependent manner during*Plasmodium chabaudi* infection

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B cell and antibody response are crucial for protective immunity to blood-stage malaria infections. The early B cell response to *Plasmodium chabaudi* results in intense production of parasite-specific low-affinity IgM and IgG and autoantibodies. According to our results, in the acute phase of the disease, T-B cell cooperation through the MHC class II molecules is essential for B cell activation, proliferation and for antibody production. The $CD4^+$ T cell population involved in the polyclonal B cell activation shows a huge increase of ICOS and OX40 molecules. $CD4^+$ T cells from acute infection also express high levels of CXCR4 but low levels of CXCR5, suggesting that T-B cell interaction occurs in the extra-follicular areas of the spleen. However, with time, the levels of CXCR5 and PD1 on the $CD4^+$ T cells increase, showing characteristics of T_{FH} cells. Moreover, PD1 expression is higher on germinal center T_{FH} than T_{FH} $GL7^-$. Our *in vitro* assays show that T cells from the acute infection are potent helpers for naïve B cells in the presence of iRBC. The IgM and IgG production contribute not only to the uptake of the iRBC exposed to phagocytes, but also to the uptake of BCG mycobacterium. In addition, our results show that during the acute infection $CD4^+ICOS^{HIGH}$ T cells are responsible for polyclonal B cell activation *in vitro* in the presence of iRBC. Our results suggest that the cooperation between conventional $CD4^+$ T cells and B cells has a central role in the polyclonal antibody response to *P. chabaudi.*

P4.09.08
Nitric oxide but not cytokine production by macrophages stimulated with malaria hemozoin is dependent on Nod2 activation

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Hemozoin (Hz) is the detoxification product of heme, released into the circulation during malaria infection. Once phagocytized by host cells, it induces several immune responses, and it is known to synergize with IFN-ɣ to produce Nitric Oxide (NO). The precise signaling leading to NO production has not been elucidated, yet. Nod1/Nod2 are intracellular receptors that have been related to the innate immune response to protozoa parasites including malaria. In the present study, we provide first evidence of the activation of Nod2 using immortalized BM derived macrophages from wild type (BM-Wt) or Nod-2 knock-out Balb/c mice. We observed that BM-Wt stimulation with whole *P. falciparum* infected red blood cells (W-IRBC) or lysates (LY-IRBC) induced higher amount of NO in IFN-ɣ pre-treated compared to non-treated cells. The response was dependent on Nod2. Similarly, IFN-ɣ pre-treatment increased NO production induced by Hz and this was also dependent on the Nod2-Rip2-Card9 signaling pathway and on the activity of iNOS, since in the presence of Apigenin, iNOS inhibitor, NO was impaired. Moreover, immunofluorescence staining showed Nod2 involvement in IFN-γ-primed macrophages stimulated with Hz. mRNA of several cytokines measured by quantitative real-time PCR in non-IFN-γ primed BM treated with Hz was found to be upregulated, but independent from Nod2. The data presented here suggest that parasite products, specifically native Hz, activate Nod2 via Rip2 and Card9 for the production of NO, upon priming with IFN-γ, but not for cytokine production. The relevance for malaria immunity and pathogenesis is discussed.

P4.09.09

High levels of heme correlate with severe malaria pathology and morbidity in patients from endemic areas in India

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The hemolysis occurring during malaria is characterized by the release of large amounts of heme, a pro-oxidant and immunomodulatory molecule. Previous studies have shown the implication of heme in the induction of experimental cerebral malaria, but its impact in the genesis of severe forms of human malaria remains unclear. Herein, we measured the levels of heme by a colorimetric reaction in the serum of uninfected and *Plasmodium falciparum*-infected patients from Orissa (India), an endemic area for malaria. *P. falciparum*-infected patients showed a panel of clinical phenotypes of the disease, ranging from uncomplicated to multi-organ dysfunctions associated to cerebral malaria. In order to estimate the trend of the immune response, the levels of 26 chemokines/cytokines were measured by flow cytometry. Our data show that heme concentrations are positively associated with the severity of the disease. However, heme levels were higher in the serum of severe non-cerebral forms of malaria when compared to cerebral outcomes. In addition, heme levels positively correlated to the concentration of pro- and anti-inflammatory cytokines, suggesting its implication in the induction of a complex immune response. When only considering severe forms of malaria, heme levels positively correlated to IL-10 concentrations. Thus, heme might favor a deleterious inflammatory environment within the host in the context of *P. falciparum* infections, but might also induce an anti-inflammatory response that would protect against cerebral forms of malaria. In this context, we suspect that host variability in heme scavenging/degrading protein activity or their concentrations may influence the susceptibility to severe malaria.

P4.09.11

Cellular cytokine and chemokine responses associated with susceptibility to severe malaria in children in Papua New Guinea

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Severe life-threatening malaria is associated with high serum levels of pro-inflammatory cytokines upon presentation. However, to date cellular responses have not been evaluated in relation to disease risk. Therefore we analyzed cellular cytokine and chemokine responses in children with severe malaria (SM; n=201), uncomplicated malaria (UM; n=172) and healthy community controls (HC; n=162) following short-term stimulation with live P. falciparum infected red blood cells in vitro. The samples from infected children were collected during convalescence to ensure normalization of peripheral cellular composition. Cytokine and chemokine outputs and cellular phenotypes were measured by ELISA, fluorescent bead assays and flow cytometry.
Compared to HC and UM, children with severe malaria had significantly higher IL-10, IP-10, MIP-1β and MCP-2 responses ($p \leq 0.05$). The median TNF and MIP-1α were also significantly higher in the severe malaria group compared to the UM group (p=0.0006 and p=0.0005 respectively). In addition, IL-10, IL-6, MIP-1α MIP-1β and MCP-2 was associated with increased odds of severe malaria (OR 1.01-5.74, $p< 0.05$). Furthermore, it was demonstrated that TNF, MIP-1β and MIP-1α were predominantly produced by monocytes and γδ T cells and IL-10 by CD4+ T cells.
The data indicate that production of high amounts of specific cytokines and chemokines in response to P. falciparum by monocytes and γδ T cells is a risk factor for severe malaria.

P4.09.12

Sexual hormones are involved in oxidative stress in mice infected with *P. berghei* ANKA

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Malaria incidence between both sexes is similar. However, men develop higher parasitaemias and more severe pathologies than women. On the other hand, both reactive oxygen and nitrogen species play an important role on malaria parasite elimination. Estradiol is a natural antioxidant and testosterone is associated with immunosuppression. In addition, expression of some immune response genes is modulated by hormones. We studied whether differences in pathology and parasitaemias could be associated with sexual hormones and analysed the effect of gonadectomy on the activity of antioxidant enzymes and on oxidative stress in CBA/Ca mice infected with *P. berghei* ANKA. Groups of male and female mice were gonadectomised and after 28 days were infected with *P. berghei* ANKA. Additional groups of mice were opened and closed to analyse the effect of surgery. Two additionally groups of mice were left intact as controls. Nine days post infection mice were sacrificed. Specific activity of: catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) was analysed. In addition, MDA levels were evaluated in blood and in spleen.
Gonadectomy in female mice significantly decreased the specific activity of catalase, SOD and GPx. In addition, levels of MDA were significantly higher in the group of gonadectomised female mice, while in male mice we did not detected significant changes.
Our results suggest that sexual hormones in females are involved in the development of oxidative stress in CBA/Ca mice infected with *P. berghei* ANKA.
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P4.09.13

Blood group O protects against complicated *Plasmodium falciparum* malaria by the mechanism of inducing high levels of anti-malarial IgG antibodies

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In a prospective clinical study in North Kordofan (Western Sudan), the possible association between blood groups and anti-malarial antibody responses with clinical outcome of *Plasmodium falciparum* malaria among Sudanese patients was investigated. A total of 184 individuals were consecutively enrolled from an outpatient clinic. Sixty six (35.9%) patients were classified as complicated malaria (CM), 53 (28.8%) with uncomplicated malaria (UM) and 65 (35.3%) were malaria free controls (MFC). Phenotypes of ABO blood groups were typed using commercial anti-sera. The antibody responses to MSP2 malaria antigens were assessed by an enzyme-linked immunosorbent assay (ELISA). The frequency of O blood group was significantly lower in those with CM when compared with MFC and UM patients (*P* value < 0.001 and 0.002 respectively). The levels of IgG1, IgG2 and IgG3 antibodies were statistically significantly higher in UM and MFC compared with CM patients. Statistical analysis indicated that higher levels of total IgG, IgG1, IgG2, and IgG3 specific to the MSP2 (both antigen forms, 3D7 and FC27) were associated with a reduced risk of complicated CM in O blood type carriers than in non-O blood type carriers, *P* value <0.001. Taken together, the current study indicates that blood group O is associated with a reduction in the risk of developing complicated malaria in western Sudan. Our results also revealed that the natural acquisition of immunity against clinical malaria appeared to be more associated with IgG1 and IgG3 antibodies, signifying their roles in parasite-neutralizing immune mechanisms.

P4.09.14
Targeting the Plasmodium chabaudi MSP1(19) protein to the DEC205 dendritic cell population induces faster parasite clearence

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Malaria is an infectious disease distributed worldwide. It's responsible for thousands of deaths every year, affecting mainly children. The acquired immune response during infection is partial and transitory. A membrane antigen of the merozoite is widely used as a vaccine target because of its immunogenic properties. This protein is expressed during schizogony as a precursor of 200kDa that undergoes successive cleavages, finally remaining anchored in the membrane as a portion of 19kDa named MSP-$1_{(19)}$, which has an important role in the reinvasion of the erythrocytes. Several studies have focused on the development of a vaccine against the parasite that leads to the maintenance of a protective memory response to different immunization schedules. A promising vaccine strategy is to target antigens directly to dendritic cells (DC) *in vivo*. This is accomplished by the administration of a monoclonal antibody that recognizes the endocytic receptor DEC205 present at the surface of DCs fused with an antigen of interest. Here we produced a hybrid αDEC205 coupled to the MSP-$1_{(19)}$ from *Plasmodium chabaudi* (AS) and used it to prime C57BL/6 mice that were subsequently boosted with the recombinant MSP-$1_{(19)}$ protein in the presence of Poly (I:C). Anti *P. chabaudi* MSP-$1_{(19)}$ antibodies were only detected in mice that received a boost with the recombinant MSP-$1_{(19)}$ protein. Protection against acute infection correlated with higher antibody titers. Adoptive transfer of blood from immunized and challenged mice showed a faster parasite clearance when MSP-$1_{(19)}$ was targeted to dendritic cells using the αDEC-MSP-$1_{(19)}$ antibody.
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P4.09.15
Placental lesions as result of TLR4 activation by Plasmodium berghei are associated with low birth weight

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Introduction: It has been described that TLR4 recognize components of Plasmodium sp. and influence the host immune response. As this activation during pregnancy can result in a range of complications affecting mother and fetus, the aim of this work was to evaluate the effects of TLR4 absence on the development of placental malaria (PM). Methods & Results: To verify the effects of TLR4 activation on the placental tissue, we infected pregnant mice with P. berghei NK65 on the 13th gestational day. Histopathological analysis of the placentas showed a decrease of vascular spaces in infected wild type (WT) mice when compared with placentas from non-infected control mice. In contrast, we did not observe differences between infected and non-infected placentas from TLR4 knockout (TLR4KO). As the reduction of vascular space can be a consequence of inflammatory processes, our next step was to analyze local immune response induced by the parasite. Our results showed that pro-inflammatory cytokines IL-6 and TNF-alpha expression were abrogated in infected TLR4KO mice and no differences were observed with IFN-gamma production. As a consequence of inflammatory process, fetuses from infected WT mother present a significant body weight reduction. Conclusion: Together, our results point out the importance of a severe local inflammatory response in the development of PM pathogenesis via TLR4 signaling and, additionally, indicate the TLR4 as potential target for future therapies.
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P4.09.16
The P2X7 receptor contributes to protection and to ameliorate the clinical manifestations of blood-stage Plasmodium chabaudi malaria

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Malaria is a potentially serious infectious disease caused by protozoa parasites of the genus *Plasmodium.* The blood stage of infection is responsible for all symptoms associated with malaria. Recently, it has been shown that innate immune receptors are able to detect signals released by damaged cells, such as ATP. The P2X7 receptor (P2X7R) detects extracellular ATP and therefore could contribute to activate the immune response to *Plasmodium*. In this study, we observed that ATP levels in the serum of C57BL/6 mice that were infected with *Plasmodium chabaudi* AS were significantly higher after the period of rupture of infected red blood cells (iRBCs). Moreover, $CD4^+$ T cells and $CD11c^+$ cells from the spleen of infected mice presented with increased P2X7R-mediated responses to ATP or supernatants from lysed iRBCs, which were measured by the permeability to ethidium bromide. Furthermore, $P2X7R^{-/-}$ (C57BL/6 background) female mice had difficulty in controlling parasitemia and failed to reestablish body temperature and weight after the peak of parasitemia. Accordingly**,** $P2X7R^{-/-}$ male mice died from day 8 to 15 postinfection. Our results suggest that ATP is released from ruptured iRBC and its recognition by the P2X7R contributes to parasite control and to ameliorate the clinical manifestations of blood-stage *P. chabaudi* malaria.

P4.09.17
Polygenic control of human antibody responses to synthetic malaria vaccine

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In nature, all the evolutionary mechanisms are related with diversity, ensuring species survival. In this way two main genetic peculiarities play an essential role: polymorphism and polygenes. The genetic regulation of innate and acquired immunities constitutes the complex network of resistance/susceptibility mechanisms. The analysis of the quantitative antibody production against a chemically synthesized malaria vaccine was performed in three human populations naturally exposed to malaria: Tumaco, a hypo-endemic area and La Tola, a region of higher endemicity, both in Colombia; Las Majadas an endemic province in Venezuela. The IgG antibody titers were determined thirty days after three vaccine dose. The mean antibody titers were 1.3 to 4.5 times higher in women than in men and the frequencies of low responders (titers < 2 log 10) were 10% in Tumaco, 23% in Las Majadas and 49% in La Tola. There is a significant inverse correlation between the IgG titers against the vaccine and the anti-*P. falciparum* pre-immune sera in both La Tola and Las Majadas. Multiple regression analysis of intra-familial antibody responsiveness showed significant correlation between mother vs. daughter, mother vs. son, father vs. son and daughter vs. son. Intra-familial variances were lower than total phenotypic variances in 50% of Las Majadas, in 54% of La Tola and in 80% in Tumaco indicating the importance of genetic constitutions and the relevance of environmental factors to this quantitative character. Moreover, the continuous frequency distributions of antibodies provide evidence for the polygenic control of the immuneresponsiveness to malaria vaccine. Support: CNPq, FAPESP

P4.09.18
Inflammatory factors and leucocytes are involved in the pathogenesis of malaria associated acute lung injury/acute respiratory distress syndrome in murine model

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INTRODUCTION: Malaria is a huge burden on global health and caused 2.4 million deaths in 2012. Severe malaria can manifest in the lungs, an illness known as acute lung injury/acute respiratory distress syndrome (ALI/ARDS). Using an experimental model previously established by our group we evaluated the immune cell profile and inflammatory response in malaria associated ALI/ARDS. METHODS AND RESULTS: DBA/2 mice were infected with *Plasmodium berghei* ANKA. Lungs and bronchoalveolar lavage (BAL) were collected 7 days post infection (7dpi). Using the respiratory patterns (enhanced pause and respiratory frequency) and parasitemia from a survival group, we established ROC curves and were able to classify experimental animals euthanized 7dpi as suffering ALI/ARDS or HP (hyperparasitemia). There was an increase in neutrophils, seen by increased *Ncf2* mRNA expression (qRTPCR) and increased numbers of Gr-1^{high} $CD11b^{+}$ cells (flow cytometry), in the lungs of ALI/ARDS mice compared to HP mice. In the lung we observed increased numbers of alveolar macrophages ($F4/80^{+}/CD11_{C}^{+}$) and fewer interstitial macrophages ($F480^{+}$) (flow cytometry) and in the BAL we observed an increase in macrophages and neutrophils (cytospin) in the ALI/ARDS mice. We also found that mRNA expression of *iCAM-1*, *HO-1, IL-10* (anti-inflammatory) and *IFNγ* (pro-inflammatory) genes was elevated (qRTPCR) in the lungs of the ALI/ARDS mice. CONCLUSION: Our data suggests that neutrophils and alveolar macrophages play a role in ALI/ARDS development. We hypothesize that the neutrophil recruitment is partly dependent on increased ICAM-1 expression and that the high *IL-10* mRNA expression is an attempt to re-establish homeostasis.

P4.09.19
Extracellular ATP accelerates malaria anemia

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Although anemia is one of the most common malaria symptoms, its molecular mechanisms still remains unclear. Extracellular ATP and its metabolites are used for intercellular communication and stimulation of immune response. The main supplier of extracellular ATP is RBCs under physiological condition. Here we show that extracellular ATP accelerates malaria anemia. First, to examine the role of extracellular ATP on malaria anemia, we injected ATP to mice infected with non-lethal strain *Plasmodium berghei* XAT. Surprisingly, the severity of malaria anemia was accelerated by injection of ATP. On the other hand, administration of carbenoxolone, the blocker of putative ATP secretion channel pannexin, ameliorated malaria anemia. These data indicated that extracellular ATP is involved with malaria anemia. Next, to determine the receptor for extracellular ATP involved with malaria anemia, various P2 receptor agonists or antagonists were administered to *P. berghei* XAT-infected mice. Injection of brilliant blue G, the selective inhibitor of P2X7 receptor, induced milder anemia, suggesting that P2X7R is involved in malaria anemia. To farther examine the role of P2X7R on malaria anemia, P2X7R deficient mice were infected with *P. berghei* XAT and severity of anemia was examined. P2X7R deficient mice exhibited milder anemia than WT mice upon non-lethal *P. berghei* XAT. Moreover, milder anemia symptom on P2X7R mice was observed by infection of not only *P. berghei* XAT but all other *Plasmodium* parasite we examined, including *P. berghei* ANKA, NK65, *P. yoelii* 17 XL, 17XNL, *P. chabaudi* AS. Collectively, extracellular ATP and its receptor P2X7R were involved with malaria anemia.

P4.09.20
The role of P2X7 receptor in hepatic necrosis caused by Plasmodium chabaudi AS infection

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The intense activation of the immune system during the erythrocytic stage of *Plasmodium* is responsible for several syndromes associated with the disease. Purinergic receptors of the P2X family detect extracellular ATP (eATP) and their interaction induces inflammasome activation in macrophages and consequent production of proinflammatory cytokines and cell death. In malaria, ATP is released upon erythrocyte rupture and T cell activation. In the murine model of malaria caused by *Plasmodium chabaudi* AS, infected erythrocytes preferentially adhere to the liver endothelium. Therefore, it is believed that the rupture of parasitized erythrocytes and consequent release of ATP mainly occur in that organ. A study performed by our research group showed that liver necrosis occurs in C57BL/6 mice infected with *P. chabaudi* AS.
To study the role of P2X7R in hepatic necrosis, we infected C57BL/6 and $P2X7R^{-/-}$ mice with *Plasmodium chabaudi chabaudi* AS 10^{6} (i.p.) and 7 days later the livers were collected for histopathological analysis. We observed that infected $P2X7R^{-/-}$ mice, contrary to C57BL/6 mice, do not present/develop liver necrosis. In another approach, we infected DBA/2 mice, which were treated with P2X7-antagonist Brilliant Blue-G (BBG) (45.5 mg/Kg) every 48 hours for 5 days. Seven days after infection the livers were collected for histopathological analysis, peripheral blood and serum were collected for hemoglobin, AST, ALT and LDH measurements. Our results indicate that BBG administration prevents hepatic necrosis.
These data demonstrate that the absence of P2X7 receptor or the administration of BBG ameliorates the severity of hepatic necrosis.

P4.09.21
S-nitrosoglutathione prevents experimental cerebral malaria

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Administration of the exogenous nitric oxide (NO) donor dipropylenetriamine-NONOate (DPTA-NO) to mice during Plasmodium berghei ANKA (PbA) infection largely prevents development of experimental cerebral malaria (ECM). In the present study we evaluated whether an alternative, physiologically relevant NO donor, S-nitrosoglutathione (GSNO), was able to prevent ECM at lower doses with minimal side effects. Prophylactic treatment with high (3.5 mg), intermediate (0.35 mg) or low (0.035 mg) doses of GSNO decreased incidence of ECM in PbA-infected mice, decreasing also edema, leukocyte accumulation and hemorrhage incidence in the brain. The high dose inhibited parasite growth and also induced transient hypotension. Low and intermediate doses had no or only mild effects on parasitemia, blood pressure, and heart rate compared to saline-treated mice. PbA infection decreased brain total and reduced (GSH) glutathione levels. Brain levels of oxidized (GSSG) glutathione and the GSH/GSSG ratio were positively correlated with temperature and motor behavior. Low and intermediate doses of GSNO failed to restore the depleted brain total glutathione and GSH levels, suggesting that ECM prevention by GSNO was probably related to other effects such as inhibition of inflammation and vascular protection. These results indicate that ECM is associated with depletion of the brain glutathione pool and that GSNO is able to prevent ECM development in a wide range of doses, decreasing brain inflammation and inducing milder cardiovascular side effects.

P4.09.22
Pys48 and Pys25 DNA Vaccine supplement medicated the inhibitory development of *Plasmodium yoelii* gametocytes into gametes

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In addition to vector control and malaria chemotherapy, immunological intervention will be needed to stop the resurgence of malaria. Transmission-blocking vaccine (TBV) is one of the new strategies for controlling malaria. It will be a major method to prevent from malaria if TBV is applicated together with other stages malaria vaccines and anti-malaria drugs. P25 is the major surface protein expressed on the zygote and ookinete of Plasmodium, which is well known as the important candidate for the TBV. In recent years, the specific protein P48 expressed on the surface of gametocytes as a transmission-blocking immune target has caused widespread interesting. Here, we have pursued a vaccination strategy, based on DNA immunization in mice with genes encoding two antigens present on the sexual stages of *Plasmodium yoelii* 17XL (P.y 17XL), Pys48 and Pys25, to induce important antibodies. The DNA vaccines of Pys25 and Pys48 were able to induce high levels of antibodies in mice. The effective transmission blocking activity was confirmed by mosquito feeds and ookinete culture *in vitro*, but the transmission-blocking activity of Pys25 and Pys48 composite immune group was lower than that for either Pys25 or Pys48 simple immune group, moreover the effect has significant antibody titer dependent. Therefore, our research will provide necessary and basic data for identify the role of P25 and P48 in transmission-blocking immune and developing effective compound malaria vaccines.
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P4.12 Microbiome, gut flora and inflammation

P4.12.01
Tuberculosis and acquired immunodeficiency syndrome

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Introduction: HIV-Tuberculosis is life threatening diseases in the world that approximately 1.1 million people have infected. The incidence of drug resistance is common in this group of patients. The immune system is very important to tuberculosis control. Thus, the purpose of this study was to evaluate the prevalence of HIV in TB patients.
Methods: In a descriptive study, 183 TB patients (99 men and 84 women) who were referred to health centers of Mazandaran province were selected after obtaining informed consent. Peripheral blood was drawn from subjects and their serum separated. HIV Ab and western blotting test were analyzed in all samples.
Results: Mean age of tuberculosis patients was 46.8 ± 19.8 years. HIV prevalence in this population showed a prevalence of 2%.The frequency of infections TB / HIV in the men was 3.03% and 1.2% in the women (OR = 2. 5, 95% CI = 0.26±25.4, P=0.3). Also, one of the 4 patients infected TB / HIV patients have a prison record.
Conclusion: According to the low prevalence of HIV among TB patients in this research, it is concluded that control of this patients can be cause to prevent of the disease progression.

P4.12.02
Characterization of commensal bacteria based on their effects on human dendritic cell-induced T-lymphocyte polarization

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Enormous diversity of commensal bacteria determines individual functions acting on the development and activities of the human immune system. These involve specialized macrophage and dendritic cell subsets, expression of unique pattern recognition receptor combinations coupled to evolutionally conserved signaling pathways, transcriptional regulation, post-translational modifications, induction of co-stimulatory molecules, secretion of cytokines, chemokines and type I interferons. This complexity can directly be translated to T-lymphocyte polarization to support tolerance induction or inflammation. We have developed a sensitive in vitro culture system for investigating the response of moDC subsets to commensal bacteria by monitoring the expression of type I/II CD1 proteins, phagocytic activity, secretion of chemokines, pro-inflammatory and T-cell polarizing cytokines. Under physiological conditions the gut microenvironment is conditioned by retinoic acid (ATRA) produced by intestinal epithelial cells and CD103+ DCs. To consider the impact of this special microenvironment on moDC-induced T-lymphocyte responses we compared the effects of selected microbes in absence and presence of ATRA. Selected microbes exerted their modulatory effects in a dose- and strain-dependent manner and ATRA had a significant impact on moDC-induced T-cell responses:
• ATRA inhibited CD1a expression in moDCs without affecting CD83 expression;
• Gene expression involved in retinoid synthesis was enhanced but microbes counter acted this effect;
• Secretion of IL-1β concommitant with NOD2, NLRC4 and NLRP12 gene expression was dramatically enhanced by ATRA but microbes inhibited this effect;
• Opposing activity of NOD2 to NLRC4 and NLRP12 pointed to an autocrine IL-1β-mediated regulation;
• ATRA increased Th17 and decreased Th1 responses significantly.
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P4.12.03
Staphylococcus aureus affect T-regulatory cell phenotype and FoxP3-expression

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Purpose: T-regulatory cells (T-regs) control peripheral and tissue-based immune responses. *Staphylococcus (S.) aureus* and *Lactobacillus (L.) reuteri* are both common bacteria of the neonatal commensal gut microbiota and can potentially interact with, and affect, immune cells. Our previous studies have shown that *S. aureus*, but not *L. reuteri*, strongly promotes inflammatory responses while *L. reuteri* can dampen *S. aureus* induced immune responses. Our aim was therefore to investigate how these bacteria affect the T-reg population.
Materials and methods: HT-29 intestinal epithelial cells (IEC) were stimulated for 24h with media containing 5% of each bacterial supernatant or both in combination. PBMCs were stimulated with the bacteria-conditioned IEC supernatants for 24h, washed and stained for T-regs using the CD4, CD25, CD127 and FoxP3 markers. T-regs were gated based on $CD127^{low}$CD25+ expression and acquired by flow cytometry.
Results: Stimulation with *S. aureus* reduced the percentage of $CD127^{low}$CD25+FoxP3+ T-cells, while increasing the total CD4+ T-cell surface expression of the activation marker CD25. *L. reuteri* alone had no effect on these parameters and it did not prevent the *S. aureus* induced reduction of FoxP3+ cells. However, the cell surface expression of CD25 was reduced by simultaneous stimulation of PBMCs with *S. aureus* and *L. reuteri* compared to *S. aureus* alone.

Conclusions: *S. aureus* may perform its pro-inflammatory activity through down-regulation of FoxP3+ T-regs and by promoting T-cell activation, while *L. reuteri* partially dampen this effect.

P4.12.04
Enterococcusdurans EP1 modulates inflammation in a DSS-induced colitis model in mice

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Enterococcus durans EP1, a gram-positive coccoid bacteria, has shown various probiotic properties. The objective of this work was to evaluate the immunomodulatory capacity of EP1 in a colitis model induced by dextran sulfate sodium (DSS) in mice. Six weeks old Swiss mice were separated in two groups of 16 animals. During 14 days, one group received daily 108 cfu/mice of EP1 and the other received PBS. On day 14, each of the mentioned groups was separated in two (eight mice each) and administration of DSS 3% w/v started for one of them and lasted seven days. Significant differences in the disease activity index were observed between DSS group and DSS treated with EP1 mice (DSS-EP1), DSS mice´s colons were shorter ($p<0.05$) than the colons from control and DSS-EP1 mice. Histological studies were consistent with these findings. Cytokines production by colon explants ex vivo showed that DSS mice secreted higher levels of IL6, IL17A, IFNγ, TNFα and GM-CSF compared with control and DSS-EP1 mice but no differences were observed for IL10. Gene expression studies demonstrated that in colon the expression of IL17A, INFγ, IL1β and CXCL1 was lower in DSS-EP1 group compared with DSS mice. In mLN, expression of IL23, IL17A, RORγT and GM-CSF was significantly decreased in DSS-EP1 group. Moreover, EP1 treatment partially recomposed the changes in the microbiota induced by DSS. These results indicate that administration of E. durans EP1 attenuates damages generated by DSS by decreasing expression and secretion of proinflammatory mediators, as well as modulating microbiota.

P4.12.05
Butyric acid in the gingival tissue induces mitochondrial oxidative stress while acutely increasing caspase-9 activity

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Butyric acid (BA) is a major extracellular metabolite produced by anaerobic periodontopathic bacteria and is commonly deposited in the gingival tissue. BA retention in the gingival tissue was previously shown to induce mitochondrial oxidative stress, however, its effects on cellular apoptosis was not fully elucidated. In this study, we determined how BA-induced increase in hydrogen peroxide (H_2O_2) amounts affect the cellular apoptosis pathway. Throughout the study, we made use of rat jugular blood taken at 0, 15, and 180 min after BA injection in the gingival tissue. We fractionated both blood mitochondria and blood cytosol, and, similarly, verified the purity of each isolate. Subsequently, we measured blood mitochondrial H_2O_2, heme, superoxide (SOD), and catalase (CAT), whereas, we determined blood cytosolic caspase-9 activity. In blood mitochondrial samples, we found that H_2O_2, heme, SOD, and CAT levels gradually increased with respect to time after BA-injection which would insinuate that mitochondrial oxidative stress was induced. In blood cytosolic samples, we detected high caspase-9 activity 15 min after BA injection which eventually normalized after 180 min which would insinuate that BA acutely increases caspase-9 activity and is somehow regulated in time. We correlated the decrease in caspase-9 activity to the sustained increase in mitochondrial H_2O_2 amounts. Moreover, we suspect that BA-induced increase in mitochondrial H_2O_2 amounts inhibited the caspase-dependent apoptosis by regulating caspase-9 activity. Thus, we hypothesize that BA in the gingival tissues does not induce cellular apoptosis attributable to an increase in mitochondrial H_2O_2 amounts.

P4.12.06
Homology modeling and structural analyses of the six Porphyromonas gingivalis FimA variants

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Porphyromonas gingivalis is a Gram-negative bacteria primarily responsible for gingivitis. P. gingivalis fimbrillin (FimA) mediates bacterial interaction with host tissues by mediating bacterial adhesion and colonization at targeted sites. FimA is classified into six variants based on nucleotide sequences and each variant differs in bacterial pathogenicity. However, to date, previous attempts to crystallize the FimA protein has failed and the absence of FimA protein structural data has hampered our understanding of this protein. With the advent of various protein prediction and structural analyses software, it is now possible to predict and characterize the FimA structure. Here, we generated FimA homology models and characterized all six FimA variants (Type 1-5 and 1b). We cloned and sequenced five P. gingivalis strains from each FimA variant. Multiple sequence alignment was performed to determine the homologous sequence and, subsequently, used to generate the mature FimA homology models. We determined the quality of all FimA models produced and found that each generated model has an X-ray crystallography quality making all FimA models suitable for structural characterization. Topology analyses showed that FimA Types 1, 1b, 3, 4, and 5 is comprised of 18 beta-sheets, whereas, FimA Type 2 have 16 beta-sheets. Furthermore, we found that all FimA models can be subdivided into three parts which we designated as upper, middle, and lower regions. Interestingly, all FimA models have a structurally conserved middle region which we suspect is important to maintain the overall FimA structure, thus, making the FimA middle region an ideal antibacterial or vaccine target.

P4.12.07
Phytol-derivative PHIS-01 as a vaccine adjuvant and a molecular probe for anti-bacterial immune microenvironment against methicillin-resistant Staphylococcus aureus

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Staphylococcus aureus (SA), a commensal Gram-positive bacterium present in about 30% adult population, can be potentially pathogenic emerging as methicillin-resistant MRSA strains and affecting nearly a million individuals every year. Despite significant efforts, there is as yet no clinically approved anti-staph vaccine. To discern underlying problems in developing such vaccine, we asked whether the synthetic diterpenoid PHIS-01 (phytanol) could act as an effective adjuvant in immunization with heat-inactivated MRSA. Our earlier studies in a mouse model have shown PHIS-01 to be effective in engendering protective immunity against methicillin-sensitive *S. aureus.* The present study was designed to evaluate the effectiveness of PHIS-01 against MRSA. PHIS-01-adjuvanted MRSA vaccine significantly reduced bacterial loads upon live challenge in the adjuvanted group relative to unadjuvanted group. Although antibody levels did not differ in the control and adjuvanted groups, significant differences were noted in the expression of antimicrobial peptides, like Bpi and Lcn2, cytokines like IL-1β and Ifnb1 and chemokines like CCl3, CCl4 and Cxcl3. Additionally, the adjuvanted groups showed enhanced expression of inflammasome and TLR associated genes. Assessment of CD4+ T-cell subset induction in terms of specific transcription factors revealed that MRSA could selectively upregulate Foxp3 (T-regulatory cells). Interestingly, PHIS-01 could effectively subvert this effect and also serve as the molecular probe to delineate changes in immune homeostasis that is pivotal in host-parasite interactions.

P4.12.08
The intestinal microbiota triggers epithelial Toll-like receptor expression and impairs neuropilin-1 processing and hedgehog signaling in the small intestinal mucosa

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A single layer of epithelial cells forms the barrier between gut microbiota and small intestinal lamina propria. Ligation of microbial-associated molecular patterns (MAMPs) to Toll-like receptors (TLRs) expressed on epithelial cells promotes proliferation and regulates intestinal homeostasis. Here, we use germfree mouse technology to explore how MAMPs derived from gut microbes impact epithelial neuropilin-1 (Nrp-1) expression. Recent reports demonstrate that Nrp-1 regulates hedgehog (HH) signaling.
Comparing mice that are colonized from birth with a microbiota (CONV-R) with germfree controls (GF) we found that small intestinal mRNA of TLR2 and its co-receptor TLR1 are increased upon colonization. This effect could be reversed by antibiotic microbiota depletion. Stimulation of the epithelial cell line MODE-K with TLR2 agonists resulted in an increase in TLR2 and TLR1 transcripts and was associated with increased cellular TNFα levels and increased proliferation. Treatment of MODE-K cells with TNFα resulted in decreased Nrp-1 levels. In line with decreased TNFα mRNA levels detected in the small intestine of GF mice we found decreased Nrp-1 levels in colonized mice. Accordingly, stimulation of MODE-K cells with TLR2 agonists decreased Nrp-1 protein levels. Co-immunoprecipitation analyses suggest that Nrp-1 interacts with TLR2. Localization of Nrp-1 to the cell membrane was reduced upon TLR2 stimulation. Similar to decreased Nrp-1 levels colonization of GF mice led to a drop in HH ligand expression.
Collectively, we found that the epithelial TLR2/Nrp1 signaling loop is regulated by MAMPs of the microbiota. This is associated with altered HH signaling which may account for changes in small intestinal morphology.

P4.12.09
Is IutA from Escherichia coli a type 1 Thymus-independent antigen?

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Certain strains of Escherichia coli contain a pColV plasmid that encodes IutA, an outer membrane receptor for the iron-aerobactin complex. Preliminary results from our group suggest that IutA is a mitogen for B cells. Here we performed direct stimulation assays of CFSE-labeled B cells with recombinant IutA (rIutA) and indirect assays with B cells cocultured separately with spleen macrophages stimulated with rIutA using transwell membranes or cultured with conditioned medium from IutA-stimulated macrophages. We showed that rIutA was not able to induce directly the proliferation of B cells, but it induced macrophages to release a molecule that acted on B cells. When we used cells from mice MyD88-/-, we determined that MyD88 is crucial for signaling induced by rIutA in B cells, but not in macrophages. A similar analysis with cells from mice TLR4-/- or TLR2-/- revealed that these receptors were not required for rIutA signaling. Since IutA induced macrophages to produce IL-1 and signaling of IL-1 receptor is dependent on MyD88, we thought that IutA stimulated macrophages to produce IL-1 that, in turn, was responsible for B cell proliferation. This hypothesis was confirmed when we observed that B cells pretreated with IL-1 receptor antagonist had a significantly decrease in proliferation in response to conditioned medium from cultures of IutA-stimulated macrophages. Altogether, our results suggest that IutA induces polyclonal B-cell proliferation independently of T cells in a mechanism mediated by macrophages. These results open perspectives for studying IutA as a molecule that stimulates the mucosa-associated lymphoid tissue.
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P4.12.10
In vivo **analysis of Staphylococcal Superantigen-Like (SSL) protein production during infection**

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Staphylococcus aureus is a major human pathogen, a leading cause of hospital-acquired infection and alarmingly a rapidly increasing cause of antibiotic-resistant community-acquired infection. It persistently colonizes 20 - 30% of the population with up to half of us colonized at any point in time. This organism can infect practically any part of the body with disease pathologies covering the full range of severity from soft tissue infections, to joint infections, through to life-threatening conditions including septicaemia, endocarditis, and toxic shock. Its remarkable ability to colonize and infect is due to the abundance of virulence factors it produces. Many host-derived influences affect the expression patterns of virulence genes in *S. aureus* including stresses placed upon the bacteria by attack from the immune system. A family of fourteen secreted molecules named the Staphylococcal Superantigen-like (SSL) proteins are becoming recognised as being amongst the virulence factors responsible for immune evasion by *S. aureus*. Neutrophils and their anti-microbial products appear to trigger regulatory systems that control *ssl* transcription. Research performed in our laboratory using a subcutaneous abscess mouse model has established that SSL proteins are massively up-regulated during the early stages of infection. This up-regulation has been determined at the transcript level using qRT-PCR with subsequent detect of SSL proteins within the abscess by Western analysis revealing that SSLs are important defence molecules produced by *S. aureus* in the face of immune attack during an infection situation.

P4.12.11
The protective role of the intestinal microbiota in the host defense against Streptococcus pneumoniae-induced pneumonia

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For many years it has been hypothesized that in the critically ill the intestine has an important detrimental role in promoting systemic inflammation and infection. More recently however, the intestinal microbiota has emerged as a key defense system by local support of mucosal defense systems and systemic priming of antibacterial effector mechanisms. In this study, we aimed to investigate the role of the intestinal microbiota in the host defense against severe pneumonia. Therefore, we inoculated mice intranasally with Streptococcus pneumonia and depleted the intestinal microbiota prior to infectious challenge using antibiotic treatment with vancomycin, ampicillin, neomycin and metronidazole. In the intestinal microbiota-depleted mice, we found significantly increased pulmonary outgrowth of S. pneumonia when compared to controls. This corresponded with increased production of interleukin (IL)-6, IL-1β, and chemokines (e.g. CXCL-1) and decreased IL-10. Interestingly however, tumor necrosis factor (TNF)-α levels were downregulated in intestinal microbiota-depleted mice. In addition, microbiota-depleted mice showed reduced survival after S. pneumonia infection. Remarkably, microbiota-depleted mice that received a fecal transplant from untreated control mice showed a largely restored host defense response to S. pneumonia, e.g. less bacterial outgrowth and restored cytokine release. Alveolar macrophages isolated from uninfected, microbiota-depleted mice were found to be less capable in phagocytosing S. pneumonia and were less able to secrete IL-6 and TNF-α in response to LPS or LTA stimulation. Moreover, neutrophils from microbiota-depleted mice were less capable of phagocytosing S. pneumonia. We conclude that the intestinal microbiota plays a protective role in the host defense against S. pneumonia induced pneumonia.

P4.12.12
Probiotics promote protective immunity against enteric bacteria infection by gut immune maturation

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Commensal bacteria play an indispensable role in gut homeostasis, not only by regulating a composition of dietary components, but also by prohibiting infection with enteric bacteria. In order to clarify the role of probiotics in gut protective immunity, we fed C57BL/6 mice for five days with probiotics including *Lactobacillus* (*L.*) *Rhamnosus* and *L. Acidophilus.* The mice were then orally infected with human-specific pathogen such as enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) strain. For the positive control, mice were treated orally with antibiotics including ampicillin, vancomycin, neomycin, and metronidazole. Significantly lower numbers of bacteria in the gut-associated lymphoid tissues were detected in probiotics-fed mice compared to those of nil and antibiotics-fed mice at three hours post infection. While oral O157 challenge resulted in severe pathology in the gut such as epithelium shedding and paneth cells distortion in the nil and antibiotic-fed mice group, pre-feeding with probiotics maintained a healthy gut. In addition, predominant levels of mucin secretion were found in the probiotics-fed mice when compared with nil and antibiotic-fed mice. Most interestingly, mRNA levels of HB-EGF, CDX2, TFF3 and Vangl1, which are known for stem cell proliferation and goblet cell activation, were significantly higher in the gut epithelium of probiotics-treated mice than in those of nil and antibiotic-fed mice. Taken together, these data suggest that probiotics play a pivotal role in promoting gut stem cell maturation and subsequently in protecting host against enteric bacteria infection.

P4.12.13
Modulation of Enteropathogen-mediated immunity by Sodium Butyrate

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Background: Sodium butyrate (SB), a short chain fatty acid produced by anaerobic gut bacteria is a major energy source for intestinal epithelial cells (IECs). SB exerts anti-inflammatory effects and inhibits the development of colon cancer through mechanisms that are not completely understood. We hypothesised that SB may modulate and promote host defence in response to enteropathogens implicated in Diarrheal and Inflammatory Bowel Diseases.
Methods: THP-1 monocytes were pre-treated for 12 hours with SB (0.5, 1 and 5mM), the pan-histone deacetylase inhibitor trichostatin-A (TSA) (10, 50 and 100nM) and 5-aza-dC, an inhibitor of DNA-methyltransferase (0.5, 1 and 5μM). Cells were then infected with Adherent invasive *E.coli* (AIEC) (MOI = 10), *Campylobacter jejuni* (CJ) (MOI = 100) and *Salmonella typhi* (S. typhi) (MOI = 5) for 6 hours and supernatants were analyzed for the presence of TNF-α, IL-8 and IL-10 cytokines by ELISA.
Results: Pre-treatment with all three inhibitors led to marked suppression in TNF-α production (> 50%). SB, but not TSA or 5-aza-dC, enhanced IL-10 cytokine levels (>20%) following infection. SB alone enhanced IL-8 production dramatically (>100%) even at low concentration, whereas TSA and 5-aza-dC did not have a significant effect.
Conclusion: Our findings show that SB can enhance the innate immune response to bacterial infection, increasing the production of chemokine and anti-inflammatory cytokines. These effects are independent of the inhibition of histone-modifying enzymes or DNA methylation events.

P4.12.14
Relation between IL-17 gene polymorphisms and *H. pylori* associated gastric diseases

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Introduction: Helicobacter pylori (H. pylori) is the main cause of chronic active gastritis, which can lead to peptic ulcer, gastric adenocarcinoma, and gastric mucosaassociated lymphoma. According to the important role of Interleukin-17 (IL-17) in pathogenesis of inflammatory diseases and the known effect of IL-17 gene polymorphisms on the production of this cytokine, we investigated the association between SNPs of IL-17A gene and the risk of H. pylori related chronic active gastritis and peptic ulcer diseases.
Materials and Methods: The study groups included 100 patients suffering from chronic active gastritis and 50 patients with peptic ulcer in addition to 226 healthy individuals as the control group. Alleles and genotypes at nine polymorphic sites of IL-17 were compared among the study groups by PCR-RFLP.
Result: The results showed that the distribution of A allele at position rs3819024 was significantly more frequent in the gastritis patients than the controls (P=0.0003) and was significantly more frequent in the ulcerative gastritis patients than the controls (P=0.0001). Moreover, homozygous rs3819024GG was significantly more frequent in the controls than the gastritis patients (P=0.0008) and also, it was significantly more frequent in the controls than the ulcerative gastritis patients (P=0.0005). Furthermore, AA (rs4711998A and rs3819024A) haplotype was significantly more frequent in ulcerative gastritis patients than the controls (P=0.0002) and GG haplotype was significantly more frequent in controls than ulcerative gastritis patients (P=0.001).
Conclusion: It could be suggested that IL-17 genetic variants at position rs3819024 can affect resistance or susceptibility to H. pylori-associated gastroduodenal diseases among Iranian population.

P4.12.15
Phenotype and function of lymphocytes in normal and inflamed appendix

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Introduction: The appendix is considered as part of the gut-associated lymphoid tissue. Appendicitis is one of the most common abdominal inflammatory diseases. The function of appendix is not clearly defined. In this study to understand better the immunological function of the appendix, we investigated the function and phenotypic pattern of lymphocyte subsets in appendix of patients with normal and inflamed appendix tissue.
Materials and Methods: Appendix tissue and appendiceal mononuclear cells obtained from 81 patients (mean age; 23±10.5), who were clinically suspected of having appendicitis. Based on histopathological examination, twenty-five patients had normal appendix while 40 and 16 were diagnosed with acute and gangrenous appendicitis, respectively. The phenotypic characteristics of lymphocyte subsets in appendix analyzed by three color-flow cytometry. The proliferative responses of tissue mononuclear cells assayed by MTT method.
Results: There were significantly different in percentage of CD19/HLA-DR , HLA-DR and CD19 cells between acute, gangrenous appendicitis patients and patients with normal appendix (p=0. 01). There is significant different in the proliferative responses of the appendiceal mononuclear cells to PHA and LPS between acute, gangrenous appendicitis patients and patients with normal appendix(p=0.012).
Conclusion: The phenotypic and function of lymphocytes are different between normal and inflamed appendix tissue. These results show that appendix tissue with special lymphocyte profile maybe effective in preventing or reducing intestinal infections.

P4.12.16
High fat diet impairs respiratory tolerance in a mouse model of allergic asthma

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Obesity is now recognised as a state of chronic low-grade systemic inflammation. Epidemiological studies show that obesity is a risk factor for allergic asthma, and the prevalence of both conditions is increasing worldwide. We hypothesized that obesity limits induction of inhalational respiratory tolerance to airborne antigens, therefore predisposing to allergic asthma. We induced acute allergic airway inflammation (AAI) by sensitising C57BL/6 mice with ovalbumin (OVA) in alum, followed by intranasal OVA challenge. Respiratory tolerance was initiated by administering OVA intranasally prior to induction of AAI. Diet-induced obesity was modelled by feeding C57BL/6 mice for 8 weeks with high fat diet (HFD) prior to tolerance induction. Our data show that respiratory tolerance in mice on normal diet (ND) caused the expected decrease in AAI, characterised by decreased airway eosinophilia and Th2 cytokine production in bronchoalveolar lavage fluid. However, respiratory tolerance was impaired in mice fed with HFD. Similarly, Th2 cytokine production by lung-draining lymph node cells and serum OVA-specific IgE were reduced by respiratory tolerance in ND but not in HFD fed mice. At the cellular level, we observed a higher percentage of plasmacytoid dendritic cells (pDCs) in lungs and spleen of OVA tolerized ND mice compared to OVA tolerized HFD mice, consistent with previous reports of pDCs being tolerogenic in asthma. These data show for the first time that HFD feeding impairs the capacity of mice to become tolerant to airborne antigens, providing a potential explanation for the observed link between diet-induced obesity and allergic asthma.

P4.12.17
Link between Western diet and intestinal inflammation: role of TLRs/NLRs

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Diet and nutritional status are among the most important modifiable determinants of human health and are influenced in part by gut microbial community. The importance of the gut microbiota in intestinal development and homeostasis raises the question of how the host senses the microbial organisms. The Toll-like and Nod-like receptors (TLRs/NLRs) are involved in the host response to bacteria. Breakdown of interaction between commensal bacteria and TLRs/NLRs induces alteration in immune response and may increase susceptibility to inflammation-induced intestinal cancer. Newmark reported that the Western diet (WD), designed to mimic the intestinal cancer risk factors in humans, induces neoplastic lesions, when fed to wild-type mice for 1 year. These findings have important public health implications and reinforce the need to further define the complex relationship between dietary factors and microbiota associated with risk of developing intestinal cancer. Given the importance of the fact that WD influences the diversity of the microbiota and TLRs/NLRs play a major role in the recognition of bacterial products and induction of inflammation, our aim is to elucidate the role of TLRs/NLRs in intestinal inflammation and related mechanisms that can increase risk for intestinal carcinogenesis associated to consumption of WD. Although several studies reported that a WD for 1 year induces intestinal tumors in normal C57Bl/6 mice there are no studies regarding TLRs/NLRs KO mice. For this reason we considered KO and WT mice fed with both WD and AIN-76A for 3, 6, 12 month and we reported data regarding mice sacrificed at each time point.

P4.12.18
Immunoregulatory mechanisms associated with Bifidobacterium infantis 35624 exopolysaccharides

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Gastrointestinal disorders are associated with a different gut microbiota composition of patients compared to that of healthy individuals. The use of probiotics to restore the microbial balance in the gut has been proposed as an immunomodulatory approach for prevention and treatment of these diseases. Many mechanisms could explain the probiotics' benefits: the competition with pathogens, the enhancement of the intestinal epithelial barrier function and the generation of regulatory responses. Nevertheless, further studies are needed to clarify these mechanisms as well as the molecular pathways involved in the signal transduction between probiotic MAMP (microorganism-associated molecular pattern) and host PRR (pattern recognition receptor) exposed by innate immune cells. In the interaction with the host, bacterial metabolic products and/or structural components can be responsible for probiotic beneficial effects. Polysaccharides have already showed their capacity to induce IL-10 and IL-12 production in in vitro and animal studies. However, studies in humans or isolated human cells are lacking.
The study on Bifidobacterium infantis exopolysaccharides (EPS) using WT and EPS knockout strains reveals the importance of this structural molecules on its capacity to induce a tolerogenic phenotype in MDDC (monocyte-derived dendritic cells) and to down-regulate their expression of activation markers. In THP-1 and HEK cells, EPS also influence the capacity to reduce the activation of NFkB/AP-1 pathway.
In addition to the administration of live microbes, the identification of microbial factor(s) responsible for immunomodulatory properties of probiotics might represent a chance to use these isolated compound(s) alone.

P4.12.19
Epigenetic control of host genes in intestinal epithelial cells by commensal bacteria

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The significance of intestinal microbiota to the maintenance of health has recently attracted considerable attention. Although commensal bacteria are not immunological "self" to the host, the intestinal immune system does not exclude them completely, resulting in a symbiotic relationship. There is now increasing evidence that these bacteria are involved in establishing the mutualism by controlling the host cells. In this study, the effect of commensal bacteria on the gene expression of intestinal epithelial cells (IECs) was investigated to clarify the mechanisms underlying the intestinal symbiosis.
IECs were prepared from the large intestine of conventional (CV) and germ-free (GF) mice. Genomic DNA fragments obtained from IECs were pulled down using methyl-CpG binding domain protein 2-coupled beads and analyzed by next generation sequencing to compare the DNA methylation pattern between these mice. The 5'-regions of 453 genes showed more than 1.5-fold change in the annotation frequency between CV and GF mice. *Gm7120* was one of the candidate genes differently methylated in the CpG motifs between CV and GF mice. The *Gm7120* gene was methylated at a higher level and expressed at a lower level in IECs of CV mice than in those of GF mice. Knockdown of *Gm7120* by RNAi resulted in decreased expression of RALDH1, an enzyme mediating the conversion of retinal, derived from vitamin A, to retinoic acid that plays a key role in the immunological homeostasis of the intestine. Collectively, these results indicate that commensal bacteria control specific host genes through epigenetic mechanisms and thereby maintain the intestinal symbiosis.

P4.12.20
Fibre, short-chain fatty acids and microbiota combat the development of asthma

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Recent studies suggest that the gut microbiota and microbial by-products play an important role in the development of asthma. Fibre and short-chain fatty acids (SCFAs) are highlighted as particularly important, however the role of these elements in the development of asthma and our understanding of the gut-lung axis is poorly defined.
Using a series of studies, we investigated the effect of fibre and SCFAs on the development of allergic airways disease (AAD) using house-dust mite-induced mouse models. Characteristics of AAD that were assessed included airways hyper-reactivity, inflammatory cells and cytokines, and serum IgE.
The development of AAD was reduced in adult mice fed a high fibre diet and exacerbated in mice receiving a low fibre diet. Interestingly, we also demonstrated that low fibre diet in early life, especially in the perinatal phase is a key driver of susceptibility. Our high fibre diet increased levels of SCFAs, particularly acetate, which when administered in the drinking water also reduced the development of AAD. Furthermore, high acetate-producing bifidobacterium suppressed the development of asthma in antibiotic treated mice and monocolonized germ-free mice. Altogether these data show beneficial effects of dietary fibre and acetate in AAD possibly through the of G-protein coupled receptors 41 and 43, histone deacetylases and regulatory T cells.
This collection of studies highlights a novel gut-lung axis involved in the regulation of asthma.

P4.12.22
Remodeling of stromal architecture in the gut mucosa as a mirror of immunological changes under various conditions (germ-free animals, colitis and cancer)

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Comparison between germ-free (GF) versus conventionally (CV) reared animals allows to enlighten the inflammatory environment regulation and cancer microenvironment development. The GF animals present larger bowel, thinner intestinal wall with poorly developed lymphatic infiltrates and follicles. Rapid acquisition of regular bowel anatomy and increased complexity of collagen structures coupled lymphatic follicle enlargement after conventionalization of GF animals. Similar remodeling of lymphatic structures was evidenced after induction of chronic colitis by dextran sodium sulphate (DSS), carcinogenesis by azoxymetane (AOM) or associating both inductions (DSS followed by AOM administration). Inflammatory background accelerated the AOM-induced tumor development in the sigmoid. In CV animals, circulating CD3+CD4+CD25high Treg cells progressively increased during the various inductions, faster after AOM. Contemporarily, collagen architecture of mucosal scaffold showed progressive thickening and distortion. In the left colon/sigmoid mucosa, VEGFa and TGFbeta expression increased but not the inhibitory VEGFb. Reduction of CD3+ cells and higher percentage of NK and NKT cells suggested prevalence of innate immunity in early anticancer responses. In GF animals the percentage of NK and NKT cell are constitutively higher than in CV, and during carcinogenesis, GF cytotoxic activity had lower reduction that in CV animals. Resistant-to-cancer animals showed higher CD8+ levels. Under either AOM-only carcinogenesis or DSS+AOM induction, GFs developed fewer and smaller tumors than CV animals. Concluding, inflammatory stimuli deregulation produces immunological changes that reflect also on tissue scaffold architecture. Acknowledgements: grants GAAV IAA500200917, RVO61388971 (CZ), Rusconi Foundation, Varese (IT), ENI Czech Republic, Prague (CZ), Manghi Czech Republic s.r.o (CZ), Paul's Bohemia s.r.o (CZ).

P4.12.23
The Microbial Landscape Of Breast Cancer: Examination At The Site Of Disease

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Microbes have been reported to influence various disease states including obesity, diabetes and cancer in humans. Breast cancer is the second leading cause of cancer deaths in women, with over 400,000 deaths annually worldwide. Though some risk factors such as diet, age and genetic predisposition have been identified, the majority of breast cancers have unknown etiology. Given the importance of host-microbiota interactions in human health and disease, we investigated the relationship between the breast microbiota and breast cancer. Here, we characterized and compared the bacterial species found in the breasts of healthy women and women with breast cancer. Quantitatively, we found that healthy tissue harbors more bacteria than tumor tissue ($p<0.001$). Qualitatively, we found that there were shifts in the microbiota associated with the diseased state, with certain bacterial genera being more abundant in healthy versus tumor tissue. Specifically, the bacterium Sphingomonas yanoikuyae is enriched in matched normal adjacent tissue compared with tumor tissue from the same patient ($p<0.01$). These data support the hypothesis that the presence of S. yanoikuyae may support a healthy breast environment. Our findings highlight a new area of research into the breast microbiome and its association with breast cancer development and progression.

P4.12.24
Synthesis and pharmacological evaluation of 8-substituted phenyl xanthines for asthma therapy

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Xanthines belongs to an important class of drugs used in the treatment of asthma and have been considered to be the most effective prophylactic agents for the control of symptoms of chronic asthma. Currently, adenosine receptor antagonists (AdOR) are reported to be novel therapeutic antiasthmatic agents. Development of receptor target inhibition is a novel approach in current drug design scenario. In the last few year's xanthines have received considerable attention as molecular targets for the development of antiasthmatic agents. Several positions of xanthine skeleton with different substituent have been explored to obtain a variety of potent derivatives.Present investigation has been made to design potent selective xanthine derivatives, synthesize and screen them in vitro (adenosine antagonism) and in vivo (BAL analysis) for antiasthmatic activity. To observe the effects of morpholino substituted group, a new series of 8-(substituted-phenyl) xanthines has been synthesized and compounds were evaluated for their affinity for A1 and A2A adenosine receptors (AR) using radioligand-binding assays. The synthesized xanthine derivatives exhibited varying degrees of affinity and selectivity towards A1 and A2A receptor subtypes. As a part of research study, we further examined the dynamic changes in cellular profile of bronchoalveolar lavage (BAL) fluid after lipopolysaccharide (LPS) challenge in the male wistar rats of synthesized xanthine derivatives.In general, the newly synthesized compounds displayed significant antiasthmatic activity. The outcome of the current work is the generation of an important pool of new xanthine derivatives.

P4.12.25
Comparative fecal microbiome of Holstein and Nelore bovines: differential bacterial composition can be related to susceptibility to ectoparasites

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The microbial composition of gastrointestinal tract plays important role in immunological status in animals. Bovine breeds present different levels of immunity to tick infestations; zebu are resistant and taurine are susceptible. Since microbial metabolites can be associated with the innate immunity of hosts, we evaluated the fecal microbiome from a zebu and taurine breed, for analyzing if the diversity found could be related to traits such as ectoparasites susceptibility. We extracted genomic DNA of fecal samples collected from rectum of 6 Nelores (zebu) and 6 Holsteins (taurine), under same feed and animal management system. The 16S rRNA genes were amplified using primers for V5-V6 region and pyrosequencing of samples was performed using Roche FLX high-thoughput sequencing. The taxonomic assignment was achieved at phylum, family and genus level. As expected, the most abundant phyla were Firmicutes and Bacteroidetes for both breeds. Some genera presented differential prevalence according to the breed (compared with test-t). We highlight the *Bacteroides* genus, significantly more frequent in Holstein (50.4% *vs* 31.1% in Nelore, p=0.02). *Bacteroides* bacteria generate metabolites from aromatic aminoacids. Noteworthy, the skatole metabolite (derived from tryptophan) is attractive to insects, therefore, we believe that the major prevalence of *Bacteroides* in feces of Holsteins might be related to attraction of ectoparasites, such as myiasis-causing flies and ticks. Thus, the odor generated in feces would be one among several factors that contribute for susceptibility to tick infestations in Holstein breed.

P4.13 Immune regulation at barrier sites

P4.13.01
Host defense peptide CRAMP acts as a potent mast cell chemoattractant and mediator release stimulus

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Cathelicidins, a family of cationic peptides, are well known for their role in innate host defense, particularly against bacteria. Apart from direct killing of microbes through the membrane disruption, cathelicidins can also indirectly regulate immune response by affecting activity of cells involved in antibacterial defense mechanisms. Considering important role of mast cells (MCs) in protection against bacterial infection, the aim of this study was to determine whether CRAMP, rat-derived cathelicidin, can induce MC degranulation and histamine release (spectrofluorometric method), cysteinyl leukotrienes (cysLTs) generation, TNF and CXCL8 synthesis (ELISA methods), as well as MC migratory response (Boyden microchamber assay). Experiments were conducted *in vitro* on fully mature rat peritoneal MCs. We have stated that MC stimulation with CRAMP resulted in a dose-dependent generation and release of significant amounts of cysLTs, but failed to induce MC degranulation and histamine release. Moreover, MC activation *via* CRAMP led to TNF, but not CXCL8 synthesis. We also documented that CRAMP acts as a strong MC chemoattractant. MC response to CRAMP stimulation was observed when cathelicidin was used at concentrations of≥ 20 μg/mL, i.e. significantly higher than physiological level. To gain some insight into CRAMP-mediated signal transduction pathways in MC, we examined the effect of the several inhibitors specific to PLC/PLA_2, PI3K, ERK and p38 MAPK signaling molecules. Our results suggest that cathelicidins may enhance antibacterial inflammatory response *via* attracting MCs to pathogen entry site and *via* induction of MC-derived mediator release. This work was supported by the Medical University of Łódź (Grant No. 502-03/6-164-01/502-64-004).

P4.13.02
Chemerin is an antimicrobial agent in human epithelium

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Chemerin, a protein ligand for the G-protein coupled receptor CMKLR1 (chemokine-like receptor 1), selectively attracts specific subsets of immunoregulatory antigen presenting cells, including dendritic cells and macrophages. C-terminal chemerin sequence, F149 S157 plays key role in mediating chemotactic activity through CMKLR1 receptor. Chemerin is predicted to belong to the structural cathelicidin/cystatin family of proteins comprised of antibacterial polypeptide cathelicidins and inhibitors of cysteine proteinases (cystatins). Chemerin is widely expressed in healthy epithelium, a natural body barrier, although the biological significance of chemerin in this tissue remains unknown. We hypothesized that chemerin may be involved in host defense by functioning as an antimicrobial agent in epithelial cells. Here we show that chemerin is abundant in human keratinocytes and bronchial epithelial cells in situ. Chemerin-replete exudates from primary epidermis cultures inhibit bacteria growth and chemerin represent a quantitatively significant fraction of anti-bacterial activity in the products of cultured keratinocytes. The internal region Val66-Pro85 of chemerin mediates the majority of the antibacterial activity that is comparable in potency to other antimicrobial proteins. Collectively, our work uncovers a novel protective function for endogenous chemerin in human epithelium that is independent of its role as a leukocyte chemoattractant.

P4.13.03
Bcl3 expression is increased in Irritable Bowel Syndrome (IBS) patients independently of IL-10

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Irritable bowel syndrome (IBS) is characterised by a state of low-grade inflammation. B-cell leukemia/lymphoma 3 (Bcl3) is a co-transcriptional gene regulator activated by the NF-kB pathway. An immunosuppressive role, exerted by IL-10, has been ascribed to Bcl3 in the intestinal mucosa. The status of Bcl3 has not been characterised in IBS. We hypothesised that altered expression of Bcl3 is relevant to inflammation reported in IBS. AIM: To compare expression levels of Bcl3 and their correlation with expression of IL-10 in intestinal mucosa of IBS patients and healthy-controls (HC). METHODS: Colonic biopsies of IBS patients and HC from Cork University Hospital and Hospital Clínico Universidad de Chile were analysed for Bcl3 and IL-10 expression by qPCR and tissue distribution of Bcl3 was defined by immunofluorescence. The effect of

supernatants from colonic biopsies, 24h cultured in the presence or absence of CD3/CD28 antibodies, on Bcl3 expression in the intestinal epithelial (IE) cell line (DLD-1) was evaluated using qPCR and immuno-blotting. RESULTS: We observed a significant increase in Bcl3 expression, but not IL-10, in IBS patients compared to HC (p=0.0115). Immunofluorescence demonstrated a predominantly nuclear distribution of Bcl3 in the IE of both groups. Bcl3 expression was more significantly induced in DLD-1 cells by the supernatants of stimulated biopsies from IBS compared with HC. CONCLUSION. Our results suggest that Bcl3 expression is increased in the IE of IBS patients, as a regulatory mechanism independent of IL-10 expression. Supported by FONDECYT-11121527, MECESUP-0608 and Science Foundation Ireland through a grant to the Alimentary Pharmabiotic Centre.

P4.13.04

Soluble factor produced by Staphylococcus aureus modulates the cell cycle of eukaryotic cells

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Staphylococcus aureus can cause life-threatening infections such as bacteremia, pneumonia, osteomyelitis, endocarditis and sepsis.
Epithelial cells are able to sense microbes, creating an early line of defense. We investigated S. aureus-host epithelial cells interaction using multidisciplinary approaches. Bacteria slowed down cell proliferation and induced a cytopathic effect. We observed an enlargement of host cells at MOI (Multiplicity of Infection) 20:1, which started being visible from 72h post-infection. Increasing MOI to 100:1 resulted in the induction of apoptosis. Using flow cytometry analysis, we demonstrated that S. aureus induces a G2/M phase delay in synchronized HeLa cells, which was associated with accumulation of the cyclin-dependent kinase Cdk1/cdc2, a key inducer of mitosis entry, and with the accumulation of unphosphorylated histone H3, which was correlated with a dramatic reduction of the mitotic cell number. This delay required the presence of live S. aureus since the addition of the heat-killed bacteria did not alter the cell cycle. Culture supernatants of S. aureus strains induced a similar G2/M phase transition delay, suggesting a soluble factor is involved in the transient cell cycle arrest. Analysis of S. aureus proliferation in asynchronous, G1- and G2-phase-enriched HeLa cells showed that the G2 phase was preferential for bacterial infective efficiency, suggesting that the G2 phase delay may be used by S. aureus for propagation within the host. Our findings reveal the potential of S. aureus in the subversion of key cellular processes such as cell cycle progression, and shed light on the biological significance of host cell cycle alteration.

P4.13.05

Characterization of a lymphocyte inhibitor from Aedes aegypti saliva

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Aedes aegypti is an important mosquito species and transmits diseases such as yellow fever, Chikungunya fever and dengue fever. In order to successfully nourish themselves, these hematophagous mosquitoes find the immunological system of their hosts as a major challenge. During the millions of years of co-evolution with their hosts, these insects developed a salivary cocktail with an arsenal of molecules presenting several immunomodulatory properties. In the present work, we characterized a lymphocyte inhibitor present in Ae. aegypti salivary gland extract (SGE). Our results reveal that Aedes aegypti saliva possesses a potent inhibitor of T cell proliferation able to induced apoptosis in naïve, but not memory T cells. Fractionation of salivary gland extract (SGE) by FPLC showed one dominant active fraction with apparent molecular mass of >400 kDa. The analysis of the fractions by mass spectrometry revealed a single candidate in which the amount detected correlated with the observed activity. Serum from mice previously immunized with the active fraction and Complete Freund Adjuvant was able to neutralize the SGE-induced modulatory activity. We also observed that cells from MRL/lpr mice (FAS mutant mice) and Bim KO mice were discreetly less affected by SGE in comparison to wild type mice, suggesting that both signaling pathway of apoptosis might be involved or acting as compensatory pathways. Activation of caspase-8 was also analyzed by Western Blot and seems to be involved in the SGE-induced apoptosis. Further studies will be required to better understand the mechanisms of action underlying such effects.

P4.13.06

Expression and regulation of TSLP in human oral mucosa and cultured oral keratinocytes

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Epithelial cells at mucosal surfaces are exposed to a variety of antigens, including pathogenic and commensal bacteria, food antigens and allergens. The ability of the immune system of the oral cavity to protect against infection while avoiding destructive inflammatory responses is
essential for maintaining oral health. Thymic stromal lymphopoietin (TSLP) is an epithelial-derived cytokine known to activate dendritic cells and regulate immune responses in several tissues. The aim of this study was to investigate the expression and regulation of the two TSLP transcript variants in oral mucosa. Oral mucosal biopsies were taken and processed for immunohistochemistry (IHC). Other biopsies were used to establish cultures of oral mucosal keratinocytes which were stimulated with various inflammatory mediators and thereafter harvested for examination by real time PCR. IHC analysis showed that TSLP was constitutively expressed in the gingival epithelium. In cultured oral keratinocytes exposed to poly(I:C), TNFα/IL-1β or IFNγ, the expression of long form TSLP (lfTSLP) transcript but not that of short form (sfTSLP) transcript was significantly increased. These results show that lfTSLP is the regulated form associated with inflammation in oral keratinocytes. Due to its extensive expression in gingival biopsies, however, TSLP might also have other, non-immunological roles in oral epithelia, e.g. participate in modulation of barrier functions or epithelial differentiation.

P4.13.07

Induction of Interleukin-10 expression by the commensal microbiota in intestinal regulatory T-cells

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Interleukin-10 (IL-10), a pleiotropic cytokine, is a crucial anti-inflammatory mediator playing a non-redundant role in the intestine. The importance of this cytokine has been illustrated by knock-out mice which develop a lethal colitis in response to colonic bacteria. Recent studies have shown that IL-10 is predominantly expressed by Foxp3+ T-cells in the colonic lamina propria. In this study we aimed to further characterize this subset of IL-10 secreting Foxp3+ T-cells and to dissect the mechanisms governing their activation in the intestine, employing a novel dual reporter mouse for Foxp3 and IL-10. We found that the induction of IL-10 expression ultimately depends on the commensal flora and colonization with the pathobiont Helicobacter hepaticus (H.h.) significantly enhanced the frequency of Foxp3+ IL-10+ T-cells in the intestine. Induction of IL-10 did not require candidate cytokines such as IL-21 or IL-27, as well as MyD88, the

universal adaptor protein for the induction of TLR signaling. Using TCR Vb-chain spectrotyping we show that IL-10+Foxp3+ T-cells contain a broad TCR Vb-chain repertoire suggesting broad specificity which did not show a particular enrichment in a single TCR Vb-chain following H.h. infection. Our data show that resident intestinal bacteria not only promote the expansion of effector T-cell subsets but are as well crucial in the induction of immune-regulatory pathways.

P4.13.08

***Helicobacter pylori* HP0175 promotes the production of IL-23, IL-6, IL-1β and TGF-β.**

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Helicobacter pylori infection induces a chronic gastric inflammatory infiltrate. *Helicobacter pylori* is a spiral-shaped Gram-negative bacterium that chronically infects the stomach of more than 50% of the human population and represents the major cause of gastric cancer, gastric lymphoma, gastric autoimmunity and peptic ulcer diseases. The World Health Organization classifies *Helicobacter pylori* as a type I carcinogen for distal gastric cancer. Eradicating the bacterium, in high risk populations, reduces incidence of gastric cancer. *Helicobacter pylori* induces an inflammatory response in the gastric mucosa characterized by polymorphonuclear and mononuclear cell infiltration. The host immune response to *Helicobacter pylori* influences the clinical outcome of the infection. This study was undertaken to evaluate the type of the innate immune responses elicited by the secreted peptidyl prolyl cis,trans-isomerase of *Helicobacter pylori* (HP0175). The cytokine production induced by HP0175 in neutrophils, monocytes and dendritic cells was evaluated. HP0175 was able to induce the expression of IL-23 in neutrophils, monocytes and dendritic cells, and IL-6, IL-1beta and TGF-beta in monocytes and dendritic cells. These findings indicate that HP0175 is able to promote the activation of innate cells and the production of a cytokine milieu that may favour the development of Th17 response.

P4.13.09

Mechanistic insights into *Helicobacter pylori* pathogenesis and host response

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Helicobacter pylori is a Gram negative bacterial gastric pathogen that infects ~50% of the world's population with some progressing to gastric cancer. The indelible link between Helicobacter pylori and severe gastric diseases coupled with the emergence of multiple-antibiotic resistant H. pylori and the high prevalence of gastric cancer necessitate urgent attention. Furthermore, the mechanistic action of pathogenesis has not been firmly established. Among the known pathogenic factors of H. pylori reported, cytotoxin associated gene A (CagA), an oncoprotein, has been cited to induce inflammatory response in host cells and cause disruption of gastric epithelial cell-cell tight junctions. We report here a surface localized heat shock protein that effects the cytoskeleton rearrangement of cells independently, and exacerbates disruption of cell-cell tight junctions through its interaction with CagA. This protein is found in all strains of H. pylori analysed, and that it aids the bacteria in colonizing BALB/c mice. The dynamics of interaction between heat shock protein and CagA intrabacterially, as well as between the bacterial pathogen and the host in the course of H. pylori pathogenesis will be discussed.

P4.13.10

Intestinal mucosal barrier in experimentally induced septic shock and gut inflammation

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BACKGROUND: Gut forms the largest and most important barrier against the external environment. This barrier is in constant interaction with both gut resident microbiota and mucosal immune system, and its dysfunction could cause the inflammatory or autoimmune disease. Our aim was to analyze how local and systemic inflammation influences the intestinal barrier function and how this deleterious effect can be counteracted by oral treatment with lysate of probiotic bacterium.
METHODS: First, before the induction of colitis we pretreated BALB/c mice with lysate of probiotic *Lactobacillus casei* DN-114001 (Lc) by oral gavage. Next, we induced either systemic or intestinal inflammation in these mice by intraperitoneal injection of lipopolysecchariide or by oral administration of dextran sulfate sodium, respectively. We measured the function of the gut barrier by FITC-labeled dextran method and its molecular properties by changes in expression and localization of tight junction proteins (occludin, ZO-1 and claudin-2) by RT-PCR and immunofluorescence, respectively.
RESULTS: We found that in both systemic and local inflammation the gut permeability for macromolecules is significantly increased. In both models, this functional change is accompanied by increase in expression of proinflammatory cytokines (e.g. IL-6) in colon mucosa. Oral treatment with Lc can prevent the changes in the localization of tight junction proteins, and decrease in their expression, caused by the systemic or intestinal inflammation.
CONCLUSIONS: We conclude that components from probiotic bacteria decrease the sensitivity to both systemic and local inflammation by strengthening the gut barrier function.
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P4.13.11

Homeostatic proliferation of naïve CD4$^+$ T cells in mesenteric lymph nodes generates gut-tropic Th17 cells

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Homeostatic proliferation of naïve T cells in the spleen supplies memory T cells. This "systemic" proliferative response divides into fast or slow cell division rates. Since the fast proliferation is critical for generation of effector memory T cells, which are abundant in the intestinal tissue, "gut-specific" homeostatic proliferation may be important for generation of intestinal effector memory T cells. However, such organ-specific homeostatic proliferation has not been addressed. In this study, we examined the gut-specific homeostatic proliferation by transferring CFSE-labeled naïve CD4$^+$ T cells into lymphopenic mice and evaluating donor cell division and differentiation in the intestine and mesenteric lymph nodes (MLNs). We found that the fast-proliferating cell population in the intestine and MLNs had a gut-tropic $\alpha_4\beta_7^+$ CCR9$^+$ CCR6$^+$ Th17 phenotype, and that their production was dependent on OX40 costimulation and foreign antigens including commensal bacteria. FTY720 treatment induced accumulation of $\alpha_4\beta_7^+$ IL-17A$^+$ fast-dividing cells in MLNs and eliminated donor cells in the intestine, and mesenteric lymphadenectomy significantly reduced the Th17 cell population in the host intestine, indicating that MLNs rather than intestinal tissues are essential for generating intestinal Th17 cells. Furthermore, the fast-dividing cells survived for at least 60 days in MLNs when they were transferred to Rag2-/- second host mice, suggesting that the fast-dividing population might include gut-tropic memory precursor cells. These results reveal that MLNs play a central role in inducing gut-tropic Th17 cells and in maintaining effector memory CD4$^+$ T cell homeostasis in the small intestine.

P4.13.12
Immunological Common Junctions: Novel Targets for Therapeutic Interventions

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Human - pathogen interaction involves a sequence of events that are pre-programmed. Diverse routes taken by pathogenic microorganisms predispose humans to a spectrum of fatal infections. Regardless of immunological barriers, the hunt for human body contact and successful establishment of infection has become an inherent property of many microorganisms. The port of entry which permits microorganisms to pass through important molecular junctions could make a nearer pathway or associated gated channel or receptor undergo functional changes. Although significant strides have been taken in exploring the pathogen entry sites and developing the pathways, the area needs some additional input in search of common immunological junctions. It is anticipated that microorganisms specific to a given target tissue or infection destination pass through these junctions. If the case is, it is unknown how many common junctions currently exist.To this end, algorithms or databases that are specially designed for modeling the pathways could throw light on such junctions.Say, a database of microorganisms that is specific to one infection category leading to skin disorders could help in understanding about the common junctions involved.This could be accomplished in a cross -platform fashion by collaboration between several countries which would submit pathway data specific to a pathogen. A sum total of all data when pooled and analyzed from different countries may furnish insights on the suspected involvement of same junction. Ultimately, novel drugs designed would be efficacious in targeting the junctions. Hence, strategies to block common immunological junctions need exploration.

P4.13.13
The flagellin sensor NLRC4 mediates protection against a mucosal pathogen

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Pattern recognition receptors (PRRs) enable mammals to discriminate self from non-self through the recognition of microbial signatures, such as bacterial flagellin. Flagellin is detected by the PRR NLRC4, which drives the activation and release of potent pro-inflammatory mediators. We aimed to characterize the role of NLRC4 in acute intestinal inflammation.
We used a model based on the murine pathogen *Citrobacter rodentium*. *Citrobacter rodentium* is a mouse homologue of enteropathogenic *E. coli* and induces acute intestinal pathology and diarrhea in the host.
We found that NLRC4$^{-/-}$ mice developed more severe weight loss, increased bacterial colonization levels and exacerbated intestinal inflammation compared to WT counterparts. Additional experiments using bone marrow chimeras revealed that the protective effects of NLRC4 were mediated by non-hematopoietic cells.
In conclusion, NLRC4 signalling in non-hematopoietic cells provided protection during acute bacterially-induced intestinal inflammation, possibly by limiting bacterial colonization. Further studies are needed to elucidate the mechanism responsible for protection.

P4.13.14
Brucella abortus 2308 induce downregulation of the activation markers in murine B cells

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Introduction. It has been demonstrated that Brucella abortus is capable to multiply into professional antigen presenting cells such as macrophages and dendritic cells. However, there are a few reports about Brucella infection into B cells. In this work, we evaluated the ability of B. abortus 2308 for infect the mouse B cell line, A20. Materials and methods. A20 lymphocytes cultured in RPMI medium supplied with L-glutamine and 10% of fetal calf serum were infected with several MOIs of Brucella abortus 2308 transformed by electroporation with the pJC43 (pBBR1MCS-2-gfp-mut3) plasmid to evaluate the entry of green fluorescent Brucella. The percentage of cells containing bacteria was evaluated by flow cytometry at three time points. To evaluate whether Brucella activated the B cells, kinetics of infection was performed and activation markers were analyzed by flow cytometry. Results. Association of Brucella abortus 2308-gfp-mut3 with A20 cells to reach a maximum of 20% of A20 infected cells. Infected cells increase expression of CD80 and MHC II and down-regulation of CD69 and CD86; CD40 was not modified. Conclusions. Brucella abortus 2308 interacts with A20 and induces change in the activation markers and costimulatory molecules.

P4.13.15
Transcription factor Spi-B-dependent and -independent pathways for the development of Peyer's patch M cells

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The mucosal immune response is triggered by antigen uptake from the lumen across the mucous and epithelial cell layers to organized mucosa-associated lymphoid tissues such as Peyer's patches (PPs) in small intestine. This antigen uptake is mediated mainly by microfold cells (M cells) with transcytosis action. Although many of the biological features of M cells, including their function, have been known for many years, the molecular mechanisms of M-cell development, antigen recognition, and transcytosis have remained unclear. Here, we report that *Umod* is a novel M cell–specific gene, the translation products of which might contribute to the uptake function of M cells. We also found that a transcription factor, Spi-B, which is expressed mainly in hematopoietic lineages, was also expressed in M cells but not in other non-hematopoetic lineages. Spi-B-deficient mice showed reduced expression of most, but not all, other M cell–specific genes and M-cell surface markers. A bone marrow transfer experiment indicated that M cell–intrinsic Spi-B activity was important for M-cell development. Whereas uptake of *Salmonella* Typhimurium via M cells into PPs was obviously reduced in Spi-B-deficient mice, the frequency of intra-tissue cohabiting bacteria found in the PPs was comparable between wild-type and Spi-B-deficient mice. These data indicate that there is a small M-cell population with developmental regulation that is Spi-B independent; however, Spi-B is probably a candidate master regulator of M-cell functional maturation and development by another pathway.

P4.13.16
Influenza virus infection induces long-term and compartmentalised derangement of airway and lung dendritic cell subsets

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Inhaled agents pose a major challenge to immunological homeostasis in the respiratory tract (RT), requiring constant screening for their risk to the host. This requires a balanced network of dendritic cells (DC) and other cells that regulate tolerance or immunity. Respiratory viruses such as Influenza pose a serious threat of long-term disruption of this balance given their potent inflammatory and cytotoxic activities. In this study, we used a mouse model of A/PR8/34 H1N1 Influenza Type A Virus (IAV) infection to examine its long-term effects on DC in airway mucosal (AM) and parenchymal lung (PL) tissue. In adult mice, we found marked differences in the kinetics of DC subsets in the AM and PL compartments, with a generally more acute responses in AMDC that resolved by day 7 after infection, but with persistent depletion of CD11blo PLDC for up to 3

weeks following infection. Similarly, upregulation of CD40 and CD80 was observed on AMDC at day 7, but this was delayed until day 14 in PLDC. A marked depletion in tissue-resident PL macrophages was observed at day 14 post-infection, with these cells showing a persistent activation state. In mice infected at 3 weeks of age, PLDC and macrophages showed persistent changes for up to 5 weeks following IAV infection. These data demonstrate that IAV has differential effects on DC populations in compartments of the RT, leading to long-term derangement in the numbers and activation states of these cells, that may permanently disrupt immunological homeostasis in this environment.

P4.13.17
Translation of Tollip is inhibited in the small but not large intestinal epithelial cells

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Immune responses against gut microbiota should be minimized to avoid unnecessary inflammation at mucosal surface. In this study, we analyzed the expression patterns of Toll-interacting protein (Tollip), an inhibitor of TLR signaling, in intestinal epithelial cells (IECs). Comparable mRNA expression was observed in small and large IECs (S-IECs and L-IECs). However, at the protein level, Tollip was only expressed in L-IECs but not in S-IECs. Similar results were obtained in germ-free mice, indicating that L-IECs-specific Tollip protein expression is not dependent on bacterial colonization. To identify the mechanisms underlying the post-transcriptional repression of the *Tollip* gene expression, 3UTR -mediated translational regulation was evaluated by luciferase assay. By deletion analysis, the region +1876/+2398 was shown to be responsible for the translational repression of *Tollip*. The inhibition of miR-31, whose target sequence was included in this region, resulted in 2-fold increase of the luciferase activity. Furthermore, miR-31 expression was significantly higher in S-IECs than in L-IECs. These data suggested that miR-31 represses the translation of Tollip mRNA in S-IECs. However, since the recovery of the luciferase activity by miR-31 inhibition was relatively modest, another mechanism was possibly involved in the regulation. Consistently, northern blotting analysis showed that the molecular mass of Tollip mRNA was unexpectedly higher in S-IECs than in L-IECs. Therefore, it was suggested that the translation of the *Tollip* gene expression could be inhibited in S-IECs by post-transcriptional modification of its mRNA. Collectively, it is concluded that the translation of Tollip is inhibited in S-IECs by miR-31 and additional mechanisms.

P4.13.18
Monoclonal intestinal IgAs are poly-reactive against commensal bacteria but recognize a single protein expressed by multiple bacteria

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Immunoglobulin A (IgA) is crucial for defense at mucosal surfaces and for intestinal homeostasis. Commensal bacteria are much diversified and their composition is easily changed by food. Therefore, to maintain gut homeostasis with limited IgA repertoire, the idea is reasonable that each monoclonal IgA recognizes multiple bacterial strains. Yet, how the monoclonal IgA recognizes multiple different bacteria is not known. We generated IgA-producing hybridomas derived from the intestinal IgA-secreting cells of wild-type mice. We selected three hybridomas producing hyper-mutated IgA. Their CDR amino acid sequences in V_H and V_L regions were not related, suggesting they were independently selected clones. Each of three monoclonal IgAs bound to more than 10 different intestinal bacteria with high-affinity. To elucidate their specific antigens, three different bacteria (*Escherichia coli*, *Pseudomonas fulva* and *Staphylococcus aureus*) were lysed and used for SDS-PAGE and Western blotting. Unexpectedly, all three monoclonal IgAs recognized a single protein at the size of about 46 kDa expressed by three different bacteria. On the contrary, three monoclonal IgAs did not recognize any proteins expressed in *Lactobacillus casei.* It suggests the intestinal microenvironment has a sophisticated mechanism to select the IgA-producing cells, whose antibody can regulate a bunch of commensal bacteria through a common antigen, while allowing the beneficial bacterial growth.

P4.13.19
Density of FOXP3+ T cells and CD11c+ dendritic cells in small bowel mucosa in children with atopic dermatitis, celiac disease and functional gastrointestinal disorders

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Aim: to evaluate Treg and dendritic cells (DCs) in intestinal mucosa of children with atopic dermatitis (AD) and celiac disease (CD) in comparison to children with functional gastrointestinal disorders (FGD).
Material and Method: 75 patients (37 male, mean age 8.4±4.8 years), who underwent small bowel biopsy were studied. AD was diagnosed in 8, AD with coexisting CD (AD+CD) in 5, CD in 9, FGD in 53 patients. Four of 8 AD and 6 of 53 FGD (p=0.006) are sensitized to grain allergens (ImmunCAP IgE fx20 assay (Phadia Oy). Staining for FOXP3, CD4 and CD11c was performed on paraffin-embedded sections of bioptates using the avidin-biotin method (Vectastain ABC Kit).
Results: The densities of all three cell populations were the lowest in AD group. The density of FOXP3 + cells was significantly higher in CD and AD+CD patients comparing with FGD and AD (p=0.004; p=0.03). CD11c+ DCs density was significantly higher in AD+CD patients comparing with AD alone and with FGD (p=0.03). However, in AD group significantly higher density of CD11c + DCs was in persons positive to sIgE fx5 as well as for sIgE fx20 (p=0.04). The number of CD4+ cells was higher in all other three groups comparing with AD being highly different between AD and AD+CD group (p=0.0007).
Conclusion: Densities of all studied cell types were lowest in AD, whereas FOXP3+ cells were the highest in CD patients. The finding of high variability of cell population densities among patients with FGD represents most probably the different etiology of FGD.

P4.13.20
Foxp3+ regulatory T cells promote a protective Th17 cell response against intestinal infection with *Citrobacter rodentium*

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Intestinal infection with the mouse pathogen *Citrobacter rodentium* induces a strong local Th17 response. While this inflammatory immune response helps to clear the pathogen, it also induces inflammation-associated pathology in the gut and thus has to be tightly controlled. In this project, we therefore studied the impact of $Foxp3^+$ regulatory T cells (Treg) on the infectious and inflammatory processes elicited by *C. rodentium*. Surprisingly, we found that depletion of Treg by diphtheria toxin in the Depletion of REGulatory (DEREG) mouse model resulted in impaired bacterial clearance in the colon, enhanced body weight loss and systemic dissemination of bacteria. Consistent with the increased susceptibility, we found that the local colonic Th17 cell response was impaired in Treg-depleted mice, suggesting that the presence of Treg is crucial for the establishment of a functional Th17 response in the gut. As a consequence of the impaired Th17 response, we also observed less inflammation-associated pathology in the colons of Treg depleted mice. Interestingly, anti-Interleukin (IL)-2 treatment of infected Treg-depleted mice fully restored the colonic Th17 response, indicating

that Tregs support the induction of a protective Th17 response during intestinal infection by consumption of local IL-2.

P4.13.21
Toward prediction of immune signatures of health

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Immune parameters are used as surrogate markers of health in studies of functional foods. Phenotypic heterogeneity within the immune system requires assessment of multiple aspects of the immune system to determine changes in immune status. We aimed to characterise variation in immune biomarkers in healthy individuals over 5 months and in relation to diet, physical activity, age, geographic location and faecal microbiota.
We analysed data from 125 healthy adult individuals (60 females, 65 males; mean age 37.6 ± 11.6 y) collected in a 150 day clinical trial. Participants completed a daily illness log. Faecal samples and fasting blood samples were collected at day 0 and day 150 for assessment of bacterial groups and inflammatory markers, the concentration of plasma cytokines, peripheral $CD4^+$ T-cell subsets, peripheral NK cell function and peripheral blood mononuclear cell (PBMC) phagocytosis.
Initial analysis indicates that there was no substantial change in faecal bacterial groups between day 0 and day 150. There was a significant increase in IL-4, IL-12p70 and macrophage inflammatory proteins while a significant decrease in IL-10 and RANTES was observed between the time points. There was a significant difference between day 0 and day 150 in $CD4^+CD25^+CD127^{lo}$ regulatory T-cells, with individuals undertaking higher levels of physical activity exhibiting a greater increase in frequency compared to those undertaking low levels of physical activity. Large variability (~30%) in the functional capacity of NK cells was observed. Our multifactorial dataset is currently being analysed to identify 'signatures' of immunology associated with illness patterns.

P5.01 Cytokine regulation in disease

P5.01.01
Study of IL-23 expression level in liver transplanted patients

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IL-23 has role in development of immune diseases. IL-23 induces Th17 cells and strong proinflammatory activity. Association of IL-23 with rejection of liver allograft was rarely investigated. Therefore, in this study the expression level of IL-23 cytokine was evaluated in liver transplant patients.
30 adult patients who received orthotopic liver were enrolled in this study. The EDTA-treated blood samples were collected in 1st, 3rd and 7th days post transplantation. In house_comparative Real-time PCR was designed for analysis of the expression level of IL-23 compared with β-actin control gene. The expression level of IL-23 was evaluated by Livak method (2-ΔΔcT).
The expression level of IL-23 was highly increased in 3rd and also 7th days relative to 1st day post liver transplantation.
Based on results, increase of IL-23 as a proinflammatory cytokine may relate to in post liver transplant inflammation. Also previously it was known that IL-17 is increased after transplantation and as IL-23 is inducer of IL-17 which this results support it.

P5.01.02
Effects of dexamethasone on IL-6 and IL-8 expression induced by TGF-β1 in colon cancer cells

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Glucocorticoid (GC), one of the most commonly used anti-inflammatory drugs, inhibits the deposition of extracellular matrix independent of its anti-inflammatory effect. The purpose of this study was to investigate the effects of dexamethasone on expression/production of IL-6 and IL-8 induced by TGF-β1 in colon cancer cell lines (Lovo). Dexamethasone significantly inhibited IL-6 and IL-8 mRNA levels induced by TGF-β1 in Lovo cells. The beneficial effect of Dexamethasone on the TGF-β1-induced IL-6 and IL-8 was mediated through the amelioration of ERK, p38 mitogen-activated protein kinase (MAPK) and JNK phosphorylation; however, this effect was not related to the TGF-β1-induced activation of Smad2/3 signaling. By inhibiting the pro-inflammatory cytokine expression, dexamethasone may contribute to therapy of colon cancer but the dexamethasone has a negative effect in colon cancer treatment because these glucocorticoids can to induce the colon cancer cell proliferation.

P5.01.03
Antitumor effect of a hydroalcoholic extract of Uncaria tomentosa in a murine C57BL/6- B16 melanoma model

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Uncaria tomentosa extracts have shown immunomodulatory, antiproliferative and antitumor activity. However, little is known about its effects in immunocompetent cells or Th1/Th2/Th17 cytokines production.Objective: To evaluate antitumor effect of an hydroalcoholic extract of *Uncaria tomentosa* (Cat's Claw) containing 5.03% pentacyclic oxindole alkaloids (UG-POA) in a murine model for melanoma C57BL/6-B16 by measuring lymphocytes, Th1/Th2/Th17 cytokines and tumor weight and volume. Methodology: Treatment with UG-POA extracts (50,500,100 mg/kg) or its dilution vehicle was given orally 7 days before and 21 days after tumor induction with 1x105 B16. Animals were sacrificed on day 22. Weight and tumor volume were assessed. Peripheral blood mononuclear cells (PBMC) were obtained, and analyzed by flow cytometry. Culture supernatants were collected to measure the production of Th1/th2/th17 using Cytometric Bead Array (CBA). +Results: We found a dose-dependent increase of CD3 + CD4 + LT, being statically significant at 50 and 1000 mg/kg compared with positive control group. However, CD4 + CD44 + LT showed a significant dose-dependent decrease compared with positive control group. CD3 + CD8 + LT population showed no differences in relation with positive control, except for a decrease in 50 mg/kg treated group. When evaluating Th1/Th2/Th17, we only found an increase in IL-6 in a dose dependent fashion, being significant at 1000 mg/kg. Tumor weight and volumes showed no significant differences.
Conclusions: It´s likely that UG-POA exhibits an anti-tumor effect modulating the increase and activation of LT CD4+ and IL6, an important cytokine in the host's immune and metabolic responses to cancer.

P5.01.04
Modulation of Oxido-Nitrosative Stress Induced Neuro-Inflammatory Cascade & Monoaminergic Pathway By Resveratrol In Reserpine Induced Pain Depression Dyad In Rats

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Background: The emerging concept for pain-depression pathogenesis includes dysfunction of biogenic amine-mediated CNS pain control and the possible involvement of oxido-nitrosative stress-induced

neurogenic inflammation. Thus the aim of the present study was to investigate the effect of resveratrol on the basis of its monoamine replenisher and antioxidant potential in reserpine-induced pain-depression in rats.
Methods and Results: Administration of reserpine (1 mg/kg; sc) for three consecutive days in male wistar rats led to a significant decrease in nociceptive threshold as evident from reduced paw withdrawal threshold in Randall Sellitto and von-Frey hair test and a marked increase in immobility time. This behavioural deficit was integrated with decrease in the biogenic amine (dopamine, norepinephrine and serotonin) levels along with increased substance-P concentration, nerve growth factor, oxido-nitrosative stress, inflammatory cytokines (TNF-α & IL-1β), NF-kβ and caspase-3 levels in different brain regions of the reserpinised rats.
Conclusion: These results reveal that common mechanisms appear plausible as co-activating factors which results in neurophysiological overlap between pain and depression. Furthermore, the study demonstrates the effectiveness of resveratrol in ameliorating the behavioral deficits associated with pain and depression by restoring behavioral, biochemical, neurochemical and molecular alterations against reserpine-induced pain depression dyad

P5.01.05
Maternal cytokine production patterns in women with pre-eclampsia

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A number of studies on unexplained recurrent spontaneous abortion, premature rupture of membranes and preterm delivery in humans have shown a close association of these conditions with increased production of some type 1 cytokines by peripheral blood cells. The objective of this study was to ascertain whether a Th1-biased cytokine production profile exists in women with pre-eclampsia as compared with normal pregnant women at the time of parturition.
PROBLEM: To determine the levels of cytokines produced upon mitogenic or antigenic stimulation of maternal peripheral blood mononuclear cells (PBMC) from women with pre-eclampsia.
METHOD OF STUDY: PBMC from 54 women with a history of successful pregnancy and 32 women undergoing pre-eclamptic delivery were stimulated with a mitogen or with autologous placental cells or with trophoblast antigens, and the levels of cytokines released into the culture supernatants then assessed by enzyme-linked immunosorbent assay. RESULTS: Significantly higher levels of the Th1 cytokines, interferon-gamma, and tumor necrosis factor-alpha were produced by the pre-eclamptic group than by the normal pregnancy group, which on the contrary showed significantly greater production of the Th2 cytokines, interleukin (IL)-4, IL-5, IL-6 and IL-10. A comparison of the ratios of Th2 to Th1 cytokines indicates a higher Th1 cytokine bias in pre-eclampsia as compared with normal pregnancy.
CONCLUSIONS: These data are suggestive of a maternal Th1 cytokine bias in pre-eclampsia.

P5.01.06
Suppressive Role of IL-27 against Pro-inflammatory Role of IL-17A in Patients with Multiple Sclerosis

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Background: Effector $CD4^+$ T cell subsets have important role in Multiple Sclerosis (MS). Interleukin-27 (IL-27) suppresses Th (Th1, Th2 and Th17) cells and dampens autoimmunity and tissue inflammation via promoting the generation of Tr1 cells. IL-27 significantly inhibits both nonpolarized and IL-23 driven IL-17 production by myelin-reactive T cells. A strong suppressive effect of IL-27 has been demonstrated in active experimental autoimmune encephalomyelitis (EAE). IL-27 can potently suppress the IL-17A effects and effective phase of EAE in vivo and may have potential therapeutic role in autoimmune diseases such as MS.
Objective: The aim of this study was identifying the role of IL-27 and whether this cytokine suppresses the inflammatory cytokine IL-17 in MS patients.
Methods: Venous blood was collected from forty MS patients and forty-three healthy subjects as control group. Serum levels of IL-27 and IL-17A were measured by ELISA method.
Results: This study showed significant difference between serum levels of IL-17A in MS patients (120.68 ± 209.85 pg/ml) and control group (67.26 ± 117.76 pg/ml), ($p < 0.05$). Serum IL-27 levels of MS patients (159.7 ± 581.4 pg/ml) were significantly lower than control subjects (180.35 ± 507.84 pg/ml), ($p < 0.05$).
Conclusion: Our findings suggest the potential inhibitory role of IL-27 on IL-17 mediated inflammatory process of MS disease.

P5.01.07
Immune mechanisms in periodontitis - effect of IL-4 gene polymorphisms on cytokine production

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Introduction: Periodontitis is a chronic inflammatory disease of the teeth-supporting tissues in which genetic predisposition, dental plaque bacteria and immune mechanisms play important roles. The aim of this study was to assess the influence of IL-4 gene polymorphisms on cytokine production
Methods: Cytokine production (IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-8 , IL-10, TNF-α, INF-γ) was studied after *in vitro* stimulation of isolated peripheral blood mononuclear cells (PBMC) by mitogens and dental plaque bacteria (*Aggregatibacter actinomycetemcomitans, Escherichia coli, Tannerrella forsythensis, Porphyromonas gingivalis* and Heat Shock Protein 60). Cytokine production was detected by *multiplex cytokine analysis* system Luminex. The results were correlated with IL-4 genotypes in patients with chronic periodontitis (CP) and healthy controls.
Results: We found that mononuclear cells isolated from peripheral blood of CP patients with investigated IL-4 polymorphisms (-589C/T, -33C/T, VNTR in intron 3) significantly changed production of IFN-γ, IL-10, IL-1β and IL-1α, TNF-α, IL-10, MCP-1, IL-6 after stimulation by all selected bacteria and HSP ($p<0.05$).
Conclusion: In patients with CP, IL-4 polymorphisms may influence the function of mononuclear cells to produce not only IL-4 but also other cytokines. The results of this study support Seymour's hypothesis (1999) about the role of TH2 produced cytokines such as IL-4 in genetic predisposition to chronic periodontitis.
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P5.01.08
Concentration Levels of IL -10 and TNF-α Cytokines in Patients with HPV DNA Positive and Negative Cervical Lesions

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The study was performed to assess the immune response in women with HPV DNA positive and negative cervical lesions. Estimation of IL-10 and TNF-α cytokines was performed through utilizing Enzyme linked immunosorrbent assay (ELISA) technique in cervical secretions and serum of the studied patients. Moreover, PCR screening kits were utilized to detect HPV DNA on cervical smears obtained from the studied cases with different cervical lesions. The detected levels of IL-10 (mean ± SE) concentration in cervical secretions of patients with HPV DNA positive and negative states and control group were 88.73 ± 16.90 pg/ml, 24.00 ± 2.84 pg/ml and 8.27 ± 0.59 pg/ml respectively with significant statistical differences

(p<0.05). . Moreover, the observed levels of TNF-α in cervical secretion of the cases with HPV DNA positive and negative states and control group were 12.18 ± 3.49 pg/ml, 9.90 ± 0.73 pg/ml, and 7.90 ± 0.87 pg/ml respectively, but with non statistical significance differences. **:** A raised production pattern of both IL-10 and TNF-α level in secretions of HPV D NA positive women with different cervical lesions were detected. However, the observed higher levels of IL-10 than TNF-α indicate down-modulation of tumor-specific immune response to HPV infected lesions via significant raised concentrations of the first cytokine than the second one. Therefore, this phenomenon seems to provide a tumor progressive microenvironment by the immunosuppressant properties of IL-10 with minimal antitumor activity of TNF-α. This was ascertained by the detected higher levels of IL-10 in lesions with advanced and progressive cytological stages.

P5.01.09
The inflammatory cytokine TNFalpha cooperates with hyper-activated Ras in promoting metastasis and turns WT-Ras into an oncogenic entity

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To improve our ability to interfere with processes of cancer growth and progression we need to better understand the relative contribution of oncogenic events vs. cells/factors of the tumor microenvironment to these processes. Our published study of non-transformed cells has shown that inflammatory cytokines had a more prominent impact, compared to oncogenic Ras^{G12V} and deregulated p53, in inducing a "cancer-related chemokine cluster", of which the angiogenic chemokine CXCL8 was a prominent member. In the present study, we extended this research direction to breast tumor cells originally expressing WT-Ras and WT-p53. In contrast to non-transformed cells, in which Ras^{G12V} had to act together with deregulated p53 to induce the chemokines, in the tumor cells, Ras^{G12V} induced CXCL8 without need for accompanying p53 down-regulation. In the tumor cells, TNF and IL-1 synergized with Ras^{G12V}, together leading to amplified elevation in CXCL8 release and mRNA expression. WT-Ras was not active in promoting CXCL8; however, TNF activation has turned WT-Ras into an active, GTP-bound prenylated form, and the joint activities of TNF+WT-Ras have led via MEK activation to increased CXCL8 release by the tumor cells. The additive effects of TNF+WT-Ras were mediated by increased activation of NF-B and AP-1. Jointly, TNF + Ras activities have given rise to increased angiogenesis and pronounced elevation in dissemination of tumor cells to lymph nodes. Thus, in breast cancer patients, where tumors are enriched with TNF, the cytokine may rescue the oncogenic potential of WT-Ras (which is the prevalent form), together giving rise to more aggressive disease.

P5.01.10
Cytokines, depression, and anxiety in patients with colorectal cancer

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Colorectal cancer (CRC) is a leading cause of cancer mortality in the world. Depression and anxiety are the most prevalent psychological disorders in patients with CRC. This study aims to investigate whether there is a correlation between anxiety, depression and serum cytokines in CRC patients. The study group consisted of 19 patients hospitalized for surgical resection of CRC and 20 healthy volunteers comprised the control group. Depression and anxiety were analyzed using the Hospital Anxiety and Depression Scale and serum levels of IL-1, IL-6, IL-12, TNF-α, TGF-β and IL-10 were measured by ELISA. We found that approximately 50% of CRC patients manifested clinically significant levels of anxiety and depression or a combination of severe anxiety and depression. Furthermore, these patients had increased serum levels of IL-1, IL-6 and TNF-α, but lower concentrations of IL-12. Analysis of correlation between HADS scores and serum levels of cytokines in CRC patients revealed a positive association of anxiety and/or depression with IL-1, IL-6 and TNF-α and a negative correlation with IL-12. These initial results indicate a correlation between serum levels of pro-inflammatory cytokines, anxiety and depression in CRC patients and suggest that cytokines are involved in the pathophysiology of these comorbidities.
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P5.01.11
IL-17 protects T cells from apoptosis and contributes to development of ALPS-like pictures

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Autoimmune/lymphoproliferative syndrome (ALPS), and its animal model MRL/Lpr mice, are characterized by autoimmunity, lymphoadenopathy and expansion of TCRαβ+ CD4/CD8 double-negative (DN) T cells and are due to mutations hitting the Fas/FasL system. Dianzani Autoimmune Lymphoproliferative Disease (DALD) is a similar picture, but lacks expansion of DN T cells. In this work, we investigated the role played by IL-17 in ALPS and DALD. We demonstrated that IL-17 (A and F) serum levels were higher, and Th-17 memory cells were expanded, in ALPS and DALD patients than in healthy controls (p<0.05). We then evaluated the effect of recombinant IL-17 (rIL-17) in Fas-induced cell death (FICD). Results showed that rIL-17 (A and F) significantly inhibited FICD of T-cells; this effect was specific since it was reversed by anti-IL-17 neutralizing Ab. Interestingly, IL-17 in the patients' sera was enough to prevent cell death of Fas-sensitive T-cells and surprisingly blocking of IL-17 in patients' sera restored their Fas-sensitivity. These data induced us to set a preclinical study to test the therapeutic effect of an IL-17 blocking Ab. MRL/Lpr treated mice showed reduced lymph nodes and spleen weight, with a decrease in DN T cells, lower dsDNA Abs, and prolonged life span (p<0.01). Collectively, these data suggest that in some ALPS/DALD patients, high levels of IL-17 contribute to the apoptotic defects typical of patients, and is a proof of concept that IL-17 may be a new therapeutic target.

P5.01.12
Haplotype analysis of interleukin-8 gene polymorphisms in chronic and aggressive periodontitis

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Objective: Periodontitis is an inflammatory disease characterized by connective tissue loss and alveolar bone destruction. Interleukine-8 (IL-8) variants are important in the regulation of the inflammatory response. The aim of this study was to analyze four polymorphisms in the IL8 gene and their haplotypes in relation to chronic (CP) and aggressive periodontitis (AgP).
Design: A total of 492 unrelated subjects were included in this case-control study. Genomic DNA of 278 patients with CP, 58 patients with AgP, and 156 healthy/non-periodontitis controls were genotyped, using the 5′ nuclease TaqMan® assay for allelic discrimination, for IL8 (rs4073, rs2227307, rs2227306, rs2227532) gene polymorphisms. Subgingival bacterial colonization was investigated by the DNA-microarray based on a periodontal pathogen detection kit in a subgroup of subjects (N=114).
Results: Allele and genotype frequencies of all investigated IL8 polymorphisms were not significantly different between the subjects

with CP and/or AgP and controls (p > 0.05). Nevertheless, the complex analysis revealed differences in IL8 haplotype frequencies. Specifically, the haplotypes A(-251)/T(+396)/T(+781) and T(-251)/G(+396)/C(+781) were significantly less frequent in patients with CP (2.1% versus 5.5%, resp. 4.9%, p < 0.05) and haplotype T(-251)/G(+396)/C(+781) was significantly less frequent in patients with AgP (0.9% versus 4.9%, p < 0.05) than in controls.
Conclusions: Although none of the investigated SNPs in the IL8 gene was individually associated with periodontitis, some haplotypes can be protective against CP and AgP in the Czech population.
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P5.01.13
Analysis of Th17 cells and IL-17, IL-23 cytokines in peripheral blood from children with autoimmune thyroid disease

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Up till now, altered balance of Th1 and Th2 immune cells has been postulated to play an important role in the pathogenesis of autoimmune thyroid diseases (AITD). However, recent studies on thyroid diseases suggest a new role for Th17 (T helper 17) cells. The aim of the study was to estimate the proporcions of circulating CD4+CD161+CD196+ and CD4+IL-17+ Th17 cells and serum concentrations of IL-17 & IL-23 in patients with Graves' disease (GD, n=22, mean age 14.3 ± 4 years), Hashimoto's thyroiditis (HT, n=37, mean age 15±2 yrs) and in healthy controls (C, n=25, mean age 15.2 ± 2 yrs). In untreated HT children we observed an increased percentage of CD4+CD161+CD196+ (p <0.04) and CD4+IL-17+ (p <0.01) Th17 lymphocytes in comparison to the healthy controls. In GD children we did not reveal such abnormalities in the population of these cells. In untreated patients with AITD we observed an increased levels of IL-23 in comparison to controls (GD, p=0.004;HT, p=0.046). Methimazole treatment in GD led to decrease these cytokine levels in a period of 6-12 months. However, during 6-24 months of L-thyroxine therapy in HT there wasn't any reduction of IL-23 concentration compared with HC. IL-17 was elevated in HT cases in comparison to the controls (p=0.021), which normalized during therapy.We conclude that the increased percentage of Th17 cells and elevated level of IL-17 and IL-23 cytokines in children with HT can suggest their role in initiation and development of immune and inflammatory processes in this endocrinopathy.

P5.01.14
Cryoglobulins are involved in pathomechanisms of altered immune response associated with schizophrenia and ischemic stroke

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The aim of this study was to investigate the effects of abnormal immune complexes, cryoglobulins (Cgs), recently found by us in the blood of patients with schizophrenia (SCZ) and ischemic stroke (IS) on the production of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), anti-inflammatory cytokine (IL-10), and chemotactic cytokines (IL-8, MCP-1) by peripheral blood mononuclear cells (PBMCs). In this study Cgs isolated from the blood of patients with SCZ and IS and PBMCs isolated from the blood of healthy subjects were used. The enzyme-linked immunosorbent assay (ELISA) and 3-(4,5)-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay were used upon study. The results obtained indicated significant increase in IL-1β, IL-6, TNF-α, IL-8, and MCP-1 production by cultured PBMCs when incubating for 24 hours with Cgs in the range of concentrations of 0.2-1.0 mg/ml. The gender difference did not affect Cgs-induced production of these cytokines by PBMCs. No influence of Cgs on production of IL-10 by PBMCs was observed. Based upon the results obtained we concluded that Cgs presented in the blood of SCZ and IS patients are implicated in the immune response alterations associated with these diseased conditions through induction of the expression of pro-inflammatory and chemotactic cytokines. The potential mechanisms of Cgs-induced alterations in pro-inflammatory and cytotoxic cytokines production by PBMCs is proposed, considering implication of the Fc and CR1 receptors as well as the complement cascade in these processes.

P5.01.15
IL-17 plays a critical pathogenic role in lung cancer

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Lung cancer development is associated with extensive pulmonary inflammation. Lack of understanding on the tumor-promoting mechanisms of inflammation in lung cancer hampers efficient and targeted tumor therapy. IL-17-producing CD4+ helper T cells (Th17 cells) play a critical role in promoting tissue inflammation. While Th17 cells are found in human lung cancer, their role in lung cancer is not known. We have applied a mouse model of lung cancer, in which an oncogenic form of K-ras (K-ras^{G12D}), frequently found in human lung cancer, is restrictedly expressed in lung epithelial cells (CCSP^{cre}). In this model, Th17 and Treg but not Th1 cells are enriched at the tumor tissues. When CCSP^{cre}/K-ras^{G12D} mice were intranasally challenged with lysates of non-typeable *Haemophilus influenza* (NTHi), which accelerated the tumor growth, they showed greatly enhanced Th17 cell infiltration in the lung tissues, suggesting a tumor-promoting role by Th17 cells. Indeed, lack of IL-17, but not IL-17F, results in significant reduction in lung tumor numbers in CCSP^{cre}/K-ras^{G12D} mice and also those treated with NTHi. Absence of IL-17 not only resulted in reduction of tumor cell proliferation and angiogenesis, but also reduced recruitment of myeloid cells and decreased the expression of pro-inflammatory mediators. Taken together, our data demonstrate a critical tumorigenic role of Th17 cell-mediated inflammation in lung adenocarcinoma and may suggest a novel way of prevention and treatment of this disease

P5.01.16
Effects of imiquimod treatment on TNF expression in two-step skin carcinogenesis model in mice

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Aldara, 5% imiquimod cream, is widely used in the clinic for treatment of virus-associated genital warts and several forms of skin cancer. There is evidence indicating a boost in the immune response against virus-infected or transformed cells following imiquimod application. Imiquimod has been initially characterized as an agonist of TLR7/8, however, most recent data suggest that it has a wider mode of action. In particular, it may be involved in regulating cAMP levels resulting in immunosuppression. We found that imiquimod dampens TNF production in both bone-marrow derived and peritoneal macrophages in response to LPS. Moreover, production of TNF is inhibited by imiquimod in vivo - in LPS/D-Gal hepatotoxicity model, as well as in LPS-induced septic shock. As TNF-mediated inflammation has been shown to support tumor formation in skin carcinogenesis, we wanted to assess effects of imiquimod treatment on inflammation during early stages of carcinogenesis. For this purpose, we performed two-step

skin carcinogenesis experiments applying DMBA/TPA to the back skin of Balb/C mice followed by imiquimod treatment. Two weeks after DMBA application followed by six TPA applications, skin samples were harvested and expression profile of several proinflammatory cytokines was analyzed. We found that animals subjected to DMBA/TPA and then treated by imiquimod had significantly decreased levels of TNF and other pro-inflammatory cytokines compared with the controls. Taken together, our data indicate that imiquimod inhibits TNF production in several experimental models, including the two-step skin carcinogenesis, thereby providing a new insight into its mode of action in patients undergoing Aldara treatment.

P5.01.17
Interferon gamma supresses collagen-induced arthritis through regulation of IL-17 : possible role of indoleamine 2,3 deoxygenase

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C57BL/6 mice are known to be resistant to the development of collagen-induced arthritis (CIA). However, they show a severe arthritic phenotype when the Ifng gene is deleted despite that IFN-γ is generally regarded as a pro-inflammatory cytokine. This study was conducted to 1) clarify that arthritogenic condition of IFN-γ knockout (KO) mice is dependent on the disinhibition of Th17 and 2) demonstrate that IFN-γ-induced indoleamine2,3dioxgenase (IDO) is engaged in the regulation of Th17. The results showed that the IFN-γ KO mice displayed increased levels of IL-17 producing T cells and the exacerbation of arthritis. When Il17 was deleted from the IFN-γ KO mice, only mild arthritis developed without progression of arthritis score. With respect to lymphocyte subtype population, the proportion of CD44highCD62Llow memory-like T cell was elevated in the spleen, draining the lymph node and mesenteric lymph node of IFN-γ KO CIA mice. When Th17 polarized CD4+ T cells of IFN-γ KO mice were co-cultured with their own antigen presenting cells (APCs), a greater increase in IL-17 production was observed than in co-culture of the cells from wild type mice. Of note, pretreatment of 1-methyl-DL-tryptophan, a specific inhibitor of IDO, abolished the inhibitory effects of IFN-γ. Given that IFN-γ is a potent inducer of IDO in APCs, these results suggest that IDO is involved in the regulation of IL-17 by IFN-γ.

P5.01.18
GLK controls Th17-mediated autoimmunity and NF-κB signaling by activating PKC-θ in T cells

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PKC-θ is required for NF-κB activation induced by T-cell receptor (TCR) signaling; however, the direct activator of PKC-θ was unknown. We found that GLK (MAP4K3) directly activates PKC-θ during TCR signaling. TCR signaling activates GLK by inducing its direct interaction with the upstream adaptor protein SLP-76. GLK-deficient mice show impaired immune responses and are resistant to Th17-mediated experimental autoimmune encephalomyelitis. Consistently, systemic lupus erythematosus (SLE) patients show dramatically enhanced GLK expression and PKC-θ-IKK activation in T cells, and the percentage of GLK-overexpressing T cells is correlated with disease severity. Our recent data indicate that GLK overexpression is also involved in adult-onset Still's disease (AOSD), rheumatoid arthritis (RA), and other autoimmune diseases. Data derived from GLK transgenic mice further indicate that GLK plays a critical role in Th17-mediated autoimmunity. IL-17 production in T cells is regulated by GLK signaling; the mechanism of GLK-induced IL-17 production will be discussed. Taken together, GLK is a direct activator of PKC-θ and activation of the GLK-PKC-θ-IKK could be used as novel diagnostic biomarkers and therapeutic targets for Th17-mediated autoimmune diseases. (Supported by NHRI-98A1-IMPP01-014)

P5.01.19
Immune response to a mixture of bacterial lysates in a rat model

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Bacterial lysates mixtures are widely used with the intention to generate a protective immune response in patients with repeated respiratory infections. We examined three study groups: group 1 received the injection of a bacterial lysate mixture. Group 2 received lipopolysaccharide (LPS), and group 3 Evans solution. All groups were injected with increasing doses at day 0 and 8. We obtained serum at time 0, 24 h, day 8 and day 15 to measure IL-1β, IL-4, IL-10 and IFN-γ by ELISA assay. In group 1, injected with bacterial lysates, there is an initial response at 24 h followed by a decrease in cytokines compared to basal levels and a final increase at day 15, being more evident for IL-4 (9.3 / 0 / 0 / 56.3pg/ml) and IL-1β (67.7 / 63.1 / 74.1 / 77.5pg/ml). The group 2 response to LPS administration shows an important initial increase at 24 h for IL-10 (39.4 / 117.9 / 53.4 / 23.6 pg/ml), IL-4 (13.4 / 49 / 52.9 / 45.9 pg/ml), IL-1β (72 / 92.6 / 82.1 / 71.8 pg/ml) and IFN-γ (13.2 / 21 / 29 / 17.5 pg/ml) and then a gradual decline practically to basal levels after 15 days. The group 3 responded to IL-10 (32.4 / 56.4 / 17 / 27.7 pg/ml). Our results show a change in cytokine levels to the immune stimulus with lysate bacterial in this rat model, identifying a progressive and gradual response especially for IL-4 and IL-1β.

P5.01.20
Regulation of lymphoma B cell proliferation by IL-31 and its receptor

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Interleukin-31 (IL-31) is a member of the IL-6 cytokine superfamily originally identified as product of activated Th2 cells. IL-31 signals through an heterodimeric receptor composed of a gp130-like (GPL) receptor chain, IL-31 receptor A (IL-31RA), and the Oncostatin M Receptor (OSMR). No information is available on the effects of IL-31 on normal or malignant B cells.
IL-31R complex was expressed in FL B cells both at mRNA and protein level and mediated IL-31 driven cell proliferation by activating the STAT/MAPK signaling pathways. In contrast, the postulated normal counterpart of FL B cells, *i.e.* GC B cells, expressed the IL-31R complex but no signal transduction was detected following exposure to IL-31. IL-31 and IL-31RA expression was investigated in FL tissue samples from patients with different histological grading and found to be significantly increased in grade III compared to grade I samples. Altogether, these findings point to
a role of IL-31/IL31R complex in FL progression. Western blot experiments showed expression of IL-31 both in the cytosol and the surface membrane fractions of FL cells and normal GC B cells, but no IL-31 secretion in culture supernatants was detected by ELISA and Western blot.
These results may suggest that in FL B cells surface IL-31 binds to its receptor and triggers signal transduction in adjacent tumor cells through paracrine loops. An alternative possibility under investigation is that IL-31 is released in fluid phase within microvesicles such as exosomes.

P5.01.21
Evaluation of potential serum biomarkers for periodontal disease in type 1 diabetes mellitus children

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The most prevalent periodontal disease among children is gingivitis, and it usually becomes more severe in adolescence. If gingivitis is not treated, it may progress to periodontitis. Diabetic patients are considered a high-risk group with greater susceptibility to severe forms of periodontal destruction. A few reports on the relationship between diabetes and periodontal disease have included children and adolescents. New data support the concept that in diabetes-associated periodontitis, the altered host inflammatory response plays a critical role. Among the pro-inflammatory biomarkers, IL1-β, TNF-α and PGE2 are considered modifiers of periodontal disease expression in adult diabetic patients. The aim of this study was to compare periodontal status and the level of serum biomarkers in children with type 1 diabetes versus healthy children. Fifty children (25 diabetic and 25 non-diabetic children) were selected and the clinical parameters, plaque index, gingival index and pocket depth, were recorded. Pro-inflammatory mediators levels (TNF, IL1-β,IL-6 and PGE2) from blood samples were measured using enzyme-linked immunosorbent assays. The results were statistically analyzed using ANOVA one way test ($p<0.05$). Regarding periodontal status, there was no statistical difference between diabetic and non-diabetic children. Among the serum biomarkers evaluated, IL1-β concentration was associated with the severity of gingivitis, but not the presence of diabetes. In conclusion, IL1-β could be an important early biomarker to periodontal disease, regardless the presence of diabetes mellitus. Supported by FAPERJ:grants no.E-26/110.508/2010; E-26/102.275/2011

P5.01.22
Influence of TLR2 in the cytokine profile after S. schenckii infection

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Sporotrichosis is a mycosis resulting from Sporothrix schenckii infection that causes skin and subcutaneous lesions. Toll like receptors play an important role in immunity, since they bind to pathogen surface antigens and initiate the immune response. However, little is known about the role of TLR and recognition of S. schenckii by immunological system. The aim of this study was to evaluate the influence of TLR2 in the cytokine profile after mice infection with S. schenckii. Female mice of C57BL/6 Wild type (WT) and C57BL/6 TLR2 Knockout (TLR2-/-) lineages were infected with S. schenckii and over a period of 10 weeks of sporotrichotic infection was measured the production of IL-1β, IL-12 and TNF-α by peritoneal macrophages and IL-4, IL-10 by spleen cells using enzyme-linked immunosorbent assays. The results were statistically analyzed using ANOVA one way test ($p<0.05$). The production of IL-1β, IL-12 and TNF-α was abolished in knockout mice. Regarding the cytokines IL-10 and IL-4, absence of the aforementioned receptor reduced the released levels to below those observed in animals with normal TLR2 expression. In conclusion, the production of these inflammatory mediators was highly dependent of the presence of TLR2 and could interfere with the course of the infection induced by S. schenckii.Supported by FAPESP: grants no. 2009/11999-3 e 2009/07529-1

P5.01.23
Lack of correlation between IL-21 and type I IFN signature suggests different roads to pathogenesis in Rheumatoid Arthritis

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IL-21 and IFNa are increased in several autoimmune syndromes including Rheumatoid Arthritis (RA). These cytokines have pleiotropic actions on cell types of lymphoid and non-lymphoid origin and both have been reported to induce plasma cell differentiation. In a cohort of RA patients (n=42), we tested if a correlation exists between IL-21 and type I IFN (IFN) and how these cytokines segregate with particular autoantibodies and immunologic mediators. In ~50% of the RA patients examined, IL-21 serum levels were elevated (up to ~500 pg/ml) vs. the majority of normal individuals under 20 pg/ml (n=30/31). Increased IL-21 was not part of general cytokine activation, as only 8/97 analytes measured associated significantly with increased IL-21 levels. Strikingly, a tight correlation existed between IL-21 levels and the presence of IgM Rheumatoid Factor (RF) $p=0.0003$ and to cyclic citrullinated peptide autoantibodies (CCP) $p=0.0155$, but not to anti-ss or -dsDNA Ab titers in which only 6/42 and 5/42, respectively of RA had titers. In this RA cohort, ~20% of the patients had a 4-gene IFN signature of whole blood. Importantly, there was no correlation between increased IL-21 levels and IFN signature score. IFN signature score was independent of RF or anti-CCP titers, but correlated to ssDNA autoantibody titers $p=0.0367$ and trended towards an association with anti-dsDNA autoantibody titers. These data suggest that while both IL-21 and IFN are believed to be involved in the pathogenesis of rheumatic disease, these pathways do not appear to intersect in RA patients, and may represent distinct targets for disease intervention.

P5.01.24
T cell activation markers and inflammatory cytokine profile in patients with diabetic nephropathy undergoing hemodialysis

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Diabetic nephropathy (DN) is a common complication disease in diabetic patients, and most of them need to be integrated into renal replacement therapy such as hemodialysis (HD). Our objective was to access the expression the activation markers on T cells, as well as the synthesis of inflammatory cytokines, before and after HD. The study was approved by the local ethics committee and involved 17 patients with DN under HD treatment which signed an informed consent. Blood samples before and after one HD session were collected, in order to analyze the expression of CD25, CD69 and CD71 in T cells. We also analyzed the synthesis of IL-12p70, IL-8, IL-10, IL-1β, TNF-α and IL-6 in serum samples by Cytometric Bead Array. After the HD session, there was an increase in CD4/CD8 ratio due to significant alterations in both subsets. The relative percentage of CD25+ T cells and CD8+CD25+ increased significantly after the HD session, while the relative percentage of CD69 T cells decreased. There was a significant decrease of the CD25 Mean Fluorescence Intensity values for CD4+ T, as well as in the case of CD71 in T cell sub-populations, after the HD session. Regarding cytokine synthesis in serum samples, we found a significant increase in IL-10 and IL-6 and a decrease in IL-8 after HD session. This study showed that a HD session in DN patients affects the T cell activation status, and differentially modulates the production of inflammatory cytokines.We thank CENTRO-07-ST24-FEDER-002015 funding.

P5.01.25
Expression of cytokine and Chemokine receptors on benign and malignant ovarian tissues

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Background: Cytokines, Chemokines and their receptors regulate the proliferation and survival of tumor cells, angiogenesis and metastasis to other organs. This network of ligand and receptors has been used in molecular targeting of cancer. Methods: We compared the mRNA expression of CXCR3, IP-10, CXCR4, SDF-1, IL-4 and IL-10 in tissues of benign and malignant ovarian tumors by qRT-PCR method. Results: Our result showed that CXCR4 expressed more in malignant tissues than in ovarian benign cysts (P.value > 0.05). However SDF-1, IP-10, IL-4, CXCR3, and IL-10 had lower mRNA expression in malignant ovarian tissues compare to benign cyst tissues. Except for IL-4 (p= 0.01) and SDF-1 (P.value = 0.02) the data for other factors were not statistically significant. Conclusion: Our data support the notion that increased expression of CXCR4 on malignant ovarian tissues may play an important role in the promotion of ovarian cancer. In addition, lower expression of IL-4 in malignant ovarian tissues requires supporting evidence to determine if this cytokine has an antitumor role in ovarian cancer.

P5.01.26
Role of transcription factor and chromatin remodeler interplay in the regulation of TNFα induced MHC-I gene expression

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Emerging evidences indicate that inflammation contributes to the initiation and/or propagation of a wide range of cancers. Key features of cancer-related inflammation involve aberrant (i) activation of signaling mediators/transcription factors, (ii) expression of inflammatory cytokines. The oxygen sensing transcription factor hypoxia inducible factor (HIF-1α) which is an important contributor of tumor growth, also serves a pivotal link between inflammation and tumorigenesis. As MHC class I is involved in glioma immune evasion and since HIF-1a is a pivotal link between inflammation and glioma progression, the role of TNFα induced inflammation in MHC-I gene regulation was investigated. TNFa induced increase in MHC-I expression and transcriptional activation was concurrent with increased HIF-1α and β-catenin activity. HIF-1α-β-catenin axis mediated increase in CREB phosphorylation was accompanied by its increased enrichment at CRE/site-α on the MHC-I promoter in TNFa treated cells. Importantly, chromatin remodeler hBrm was replaced for Brg-1 as the binding partner for CREB at CRE site, in a β-catenin dependent manner, thus driving MHC-1 gene expression. As MHC-I clustering on cell surface enhance their recognition by T cells, the effect of TNFα on MHC-I clustering was investigated. Increased MHC-I clusters in TNFα treated cells was also regulated by HIF-1α-β-catenin axis. As elevated MHC-I levels have a direct bearing on immune escape mechanism in glioma and as HIF-1a is considered a potential anti-glioma target, future endeavoursto inhibit MHC-I by targeting the HIF-1α-β-catenin axis, may provide a novel therapeutic approach for modulating immune-surveillance in these tumors.

P5.01.27
BET bromodomain inhibition by JQ1 suppresses interleukin-6 secretion in myeloma cells

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Background: Multiple myeloma (MM) is a malignant and progressive tumour of plasma cells. Overproduction of interleukin-6 (IL-6), a pleiotropic cytokine, is believed to play an important role in the pathogenesis of MM. Consequently, research has focused on targeting IL-6 as a therapeutic strategy for MM. Bromodomains are a diverse family of protein interaction modules that have a vital role in transcriptional regulation. Recent studies using well-established models of inflammation have shown that inhibitors of the bromodomain and extraterminal (BET) family of proteins suppress the expression of several pro-inflammatory cytokines including IL-6. The aim of this study is to investigate the effect of JQ1, a small molecule bromodomain inhibitor, on human MM cells and IL-6 secretion.
Methods: Primary MM cells were isolated from venous blood of a patient with refractory MM. To explore the effect on IL-6 secretion, primary MM cells were pre-treated with various concentrations of JQ1 then stimulated with lipopolysaccharide (LPS) and an IL-6 ELISA was performed. The effect on cell viability was measured using the WST-1 assay.
Results: Pre-treatment of LPS stimulated primary MM cells with JQ1 led to a dose-dependent suppression of IL-6 secretion. In addition, JQ1 exposure caused a time-dependent decrease in primary MM cell viability.
Conclusion: Treatment with JQ1 is not only cytotoxic to primary MM cells but also suppresses secretion of IL-6. These findings suggest that bromodomain inhibitors such as JQ1 represent a promising new class of immunomodulatory compounds that are highly active against MM.

P5.01.28
Dynamics of IL-1ά, IL-4 and IFN-γ in children with atopic asthma in therapy Leukinferon

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Intoduction: The immunological mechanisms of atopic inflammation in the pathogenesis of asthma have the attention of researchers. In the inflammation proses variety of soluble mediators and cytokines play a key role. We conducted a study of the levels IL-1ά, IL-4 and IFN-γ in the serum during Leukinferon immunotherapy in children with atopic asthma (BA).
Objective and methods: We observed 42 children with atopic asthma, 28 of them with moderate asthma and 13 children with severe disease. We found that in severe asthma is a significant increase in serum IL-1ά and IL-4 - 4-6 times higher than in the control group (p < 0,001). Serum levels of IFN-γ decreased by 5 times compared with healthy children. In patients with moderate asthma serum levels of IL-1ά and IL-4 is higher than in the control group (p < 0,001). Serum IFN-γ is 3 times lower than in healthy children. For the normalization of imbalance of cytokines in children with asthma, we described immune modulator Leukinferon, containing human leukocyte interferon in dose 10 000TB-6 injections.
Results: After treatment, it was determined significant reduction in asthma exacerbations, longer remission period, a reduction in dose and range of basic therapy. It was determined the significant increase of serum IFN-γ and the decrease of IL-4 and IL-1ά than in the comparison group. Thus, it was demonstrated a significant increase in IFN-γ and a positive clinical dynamics as a positive effect of Leukinferon.

P5.01.29
Cellular responses to *Staphylococcus aureus* alpha-toxin in chronic rhinosinnusitis with nasal polyps

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Background: In contrast to *Staphylococcus aureus*-derived superantigenic exotoxins, the role of non-superantigenic exotoxins in the pathogenesis of eosinophilic airway diseases remains obscure.
Objective: We sought to characterize *S. aureus* alpha-toxin-induced Th1-, Th2-, Th17-, and Treg-associated cellular responses in chronic rhinosinusitis with nasal polyps (CRSwNP).
Methods: Dispersed nasal polyp cells (DNPCs) and dispersed uncinate tissue cells (DUTCs) were prepared from patients with CRS with and without nasal polyps, respectively. Cells were incubated with various concentrations of alpha-toxin or staphylococcal enterotoxin B (SEB) and then the levels of IL-5, IL-13, IFN-γ, IL-17A, and IL-10 in

the cell supernatants were determined. The effect of blocking the COX pathway and neutralizing HLA-DR and ICAM-1 was examined.
Results: DNPCs produced substantial amounts of IL-5, IL-13, IFN-γ, IL-17A, and IL-10 in response to alpha-toxin. Cytokine production was higher in DNPCs than in DUTCs. The potency of alpha-toxin in stimulating IL-5, IL-13, and IL-10 production was comparable to that of SEB. Neutralization of HLA-DR and ICAM-1 suppressed cytokine production. Inhibition of the COX pathway increased and decreased alpha-toxin-induced production of IL-5/IL-13 and IL-17A/IL-10, respectively.
Conclusions: In addition to *S. aureus*-derived superantigens, non-superantigenic alpha-toxin can provoke inflammation in chronic rhinosinusitis with nasal polyps.

P5.01.30
Imbalance of homeostatic cytokines in APECED

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Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is an isolated human autoimmune disease, caused by loss-of-function mutations in the Autoimmune Regulator (AIRE) gene. AIRE is a transcriptional factor that enhances presentation of tissue-specific antigens to developing thymocytes. In the absence of AIRE, the negative selection fails and autoreactive cells escape to the periphery. Patients suffer from chronic candidiasis and autoimmunity targeting multiple endocrine organs. Dysregulation in interleukin-7 (IL-7) pathway has recently appeared as a factor contributing to the loss of self-tolerance in APECED. Our study elucidates the role of three IL-7-related cytokines: IL-15, IL-2 and thymic stromal lymphopoietin (TSLP) in APECED. IL-7, IL-15 and IL-2 use the common γ chain in signaling, whereas TSLP signals through the same α chain as IL-7.
Plasma IL-2 and IL-15 concentrations were moderately elevated in patients, while no TSLP was detected in patient or control plasma samples. Patient CD4+ T cells expressed more IL-15 receptor α chain (IL-15Rα) than control cells. Overnight incubation with IL-15 decreased IL-15Rα expression in controls but not in patients. TSLP receptor was equally expressed in both groups and remained unaffected by incubation with TSLP. Elevated IL-15 concentration was inversely proportional to the expression of CD5, a negative T cell regulator, in patient T cells. Furthermore, IL-2 concentration correlated inversely to the proportion of patient cells bearing markers of activation.
These results suggest a role for IL-2 and IL-15, but not for TSLP, in APECED pathogenesis. An interesting question is whether they only operate in the periphery or already during the thymic development.

P5.01.31
Substantially elevated plasma IL-1β levels and hyporeactivity to IL-1β in APECED

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APECED is a rare monogenic autoimmune disease, a model for autoimmunity. We set out to study the role of IL-1β, one of the most profound proinflammatory cytokines, in the inflammatory process of APECED. Surprisingly 3 of our patients exhibited substantially elevated plasma IL-1β levels. One patient had over 3000 pg/ml of IL-1b in his plasma, which is more than six times the level normally depicted in sepsis. Older samples from the same patient revealed that he had had equal amounts of IL-1β for the past 10 years.
To find out how the patient survives in such a proinflammatory environment we measured the IL-1 receptor antagonist levels from plasma. No significant differences were observed between the patient and controls. However when the patient's PBMCs were stimulated with recombinant IL-1β and the subsequent expression of IL-6 after 6 hours stimulation was measured by qPCR his PBMCs were markedly hyporesponsive to inflammatory stimulus.
In addition to giving more information about the role of IL-1β in autoimmunity this data opens the first possibility of intervening the autoinflammatory process in APECED with biological IL-1β antagonising treatments. The exact mechanism of the hyporesponsiveness remains to be established.

P5.01.32
Effects of Siegesbeckia orientalis ethanol extract on systemic and local inflammatory reactions

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This study aimed to investigate if herbal components that exert anti-inflammatory effects may be used for inflammatory disorders by examining *Siegesbeckia orientalis* ethanol extract(SOEE). The *in vitro* experiments showed that SOEE significantly reduced nitric oxide, interleukin(IL)-6 and PGE_2 production in LPS-stimulated RAW264.7 cells. To further evaluate the anti-inflammatory effects of SOEE *in vivo*, BALB/c or ICR mice were tube-fed with 32 mg SOEE/kg BW/day in 100 μL peanut oil, while the control and PDTC (pyrrolidine dithiocarbamate, an anti-inflammatory agent) groups were tube-fed with 100 μL peanut oil/day only. After one week of tube-feeding, the PDTC group was injected with 50 mg/kg BW PDTC and one hour later, all BALB/c mice were intraperitoneal-injected with LPS for systemic inflammation; all ICR mice were subcutaneous-injected with β-carrageenan(the plantar of right hind paw) for local inflammation. The results showed that the SOEE group had significantly lower serum IL-6 level at 9 hr after LPS challenge, and significantly higher survival rates than the control group. In the local inflammation model, the SOEE group had the reduction in the degree of paw swelling at 4 hr after carrageenan challenge (1.47 ± 0.14 mm^3 vs. 1.74 ± 0.29 mm^3, $P = 0.03$).This study suggests that SOEE can suppress the production of proinflammatory cytokines and alleviate acute inflammatory hazards.

P5.01.33
Metformin modulates inflammatory response through AMPK-NF-κB signaling and restores atherogenic effects

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Metformin is one of the most widely prescribed drugs for the treatment of type II diabetes. Metformin has been reported to mediate inflammatory responses and attenuate development of atherosclerosis besides its antihyperglycemic effect. But the pharmacological and biochemical mechanisms of metformin in immune responses and atherogenesis have not been clearly elucidated. Here we demonstrate that metformin down-regulates pro-inflammatory cytokines and foam cell formation in macrophages. Metformin reduced the production of NO through the suppressed gene and protein expression of iNOS in LPS-stimulated RAW 264.7 cells and peritoneal macrophages. Metformin antagonized the production of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, leading to suppressed the phosphorylation of IκBα and the translocation of NF-κB p65 subunit into nuclear. Furthermore, scavenger receptors (SR-A, Lox-1) mRNA levels were down-regulated by metformin. These results show that metformin attenuates inflammatory mediators via AMPK-NF-κB signaling, suggesting that metformin would be a potential immunemodulatory agent for treating vascular inflammatory diseases.

P5.01.34
Immunomodulatory effects of N-(2-hydroxy phenyl) acetamide on pro-inflammatory cytokines and ROS in adjuvant-induced arthritic rats

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Rheumatoid Arthritis (RA) is characterized by inflammation, tissue injury, pain hypersensitivity and proliferation of synovial cells causing bone and cartilage destruction and ultimate disability of the joints. In the present study, N-(2-hydroxy phenyl) acetamide (NA-2) was evaluated for its disease-modifying effects in the rat model of adjuvant-induced arthritis (AIA) which closely resembles the human rheumatoid arthritis. Gait analysis was used to examine the role of NA-2 in the development of chronic pain associated with arthritis. The pro-inflammatory markers including cytokines IL-1β and TNF-α and reactive oxygen species were also measured along with the cellular immediate-early genes (*c-fos*) which reflects the pattern of neuronal activity and directly regulate the expression of these pro-inflammatory cytokines. Our results showed that NA-2 treatment inhibits not only the macroscopic inflammatory changes but also significantly reverses gait deficits in AIA ($p < 0.002$). Furthermore, expression of *c-fos* gene and c-Fos protein was also markedly decreased with the parallel significant reduction in pro-inflammatory cytokines IL-1β ($p < 0.004$) and TNF-α ($p < 0.009$) and oxidative stress markers i.e., nitric oxide and peroxide ($p < 0.00$ and $p < 0.05$ respectively). The findings of this work study suggest that administration of NA-2 is effective in preventing the chronic inflammation and pain related neuronal hyperactivity/ excitability. It may imply that beside the inhibition of local inflammation, inhibition of central inflammation/sensitization may be involved in the anti-inflammatory and anti-arthritic activity of the salicylic acid derivative N-(2-hydroxy phenyl) acetamide.

P5.01.35
Redox balance of mouse medullary CD4 single positive thymocytes

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After positive selection, the newly differentiated single positive (SP) thymocytes undergo negative selection to eliminate autoreactive T cells, functional maturation to acquire immunocompetence and egress capability. To investigate whether the intracellular reduction/oxidation (redox) balance plays an important role on SP maturation, the levels intracellular reactive oxygen species (ROS) and the expression of proteins that regulate ROS were compared among the four subsets of mouse $TCR\alpha\beta^{+}CD4^{+}CD8^{-}$ thymocytes (SP1 to SP4) that represent sequential stages of SP differentiation program. A gradual increase of ROS and a gradual decrease of thioredoxin were revealed along the SP maturation process. An early increase of ROS in the immature SP1 thymocytes resulted in enhanced apoptosis whereas an increase in the most mature SP4 cells resulted in enhanced cytokine production upon stimulation. The coculture with thymic epithelial cells increased the ROS level in SP thymocytes. *Aire*$^{-/-}$ mice have a developmental blockage at the SP3-to-SP4 transition and defects in negative selection and showed significantly less ROS in SP3 thymocytes. These results suggest that ROS may be involved in promoting the functional maturation of $CD4^{+}$ SPs and thymic medullary microenvironment contributes to the pro-oxidant shift of SP thymocytes.

P5.01.36
Low level of TNF-alpa in cystic fluid is accompanied with low number of macrophages in cystic wall of odontogenic tumor

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Aims: The aggressive behaviour and high recurrence rate of odontogenic keratocyst suggests their neoplastic potential and prompted the World Health Organization Working Group to classify it as a benign tumour with odontogenic epithelium. Since, TNF-α is a pleiotropic cytokine, that is considered as a primary modifier of inflammatory and immune reaction in response to various inflammatory diseases and tumor as well, it is very interesting to analyzed their concentration in our type of tumor.
Methods: We analyzed concentration of TNF-α in cystic fluid of 25 odontogenic keratocysts, obtained from patients undergoing surgery, under local anaesthesia, and after aspiration of cystic fluid from non-ruptured cysts. TNF was determined by ELISA assay. Presence of inflammatory cells in peri-cystic tissues and macrophages was analyzed immunohistochemistry using monoclonal antibodies. Degree of vascularisation and presence of macrophages was estimated by enumeration using light microscopy.
Results: TNF-α is detectable in cysts fluid, but in low values in all investigated cyst (range from 20-50 pg/ml). All cysts have also low number of macrophages in cystic wall. All cysts have a similar concentration of TNF irrespective of the cystic wall,the number of inflamed cells including macrophages, the epithelium proliferation and presence of vascularisation having been analyzed histologically. Also, no significant correlation was found in ration to cyst size and protein concentration (Mann-Whitney U-test, $p > 0.05$).
Conclusions: These results indicated that no inflammation has significant influence on growth as well appearance of these cysts, like to other, inflammatory cystic type in oral region.

P5.01.37
Effect of immunoglobulin E on plasmacytoid dendritic cells in systemic lupus erythematosus pathogenesis through interferon-alpha downregulation

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Plasmacytoid dendritic cells (pDCs) are dendritic cells characterized by their unique ability to produce large amounts of type I interferon (IFNalpha/beta) upon Toll-like receptor (TLR) 7/9 triggering. Blood type I interferon signature is the hallmark of several autoimmune diseases including systemic lupus erythematosus (SLE) and pDCs have been shown to play a central role. pDCs express surface regulatory receptors including the high affinity Fc receptor for immunoglobulin E (IgE), FcepsRI, involved in the negative regulation of IFNalpha secretion. We hypothesized that IgE engagement of FcepsRI on pDCs may have an effect on their functions in SLE patients.
First, we observed significantly higher IgE levels in SLE patients (independently of allergy, or parasitic infection) with quiescent disease than with active disease and healthy donors ($p<0.05$). In vitro, blood purified pDCs cultured with monoclonal IgE upregulate the surface expression of FcepsRI in an IgE dose-dependent manner ($p<0.005$). IgE-treated pDCs significantly downregulate IFNalpha secretion upon TLR7, 9 and immune complexes triggering ($p<0.05$). Finally, the coculture of IgE pretreated pDCs with allogeneic CD4+ T-lymphocytes promotes their differentiation into IL-10 -secreting-Tcells. All together, these data suggest a role for IgE in modulating the inflammatory response by pDCs and promoting a tolerance induction. Recently, use of parasitic worms as a therapy in autoimmune disease has been proposed. Our results highlight a complementary mechanism that could be involved in the protective effect of worm infection in SLE.

P5.01.38
Correlation of some Inflammation related cytokines and biochemical markers amongst individuals with high, medium and normal serum FBS levels

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Introduction- Inflammation symptoms are detectable before the onset of diabetes even when only the balance of FBS is changed. The current study aimed to evaluate inflammatory cytokines with different levels of FBS to predict the diabetes status.
Materials and Methods- At this case-control study a total of 41 individuals with FBS higher than normal together with 41 with normal FBS were included. After taking a blood sample from each participant IL-6, IL-1 β and IL-6 and FBS, LDL,HDL, TG, Cholesterol, Albumin and WBC, RBC, Hb, MCV, MCHC and RDW were. Data were analyzed with Logistic Regression Analysis and Pearson correlation tests.
Results- According to the FBS level participants were placed in 3 groups; normal FBS(normal group), FBS of 100-125(middle group) and FBS more than 126 group (high FBS group). The mean HDL was 43.34, 41.33 and 40.32 while the mean, IL-1 β was 85.28, 329.33 and 333.04 in normal, middle and high FBS groups respectively. The mean IL-6 was 315.47, 317.06 and 321.45 and the mean CRP was 4.38, 2.2 and 2.4 respectively. Albumin ranged from 5.09, 5.07 and 4.7 among different groups respectively.
Conclusion- There was a good association between the inflammatory, biochemical and FBS in case and control groups. Regression analysis found that increasing the IL-6 and IL-1 β levels is proportional to increasing in FBS level so that the prediction of FBS can be made by these markers. Evaluation of inflammatory cytokines even in health people can elucidate the status of FBS imbalance and diabetes in future.

P5.01.39
Critical roles of oncostatin M receptor signaling on the polarization of macrophage phenotypes, adipose tissue inflammation, and the development of insulin resistance

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Obesity-induced adipose tissue inflammation is one of the important causes for the development of insulin resistance. Although oncostatin M (OSM), a member of the IL-6 family of cytokines, has various biological functions including the regulation of inflammatory responses, the relationship between OSM and adipose tissue inflammation remains unclear. OSM receptor β subunit (OSMRβ) was expressed in the adipose tissue macrophages (ATMs) of both lean and obese mice, suggesting some important roles of OSM in the regulation of adipose tissue inflammation. OSMRβ-deficient (OSMRβ-/-) mice showed moderate insulin resistance without obesity under normal diet conditions. The percentage of M1-type in total ATMs and the expression of pro-inflammatory markers, such as TNF-α, in the adipose tissue were increased compared to wild-type (WT) mice. Under high-fat diet (HFD) conditions, OSMRβ-/- mice exhibited the increased percentage of M1-type ATMs, the increased expression of pro-inflammatory markers in the adipose tissue, the increase in the body weight, and severer insulin resistance compared to WT mice. To investigate the direct effects of OSM on macrophages, a macrophage cell line, RAW264.7 cells, and mouse peritoneal exudate macrophages (PEMs) were treated with OSM. The expression of M2 markers, including arginase-1 and CD206, were increased by OSM in both RAW264.7 cells and PEMs from WT mice, but failed to increase in PEMs from OSMRβ-/- mice. Thus, OSMR signaling plays some important roles in the suppression of adipose tissue inflammation and the amelioration of insulin resistance, at least in part, through the polarization of phenotypes of macrophages to M2-type.

P5.01.40
Serum soluble IFNAR2 levels in autoimmune diseases suggest that chronic IFN signaling leads to elevated levels of sIFNAR2

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The Type I Interferon (IFN) receptor consists of a heterodimer of chain 1 (IFNAR1) which is required for signaling and chain 2 (IFNAR2) which binds tightly to IFN. Soluble forms of IFNAR2 can be produced either by proteolytic cleavage or production of an alternatively spliced (?) transcript. Soluble IFNAR2 can be found in serum and urine however its role and regulation are poorly understood. Studies have suggested that sIFNAR2 is elevated in MS patients, advanced cancer patients and hepatitis C infected patients. Using a newly developed ELISA for sIFNAR2 we have examined commercially sourced serum/plasma samples from autoimmune patients and normal healthy donors. In comparison to 35 normal donors, MS patients on IFN therapy (n=29) had significantly (p=0.0005) increased levels of sIFNAR2. Those on other therapies (n=24) were not different from the normal population but were significantly different than the MS patients on IFN therapy (p=<0.0001). sIFNAR2 levels showed no correlation with treatments such as vitamin-D, copaxone or natalizumab. Systemic Lupus patients (n=67) had elevated sIFNAR2 (p=0.009) while rheumatoid arthritis (n=16) were not significantly different from normal. Sjogren's patients (n=11) and scleroderma patients (n=10) - is there something missing here? Although evidence of Type I IFN activation has been observed in RA, Sjogren's and Scleroderma patients, the frequency and intensity is thought to be less than that observed for MS patients on IFN-therapy and in Systemic Lupus. These data suggest a model where long term IFN signaling above a certain threshold leads to elevated sIFNAR2.

P5.01.41
IL-4: a key cytokine in acutely rejected neo-antigen specific skin grafts

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Null-mutations in genes coding for structural proteins of the skin are quite common in patients suffering from genodermatoses. Correcting defective genes by *ex vivo* skin gene therapy is becoming a promising therapeutic modality for the treatment of epidermolysis bullosa, however, bearing the risk of provoking unwanted immune responses against the introduced neo-antigen expressed by the skin graft. The aim of this study is the characterization of the immune response towards the neo-antigen, human type XVII collagen (hBPAG2), in order to selectively block rejection of gene-corrected cells. In our model system we graft full-thickness skin from a transgenic mouse strain expressing hBPAG2 in the murine epidermal basement membrane onto syngeneic wild-type C57BL/6 recipients. To analyze the immunological reaction in rejected grafts a mouse Th-RT[2] Profiler PCR array was performed. In cytokine blocking studies transplanted mice were injected rat anti-mouse IL-4 i.p. to neutralize IL-4 activity. The cytokine profile array of acutely rejected grafts expressing hBPAG2 was characterized by increased levels of IFNgamma, IL-4, IL-6 and TGF-beta1 compared to controls. Interestingly, expression of the Th2 cytokine IL-4 was restricted to mice grafted with hBPAG2-tg skin. In a transplantation study we demonstrated that the rate of graft acceptance could be improved by blocking IL-4 activity. Since histological assays show infiltration of mast cells we plan to further characterize the role of mast cells in transplantation.

P5.01.42
Aryl hydrocarbon receptor negatively regulates type I interferon production in murine lupus

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Systemic lupus erythematosus is a highly heterogeneous multi-organ disorder and is characterized by the production of diverse autoantibodies. Recent studies suggest an important role for type I interferon in the pathogenesis of this disease. Production of type I interferon is under the control of the transcription factor IRF7, while type I interferon signaling requires the STAT1 transcription factor. Here, we show that production of type I interferon in cultured pDCs from aryl hydrocarbon receptor knockout mice is significantly higher compared to wild-type mice stimulated with TLR 7/9 agonists. Furthermore, treatment of pDCs from wild-type mice treated with the aryl hydrocarbon receptor agonist L-kynurenine significantly inhibits TLR 7/9-mediated type I interferon production. We demonstrate that through TLR 7/9 signaling, expression of aryl hydrocarbon receptor is induced, which in-turn forms an inhibitory interaction with IRF7 and STAT1, thus attenuating both type I interferon production and signaling. In addition, we found that production of type I interferon and expression of interferon stimulation genes in aryl hydrocarbon receptor knockout mice is much higher than in wild-type mice when we induce lupus-like disease by pristane treatment. Our results show that aryl hydrocarbon receptor is a critical negative regulator of TLR-mediated type I interferon production and inhibits type I interferon signaling in murine lupus. We are currently evaluating protective effects of aryl hydrocarbon receptor agonists in the development of murine lupus.

P5.01.43
Proinflammatory cytokines in patients with chronic traumatic encephalopathy

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Cytokines could be important in mechanisms of chronic traumatic encephalopathy (CTE) development as they can activate microglia-network to release a number of neurotoxic mediators, chemokines, prostaglandins involved in alteration of endothelial dysfunction, astrocytes respond of dysfunctional brain blood barrier, and neuronal dysfunction. The aim was to examine the proinflammatory cytokine profile in patients with CTE.
Methods. Patients with CTE (n=26; 19-46 years) after closed brain trauma (mean disease duration: 8.62±5.73 years) and controls (n=24; 21-44 years) were investigated. Serum interleukins (IL) IL-6, IL-1b, IL-8 were measured by standard immunoenzyme assays using Vector Best reagents.
Results. As compared to controls, CTE showed an elevated percentage of IL-6 level suggesting that increased IL-6 (mean±SD: 26.65±6.29 pg/ml; range 14.5-38.6 vs. 4.4±1.5; range: 1.5-6.9) might be generated from activation of cytokine microglia-astrocyte network ($P<0.01$; $t=2.69$). IL-6 is an immunoregulatory cytokine acting as proinflammatory and anti-inflammatory secreted by T-cells, macrophages to stimulate immune response, by neurons, activated microglia and hypertrophic astrocytes. General IL-1b range in all patients: 19.3-45.5 pg/mL where 11 CTE (42.31 %) had higher IL-1b level with mean 32.16 vs 24.84 pg/mL ($P<0.01$; $t=2.74$), these patients had partial epilepsy (3), neurological deficit (5), severe headaches (9), cognitive decline (11) and IL-1b levels were positively correlated with high seizure frequency ($P<0.05$). CTE showed no differences in IL-8: mean 6.87; range: 4.1-22.7 pg/ml vs 4.64; range: 3.0-5.2.
Conclusions. Neuroinflammatory processes are important in clinical understanding of CTE outcome; CTE revealed a highly proinflammatory serum IL-6 level condition that could play role in disease progression.

P5.01.44
Effect of lithium carbonate treatment in mRNA levels of pro- and anti-inflammatory cytokines and expression of Wnt/beta-catenin pathway genes in PBMC of bipolar patients

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Bipolar disorder (BD) manifests as episodes of depression and mania. Many aspects of its pathophysiology are not thoroughly understood. Pro-inflammatory cytokines and the Wnt/β-catenin signaling pathway may play important roles. Lithium salts, used in the treatment of BD, modulate this signaling pathway. We analyzed the expression of pro- and anti-inflammatory cytokines in BD patients and healthy control subjects and whether lithium administration modulates cytokine expression in peripheral blood mononuclear cells (PBMC) in BD subjects, as well as the expression of the Wnt/β-catenin pathway genes.
We used PBMC from BD patients (n=7) prior to and after an eight-week lithium carbonate treatment, and healthy controls (n=12). Real-Time RT-PCR data were calculated using the 2-ΔCt method and the Kruskal-Wallis test with Dunn's post-test for statistical analysis. Inflammatory (TNF-α, IFN-γ and IL-8) and anti-inflammatory (IL-10 and IL-4) cytokines were analyzed. IL-10 levels were significantly lower in BD patients when compared to healthy controls, regardless of treatment. TNF-α was significantly lower in untreated BD patients, while IFN-γ was significantly lower in treated BD patients when compared to healthy controls. We failed to find differences due to treatment in BD patients. We analyzed the modulation of Wnt pathway genes comparing BD patients and control subjects. Our results show significant differences in GSK-3β, TCF4 and cyclin D1 expression between pre-treated bipolar patients and controls. We also observed significant differences between BD patients under treatment and controls (GSK-3β, TCF4 and HIG2) indicating that not only the manifestation of the disorder but also its treatment can affect Wnt signaling.

P5.01.45
Elevated concentrations of interleukin-12 and 18 in chronic kidney disease patients synergistically induce T cell production of interferon-gamma and proliferation *in vitro*

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Background: Elevated pro-inflammatory cytokines interleukin (IL)-12 and IL-18 have been observed in CKD patients, which may contribute in the pathogenesis of CVD through T cell activation. We aim to examine whether CKD concentrations of IL-12 and 18 are capable of inducing T cell activation *in vitro*.
Methods: Serum levels of IL-12 and 18 were quantified in a cross-sectional study of 69 healthy controls and 139 stage 3-5 pre-dialysis CKD patients by ELISA. CD3-positive T cells were cultured in the presence of IL-12 (0.1ng/mL) and/or 18 (0.5ng/mL) in complete medium (10% v/v fetal calf serum) and the levels of interferon-gamma (IFN-γ) in supernatant (ELISA) and T cell proliferation (bromodeoxyuridine [BrdU]-incorporation) determined.
Results: CKD patients had higher serum concentrations of IL-12 and 18 compared to healthy controls (IL-12: 81 vs 30 x pg/mL and IL-18: 455 vs 357, *Mann-Whitney U test, p<0.001)*. T cells cultured for 48-72hrs with IL-12/18 produced a 75-fold and 138-fold increase in IFN-γ (*t-test,* $p<0.01$), compared to unstimulated cells (below detection threshold). IL-12/18
stimulation is suppressed 3-fold by IL-12 and IL-18 blockers (both 10µg/mL) *in vitro* (*t-test,* $p=0.01$). There was a 1.3-fold increase in T cell
proliferation compared to unstimulated cells at 48-72hrs (*t-test,* $p<0.02$).
IL-12 or IL-18 alone did not induce T cell activation.
Conclusions: Serum concentrations of IL-12 and 18 are 1.5-3-fold greater in stage 3-5 pre-dialysis CKD patients compared with healthy controls. Concentrations of IL-12/18 detected in CKD patients are capable of inducing a strong T cell response *in vitro*.

P5.01.46
T cell production of interferon-gamma and proliferation is suppressed in vitro when stimulated with interleukin-12 and 18 or anti-CD3/CD28 under uremic conditions *in vitro*

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Background: It has been shown that CKD patients have elevated concentrations of IL-12 and 18 compared to healthy individuals. Previous in vitro studies shave demonstrated that uraemic medium-cultured dendritic cells (DC) induced T cell activation and proliferation, which may contribute the higher risk of cardiovascular disease in this population. However, the effect of uraemic serum on T cell function in the absence of DC interaction remains unclear.
Methods: Immunomagnetic-bead isolated CD3-positive T cells from healthy blood donors were cultured in uraemic medium (10% v/v uraemic serum from pooled stage 3-5 CKD patients) compared to normal medium (10% v/v pooled healthy individuals) stimulated with IL-12 and 18 (2.5 and 12.5ng/mL), and anti-CD3/28 (1µg/mL each). T cell production of interferon-gamma (IFN-γ) from culture supernatant and proliferation were determined by ELISA and bromodeoxyuridine [BrdU]-incorporation respectively.
Results: T cells cultured in uraemic medium stimulated with IL-12/18 or anti-CD3/28 produced a 1.8-fold and 1.5-fold decrease in IFN-γ respectively (*t-test, p<0.005)*, corresponding to 1.2 -fold and 1.3-fold decrease in proliferation (*t-test, p<0.02)* compared to T cells cultured in complete medium. T cells stimulated with IL-12/18 for 24 - 72hrs *in vitro* maintained lower proliferation and IFN-γ production for each given time-point when cultured in uraemic medium compared to those cultured in normal medium (*t-test,* p<0.03).
Conclusions:
Stimulated T cells cultured in the presence of uraemic medium in the absence of DC exhibited a reduction of IFN-γ production and proliferation *in vitro*.

P5.01.48
Anti-inflammatory effects of high-dose IgG on TNF-α-, but not IL-1β-activated human coronary artery endothelial cells

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[Background and Purpose] Kawasaki disease (KD) is an acute systemic vasculitis of unknown etiology in infants and young children, and it is frequently associated with coronary artery aneurysms. High-dose infusion of IgG (IVIG) effectively reduces systemic inflammation and the incidence of coronary artery lesions in 80-90% of KD. However, for reasons that remain unclear, some patients do not respond to IVIG. In order to obtain clues to the cause of IVIG resistance, we compared the in vitro effects of high-dose IgG on cultured human coronary artery endothelial cells (HCAECs) exposed to various inflammatory stimuli.
[Methods] HCAECs were stimulated with TNF-α, IL-1β or Poly (I:C) in the presence and absence of 20 mg/ml human polyclonal IgG for 48 hours. G-CSF and IL-6 mRNAs and proteins were measured by qPCR and ELISA, respectively.
[Results] High-dose IgG completely inhibited TNF-α-induced expression of G-CSF and IL-6, which are known to be crucially associated with the pathogenesis of acute KD. In contrast, high-dose IgG only partially inhibited Poly (I:C)-induced expression of these cytokines and did not inhibit IL-1β induced expression at all.
[Conclusion] The pathogenesis of IVIG-resistant KD may be associated with viral-induced or IL-1β-induced inflammatory reactions, and blockade of those pathways may be a new therapeutic target for IVIG-resistant KD.

P5.01.49
Association of modulatory cytokine gene polymorphisms in resistance and susceptibility to polyparasitism infection in Zimbabwe

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Single nucleotide polymorphisms within the cytokine genes, TNF-α (-308 G/A), IFN-γ (+874 A/T), TGF-β (T/C codon 10 and G/C codon 25) and IL-10 (-1082 G/A and -819 T/C) associated with moderation or severity of parasitic infections were examined in samples from school children aged between 5 - 16 years. About 72.8% were infected with either malaria and/or different helminths. Genotyping was carried out using the ARMS-PCR method. The frequency of TNF-α (GG) associated with low cytokine production was 76.1%, while 22.2% and 1.6% were predictors of medium and high production of TNF-α, respectively. For IFN-γ (+874 A/T), 70.5% were (AA) associated with high cytokine secretion, 4.4% (TT) and 25.1% (AT) associated with low cytokine production. Limited analysis on the samples also revealed that at the TGF-β locus (T/C codon 10) 88.5% were TT, which predicts high production of the cytokine, whereas 9.2% were CC. Similar analysis at another locus of TGF-β (G/C codon 25) showed that only 2.3% showed GC predicting high TGF-β production. Equal distribution of IL-10 (-819 G/A) and the rare occurrence of allele associated with low IL-10 (-1082 AA) production would suggest moderate to high IL-10 responses in the population. Finally, the high prevalence of TGF-β genotype (TT) predicting high cytokine production and the existence of IL-10 (high producer) might suggest the dominance of an anti-inflammatory environment when faced with acute P.falciparum infection in the population. These observations may also suggest a complex interaction between various cytokine gene polymorphisms and high burden parasitic infections in the area.

P5.01.50
Interleukin-17A involvement in host defense against Echinococcus granulosus infection

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Human hydatidosis is severe chronic parasitic disease, caused by the larval stage of Echinococcus granulosus. It constitutes a serious public health problem in various parts of the world, particularly in Algeria. The clinical evolution of these cysts is silent for several months, and the symptoms are not specific. Diagnosis is difficult and surgery constitutes the only therapy. We have previously shown the role of cytokines Th1 and Th2 in human hydatidosis. Our analysis of circulating cytokines production in sera from hydatid patients showed the immuno protective role of Th1 cytokines, especially IFN-γ and IL-12, and pathological role of Th2 cytokines during Echinococcus granulosus infection.The present study was focused on determining the role of IL-17A in the immune response against Echinococcus granulosus infection. We investigated IL-6, IFN-γ and IL-17A production in sera from Algerian hydatic patients with liver and lung hydatid cyst. IL-6, IFN-γ and IL-17A were also determined in PBMC culture supernatants from patients stimulated by a major parasitic antigen. The elevated activity of IL-6, IFN-γ and IL-17A were observed in most sera from patients. Our in vitro results indicate a positive correlation between IL-6, IFN-γ and IL-17A production in PBMC culture supernatants. However, IL-6, IFN-γ and IL-17A activity was low in sera and supernatants of PBMC cultures from relapsing patients and did not display any immune response against parasitic antigen. Collectively, our results show that IL-17A was involved in the host defense mechanisms against Echinococcus granulosus. Our data suggest that IL-17A play immunoprotective role in this parasitic helminth infection.

P5.01.51
Interleukin-17A involvement in host defense against Echinococcus granulosus infection

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P5.01.52
EBI3 functions as an intracellular molecule to regulate the development of colitis in naive CD4+CD25- T cells

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Epstein-Barr virus-induced gene 3 (EBI3) encodes a protein, which associates with IL-27p28 and IL-12p35 to form heterodimeric cytokines, IL-27 and IL-35, respectively. IL-27 is expressed in myeloid cells and IL-35 is expressed in CD4+CD25+ Treg cells. Since we recently found that EBI3 expression is induced in naive CD4+CD25-CD62L+ T cells by the stimulation with anti-CD3/anti-CD28, we herein investigated the role of EBI3 in naive CD4+ T cells. EBI3-decificent naive CD4+CD25-CD62L+ T cells failed to induce the colitis after adoptive transfer into RAG-deficient mice. Intracellular cytokine staining revealed that the production of IFN-γ but not IL-17 was significantly reduced in intestinal lamina propria of EBI3-deficient mice. Similarly reduced production of IFN-γ but not IL-17 was also observed when lymphocytes in the intestinal lamina propria were restimulated in vitro. Moreover, the production of IFN-γ but not IL-17 in EBI3-deficient CD4+ T cells differentiated in vitro under Th17 polarizing conditions were significantly reduced compared with that in wild-type CD4+ T cells. To examine whether EBI3 functions as a soluble factor like a cytokine or an intracellular factor, equal number of naive CD4+ T cells obtained from EBI3-deficient and EBI3-intact GFP-transgenic mice were mixed and differentiated under Th17 polarizing conditions. Intriguingly, the IFN-γ production was significantly reduced in only EBI3-deficient GFP-CD4+ T cells but not in EBI3-intact GFP+CD4+ T cells. Taken together, the present results suggest that EBI3 functions as an intracellular molecule to regulate the development of colitis by regulating IFN-γ production in naive CD4+CD25- T cells.

P5.01.53
Prostaglandin D_2 represents contradicting roles in dermatitis

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Prostaglandin D_2 (PGD_2) is one of cyclooxgenase-metabolites and implicated in the pathogenesis of various inflammatory diseases. However there have been controversial reports showing its pro-inflammatory or anti-inflammatory role in various inflammatory disease models. The role of PGD_2 in inflammation is still elusive. In the present study, we investigated the role of PGD_2 in croton oil-induced dermatitis using transgenic mice (TG) overexpressing hematopoietic PGD synthase (H-PGDS). Administration of 2.5% croton oil caused tissue swelling and vascular leak in mice ears. Comparison with WT, TG ears produced more PGD_2 and showed decreased inflammatory responses at early phase (2 h), but showed more severe manifestations at late phase (6 h). The data obtained from the bone marrow transplantation between WT and TG suggested that the tissue resident cell-derived PGD_2 attenuated the early-phase inflammation, while the PGD_2 produced from hematopoietic cells exacerbated the late-phase inflammation. There are two distinct PGD_2 receptors, DP and CRTH2. In TG, treatment with a DP antagonist reinstated the inhibited inflammation at early phase, while treatment with a CRTH2 antagonist inhibited the aggravated inflammation at late phase. *In vitro* experiment showed that treatment with PGD_2 or a DP agonist, but not a CRTH2 agonist enhanced vascular endothelial barrier formation. Collectively, in H-PGDS-overexpressing mice ears, tissue resident cell-derived PGD_2 suppresses dermatitis via DP at early phase, while hematopoietic cell-derived PGD_2 stimulates CRTH2 and promotes inflammation at late phase. This is the first report showing that PGD_2 represents opposite roles in inflammation depending on disease phase *in vivo*.

P5.01.54
ST2/IL-33 signaling worsens immune-mediated hepatitis from drug haptens

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Susceptible persons develop immune-mediated drug-induced liver disease (Im-DILI) following exposure to certain drugs. Antibodies in Im-DILI are detectable in other forms of hepatitis suggesting that animal models of Im-DILI may uncover innovative therapeutic options for Im-DILI and other forms of hepatitis. IL-33 is an IL-1 family member that drives Th2 cytokines, cells and injury in asthma (Verri *et al*., 2010), but protects in atherosclerosis and Con A hepatitis. In experimental anesthetic Im-DILI, IL-1β, IL-2, IL-6 and IL-13, were demonstrated, in addition to neutrophils, mast cells, eosinophils, NK and NKT cells (Njoku *et al*., 2005). Furthermore, hepatitis was successfully transferred to naive mice using CD4+T cells. We hypothesized that signaling via ST2/IL-33 worsens experimental Im-DILI. To test our hypothesis we immunized female BALB/c mice with our catalytic site epitope of CYP2E1 (JHDN-5) that was covalently altered by a trifluroacetyl hapten (TFA) on days 0 and 7 ± IL-33 blocking antibody. Three weeks later liver supernatants were tested for IL-6, TARC, IL-33 and ST2 by ELISA. Hematoxylin and eosin-stained slides were scored for inflammation. Sera were tested for TFA, S100 and CYP2E1 antibodies. We found significantly elevated IL-6, TARC and IL-33 in TFA-JHDN-5 - immunized mice; additionally, anti-IL-33 diminished inflammation scores from 2.8 ± 1.1 to 1.6 ± 0.6 but increased TFA, S100 and CYP2E1 antibodies ($p<0.05$, Mann-Whitney*U*). We confirm that IL-33 worsens Im-DILI and suggest protective roles for antibodies. This preliminary study suggests that anti-IL-33 and other antibodies may offer therapeutic options to diminish Im-DILI and possibly other forms of hepatitis.

P5.01.55
Lack of TNF-Rp55 delays thrombus resolution in a stasis-induced DVT model through reduced MMPs and uPA expression

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Deep vein thrombosis (DVT) is multifactorial and often results from a combination of risk factors such as genetic conditions, obesity, drugs, pregnancy, aging, trauma, and malignancy. We examined the pathophysiological roles of TNF-α-TNF-Rp55 axis in the thrombus resolution using *Tnfrp55* KO mice. Upon the ligation of the inferior vena cava (IVC) of wild type (WT) mice, venous thrombi formed and grew progressively until 5 days, and the thrombus weight decreased less than 50% at 14 days. Concomitantly, intrathrombotic gene expression of *Tnfa* and *Tnfrp55* was enhanced as post-ligation intervals were extended. When *Tnfrp55* KO mice were treated in the same manner, thrombus mass was larger than WT mice. Moreover, the thrombosed IVC blood flow was less recovered in *Tnfrp55* KO than in WT mice. And intrathrombotic *Plau*, *Mmp9* and *Mmp2* mRNA expression were significantly reduced in *Tnfrp55* KO mice than WT ones. Supportingly, the administration of anti-TNF-α monoclonal antibody delayed the thrombus resolution in WT mice. On double-color immunofluorescence analysis, we found out the MMP-2, MMP-9 and uPA were produced mainly by intrathrombotic infiltrating macrophages. Furthermore, TNF-α treatment enhanced gene expression of *Plau*, *Mmp9* and *Mmp2* in WT-derived macrophages but not *Tnfrp55* KO-derived ones. And the gene expression of *Mmp9* and *Mmp2* was reduced respectively by ERK and NFkB inhibitor in WT-derived macrophages treated by TNF-α. Collectively, the lack of *Tnfrp55* can have a detrimental role in the thrombus resolution by suppressing uPA, MMP-2 and MMP-9 expression. Thus, TNF-α-TNF-Rp55 axis can be a good molecular target for the DVT treatment.

P5.01.56
Impaired production of IL-10 in response to *Staphylococcus aureus* alpha-toxin is closely associated with pathophysiology and postoperative outcome in chronic rhinosinusitis with nasal polyps

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Background: *Staphylococcus aureus* can modulate chronic airway inflammation by producing various toxins. However, in contrast to *S. aureus*-derived superantigenic toxins such as enterotoxins, the role of non-superantigenic toxins in the pathogenesis of chronic rhinosinusitis with nasal polyps, one of the major inflammatory upper airway diseases in humans, remains obscure.
Objective: We sought to characterize Th1-, Th2-, Th17-, and Treg-associated cytokine productions following the exposure to *S. aureus* alpha-toxin, the major non-superantigenic toxin, in chronic rhinosinusitis with nasal polyps using a human *ex vivo* model.
Methods: Dispersed nasal polyp cells were prepared from patients with chronic rhinosinusitis with and without nasal polyps. Cells were incubated with alpha-toxin, and the levels of IL-5, IL-13, IFN-γ, IL-17A, and IL-10 in the cell supernatants were determined. The pathophysiological significance of alpha-toxin-induced cytokine production by nasal polyp cells was also determined.
Results: Nasal polyp cells produced substantial amounts of IL-5, IL-13, IFN-γ, IL-17A, and IL-10 in response to alpha-toxin. Alpha-toxin-induced IFN-γ, IL-17A, and IL-10 production by nasal polyp cells negatively correlated with the degree of eosinophil infiltration into nasal polyps. Furthermore, alpha-toxin-induced IL-10 production by nasal polyp cells correlated negatively with postoperative CT score and positively with radiological improvement assessed 6 months after sinus surgery.
Conclusions: Th1-, Th2-, Th17-, and Treg-associated cellular responses were provoked following the exposure to non-superantigenic alpha-toxin in chronic rhiosinusitis with nasal polyps. These responses, especially failure to synthesize IL-10, regulate the pathophysiology of this disease including local eosinophila and postoperative outcome.

P5.01.57
IL6 trans-signaling promotes functional recovery of hypofunctional phagocytes through STAT3 activation during peritonitis

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Objective: The role of high interleukin 6 (IL6) levels has not been clearly explained in severe sepsis. We show that the augmentation of the IL6 signal by recombinant IL6 receptors (rIL6R) delivery allows the functional recovery of phagocytes in a peritonitis mouse model.
Materials and Methods: Mice were challenged intraperitoneally (i.p.) of live *Staphylococcus aureus* for effect of IL6R delivery on the 24h-survival, bacterial clearance and cellular responses. In additional experiments to assess of effect of IL6R delivery on phagocytosis, the model was i.p. inoculated with heat-killed *S. aureus* with or without rIL6R and the peritoneal lavage fluid and cells were collected at 1 h after the i.p. inoculation of *S. aureus*.
Results: The IL6R delivery tended to improve 24-h survival and increase bacteria clearance from the septic mice. The rIL6R treatment to heat-killed bacteria challenged mice augmented the uptake of bacteria and phagosome acidification, inducing the phosphorylation of STAT3 in peritoneal cells within 1 hour after the IL6R delivery. Furthermore, the rIL6R delivery prevented the extracellular release of neutrophil elastase activity and myeloperoxidase (harmful factors).
Conclusions: These results indicate that augmentation of IL6 signaling appears to be critical for the effective management of hypofunctional neutrophils during severe inflammation, such as sepsis.

P5.01.58
Cytokines as molecular biomarkers for cancer cachexia

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Introduction: Cachexia is a multifactorial syndrome defined by irreversible loss of skeletal muscle mass with or without loss of fat mass. Cachexia is characterized by a negative protein and energy balance that causes disorders in homeostasis such as progressive wasting, weakness, anorexia and anemia. Solid malignancies trigger an intrinsic undesired chronic inflammatory state that leads to an abnormal increase of inflammatory factors that induce cachexia. In the present clinical study, we have analyzed and correlated the cytokine profile and muscle function data of 71 patients with advanced cancer.
Methods: Cancer patients include cases of head and neck cancer, non-small cell lung cancer, pancreatic cancer, hepatobilliary cancer, colorectal cancer and upper gastro-intestinal cancer. Human blood samples were drawn into EDTA-coated collection tubes and 16 cytokines in plasma were measured using Human Cytokine bio-plex technology. The cytokine profile and muscle function data were correlated using Partial Least Squares (PLS) regression.
Results: Cancer patients with impaired muscle function are characterized by elevated levels of IL-1β, IL-6, IL-4, IFNγ, CRP (C-reactive protein) and LDH (lactate dehydrogenase). IL-6 and IL-1β induce protein catabolism and inhibit protein anabolism through the activation of NF-kB signaling pathway. IL-6 also induces an acute phase response (APR) characterized by elevated levels of CRP. On the other hand, IL-1β and tumor-induced hypoxia may induce HIF-1 (hypoxia inducible factor 1) and increase the transcription of LDH causing the accumulation of lactate. **Conclusion:** Abnormal levels of IL-1β, IL-6, IFNγ, CRP and LDH could be used as biomarkers for cancer cachexia.

P5.01.59
Cytokine profile changes following exposure to Hemiscorpius lepturus scorpion venom under experimental conditions

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Introduction: A previous clinical study showed that it produces a marked increase in TNFα level in patients envenomed by this scorpion. The aim of the present study was to further assess the changes in change other cytokine levels following in vitro exposure to this venom.
Method: Heparinized blood and peripheral blood mononuclear cells (PBMC) samples were exposed to 1 µg/ml of H. lepturus venom for 24 hr. Control samples of both preparations were treated similarly in the absence of the venom. The cytokine levels in the supernatant media and serum from whole blood samples were measured by MSD multi-array 96-well 10 small spot plates. The collected data were analyzed by WINMDI software.
Results: IFNγ and IL2 show significant 2.5 and 10 fold increased respectively. While IL10, showed a significant increase (by 4.5 fold) and IL-5 and IL-3 by 2 fold. TNF-α IL-12p70 increased by 4 fold. Overall trends of changes following exposure to H Lepturus venom in both whole blood and PBMCs had similar trends.
Conclusion: Besides its cytotoxic properties which were shown by the increase in TNF-α and IL-12p70; this venom has direct immune suppressive and possible indirect immune stimulating actions which may be utilized in development of novel agents that may be identified and used for treatment of different immune mediated illnesses.

P5.01.60
The knock-out of TWEAK receptor/Fn14 ameliorates lupus nephritis in MRL/lpr mice

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Renal mesangial cells, podocytes, and tubular cells express Fn14, the receptor for the TNF-family member cytokine TWEAK. TWEAK engagement of Fn14, the sole known biologic receptor for TWEAK, induces kidney resident cells to produce multiple inflammatory mediators including MCP-1 and RANTES. In the chronic graft-versus-host and nephrotoxic nephritis models of antibody-mediated renal disease, genetic deficiency of Fn14, or treatment with an anti-TWEAK monoclonal antibody, decreases kidney inflammation and proteinuria without affecting systemic autoantibody titers. These studies suggest that inhibition of TWEAK/Fn14 interactions might be efficacious in spontaneous models of lupus nephritis (LN). We assessed the role of the TWEAK/Fn14 pathway in the well-established MRL/lpr lupus prone mouse strain. We found that at 26 weeks of age, MRL/lpr Fn14 wild-type (WT) mice had significantly higher levels of proteinuria as compared to MRL/lpr Fn14 knockout (KO) mice. Furthermore, MRL/lpr Fn14 KO mice had significantly improved renal histopathology, with less glomerular immune deposition, endocapillary hypercellularity, mesangial proliferation, tubulointerstitial lesions, and perivascular inflammation. By immunohistochemistry, there were significantly fewer Ki-67+ proliferating cells in both glomerular and tubular compartments, as well as decreased KIM-1 staining, in MRL/lpr Fn14 KO mice. However, there were no differences between the strains in autoantibody titers at several time points, suggesting that TWEAK likely acts by modulating events locally in the kidney. Our results convincingly demonstrate that inhibition of TWEAK signaling significantly attenuates renal disease in murine lupus, suggesting that the TWEAK/Fn14 axis may be a novel therapeutic target for LN.

P5.01.61
Inhibiting TWEAK (TNF-like weak inducer of apoptosis) signaling ameliorates neuropsychiatric disease in MRL/lpr mice

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Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by involvement of multiple organs, including the central nervous system. While neuropsychiatric involvement in systemic lupus erythematosus (NPSLE) is relatively common and appears early, the underlying mechanisms are not fully understood. TWEAK is a cytokine member of the TNF superfamily; the TWEAK receptor, Fn14, is expressed in the brain in endothelial cells, astrocytes, microglia and neurons. TWEAK/Fn14 interactions can lead to cytokine production, neurodegeneration, and increases in blood brain barrier (BBB) permeability. Furthermore, patients with neuropsychiatric SLE demonstrate high levels of TWEAK in the cerebrospinal fluid. To elucidate a possible role for TWEAK signaling in the pathogenesis of NPSLE, comprehensive neurobehavioral tests were employed to assess neuropsychiatric manifestations in MRL/lpr Fn14 wild type (WT) and MRL/lpr Fn14 knockout (KO) mice. We found that Fn14 deficiency significantly ameliorates NPSLE, as seen by dramatically less depressive-like behavior and improved cognitive function (memory) in MRL/lpr Fn14 KO mice. Moreover, RANTES, C3 and IgG were reduced in the brains of MRL/lpr Fn14KO mice. Additionally, MPRL/lpr Fn14KO mice had decreased albumin concentrations in the cerebrospinal fluid, indicating preserved BBB integrity. However, there were no differences between MRL/lpr Fn14 WT and KO mice in the serum levels of autoantibodies associated with NPSLE. Finally, Fn14 KO mice display reduced apoptosis of brain cells. Thus, TWEAK/Fn14 interactions play a central role in the pathogenesis of lupus associated neuropsychiatric disease, suggesting a novel target for therapeutic intervention for this major disease manifestation.

P5.01.62
All trans retinoic acid enhanced the markers of T regulatory cells while reducing nitric oxide synthase 2 expression and proinflammatoy cytokines production in peripheral blood mononuclear cells from Algerian patients with inflammatory bowel disease

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Inflammatory bowel diseases (IBDs) are chronic inflammatory diseases of the gastrointestinal tract, which are clinically present as one of two disorders, Crohn's disease (CD) or ulcerative colitis (UC). The immune dysregulation in the intestine plays a critical role in the pathogenesis of inflammatory bowel disease, involving a wide range of molecules including cytokines and Nitric Oxide (NO). The maintenance of host integrity relies on the constant resetting of tissue homeo¬stasis by regulatory networks. One cardinal feature of this regulation is associated with the ability of lymphocytes with constitutive or induced regulatory properties to accu¬mulate at the disrupted site and limit tissue damage. This study was designed to investigate the effects of All trans Retinoic Acid (AtRA) on NO, Urea and cytokines (IL-10, TGF-β, IL-2, TNFα, IFNγ) production in periphal blood mononuclear cells (PBMC) cultures from Algerian patients with IBD. We also investigate the effect of AtRA on Nitric oxide synthase 2 (NOS2) and Treg markers expression using transcriptomic analysis. Our results suggest that AtRA is potent inhibitors of NO and proinflammatory cytokines. In contrast, AtRA increased Urea and TGF-β production, but not IL10 production. AtRA up regulated mRNA transcripts expression of Treg markers in PBMC of all IBD groups. Our study also shows that AtRA down regulated mRNA transcripts expression of NOS2 in PBMC. Collectively, our study indicates that AtRA plays pivotal role in regulatory networks of immune system in IBD patients.

P5.01.63
Serum level of immunoglobulins in patients with respiratory allergic diseases

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Allergic diseases are immunologically common diseases in which the immunoglobulins, as important components of the immunity system play an effective role in their pathogenesis. Allergic diseases have a great range from allergic dermatitis, allergic rhinitis to allergic asthma and so on. The aim of this research was to investigate the relation of serum level of IgE, IgA, IgM, IgG in patients with allergic rhinitis and asthma separately and both together.
In this research the serum levels of immunoglobulins including IgG, IgM, IgA, IgE was measured in 130 patients suffering from respiratory allergic disease, which who were referred to Isfahane Issabne Maryam Hospital Allergy And Asthma Clinic and in 120 healthy individuals. Both patients and control groups were matched considering age and sex.
A significant reverse relation was observed between the serum IgA and IgE in patients with both asthma and allergic rhinitis ($r=-0.396, p<0.05$). A direct and significant relation between IgE and IgM serum level in patients with both asthma and allergic rhinitis ($r=0.448, p<0.05$). In this study, a significant reverse relation was observed between serum IgA and IgM in patients with asthma and both asthma and allergic rhinitis ($r=-0.252$, $p<0.05$) and ($r=-0.423$, $p<0.05$) but not in patients with allergic rhinitis($p>0.1$). As conclusion, our results show that IgA deficiency is in relation with an increase in IgE and respiratory allergic diseases.

P5.01.64
The electromagnetic fields affect T-helper balance in rats

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The biologic influences of ElectroMagnetic Fields (EMFs) are being investigated for decades and the World Health Organization (WHO) established the International EMF Project, to determine the scientific evidence of possible health effects of EMFs. These fields have been classified as probable human carcinogen. Among all investigations, immunologic experiments, especially assessing cytokines, are rare. The present study undertakes to search for the T-helper (Th) balance otucome of exposure to these fields. Thirty Wistar rats were divided to three groups, one group included the control rats, another, rats for one month exposure, and the last group rats for 2 month exposure. An EMF in the frequency of 50 Hertz, 0.5 mili-Tesla was used, and the serum levels of cytokines of IL-4 (indicating Th2 activity) and INFγ (indicating Th1 activity) were measured. The results showed that after one month, the serum levels of IL-4 increased highly significantly (P value: 0.009), and the levels of INFγ decreased significantly (P value: 0.032). After 2 months these levels returned to the levels statistically similar to the control group (P value: 0.627 & 0.402 respectively for IL-4 & INFγ). As a conclusion of our results, in short periods, as much as one month, EMFs weigh the T-helper balance to the Th2 side in rats, however, in longer periods, as much as 2 months, the biologic homeostasis of rats causes *tolerance* to this influence of the EMFs.
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P5.01.65
TWEAK Affects Keratinocyte G2/M Growth Arrest and Induces Apoptosis through the Translocation of the AIF Protein to the nucleus

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The soluble TNF-like weak inducer of apoptosis (TWEAK, TNFSF12) binds to the fibroblast growth factor-inducible 14 receptor (FN14, TNFRSF12A) on the cell membrane and induces multiple biological responses, such as proliferation, migration, differentiation, angiogenesis and apoptosis. Previous reports show that TWEAK, which does not contain a death domain in its cytoplasmic tail, induces the apoptosis of tumor cell lines through the induction of TNFα secretion. TWEAK induces apoptosis in human keratinocytes. Our experiments clearly demonstrate that TWEAK does not induce the secretion of TNFα or TRAIL proteins. The use of specific inhibitors and the absence of procaspase-3 cleavage suggest that the apoptosis of keratinocytes follows a caspase- and cathepsin B-independent pathway. Further investigation showed that TWEAK induces a decrease in the mitochondrial membrane potential of keratinocytes. Confocal microscopy showed that TWEAK induces the cleavage and the translocation of apoptosis inducing factor (AIF) from the mitochondria to the nucleus, thus initiating caspase-independent apoptosis. Moreover, TWEAK induces FOXO3 and GADD45 expression, cdc2 phosphorylation and cdc2 and cyclinB1 degradation, resulting in the arrest of cell growth at the G2/M phase. Finally, we report that TWEAK and FN14 are normally expressed in the basal layer of the physiological epidermis and are greatly enhanced in benign (psoriasis) and malignant (squamous cell carcinoma) skin pathologies that are characterized by an inflammatory component. TWEAK might play an essential role in skin homeostasis and pathology.

P5.01.66
PI3K/PTEN axis in APCs is regulating Th17-dependent autoimmune pathology

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Background: $CD4^+$ T cells, especially those polarized towards the Th1/Th17 subsets, play a critical role in the pathogenesis of autoimmune diseases. Therefore, it is important to understand pathways controlling Th17-mediated autoimmunity. Antigen-presenting-cells (APCs) control adaptive immune responses by activation of antigen-specific T cells. However, signal transduction pathways in APCs that govern the development of Th17 cells are incompletely understood. The Phosphatidylinositol-3-kinase (PI3K) pathway is one of the most important signal transduction pathways, regulating fundamental processes. Here we report that control of PI3K signalling by PTEN specifically in APCs is essential for the development of Th17 mediated autoimmune diseases such as experimental-autoimmune-encephalomyelitis (EAE).
Methods/Results: We studied EAE, a model for the early inflammatory stage of human multiple sclerosis that is strictly mediated by pathogenic T cells polarized towards the Th17 linage. We induced EAE in animals with a celltype-specific (LysM, CD11c-cre) deletion of PTEN ($pten^{-/-}$) to test the selective role of PTEN-deficient APCs on the induction of autoimmunity without affecting other cell types, especially T cells. $Pten^{-/-}$ mice had almost no clinical signs of EAE, in contrast wt mice developed typical symptoms of paralysis and had a significant weight loss. Splenocytes and lymphocytes isolated at day 8, 12, 16 and 21 after EAE induction and restimulated *in-vitro* with myelin-oligodendrocyte-glycoprotein (MOG) had reduced amounts of Th17 polarising factors as a result of reduced generation of IL-17 and IL-22 producing T-cells determined by ELISPOT and ELISA.
Conclusion: Our findings show that the PI3K/PTEN axis in APCs is regulating Th17-dependent autoimmune pathology.

P5.01.67
Cytokine patterns during high-risk human papillomavirus and HIV co-infection in cervical cancer patients and controls in Kwazulu-Natal (South Africa).

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Cytokines including IL2, IL6 and TNFα production is reported correlates with response to therapy in cervical cancer and in HIV disease.
Aim: To analyse cytokines levels in cervical cancer patients and controls according to HIV status.
Materials and Methods: Serum samples from 134 cancer patients and 84 controls from KwaZulu-Natal were analysed for the cytokines, IL2, IL6 and TNFα using commercially available ELISA.
Results: IL2 levels were higher in the control group (9.55±1.92) than in the cancer group (4.98±1.45) (p=0.0001). IL-6 concentration was higher in cervical patients than in controls, while the opposite was found for TNFα (Table 1). HIV infection had no additional effect on the production of IL-2, IL-6 and TNFα in cervical cancer patients. The same was true for controls, with the exception of IL-6 levels found higher in HIV positive than in HIV negative controls.
Table 1 Levels of cytokine IL2, IL6 and TNFα in Cervical Cancer Patients and in Controls
cytokine cervical cancer controls
n x±SEM n x±SEM
p value
IL2 134 4.98 ± 1.45 83 9.55 ± 1.92 0.0001٭
IL6 134 52.47 ± 22.81 83 7.48 ± 1.99 0.0038٭
TNFα 134 1.69 ± 1.01 83 8.28 ± 3.05 0.0368٭
٭ statistically significant (Mann Whitney test)
Discussion: This study confirms the shift from Th1 to Th2 immune response in cervical cancer as reported elsewhere. The lack of HIV influence on the above cytokines levels in cervical cancer patients suggests that cervical cancer might have maximally stimulated them such that HIV has no further impact.

P5.01.68
Mechanisms of immunosuppression and interleukin 2 in complex treatment of severe acute pancreatitis

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Introduction. In pathogenesis of acute pancreatitis the important role are play pro- and anti-inflammatory cytokines. Their disbalance leads to depletion of immunocompetent cells and defines the expressed secondary immunodeficiency, which after 7-14 days becomes a leading pathogenic link of severe acute pancreatitis (SAP). Therefore adequate immunotherapy may play obvious role in complex treatment of this severe pathology.
Materials and Methods. Plasma levels of IL-2, IL-4, IL-8, TNF-α, populations of lymphocytes were measured in 36 patients with SAP. Twenty patients received in complex therapy recombinant interleukin-2 in a dose of 3 million MO. Sixteen patients examined as control.
Results. The mean value of serum all cytokines levels in patients with SAP was significantly higher than in healthy volunteers. The lymphocyte count was decreased below the normal range, and was significantly negatively correlated with severe score. CD4- and CD8-positive lymphocyte counts on admission and the lymphocyte count on day 14 after admission may be useful for predicting infection. Changes of cytokines status after interleukin 2 therapy are noted too. Authentic levels depression of all cytokines in peripheral blood is established. The level of IL-2 tended to normalisation in comparison with a disease debut, but remains lower, than in healthy donors. After therapy normalization of T lymphocytes level is noticed.
Conclusion. A significant depletion of circulating lymphocytes was found in severe acute pancreatitis with infectious complications. Use of interleukin 2 in complex treatment of severe pancreatitis is expediently for correction immunoparalysis and prevention of purulent-septic complications.

P5.01.69
Tuberculosis infection diagnosis by Quantiferon TB Gold In-Tube Test assessment in Namibian patients

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Introduction: Conventional diagnostic methods take long for results to come out, and active TB patients can spread infection while waiting laboratory confirmed results. The QFN TB Gold In-Tube method shortens the time need for TB diagnosis, especial in country with low TB prevalence. It was important to assess the usefulness of bio-markers in QFN supernatant plasma diagnostic approach for TB in Namibia context. The research has component to monitor the levels of IFN-γ in QFN supernatants of TB patients as a measure of response to treatment in the Namibian settings. Methodology: The study population include 84 TB suspects, 18 years and older, HIV negative and positive suspects, living in Windhoek and meeting AE-TBC requirements, at the moment of visiting Katutura State Hospital. The main target number is 100 TB suspects, the samples collected goes through the routine testing (AFB Auramine microscopy and Culture), and Quantiferon test to detect the level of IFN-γ, suspected to be released in infected individuals. The effect of treatment on the level of IFN-γ in plasma was also monitor in patient's at 2 months and 6 months after treatment.
Results: A total of 84 suspects was recruited,tested using culture and Quantiferon test. All recruited 21.4% were TB positive in microscopy, 14.3% TB positive in culture and 53.6% were positive in Quantiferon TB Gold In-Tube.
Conclusion: The use of Quantiferon TB Gold In-Tube for TB diagnosis in Namibia, has shown unreliably false positive results in comparison to TB culture.

P5.01.70
Antiplatelets drugs reduces the immunoinflammatory response in a rat model of periodontal disease

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Background: Platelets are cellular corpuscles that once activated express biological mediators that modulate the inflammatory process including pro-inflammatory cytokines, chemokines and growth factors. We hypothesized that drug-induced platelet inactivation may interfere in the inflammatory process in experimental periodontal disease in rats by suppressing the release of biological mediators from platelets to the site of injury.
Methods: Effects of treatment of rats with Asp (30 mg/kg) and Clo (75 mg/kg) given intragastrically for 3 days on ligature-induced periodontal disease were assessed. After experimental period the animals were sacrificed and right and left hemi-mandibles were used to the hystologic and histometric evaluation as well as mieloperoxidase (MPO) activity. Expression of CCL5 and CXCL4 levels were measured by enzyme-linked immunosorbent assay.
Results: Significant anti-inflammatory effects and consequently fall of alveolar bone loss were observed at doses of the drugs-induced platelet inactivation. The degree of anti-inflammatory effects at the site of inflammation correlated significantly with reduction of MPO activity. At these doses, Clo significantly increased the expression of CXCL4 and decreased CCL5 ($P<0.001$) whereas Asp only decreased in the expression of CXCL4 ($p<0.05$). Treatment with antiplatelet drugs did not influence the expression of CCL5 at the site of inflammation.
Conclusion: Systemic administration of Clo could therefore play a crucial role in platelet-expressed biological mediators and in turn could regulate the process of immunoinflammation associated with periodontitis.

P5.01.71

Vitamin D and Interferon-beta act synergistically at physiological levels to modulate cytokine expression *in vitro* in peripheral blood mononuclear cells

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Background: Current treatments for the autoimmune disease Relapse Remitting Multiple Sclerosis (RRMS) are limited, with Interferon-beta (IFNβ) being one of the most effective. Part of its efficacy is due to its effect on the cytokine profile, suppressing inflammatory cytokines and increasing anti-inflammatory Interleukin-10 (IL-10). Vitamin D is also known to have immunomodulatory effects, therefore we asked whether a combination of IFNβ and calcitriol (the physiologically active form of vitamin D) has synergistic effects on cytokine secretion by peripheral blood mononuclear cells (PBMC) from healthy people.
Methods: We separated the PBMC from the blood of 22 healthy people, and treated them as follows: stimulated (20ug/ml Concanavilin A); stimulated + 100pg/ml calcitriol; stimulated + 100 IU or 400 IU IFNβ; stimulated + 100pg/ml calcitriol + 100 IU or 400 IU IFNβ. The cytokines IL-2, -4, -6, -10, TNFα, IFNβ and IL-17 were measured flow cytometrically (BD Scientific).
Results: The majority of subjects were female (59%), and the median serum 25(OH)D was 69.5 nmol/L. Calcitriol alone suppressed IL-2, -4, -6 and 17. IFNβ alone suppressed IL-2, -6 and TNFα, but increased IL-10 four fold. Calctriol and IFNβ acted synergistically to further suppress IL-2, -6, TNFα and IFNγ. No consistent effect of age or serum 25(OH)D on cytokine expression was observed.
Significance: Co-administration of vitamin D with IFNβ could aid in RRMS by suppressing inflammatory cytokine responses and promoting IL-10. This may also have implications for other inflammatory conditions

P5.01.72

Carbon monoxide (CO) as a novel approach for treatment of islet-directed autoimmunity

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Recent studies identified carbon monoxide (CO) as a potential therapeutic molecule due to its anti-inflammatory and anti-apoptotic abilities. Since a pharmacologically designed CO-releasing molecule CORM-A1 liberates controlled quantities of CO in the cellular systems, in this study we investigated possible therapeutic value of CORM-A1 in the model of autoimmune diabetes. We have already shown that CORM-A1 administration during diabetes induction in C57BL/6 mice by multiple low doses of streptozotocin (MLDS) improves clinical and histological signs of the disease. To determine the effect of CORM-A1 within the local environment of the endocrine pancreas, infiltrated mononuclear cells were isolated from pancreata at days 8-10 of disease induction and phenotype of cell infiltrates was determined by flow cytometry and real-time PCR. The percentage of $CD4^+$, $CD8^+$, $B220^+$ and pro-inflammatory $F4/80^+CD40^+$ macrophages was reduced, while anti-inflammatory $F4/80^+CD206^+$ macrophage cell number was unchanged in pancreatic infiltrates of CORM-A1-treated mice compared to diabetic mice. Also, the proportion of Th1 ($CD4^+$IFN-γ^+) infiltrated cells was reduced and of Th2 ($CD4^+$IL-4^+) cells was increased in CORM-A1-treated mice compared to control MLDS-treated mice. However, Th17 cells remained unchanged in both groups. In line with this, mRNA expression of Th signature cytokines, IFN-γ and IL-4, was inversely regulated resulting in lower IFN-γ/IL-4 ratio in CORM-A1-treated mice. Our study suggests that CORM-A1 suppresses autoimmune response by preventing the infiltration of cells and shifting the balance towards protective Th2 cells, thus interfering with cytokine-mediated pro-apoptotic stimuli within endocrine pancreas. CORM-A1 may thus represent a novel candidate drug for treatment of autoimmune diabetes.

P5.01.73

IL-2 induces robust inflammatory responses in the lung that synergize with influenza virus to exacerbate immunopathology

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Interleukin-2 (IL-2) is an important T cell growth factor central for the homeostasis and function of regulatory CD4 T cells (Tregs) that maintain tolerance and dampen inflammation. Systemic administration of IL-2 expands Tregs and improves autoimmune disease and transplant acceptance. On the other hand, IL-2 expands CD8 T and NK cells and cancer therapies aimed at improving the function of these subsets through administration of IL-2 are often complicated by severe side effects including systemic inflammatory cytokine (IFN-γ, TNF, and IL-5) production, eosinophelia, and death. To better understand the pro versus anti-inflammatory roles of IL-2 in vivo, we administered soluble cytokine, or IL-2 complexed with antibodies that target its delivery to specific cell types to unmanipulated mice and analyzed protein expression of over 40 inflammatory cytokines and chemokines. IL-2 up-regulates a remarkably broad array of these molecules, unexpectedly, even when targeted to Tregs. The IL-2 driven inflammation is as dramatic in lungs as it is systemically, and multiple T cell subsets, NK cells, and neutrophils, but not eosinophils, are involved in the inflammatory response. We next assessed whether IL-2 driven pulmonary inflammation is beneficial or detrimental during respiratory influenza A virus (IAV) infection as it is unclear whether concurrent infections exacerbate complications that arise during IL-2 therapy. IL-2 and virus synergized to markedly enhance inflammation that correlates with lower IAV titers, but morbidity was also strikingly increased. Our findings highlight the need to better understand the regulation of inflammation during pathogen infection and have implications for improving IL-2 based therapies.

P5.01.74

Lnk/Sh2b3, an intracellular adaptor associated with celiac disease and autoimmune diabetes, regulates accumulation of inflammatory T cells and prevents intestinal villous atrophy

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The intracellular adaptor Lnk/Sh2b3 regulates cytokine signals that control lymphohematopoiesis, and Lnk-/- mice show overproduction of B cells, megakaryocytes and hematopoietic stem cells. In humans, mutations in the LNK/SH2B3 gene are found in myeloproliferative disease patients, and LNK/SH2B3 polymorphisms have been demonstrated to be associated with celiac disease and autoimmune diabetes. However, no data linking Lnk/Sh2b3 function with a risk for autoimmune-mediated inflammation have been reported so far. In this study, we revealed a previously unrecognized function of Lnk/Sh2b3 in preventing the accumulation of inflammatory CD8+ T cells and intestinal villous atrophy. Generation of an Lnk-Venus reporter mouse revealed low but apparent expression of Lnk/Sh2b3 in mature T cells. CD44hiIFN-γ+CD8+ effector/memory T cells were increased in Lnk-/- mice, and many mice exhibited villous atrophy in the small intestine. Lnk-/- CD8+ T cells survived longer and proliferated even in non-lymphopenic host animals in response to IL-15. Transfer of Lnk-/- CD44hiCD8+ T cells together with wild-type CD4+ T cells into RAG2-deficient mice recapitulated villous atrophy in ileum. In addition, DCs in spleen and peripheral lymph nodes were increased in the absence of Lnk/Sh2b3, and were hypersensitive to IL-15 and GM-CSF. Lnk-/- DCs supported differentiation of IFN-γ producing T cells from naïve CD4+ T or CD8+ T cells compared to normal DCs. Our results provide for the first time a missing link between Lnk/Sh2b3 and autoimmune-like tissue destruction or inflammatory immune responses.

P5.01.75
Molecular Regulation of IL-10 during Antibody-dependent Enhancement of Dengue Virus Infection is Important for Viral Replication

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Dengue virus (DENV) infection is a emergency global public health issues, and so far there is no effect of vaccines and therapeutic drugs. Interleukin (IL)-10 levels are increased in DENV-infected patients with severe disorders. A hypothetical intrinsic pathway has been proposed for the IL-10 response during antibody-dependent enhancement (ADE) of DENV infection; however, the mechanisms of IL-10 regulation remain unclear. We found that DENV infection alone was sufficient to induce increased expression of IL-10 and its downstream regulator suppressor of cytokine signaling 3 in human monocytic THP-1 cells. IL-10 production was controlled by activation of cyclic adenosine monophosphate response element-binding (CREB), primarily through protein kinase A (PKA)- and phosphoinositide (PI) 3-kinase/PKB-regulated pathways, with PKA activation acting upstream of PI3K/PKB. DENV infection also caused glycogen synthase kinase (GSK)-3β inactivation in a PKA/PI3K/PKB-regulated manner, and inhibition of GSK-3β significantly increased DENV-induced IL-10 production following CREB activation. Silencing of C-type lectin domain family 5 member A (CLEC5A) expression and pharmacological inhibition of spleen tyrosine kinase (Syk) activity decreased DENV-induced IL-10 production. ADE of DENV infection greatly increased IL-10 expression by enhancing Syk-regulated PI3K/PKB/GSK-3β/CREB signaling. We also found that viral load, but not serotype, affected the IL-10 response. Finally, modulation of IL-10 expression could affect DENV replication. These results demonstrate that IL-10 production is regulated by ADE through both an extrinsic DENV/CLEC5A/Syk- and an intrinsic DENV/Fcγ receptor/Syk-regulated PI3K/PKB/GSK-3β/CREB pathway, both of which impact viral replication.

P5.01.76
Role of TNF-α, their receptors and gene‘s polymorphism in patients with coronary atherosclerosis

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Under supervision were 138 patients aged 45-75 years with the diagnosis: coronary atherosclerosis, ischemic heart disease. As control has been used 100 healthy donors. The content of TNF-α, receptors: TNF-αRI and TNF-αRII has been carried out in blood serum by the immune-enzyme analysis (R&D Diagnostics Inc. reactants, USA). Gene‘s polymorphic loci of TNF-α rs1800629 (308G/A) have been carried out by chain polymerization. The statistical analysis of results have been used by Stat Plus (2009) applied program. Results: increasing of TNF-αRI and TNF-αRII has been registered in patients. However the patients aged 45 - 59 years had lower level TNF-αRI than more senior patients. At high angina pectoris functional class decreasing of TNF-α level has been registered. Within three months after the myocardial infarction incident the highest level of TNF-αRII has been detected. At atrial extrasystole the TNF-αRII level was higher than without violation rhythm. At unstable angina pectoris the level of TNF-αRII was higher than at stable one current. At high level of blood general cholesterol and triglycerides the TNF-αRI level was lower, than at normal their indices. At coronary atherosclerosis G/G variant of gene TNF-α rs 1800629 was prevalent (79,8%). The high level of TNF-α was also associated with presence at a genome of G/G allele gene TNF-α.

P5.01.77
Abnormal peripheral cytokine profile detectable before clinical onset of Huntington’s disease

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Huntington’s disease (HD) is a hereditary progressive neurodegenerative disorder with no cure available. Although it is not thought of as an inflammatory disease, it has been shown that there is a parallel immune activation in CNS and the periphery. The cytokines that are increased earliest in the disease course, before the onset of neurological symptoms, are involved in the innate immune response. This phenomenon designates components of innate immunity as potential biomarkers of HD progress.
To monitor the early HD progression, we have established a biomedical model of HD - a miniature pig transgenic for N-terminal part of mutated human huntingtin. The immunological characteristics have been studied on age-matched control and transgenic minipigs with the same genetic background. The levels of chosen cytokines in the blood serum were examined by targeted proteomic profiling using porcine antibody microarrays and immunoassay. Aims: (i) characterization of immune activation in control and transgenic animals, (ii) monitoring the cytokine levels during ageing and disease course, (iii) detection of monocyte activation as a possible source of peripheral cytokines.
Our data indicate the significant changes between control and transgenic minipigs mainly in levels of IL-8, both in blood serum and in monocytes extracts. The cytokine profiling together with monitoring of monocyte activation will longitudinally continue in three month intervals to follow up the disease progression.
Early changes founded in HD transgenic pigs may help to clarify development mechanisms of pathological changes in the disease, and so they can provide new opportunities for therapeutic effect of adverse developments.

P5.01.78
Aberrant CD200/CD200R1 expression and function in systemic lupus erythematosus contributes to abnormal T-cell responsiveness and dendritic cell activity

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Introduction: CD200 is a transmembrane glycoprotein that can regulate the activation threshold of inflammatory immune responses and maintain immune homeostasis. We therefore evaluated the functional status of CD200/CD200 receptor 1 (CD200R1) interactions in subjects with systemic lupus erythematosus (SLE).
Methods: Serum CD200 level was detected by ELISA. The expression of CD200/CD200R1 by CD4+ T cells and dendritic cells (DCs) was examined by flow cytometry, and then compared between SLE patients and healthy controls. Peripheral blood mononuclear cells were stained with CFSE and annexin V/propidium iodide for evaluation of the effect of CD200 on cell proliferation and apoptosis. In addition, the effect of CD200 on DC function was determined by transwell migration assay as well as by measurement of binding and phagocytosis of apoptotic cells.
Results: In SLE patients, the number of CD200+ cells and the level of soluble CD200 were significantly higher than in healthy controls, whereas the expression of CD200R1 by CD4+T cells and DCs was decreased. Furthermore, the increased CD200 expression by early apoptotic cells contributed to their diminished binding and phagocytosis by DCs in SLE. Importantly, the engagement of CD200 receptor on CD4+ T cells with CD200-Fc fusion protein in vitro reduced the differentiation of Th 17 cells and reversed the defective induction of CD4+CD25highFoxP3+ T cells by transforming growth

factor beta in SLE patients. Conversely, blockade of CD200-CD200R1 interaction with anti-CD200R1 antibody promoted CD4+ T cell proliferation.
Conclusion: CD200/CD200R1 expression and function are abnormal in SLE and may contribute to the immunologic abnormalities in SLE.

P5.01.79
BAFF induces IL-6 production in human monocytes through signaling pathways that involve NF-kB and PI3 kinase

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Background and Purpose: BAFF plays an important role in the development of autoimmune diseases such as Sjögren's syndrome (SS). In our previous study, we found that soluble BAFF (sBAFF) induced abnormal increase in the production of IL-6 by SS monocytes as compared to normal monocytes. Therefore, elucidation of the regulatory mechanism of the abnormality is important to understand the mechanism of pathogenesis of SS. For this purpose, we investigated signaling pathway for sBAFF in THP-1, a human monocytic leukemia cell line that recapitulates the characteristics of monocytes of autoimmune diseases.
Materials and Methods: THP-1 stimulated with recombinant human IFN-gamma (rIFN-gamma) were cultured in the presence of sBAFF as well as inhibitors against NF-kB or PI3 kinase. The expression levels of IL-6 and a BAFF receptor (BR3) were measured by ELISA and/or quantitative RT-PCR.
Results and Discussion: rIFN-gamma remarkably enhanced the expression of BR3 in THP-1. In addition, stimulation of rIFN-gamma-primed-THP-1 with sBAFF induced robust increase in the production of IL-6. These data strongly suggest that sBAFF is involved in the production of IL-6 by monocytes. When these cells were further treated with NF-kB inhibitors (BAY11-7082, BAY11-7085) or a PI3 kinase inhibitor (LY294002), the production of IL-6 was significantly decreased in a dose dependent manner. These data suggest that sBAFF plays a role in the induction of IL-6, which is one of major players in inflammatory responses, through NF-kB and/or PI3 kinase pathways.

P5.01.80
Increased levels of IL-6, TNF-a, MIF, IL-8, and MCP-1 in schizophrenia

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Schizophrenia is a serious psychiatric disease with chronic symptoms and significant impairment in psychosocial functioning. Although numerous antipsychotic drugs have been developed, the main symptoms of this disease are still untreated. Inflammatory and immune response regulating proteins are thought to play an important role in the generation and progression of schizophrenia. However, recent findings on the role of proinflammatory and chemotactic cytokines in schizophrenia remain controversial and required further clarification. In this study we evaluated the levels of the IL-6, TNF-a, MIF, IL-8, and MCP-1 in patients with paranoid schizophrenia and age-, sex-matched healthy volunteers (controls) using enzyme-linked immunsorbent assay. According to the results obtained, all studied cytokines were overexpressed in the blood of schizophrenia patients when compared to controls ($p<0.0001$). No significant difference in the levels of cytokkines between neuroleptic-treated and non-treated patients was detected ($p>0.05$). These findings suggested that schizophrenia is characterized by upregulation of chemo-cytokine network that can be further used for the development of cytokine-targeted therapeutic approaches.

P5.01.81
Assay of IL-22/IL-25 in serum, whole blood and peripheral blood mononuclear cells cultures of patients with severe asthma

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Although some studies on the airways show IL-22/IL-25 playing a critical role in the pathogenesis of asthma, limited evidence about the systemic production of these cytokines is available. Therefore, the aim of this study was to assay IL-22/IL-25 levels in serum, in mitogen-activated whole blood (WB) and in mitogen-activated peripheral blood mononuclear cells (PBMCs) cultures of patients with severe asthma. In this cross-sectional study, to determine the severity of the asthma, a questionnaire was administered. The questionnaire asked information including clinical signs, clinical symptoms, and past medical history so all active or ex-smoker patients were excluded. Then, a trained observer assessed airway reversibility, peak flowmetry and spirometry in the patients. Twenty one patients with severe asthma and simultaneously, twenty age and sex-matched healthy controls were selected. Ten ml sterile blood was taken from each person. The sera were isolated and anticoagulant bloods used to WB and PBMCs cultures and hematological tests. Phytohemagglutinin (PHA) and Lipopolysaccharide (LPS) used to activate WB and PBMCs. Data of two groups were compared with Student's t-test and nonparametrical statistic test. Except total white blood cells count that was increased in the asthmatic group, other hematological indices and IL-22/IL-25 levels in the two groups were not significantly ($p<0.05$) different. The levels of IL-22/IL-25 in patients with severe asthma are not higher than in healthy people and their roles in asthma could be related to local immunological process.

P5.01.82
Status of cytokine line and morphological changes in endocrine glands of rats with tentative thyrotoxicosis

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Model of experimental exogenous thyrotoxicosis was implemented on six-month-old Wistar rats with application of levothyroxine and was sustained with estimation of thyroid hormones rate. Cytokine level in biomaterial of rats was specified by IFA method with specific diagnostic sets of "R&D Diagnostics Inc.", USA. Histological assay samples were stained with hematoxylin/eosin and silver nitrate. Observed rats were found out significant elevation in serum rates of pro-and-anti-inflammatory cytokines: IL-1β (Me 15,42 pg/ml as compared with check group Me 1,90 pg/ml, $p<0,05$), IFNγ was Me 19,58 pg/ml against 5,96 pg/ml ($p<0,01$) and IL10 - 16,59 pg/ml against 5,62 pg/ml($p<0,01$) in control. IFN-γ / IL-10 correlation both in system and organs were determined close to1with minor dominance of Th2 marker cytokines in situ of healthy rats and tenfold ratio change towards Th1 marker cytokines at organ level in thyrotoxicosis. Morphological changes in thyroid were evinced in mastocytar focal infiltration of follicles stroma indicating activation of T-cell population of lymphocytes. Augmentation of cells with proliferative phase of cell cycle with amitotic activity was detected in glomerular and fasciculate zone of adrenal glands and in adenohypophysis. Hereby findings prove interdependence of hormone, morphological and immune changes in endocrine system under thyrotoxicosis and enlarge our view about the conception of Grave's Disease development.

P5.01.83
Lithium treatment reduced microglia activation and inflammation after irradiation to the immature brain

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To evaluate the effects of lithium on microglia activation and inflammation after irradiation to the immature brain, male rat pups were injected 2 mmol/kg lithium chloride i.p. on postnatal day 7 (P7), additional lithium injections, 1 mmol/kg, were administered at 24 h intervals. Pups were subjected to whole brain 6Gy irradiation on P11. The pups were sacrificed at 6h and 24h after IR. Microglia scattered in the brain can be detected by counting their numbers, their size, engulfment of cell debris or by the production of cytokines and chemokines. Microglia were stained using the marker Iba-1, revealing the presence of these cells throughout the brain under normal conditions, and apparently higher numbers and bigger sizes after IR, particularly in areas where cell death occurred. Quantification of Iba-1+ cells in the GCL of DG showed a significant increase after IR, but with a lower increase in the lithium-treated brains. The concentration of MCP-1, IL-1α, IL-1β, GRO/KC in the hippocampus were increased significantly at 6h after IR compared with non-irradiation control. Lithium treatment significantly inhibited the increase. There was no significantly difference with these cytokines/chemokines at 24h after IR between lithium and vehicle treated group. These data indicate that lithium can specifically reduce inflammation through GSK3-ß inhibition or modified microglia activation.

P5.02 Chronic inflammation and fibrosis

P5.02.01
IL-17 and IFNγ decrease in the gut of rats with experimental fibrosis. "Possible association with bacterial translocation"

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Background. Bacterial translocation (BT) in cirrhotic patients, is a trigger of peritonitis and septicemia, with a mortality rate of 30-50%. Alterations in the "intestine" immune response is a new field of study. BT has been reported in experimental models of liver fibrosis CCl4 and Thioacetamide (TAA). However, Th1 cytokines (IFN γ) and Th17 (IL-17) have been little explored in the gut of rats with experimental liver fibrosis. Aim. To analyze the expression of IL-17 and IFNγ in the gut of rats with fibrosis by TAA. Methods. Liver fibrosis was induced by thioacetamide administration (200 mg/kgi.p.). Fibrotic animals were euthanized after 3.5 and 8 weeks. Small and large gut tissue homogenates were used to detect IL-17 and IFNγ by Western-blotting. Gut sections were stained with hematoxylin/eosin to evaluate histological alterations. Results. Intestine IL-17 showed two molecular forms in the blots; 25KDa (glycosylated form) and 18KDa (non-glycosylated form). Both forms decreased 50% and 80% respectively compared with control group. These changes were evident in both early fibrosis and established cirrhosis. IFNγ decreased 65% compared to control group in the cirrhotic stage. Histological analyses showed hyperplasia of Peyer's patches, epithelial inflammatory infiltrate and mucus hypersecretion.
Conclusion. Gut bacterial overgrowth is associated with bacterial translocation process, which is closely linked to the dramatic decrease of IL-17 and IFNγ in the gut of rats with experimental fibrosis. These cytokines orchestrates antiparasitic important events at intestinal mucosal level and also activation of macrophages and neutrophil recruitment.

P5.02.02
Experimenrtal cholestasis is associated with increased IL-17 and IFN-γ expression

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Background. IL-17 play a central role in the pathogenesis of liver fibrosis through production of type-I collagen in hepatic stellate cells. However, IL-17 and RORγ have been little explored in experimental fibrosis by cholestasis. Also, the classical Th1 cytokine IFN-γ is poorly known in this model.
Aim. To analyze the expression of IL-17 and IFN-γ∀ and the transcriptional factors RORγ and T-bet in liver of rats with experimental fibrosis by bile duct ligation (BDL).
Methods. Liver fibrosis was induced by BDL. Fibrotic animals were sacrificed after 8 and 30 days. qRT-PCR for RORγ and T-bet was achieved with total RNA. Liver homogenates were used to detect IL-17 and IFN-γ by Western-blotting. Liver sections were stained with hematoxylin/eosin and Masson trichrome to verify chronic injury and collagen deposition
Results. At protein level, IL-17 and IFN-γ showed important changes during progression of liver injury by BDL. A significant increase was observed accordingly to the fibrosis intensity when compared with control group. Interestingly, RORγ and T-bet gene expresion was significantly increased only at 8 days after BDL. Histological analyses showed characteristic alterations of the cholestatic process, such as inflammation, bile duct proliferation and collagen deposition.
Conclusions. Our results suggest that IL-17 and IFN-γ are involved in the cholestasis-induced liver fibrosis pathogenesis. Additionally, Th1 and Th17 cells appear to be important at the beggining of the liver fibrosis, at least under our experimental model. Finally, other sources of IL-17 e IFNγ, such as NKT and NK cells remain to be explored in liver fibrosis.

P5.02.03
ITF2357 inhibits corneal fibrosis and inflammation via JAK/STAT3 signaling pathway

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Purpose: In this study, we investigated the mechanism of action of a histone deacetylase inhibitor, ITF2357 on fibrosis and inflammation in a corneal wound healing model.
Methods: In vivo experiments were conducted with 20 rabbits that underwent -9.0D photorefractive keratectomy (PRK) surgery in one eye and were divided into 3 groups based on post-op treatment with a single dose of saline (3 days), ITF2357 (0.02% for 3 days) or MMC (0.02% for 60 sec post-surgery). Post-op clinical examination was made for 4 weeks using slit lamp microscopy and in vivo confocal microscopy (IVCM). In vitro experiments were performed with cultured primary human corneal fibroblasts (pHCFs) to study the ITF2357-induced activation of IL-10 and JAK/STAT signaling pathway using specific signaling inhibitors by immunocytochemistry, western blot and ELISA.
Results: ITF2357 is non-toxic and effective in nM concentrations to significantly reduce corneal haze and extracellular matrix formation on IVCM and immunohistochemitry. IL-10 expression was up-regulated in the ITF2357 treated PRK corneas compared to the MMC treated. Cultured pHCFs with ITF2357 produced elevated levels of IL-10 in a time-dependent manner. This in turn, resulted in the activation of pSTAT3 and downstream signaling with overexpression of SOCS3 expression. Inhibition of recruitment of STAT3 to the receptor complex with a specific inhibitor blocked the phosphorylation of STAT3, preventing its nuclear entry and hence decreased IL-10 production.
Conclusions:ITF2357 upregultes IL-10 levels via activation of JAK/STAT signaling pathway to inhibit fibrotic and inflammatory activity in corneal disease model

P5.02.04
Osteopenia and inflammation in postmenopausal women

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Osteoporosis is a condition characterized by low bone mass and increased bone fragility, putting patients at risk of fractures. Osteoporosis is currently attributed to various endocrine, metabolic and mechanical factors. However, emerging clinical and molecular evidences suggest that inflammation also exerts significant influence on bone turnover, inducing osteoporosis. A shift towards an activated immune profile has been hypothesized as important risk factor, even in the absence of a manifest autoimmune, rheumatic or inflammatory disease. Subclinical chronic inflammation may be a determinant pathogenetic factor. The purpose of this study was to examine the relationship between bone mineral density (BMD), evaluated by calcanear ultrasonography and/or dual x-ray absorptiometry, expressed as T-score, and serological markers of inflammation, such as C reactive protein (CRP), erythrocyte sedimentation rate (ESR), autoantibodies (rheumatoid factor-RF, antinuclear antibodies-ANA, anti-thyroid peroxydase-TPO, and vitamin D levels, in 551 postmenopausal women (mean age 64) without overt diseases. The study sample included 101women with T-score into the normal range and 240 with osteopenia/osteoporosis), None of them were taking osteoporosis inducing drugs. CRP and ESR mean levels were significantly higher in osteopenic/osteoporotic compared to control women (9 and 32 vs 5 and 19 respectively). Serum autoantibodies were present in 27% of women with T-score<-1,5 and 23% of controls. Vitamin D levels in postmenopausal women were lower than controls (21 and 26 respectively). These data support the view that inflammation significantly contributes to the aetiopathogenesis of osteoporosis.

P5.02.05
Type 2 Innate Lymphoid Cells (ILC2) are present in mouse models of lung and skin fibrosis and their numbers correlate with disease severity

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Fibrosis is a physiological tissue repair process that becomes unchecked in pathological conditions such as scleroderma and idiopathic pulmonary fibrosis (IPF), leading to overproduction of extracellular matrix (ECM). The T_H2 cytokine, IL-13, is a key fibrogenic effector mediating its effects by inducing and activating TGFβ. IL-13 can also directly stimulate fibroblasts, epithelial cells and smooth muscle cells, promoting fibrosis. Recently, several groups have demonstrated that type 2 innate lymphoid cells (ILC2) secrete IL-13 and regulate allergic airway inflammation as well as lung homeostasis. Since IL-13 plays an important role in fibrosis, we hypothesized that ILC2 cells contribute to this disease as well. Here we show that ILC2 cells were present in the lung during the fibrotic phase but not the inflammatory phase of the murine bleomycin model of lung fibrosis. Moreover, there was a 3 fold increase in ILC2 numbers in lungs of bleomycin treated mice as compared to PBS treated mice. ILC2 numbers strongly correlated with the levels of hydroxyproline in the lung. When cultured *in vitro*, ILC2s expressed the IL-33R (T1/ST2) and IL-13. Interestingly, ILC2 conditioned media induced expression of fibronectin and the myofibroblast marker, smooth muscle actin (SMA), in cultured murine fibroblasts suggesting that ILC2s can directly influence fibroblast function. Finally, ILC2 cells were also detected in the skin and their numbers significantly increased during the fibrotic phase in a murine graft-versus-host disease model of skin fibrosis. These data pave the way for further characterization of ILC2 and elucidation of their function in fibrosis.

P5.02.06
Prevalence of skin sensitivity to aeroallergens in Iranian patients with nasal polyposis

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Background: Sinonasal polyposis (SNP) is a chronic inflammation with unknown origin. There are some evidences about the role of allergy in pathogenesis of nasal polyposis although there is some controversy surrounding this hypothesis. The aim of this study was to assess the prevalence of allergy to common regional aeroallergens among a group of Iranian patients with SNPs with or without asthma.
Methods: Diagnosis of nasal polyposis was done based on physical examination as well as CT scan. Skin prick tests were performed with a battery of twenty common regional aeroallergens including moulds, mites, cockroach, fungus, grasses and trees in ninety three patients with SNPs. All patients were evaluated for the presence of asthma and aspirin sensitivity based on history and physical examination.
Results: Ninety percent of patients showed asthma-related symptoms. None of the patients had aspirin sensitivity. Prevalence of skin sensitivity to one or more allergen was 32% in SNP without asthma SNP patients with asthma showed higher rate of skin sensitivity (32% vs. 40%, P<0.05). Grasses, mites and fungus were the most common allergens (32%, 16 % and 13%, respectively) in both SNP without asthma and SNP with asthma.
Conclusion: The results of this study suggest that allergy to common aeroallergen is fairly high among patients with SNPs, especially in patients with SNPs and asthma and allergy to aeroallergens may contribute to nasal polyposis pathogenesis.

P5.02.07
Crucial role of basal p53 in attenuation of inflammation and fibrosis in hereditary glomerular nephritis, Alport syndrome

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Alport Syndrome (AS) is one of the hereditary progressive kidney diseases. AS is caused by mutations in Col4a3, 4 or 5 genes that compose the glomerular basement membrane (GBM). Aberrant GBM structure results in podocyte detachment from GBM and proteinuria leakage. Persistent proteinuria leakage induces chronic inflammation and fibrosis of kidney. Continuous exposure to inflammation and fibrosis-inducing cytokines leads to development of kidney failure. We found that the protein expression of tumor suppressor gene p53 in the kidney is reduced as AS progresses. p53 is known to induce various effects such as cell cycle arrest, cell apoptosis, DNA repair and anti-inflammation by regulating functional genes expression. To determine the role of p53 in AS, we made p53 knock out (-/-) and heterozygote (+/-) AS mouse model and compared them with p53 wild type (+/+) AS mouse model. p53-/- and +/- AS mice show rapid progression of proteinuria and significantly lower rate of survival compared to p53+/+ AS mice. p53+/- AS mice also showed higher glomerular injury score than p53+/+ AS mice. Consistent with these results, inflammatory cytokine genes (IL-6, KC, IL-1β and TNF-α) expression and fibrotic region were significantly increased in p53+/- AS mice kidney than in p53+/+ AS mice. Furthermore, mRNA expression of BMP-7, which is important for renal anti- fibrosis, was lower in p53-deficient kidney and primary podocytes. These results suggest that basal p53 expression is essential for suppression of glomerular nephritis progression.

P5.02.08
Intramuscular administration of MMP-8 adenoviral vector reduces inflammation up-grade induced by vector delivery and allows prevention of liver fibrosis

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BACKGROUND. Multiple protocols regarding liver fibrosis gene therapy using adenoviral vectors systemically delivered, have been reported. Unfortunately, a decrease in the efficiency of liver transduction and increase in proinflammatory cytokines have been exhibited when utilizing these delivering strategies. AIM. To establish a model to deliver therapeutic genes, focused in the prevention of liver fibrosis without promoting an inflammatory process by intramuscular administration using an adenoviral vector containing MMP8 gene (AdMMP8). MATERIAL AND METHODS. Experimental liver fibrosis was induced in male Wistar rats by TTA administration for 7 weeks. Four groups were included: control (no fibrosis), TAA induced-cirrhosis (TAA), TAA+AdGFP (irrelevant gene) and TAA+AdMMP8 (therapeutic gene). At the fifth week of TAA intoxication, administration of vectors in soleum muscle was accomplished. Subgroups of rats (n=5) at first, second and third week after vector administration were sacrificed. Percentage of fibrosis, liver function, MMP8 gene expression, proinflammatory (IL1β, TNFα), profibrogenic (COLIA1, TGFβ) and antifibrogenic (MMP1, MMP9) genes were determined. RESULTS. Liver and serum expression of MMP8 protein was sustained, fibrosis decreased up to 48%, proinflammatory genes expression was not modified, profibrogenic gene expression decreased, antifibrogenic genes expression increased, reduction of liver function was not statistically significant. According to Knodell score, intramuscular administration of AdMMP8, shows a diminishment of inflammatory cells infiltration in comparison with animals treated with AdGFP. CONCLUSION. AdMMP8 delivered in muscle did not generate liver inflammation in fibrotic animals, MMP8 gene was expressed in muscle with satisfactory functions in the liver.

P5.02.09
Mac-3 positive and enlarged foamy macrophage as an effector in radiation-induced pulmonary fibrosis

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Thoracic radiation therapy is used to treat lung, breast, and lymphoid cancers. However, radiation-induced pneumonitis and pulmonary fibrosis are common dose-limiting complications in the radiotherapy. In this study, we investigated the characteristics of effective immune cells that related to pneumonitis and fibrosis after irradiation. After anesthesia, the whole thorax of C57BL/6 mice was irradiated at 14 Gy. As expected, the deposition of collagen was increased in the lung of irradiated mice. At 4 months after irradiation, the numbers of macrophages and lymphocytes as well as neutrophils were increased dramatically in the lung. Interestingly, the macrophages recruited post-irradiation had enlarged foamy morphology. In addition, the expressions of chemokines (CCL-2, CCL-3, CXCL-10 and CXCL1) for attraction of macrophages, T cells, and neutrophils, were higher in the lung of irradiated mice. The high expressions of these chemokines except CXCL1were sustained up to 6 months following irradiation. Next, we found that the ratio of Mac-3 positive macrophages was increased in the lung after irradiation. Interestingly, the expressions of Arg-1 and MMR, as the hallmark of alternatively activated macrophages, were increased in the lung of irradiated mice and the levels of IL-4 and IL-13 were higher in BALF of irradiated mice. Taken together, we reached the conclusion that thoracic irradiation might lead to pulmonary fibrosis at least partially through increasing the influx of Mac-3 positive and enlarged foamy macrophages, i.e. alternatively activated macrophages.

P5.02.10
Mild electrical stimulation with heat shock ameliorates nephritis and proteinuria in mouse model of X-linked alport syndrome

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Alport Syndrome (AS) is a hereditary nephritis caused by defects in the genes encoding type IV collagen (COL4A3, COL4A4, COL4A5) in the glomerular basement membrane. Previously, we showed that the combination treatment of mild electrical stimulation (MES) and heat shock (HS) suppressed pro-inflammatory cytokine expression in mouse model of hepatic ischemia/reperfusion (I/R) injury. Here we evaluated the effect of MES+HS on progressive nephritis and renal injury in mouse model of X-linked AS. Six- to seven-week-old male X-linked AS mice were treated with MES+HS for 10 min twice a week. As a result, MES+HS improved histopathologic abnormalities such as glomerular atrophy and widespread infiltration of inflammatory cells. The suppression of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β, KC), pro-fibrotic gene (TGF-β1), and kidney injury marker (Lcn-2) were observed in kidney of MES+HS-treated mice. Moreover, MES+HS ameliorated progressive proteinuria and serum creatinine in AS mice. Next, to determine the mechanism of how MES+HS improves various patho-phenotypes in AS mice, we analyzed the effect of MES+HS in vitro and ex vivo. The anti-inflammatory effect of MES+HS was mediated by glomerular activation of JNK1/2 and p38-dependent pathways ex vivo. Interestingly, the anti-proteinuric effect of MES+HS treatment was mediated by podocytic activation of PI3K-Akt and HSP72-dependent pathways in vitro and in vivo. Collectively, our studies show that combination MES+HS ameliorates progressive nephritis and proteinuria through the activation of multiple signaling pathways including PI3K-Akt, Hsp72, JNK1/2, and p38 pathways, suggesting a novel therapeutic strategy for the treatment of inflammatory disease like AS.

P5.02.11
Interleukin-17 isoforms are differentially expressed in the skin of Systemic Sclerosis and Morphea patients compared to healthy skin

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Interleukin (IL)-17A, a proinflammatory cytokine, is increased in biological fluids and skin of systemic sclerosis (SSc) individuals. Our aim was to investigate whether IL-17 family members other than IL-17A were expressed in the skin of SSc and Morphea patients compared to healthy donors (HD).
Skin biopsies were obtained from the involved skin of 14 SSc and 5 Morphea and from 8 HD undergoing plastic surgery. We set up immunohistochemistry and immunofluorescence techniques to identify the presence of IL-17A+, IL-17C+, IL-17F+ and IL-17E/IL-25+ cells, and we developed an application of the Metamorph/MetaXpress software to quantify the number of positive cells in the tissue.
Positive cells for each of the IL-17 isoforms were present in the dermis of all the individuals tested, though with variable frequencies. IL-17A+ cells were distinctly more numerous in SSc than Morphea or HD. IL-17C+ cells were less abundant in SSc and Morphea than in HD. Similarly, IL-17F+ cells were less abundant in SSc than in HD. Finally, IL-17E/IL-25+ cells tended to be more numerous in SSc and Morphea than in HD. Single cells could be positive for one or more of the isoforms, with no preferential association, nor correlation.
Concordant low IL-17C and high IL-17E/IL-25 frequency of positive cells specifically characterizes both SSc and Morphea when compared to HD. This particular combination of IL-17 family members may participate to enhanced extracellular matrix accumulation, thus playing a role in the development of fibrosis. Functional studies are needed to provide support to this hypothesis.

P5.02.12
IL-33 precursor drives inflammation without engaging T1/ST2 or Th2

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Interstitial lung disease, a combination of chronic pulmonary inflammation and excessive scarring, is debilitating and deadly, particularly in patients with systemic sclerosis (SSc) or idiopathic pulmonary fibrosis (IPF). Immunohistochemical analyses of lung sections from five patients with SSc and nine patients with IPF revealed a marked increase in the numbers of IL-33-expressing cells compared with four healthy controls. Much of the elevated IL-33 was intracellular, and there were minimal signs of Th2 activation, suggesting that precursor IL-33 (PRIL33), rather than mature IL-33 cytokine (MIL33), was increased. Western blot assays of lung tissue homogenates confirmed minimal maturation of PRIL33 into MIL33. Analysis of the subcellular distribution of overexpressed IL-33 forms in normal primary fibroblast cell cultures revealed that PRIL33 is localized almost exclusively (>95%) in the cell nucleus and is not secreted from the cells, whereas MIL33 is predominantly cytoplasmic and secreted. Recombinant adenoviral gene delivery of PRIL33 and MIL33 to mouse lungs resulted in strikingly different phenotypes: PRIL33 induced pulmonary lymphocytosis and neutrophilia, whereas MIL33 induced eosinophilia, goblet cell hyperplasia, and increases in IL-4, IL-5, IL-13, and IL-17. Germline deficiency of the MIL33 receptor T1/ST2 abrogated the effects of MIL33 gene delivery but not the effects of PRIL33 delivery. Gene delivery of MIL33 in cell culture and in vivo caused elevation of the expression of CCL2, IL-6, MMP3, MMP10, MMP13, and HSP70 mRNAs and proteins, which are all known to contribute to inflammation and scarring. Thus, the IL-33 precursor can drive inflammation by remaining intranuclear and regulating gene expression.

P5.02.13
5-methyl-1-phenyl-2-(1H)-pyridone reveals immunomodulatory anti-fibrotic effects associated to antioxidant system activation in primary culture of human Hepatic Stellate Cells

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Background. Hepatic stellate cells (HSC) profibrogenic cytokines are key targets of anti-fibrotic therapies. 5-methyl-1-phenyl-2-(1H)-pyridone or pirfenidone (PFD) is a small molecule indicated for treatment of chronic inflammation and fibrogenesis. Oxidative stress is directly involved in the onset of hepatic fibrosis by HSC activation. Aim. In order to identify whether anti-inflammatory and anti-fibrogenic effects of PFD are related to activation of the endogenus antioxidant system, HSC were incubated with PDGF or 2-methyl-1,4-naphthoquinone (MEN) a ROS-inducer. Methods and Results. PFD was able to inhibit PDGF or MEN-induced pro-fibrogenic actions, including cell proliferation, cell motility and *de novo* synthesis of Collagen type I, TGFβ, TIMP-1, IL-1 and TNFα. These effects were associated with an increase of nuclear Nrf2 assessed by western blotting and confocal microscopy. Because PFD activates JNK, which stimulates Nrf2 transcriptional factor, through siRNA-mediated silencing we examined downstream antioxidant targets as antioxidant enzimes. JNK blockade by siRNA and SP600125 down-regulates Nrf2 activation. Also PFD induced a dose- and time-dependent activation of several antioxidant genes, (Glutamyl cysteine synthetase catalytic subunit, Glutamyl cysteine synthetase regulatory subunit and Heme oxygenase 1) and increase glutathione content, whose activity may contribute to the down-regulation of ROS-induced pro-fibrogenic and pro-inflammatory effects. Conclusion. These results provide molecular insights in anti-fibrogenic immunmodulatory action of PFD by counteracting ROS-induced pro-fibrogenic signalling, and by regulation of the biosynthesis of antioxidant proteins. This study indicates that activation of the antioxidant system plays an essential role in the modulation of inflamatory and fibrogenic cytokines in HSC.

P5.02.14
Phenotypical characterization of the peripheral blood T cells in celiac disease patients

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Background and objective: Celiac disease (CD) is a small intestine enteropathy caused by permanent wheat gluten intolerance. One of the earliest signs of mucosal immune activation in CD is an increase in the intestinal intraepithelial lymphocytes (iIELs) count in the small intestinal epithelium. Though most of those iIELs express αβTCR, CD is characterized by an increase in $\gamma\delta TCR^+$ iIELs. The present study aimed to establish whether these immunological changes seen in the intestinal epithelium of CD patients could also be detected in the peripheral blood lymphocyte populations with special emphasis on the $\gamma\delta TCR^+$ T cells. Methods: Peripheral blood T cells were analyzed by two color flow cytometry in 12 untreated patients with CD and 16 healthy controls. Results: There were significant differences between the mean percentages of $\gamma\delta TCR^+$ T cells in the patients and the controls (7.5 ± 3.7% in the patients versus 4.9 ± 2.5% in the controls, $P < 0.05$). However, the mean percentages of the $\alpha\beta TCR^+$ T cells were significantly lowered in the untreated patients (90 ± 3.5% in the patients versus 93.3 ± 2.8% in the controls, $P < 0.05$). There were no significant difference between the mean percentages of T cells expressing the CD4 and CD8 molecules in the patients and the controls. Conclusions: The change in the peripheral blood T cells expressing the γδTCR and αβTCR markers in the celiac patients is, therefore, a consequence of an ongoing immunological process.

P5.02.15
GSK-3: a key mediator of the balance between matrix-metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in bleomycin (BLM) - induced pulmonary fibrosis.

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Background: Airway remodelling occurs during lung fibrosis and an imbalance between MMPs and TIMPs could be involved in the aberrant tissue repair leading to lung function impairment. We previously demonstrated the anti-inflammatory and anti-fibrotic effects of the pharmacologic inhibition of the kinase GSK-3 in a mouse model of BLM-induced pulmonary fibrosis.
Aims of the study: We want to assess the trend of the expression of MMP-2, MMP-9, TIMP-1 and TIMP-2, in the inflammatory and fibrotic phases of our BLM mouse model. Next, we want to study whether *in vivo* inhibition of GSK-3 with SB216763 could modulate MMP activity and their balance with TIMPs.
Materials and Methods: MMP activity was assessed by zymographic analysis in supernatant of BALFs and in lung homogenates. MMP and TIMP expression levels were evaluated by Real Time PCR and Western Blotting in BALF cells and in lung homogenates. Their tissue localization was evaluated by immunostaining analysis.
Results and Conclusions: We found strong activity of MMP-9 and -2 in BALFs during the inflammatory response. In particular, interstitial alveolar macrophages (IAMs) showed very high expression levels of MMP-9 and -2 and of their TIMPs while, in lung parenchyma, only epithelial injured cells were found positive for these markers. During fibrotic phase, we found very high positivity for MMP-2 in lung parenchyma. Importantly, SB216763 down-modulated MMP-9 and -2 expression in inflammatory and epithelial cells during inflammation and fibrosis, suggesting that GSK3 inhibition could play an important role in restoring the extracellular matrix turnover altered in response to lung injury.

P5.02.16
Genes affecting MMP expression are down-regulated in patients with COPD and bronchial asthma

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Proteolytic enzymes such as matrix metalloproteinases (MMPs) and others are up-regulated in chronic obstructive pulmonary disease (COPD) and bronchial asthma (BA). Their exceeding expression and proteolytic activity could result from insufficient inhibition and deregulated mRNA stability. We, therefore, investigated two inhibitors of MMPs (RECK and PTEN) and also RNA-binding proteins affecting mRNA stability (AUF1, HuR and PCBP2) in the both diseases. Real time-PCR method with PSMB2 as housekeeping gene was used to quantify the relative expression of RECK, PTEN, AUF1, HuR and PCBP2 in bronchoalveolar cells from 22 control subjects, 30 COPD patients and 19 patients with BA. The numbers of RECK (p=0.004, p=0.03)and AUF1 (p=0.01, p=0.02) mRNA transcripts were lower in both groups of COPD and BA patients than those in healthy control subjects. In comparison to controls, mRNA expression of HuR was decreased in COPD patients (p=0.03). There was a positive correlation between RECK expression and AUF1 (p<0.001), PCBP2 (p<0.001) and HuR (p=0.02), respectively. Overall, expression of the investigated genes did not differ between COPD patients and those with BA (p>0.05), smoking status was reflected in our analyses. In conclusion, the down-regulation of RECK observed in this study may contribute to protease/anti-protease imbalance in COPD and BA. As this has been the first investigation of RNA-binding proteins in these inflammatory diseases, further studies are needed to clarify the involvement of these molecules in COPD and/or BA pathomechanisms. Grant support IGA PU LF 2013_009 and CZ.1.05./2.1.00/01.0030

P5.02.17
Altered response of tympanosclerotic fibroblasts to interacting mast cells: implication for tissue remodeling

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Background: Inflammation is increasingly recognized as an important factor in development of tympanosclerosis, which finally results in hearing loss. The exact pathomechanisms of tympanosclerosis (TS) is not yet resolved, but the typical pathologic route of TS proceeds through destruction of the connective tissue, fibroblast invasion, and calcification. Thus, the objective of our study was to investigate the response of fibroblasts to mast cells interaction.
Methods: Experiments were performed on primary fibroblasts and human mast cells (HMC-1) co-cultured at 1:1 ratio. Fibroblasts were isolated from tympanic membranes (TM) of patients with tympanosclerosis and from morphologically normal TM of persons died of sudden cause (control). HMC-1 cells were induced with NGF (20 ng/ml) to express the FcepsilonR1alfa and stimulated by crosslinking with antibody to FcepsilonR1alfa.
Results: Addition of stimulated HMC-1 cells to fibroblast culture resulted in an increase of TNF-alfa, IL-6, bFGF, and MMP-9 levels in cell culture media, but this changes were ~2-fold higher in culture of fibroblast from TS patients. No changes in TGF-beta were evident. We observed that the level of collagen types 1-4 expression in control fibroblasts were not affected by HMC-1 cells, whereas in fibroblast from TS patients 2-fold and 4-fold higher level of COL2A1 and COL4A1 transcripts were observed, respectively. The changes in transcript levels correlated with the proteins content.
Conclusion: The sensitivity of fibroblast isolated from TM of patients with tympanosclerosis to stimulation by interacting mast cells is significantly increased. Moreover, altered interaction of fibroblasts and mast cells might greatly contribute to pathomechanisms of tympanosclerosis.

P5.02.18
The crosstalk of kinin receptors on Experimental Focal and Segmental Glomerulosclerosis

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Focal and Segmental Glomerulosclerosis (FSGS) is one of the most important causes of chronic renal disease. Experimental FSGS models consist in reproduce in rodents the main signals of the human disease, like podocyte foot process effacement associated with albuminuria. New targets of the disease have been studied in recent literature, like kinin receptor 1 (B1RBK). On the other hand the role of the second kinin receptor, B2RBK has showed no data. The role of both receptors has been studied in also other renal diseases. The lack of effective treatments for FSGS in clinic encourages the search for new targets of treatment; we propose to evaluate the role of B2RBK, a receptor deeply involved with inflammatory disorders associated with kidney diseases.
We induce FSGS in mice by a single injection of Adriamycin (ADM) and treat them with specific kinin receptors antagonists in order to evaluate FSGS progression.
We observed that treatment with HOE-140 (B2RBK antagonist) protected mice from FSGS, diminishing albuminuria, renal fibrotic and pro-inflammatory proteins and mRNA expression and prevented podocyte damage. On the other hand B2KO mice induced FSGS showed a worse prognosis associated with higher proteinuria and podocyte damage. In order to solve this possible contradictory result we treated B2KO mice with DALBK (B1RBK antagonist), that surprisingly revert most ADM signs of the disease. We observed that differently from WT, the B2KO mice presented up regulation of B1RBK, showing a possible crosstalk of kinin receptors in FSGS.

P5.02.19
M2 muscarinic acetylcholine receptor modulates rat airway smooth muscle cell Proliferation

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Airways chronic inflammatory conditions in asthma and COPD are characterized by tissue remodeling, being smooth muscle hyperplasia, the most important feature. Non-neuronal and neuronal acetylcholine acting on muscarinic receptors (MAChRs) has been postulated as determinant of tissue remodeling in asthma and COPD by promoting proliferation and phenotypic changes of airway smooth muscle-cells (ASMC). The objective was to evaluate proliferative responses to muscarinic agonist as carbamylcholine (Cch) and to identify the MAchR subtype involved. ASMC were isolated from tracheal fragments of Sprague-Dawley rats by enzymatic digestion. Proliferation assays were performed by MTS-PMS method. Viability was confirmed by trypan blue exclusion method. Mitogens, as epidermal growth factor (EGF), tumor necrosis factor-alpha (TNF-α) and fetal bovine serum (FBS) increased ASMC proliferation (p<0.05, n=5). Cch alone increased ASMC proliferation at 24 and 48 hrs. However, combination of Cch with other mitogens exhibited a dual effect, a synergistic proliferative effect in the presence of EGF (5ng/mL) and 5% FBS, and an inhibitory effect induced by 10% FBS, EGF (10ng/mL) and TNF-α (10ng/mL). To determine the MAChR subtype involved in these biological responses, a titration curve of selective muscarinic antagonists were performed. The Cch stimulatory and inhibitory effects on ASCM proliferation was blocked by AF-DX-116 (M2AChR selective antagonist), in greater proportion than 4-DAMP (M3AChR selective antagonist), suggesting that the modulation of muscarinic agonist-induced proliferation is mediated by M2AChR. Thus, M2AChR can activate multiple signal transduction systems and mediate both effects on ASMC proliferation depending on the plethora and variable airway microenvironments existing in asthma and COPD.

P5.02.20
Periostin modulates the course of IL-13 dependent chronic inflammation and liver fibrosis

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Chronic progressive liver fibrosis (cirrhosis) and post cirrhotic complications are a leading cause of morbidity and mortality worldwide. Periostin is a matricellular protein that has been studied in the context of several chronic inflammatory, fibrotic and neoplastic diseases. Periostin has shown potential as a surrogate for disease severity and study stratification and even been suggested as a therapeutic target in some of these conditions. We used a murine model of Th2-mediated inflammation induced by the helminth, Schistosoma mansoni, to explore the regulation and function of periostin during liver fibrosis. In wild type mice, serum periostin levels increased between 9 to 24 wks post infection — but this increase was abrogated in mice lacking the functional IL-13 receptor (IL-13Ra1), suggesting a requirement for IL-13 signaling. Correspondingly, in mice engineered to mount an uncontrolled IL-13 response, significantly increased periostin expression was detected in the liver, correlating well with the degree of fibrosis and procollagen VI expression. Microscopically, periostin was abundantly found within the fibrotic granuloma, radiating to surrounding parenchyma. In mice deficient in periostin, we found that the granuloma formation around S. mansoni eggs was unimpaired, but liver fibrosis was significantly increased compared to littermate controls. In vitro, macrophage response to IL-13 was diminished in the presence of recombinant periostin. Our data show that periostin is induced by IL-13 in the liver, where it can serve as a robust biomarker for fibrosis and is also capable of modulating IL-13 activity in a feedback regulatory loop.

P5.02.21
In vivo multi-photon molecular imaging technique visualizes immune and inflammatory cell cross-talks in metabolic diseases

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To elucidate the underlying mechanisms of adult common diseases based on chronic inflammation, it is vital to examine the immune and inflammatory cell kinetics in living animals. Therefore, we developed in vivo imaging technique based on single- and multi-photon microscopy, and we assessed dynamic cellular interplay in diseased conditions.
We found that large numbers of CD8+ effector T cells infiltrated into obese adipose, and these cells were essential for the initiation and development of adipose inflammation and fibrotic reponses. In addition, we identified a novel B cell subset that is abundant in adipose tissue. Immunological or genetic B cell depletion, and B cell-specific Il10 deletion enhanced CD8+ T cell activation and adipose inflammation, and exacerbated insulin resistance in obesity. Adipose environmental factors supported B cell survival and IL-10 production. Adipose B cells also exhibited unique surface phenotypes, distinct from those of known regulatory B cell subsets. Our findings indicate that adipose B cells are a naturally occurring regulatory B cell subset that is essential for negative regulation of diet-induced adipose inflammation and maintenance of homeostasis within adipose tissue, and that B cell dysfunction pivotally contributes to the progression of inflammatory and fibrotic processes.
Our results also clearly demonstrated the power of our imaging technique to analyze complex cellular interplays in inflammatory diseases, especially parenchymal and stromal cell cross talks, and to evaluate new therapeutic interventions against them.

P5.02.22
Metformin modulates lipolysis and inflammation through the modulation of T cell priming in obese-induced Type II diabetic mice

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Inflammation is regarded as a key process underlying metabolic diseases in obese individuals where adipose tissue shows features characteristic of active local inflammation. Metformin is used for treating of T2D and have been suggested that metformin has anti-inflammatory properties. This study investigated the therapeutic effect of metformin on diabetic symptoms in DIO mice. Here we show metformin attenuates metabolic disorder in DIO mice and suppresses MHC-restricted antigen presentation in DCs. Metformin decreased aipogenesis related genes, FAS, SCD-1, GPAT, SREBP1a, and lipases, LPL, ATGL, HSL, but also suppressed innate cytokines expression through APPL1-AMPK-sirt1 signaling. Metformin decreased both class I- and class II-restricted presentation of exogenous OVA in DCs. Metformin also decreased expression of the co-stimulatory molecules, ICAM-1 and B7-1/-2, and MHC class I and II molecules but not phagocytic activity. Metformin decreased MHC class II-restricted exogenous antigen presentation in peritoneal macrophages *in vivo*. These results show that metformin attenuates adipogenesis and suppresses immune responses through APPL1-AMPK-sirt1-NF-κB signaling and followed by decreases MHC-restricted presentation of exogenous antigen. Theses finding suggests that modulation of the antigen presentation pathway by metformin could provide a novel means for regulating T cell responses in terms of controlling of inflammation in metabolic diseases.

P5.02.23
The role of surfactant protein D in cigarette smoke-induced pulmonary pathology

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Background: Surfactant protein D (SP-D) is an innate immune factor involved in clearance of microbes and apoptotic host cells. SP-D deficient mice (*Sftpd-/-*) develop pulmonary emphysema-like pathology accompanied by increased numbers of apoptotic alveolar macrophages and phospholipid accumulation. Cigarette smoke (CS) is the main risk factor for development of emphysema.
Hypothesis: We hypothesize that genetic ablation of SP-D leads to increased lung inflammation and decreased lung function in response to CS exposure in mice.
Methods: C57BL/6N *Sftpd+/+* and *Sftpd-/-* littermate male mice where exposed to CS for 2x50 min/5 days/12 weeks and control mice received room air. Inflammatory cell counts were performed in bronchoalveolar lavage (BAL) and lung mechanics were performed with a computer-controlled respirator (FlexiVent, Scireq Inc). Chemokines and cytokines were measured by a multiplex immunoassay, and phospholipids were measured by mass spectrometry.
Results: Total cell count in BAL fluid showed significant induction in CS exposed Sftpd-/- mice relative to Sftpd+/+ mice. Alveolar macrophages were increased approximately 3-fold. BAL levels of macrophage inflammatory protein (MIP-1) were significantly induced, but there were no detectable *Sftpd* dependent changes in BAL cytokines, matrix metalloproteinases 2 and 9, phosphatidycholine, or dipalmitoylphosphatidylcholine. Respiratory resistance, but not compliance, was increased in mice exposed to CS-exposure and was independent of *Sftpd*.
Conclusion: SP-D is predominantly involved in pulmonary macrophage infiltration or removal after CS exposure. The increased level of alveolar macrophages did not affect the inflammatory profile in BAL indicating that the cells might be malfunctioning. CS induced increase of airway resistance appeared independent of SP-D.

P5.02.24

High-mobility group box 1, actively secreted by cardiac fibroblasts, might be a novel culprit of myocardial contractility dysfunction and cardiac collagen deposition

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High mobility group box 1 (HMGB1), a non-histone nuclear protein, is actively secreted by immune cells, cardiomyocytes, hepatocytes, pituicytes or passively released by necrotic cells. HMGB1 has been implicated in inflammatory diseases. The present work was to assess whether viable cardiac fibroblasts could secrete HMGB1 and whether HMGB1 could affect myocardial function. Primary cardiac fibroblasts were isolated from BALB/c mice and challenged by LPS; the HMGB1 levels of mRNA and protein significantly increased after 24h; active secretion of HMGB1 has been demonstrated through activation of PKCβ and Erk1/2 pathway; and after LPS challenge, the collagen type I, III (Col1/3) and osteopontin (OPN) expression were obviously increased. *In vivo*, no inflammatory cells infiltration was noted and ultrasound diagnosis showed that actively secreted HMGB1 contributed to myocardial contractility dysfunction and cardiac fibrosis. Therefore, cardiac fibroblasts, a non-immune cell type, could actively secrete HMGB1, which was associated with myocardial function.

P5.02.25

Knee injury with concomitant osteochondral fracture is associated with increased synovial fluid concentrations of SPARC, IL-8 and TNF-α

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Purpose: Approximately 50% of patients with an anterior cruciate ligament tear also sustain an osteochondral (OC) fracture in association with the injury. The aim of the present study was to investigate if concentrations of cartilage and bone markers and pro-inflammatory cytokines in synovial fluid (SF) collected from acutely injured knees were different between acutely injured knees with/without an OC fracture.
Methods: Synovial fluid with hemarthrosis was aspirated from acutely injured knees of 111 individuals (mean age 27 years, range 13-64 years, 22% women) in median 1 (range 0-23) day after the injury. Concentrations of sulphated glycosaminoglycan, cartilage ARGS, COMP osteocalcin, secreted protein acidic and rich in cysteine (SPARC), osteopontin and pro-inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) were analyzed. Ninety-eight of the 111 patients underwent MRI (1.5 Tesla) of the injured knee in median 8 (range 1-38) days after the injury.
Results: Thirty-eight of the acutely knee injured patients had an OC fracture with discontinuity of the cortical bone and 29 had an OC fracture without discontinuity of the cortical bone in the knee joint. Thirty-one patients did not sustain an OC fracture. Knees with an OC fracture with discontinuity of the cortical bone had significantly higher SF concentrations of SPARC ($p = 0.034$), IL-8 ($p = 0.030$) and TNF-α ($p=0.005$) compared to knees without an OC fracture after adjusting for days between injury and SF aspiration, age, sex and type of knee injury.
Conclusions: An associated OC fracture may cause a higher degree of joint inflammation after knee injury.

P5.02.26

Implication of antigen-driven mechanisms in the pathogenesis of interstitial pneumonia associated with polymyositis

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Objective: Polymyositis (PM) is a major CTD characterized by chronic inflammatory lesions of muscle and other organs, including critical pulmonary involvement. Interstitial lung diseases, mainly interstitial pneumonia (IP), have been recognized in 30% - 70% of PM patients and have a poor prognosis. While the presence of myositis-specific autoantibodies suggests an autoimmune etiology of PM, the pathogenesis of PM-associated IP remains unclear. The aim of this study was to elucidate the role of T cells in this pulmonary complication.
Methods: We took advantage of a rare opportunity to carefully study two cases of earliest-stage IP associated with PM by utilizing lung biopsy tissue. We characterized the phenotype of lung-infiltrating lymphocytes from lung biopsy specimens obatained by video-assisted thoracoscopy, and analyzed T-cell receptor (TCR) variable region repertoires of T-cells infiltrating the lung tissues using a validated adaptor ligation polymerase chain reaction (PCR)-based microplate hybridization assay, comparing these to peripheral blood lymphocytes (PBL).
Results: Both cases demonstrated substantial CD3+ T cell lung infiltrates. The usage of repertoires of TCR in the lung differed from those in PBL, with certain TCR V gene families detected more frequently in lung tissue, suggesting a pivotal role for T cells in the pathogenesis of IP associated with PM. This is the first robust demonstration of selective TCR repertoire usage and its differential expression in lung tissue versus PBL.
Conclusion: These findings strongly suggest a pathogenic contribution of organ-specific oligoclonal T cell accumulation through antigen-driven mechanisms, implying potential development of immunospecific treatments such as molecular-targeted therapies.

P5.02.27

Intrinsic epithelial activation in Primary Sjogren's syndrome: Evidence of significantly reduced constitutive expression of PPAR-gamma in cultured non-neoplastic salivary gland epithelial cell lines

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PPAR-gamma is an essential transcription factor that participates in the regulation of lipogenesis, whereas it also exerts significant anti-inflammatory actions. Primary Sjogren's syndrome (SS) is characterized by chronic inflammatory lesions of epithelial tissues and experimental evidence has indicated the occurrence of chronic intrinsic activation of salivary gland epithelial cells (SGEC). Thus, SGEC are probably both the target and the inducer of inflammatory responses. Here, we sought to investigate the constitutive expression of PPAR-g in cultured non neoplastic SGEC lines from SS patients and non-SS controls, as well as to define potential cellular activators that affect PPAR-g expression. The
constitutive PPAR-g mRNA (Real-time PCR) and protein expression (Western Blot) in the SGEC from patients with SS (n=19), was significantly reduced compared to controls (n=11) ($p=0.0001$). The activation of cultured SGEC by stimulation with PolyI:C, LPS, IFN-g and IL-1β resulted in significant down-regulation of PPAR-g mRNA expression (in all cases; by 80 -85% at 12 hours, $p<0.05$), whereas IL-4 caused a two-fold increase in basal PPAR-g expression levels. Our results indicate that PPAR-g expression in human SGEC is significantly reduced following activation via TLRs and the pro-inflammatory cytokines INF-g and IL-1β. Furthermore, the present study demonstrates for the first time the significantly reduced expression of PPAR-g in the SGEC of SS patients. This finding likely

owes to the chronic intrinsic activation, which characterizes the epithelia of SS patients.

P5.02.28
IL28B polymorphisms may be associated with Chlamydia pneumoniae infection in patients with cardiac disease

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The present study aimed to investigate whether three polymorphisms in the IL28B gene (rs12979860, rs8099917, rs8103142) are associated with the Chlamydia pneumoniae infection, as well as, with the outcome of cardiac disease. The blood samples were collected from 159 patients with chronic cardiac disturbs submitted to revascularization surgery and 300 healthy subjects (control group). All samples were previously tested for the presence of antibodies to C. pneumoniae using an enzyme immune assay. In order to identify the frequencies of the polymorphisms, DNA samples were extracted from PBMCs and analyzed by real time polymerase chain reaction (qPCR). There was a higher prevalence of mutant genotypes (rs12979860TT, rs8099917GG, rs8103142CC) among the control group than within patients with cardiac disease (p=0.0008, p=0.0073 and p= 0.0049, respectively). On the other hand, as both groups were classified according to the presence of antibodies to C. pneumoniae, a significantly higher prevalence of rs12979860CC, rs8099917TT and rs8103142TT genotypes were recorded among cardiac patients as compared to the infected control group (p=0.0011, p=0.0050 and p=0.0063). The polymorphism rs8099917 showed the presence of genotype GG only among seronegative controls (15,6%) as compared to the patient who were antibody positive to C. pneumoniae (p=0.0048). Distribution of genotype frequencies were not significant when both groups were negative to the presence of antibodies. The present results suggest that the IL28B polymorphisms investigated herein may be associated with the susceptibility to C. pneumoniae, as well as, with the possible outcome of cardiac disease, but further studies should confirm this preliminary finding.

P5.02.29
Accumulation of tolerogenic cells at the site of granulomatous foreign-body reaction

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The granulomatous foreign-body reaction (GFBR) is a special type of chronic inflammation characterised by infiltration of inflammatory cells (ICs). Using a model of subcutaneous implantation of polyvinyl sponges in rats, we studied the phenotypic and functional characteristics of these cells, with particular reference to the properties of antigen presenting cells and lymphocytes. ICs were isolated from sponge exudates at days 1-21 after implantation. Dendritic cells (DCs) and lymphocytes were purified using a combination of separation gradients, adherence to plastics, and immunomagnetic sorting. We showed that dominant population of ICs at day 1 and 3 were granulocytes, followed by their decrease thereafter. The number of mononuclear cells (MNCs), macrophages and DCs, progressively increased after day 3, reaching maximal values at day 7. Maximal number of lymphocytes was detected at day 10. In addition, total ICs isolated from day 7 till day 10 exerted significant suppressive activity in co-culture with autologous ConA-stimulated thymocytes and alogeneic lymph node T cells. This finding correlated with the increased level of IL-10 in culture supernatants and the increased proportion MHC class II+ IDO+ DCs and CD3+ CD25+ Foxp3+ lymphocytes at day 7 and day 10, respectively. We did not find any significant difference in the number of these cell populations between regional lymph nodes MNCs and peripheral blood MNCs of experimental animals compared to controls. In conclusion, accumulation of cells with tolerogenic characteristics in GFBR might be relevant for the suppression of inflammation and the prevention of unwanted immune response.

P5.02.30
EBP-1 specific T cell responses correlate with liver damage in chronic hepatitis B

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The immune mechanisms especially the autoreactivity that cause liver damage in chronic hepatitis B virus (HBV) infection are not completely understood. The presence of HBV-specific $CD8^+$ T cells does not correlate with liver damage, and liver pathology is deemed to be largely caused by the non-HBV-specific immune cells including bystander $CD8^+$ and $CD4^+$ T cells. Chronic HBV infections have been linked to autoimmune phenomena. Although autoantibodies are commonly detected in chronic HBV infection, autoreactive T-cell responses have not been characterized yet. By using serologic proteome analysis, ErbB-3-binding protein-1(EBP-1) was identified as a novel autoantigen in Chinese patients with chronic hepatitis B (CHB). The prevalence and levels of serum anti-EBP-1 autoantibodies, which were detected by ELISA, were closely associated with the clinical severity of liver disease in patients with chronic HBV infection. Notably, the EBP-1 autoreactive T cells producing high levels of interferon-γ and interleukin (IL)-17, but not IL-10 and IL-4, were obviously detectable in CHB patients but not healthy controls. In addition, enhanced EBP-1 specific T-cell responses significantly correlated with features of more severe liver diseases. The expression level of EBP-1 in HBV-infected hepatocytes correlated with the histological inflammation grade of liver tissue. Thus, we assume that the majority of bystander T cells in the inflamed liver tissue may be autoreactive T cells targeting the local upregulated autoantigens e.g. EBP-1, which contribute liver damage in chronic HBV infection.

P5.02.31
Human kidney proximal tubule epithelial cells (PTEC) modulate autologous immune responses by contact dependent mechanisms

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We have demonstrated that human kidney proximal tubule epithelial cells (PTEC) down modulate autologous human T and B cell proliferative responses and modulate the development and activation of autologous dendritic cells (Wilkinson et al NDT 2011, 2012). We have extended this research by analyzing PTEC effects on B cell antibody (Ab) production and culturing T cells and DC in the presence of autologous PTEC using contact dependent and independent culture systems in an effort to define the mechanisms of this modulation. B cells, T cells and DC were cultured with or without PTEC in the presence of various stimuli. The numbers of B cells producing Ab was analysed using isotype specific ELISPOT, whilst readouts for T cells and DC included proliferation, Ag expression and cytokine secretion. PTEC significantly reduced the number of B cells that produced Ab in response to Toll-like receptor stimulation. PTEC also down modulated T cell responses and this modulation was ablated when the cells were separated by a transwell membrane. Similarly, PTEC down modulation of DC differentiation was ablated when the DC and PTEC were separated by a transwell membrane resulting in mature MoDC development and low levels of IL-10 expression. These results demonstrate for the first time that human autologous PTEC can regulate B cell Ab production and that their modulatory effects on T cells and DC are, at least in part, contact dependent. We will elucidate these mechanisms further in an effort to identify novel clinical targets for therapy in renal disease.

P5.02.32
Systemic inflammation in patients with end-stage renal disease (ESRD) receiving different types of renal replacement therapy

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Objective. To assess the degree of chronic SI (CSI) in patients with ESRD receiving different types of renal replacement therapy.
The study included the following groups: patients with ESRD receiving program hemodialysis (12 hours per week, blood samples taken prior to dialysis session (n=42), renal transplant patients with normal allograft function (n=23), patients with chronic allograft dysfunction (CAD, n=24). The control group included 50 healthy people.
Chronic glomerulonephritis was the condition that resulted in the development of ESRD in all the patients. Blood plasma was tested for the following SI indicators: CRP, IL-6, IL-8, IL-10, TNFα, D-dimers, cortisol, myoglobin, troponin I. On the basis of these markers using an original method we calculated the integral index of intensity of chronic SI (from 0 to 8 points).
Results. CSI was diagnosed only in hemodialysis patients (90.9%) and in the CAD group (43.5%). The incidence of systemic inflammation reaction and tissue alteration was the highest (90.9% and 90.9%) in hemodialysis patients. Patients in the CAD group most often presented systemic inflammation reaction (91.3%) and distress of neuroendocrine system (56.5%). While elevated levels of proinflammatory cytokines and CRP (41.7%), distress of neuroendocrine system (29.2%), and microthrombosis (4.2%) were recorded in the normal allograft function group, on the whole, there were no cases of CSI.
Conclusion. CSI is a severe ESRD complication in hemodialysis patients. Following the renal transplantation CSI is found only in patients with CAD, proving that CSI is an additional link in the CAD pathogenesis.

P5.03 Genetics of autoimmunity

P5.03.01
Contribution of Lewis blood group types and their genes to genetic susceptibility and autoimmune response in Grave's disease

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Lewis blood groups antigens are structurally and genetically defined molecules. Their association with autoimmune diseases is increasingly reported. One hundred women patients having Grave's disease and a similar number of healthy controls were studied. The Lewis antigens typing was carried out on erythrocytes, while Lewis genes frequently were derived using Hardy-Weinberg Equilibrium Test.The results showed that Le(a) carrying phenotypes were significantly associated with Grave's disease. Among these phenotypes, Lea+b+ was detected in 47% patients compared to 9% in controls (Odds ratio 8.97,95% Cl 3.85-21.44, p=0.00000002).On the other hand, the genotype frequencies of Lewis (Le) genes showed a non-significant association with the disease. Importantly, secretor homozygous genotype (SeSe) was detected in 19% of patients versus 36% in control (OR 0.42, 95% Cl 0.21-0.83, Chi-square=7.248, P 0.007).Non-secretor homozygous genotype (sese) was seen among 32% versus 16% in control (OR 2.47, 95%Cl 1.19-5.17,Chi-square=7.018,P 0.008).Heterozygous (Sese) showed a non-significant differences. The presence of anti-O-Lewis antigens was studied, the results showed that Lewis (a) patients demonstrated a hem-agglutinating anti-O-Le (a) with a mean titer of 232+/-67.5 versus a mean titer of 17.25+/-70 of control, (p=0.007),furthermore those patients gave a mean anti-O-Le a +b+ titer of 84+/-17.10 versus 15.5+/-3.90 mean titer in control (P=0.002). Our findings provide evidence suggestive of an association of Lewis antigens and their genes in susceptibility to Grave's disease.

P5.03.02
Functional study of TNF-α gene promoter polymorphisms in Algerian patients with ankylosing spondylitis

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Objective: the aim of our study was to correlate the variability of TNF-α production to single nucleotid polymorphisms (SNPs) located at four positions: -238, -308, -857 and -1031 in the TNF gene.
Patients and Methods: Twenty patients with ankylosing spondylitis (AS) and twenty healthy subjects were included in this study. TNF polymorphisms (G/A-308, G/A-238) were genotyped by Life Codes Cytokine SSO Typing kit and TaqMan technology for TNFα C/T-857, T/C-1031 polymorphisms. TNF-α production after LPS stimulation was evaluated using a whole blood culture model and TNF-α levels were measured by multiplex immunoassay FIDIS cytokine.
Results: In healthy subjects, TNF-α production after LPS stimulation of whole blood cell culture was higher among -857 CC carriers than among -857 CT heterozygotes (169.126 pg /ml vs. 31.25 pg /ml with p = 0.04). This finding is consistent with that obtained in association studies, where this genotype was found protective (50% in patients and 87% in controls with $p=7.2\ 10^{-7}$). For polymorphisms -308G/A, -238 G/A and -1031T/C TNF-α, no statistically significant result was found. In AS patients, no association was observed for any of the four polymorphisms studied (-238 G/A, -308 G/A,-857C/T and -1031 T/C) and the rate of production of the cytokine. No significant difference was observed between controls and patients for different genotypes of the four polymorphisms. In conclusion, the SNP at position -857 in the TNF gene may influence TNF-α production in healthy individuals.

P5.03.03
Association of LGALS3 gene polymorphism and Rheumatoid arthritis in Zahedan, southeast Iran

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Introduction: Rheumatoid arthritis (RA) is a systemic, multi-factorial autoimmune disease characterized by chronic inflammation and destruction of joints. Enhanced infiltration of lymphocytes and macrophages can be observed in inflamed synovium. Up-regulation of pro-inflammatory cytokines is evident in the synovial fluids. In this study we aimed to evaluate the possible association of LGALS3 rs4652 gene polymorphism and rheumatoid arthritis (RA) in a sample of Iranian population.
Method: This case-control study was performed on 120 patients (104 female, 16 male) with rheumatoid arthritis with an average age of 44.6 ± 12.9 years fulfilling the American College of Rheumatology (ACR) criteria for RA. The control group consisted of 120 healthy individual (76 female, 44 male) with a mean age of 43.2 ± 10.3 years and unrelated to RA patients. Genomic DNA was extracted from whole blood and LGALS3 polymorphism were determined using tetra amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR).
Results: The results showed that LGALS3 AC genotype was a risk factor for susceptibility to RA (OR=11.622 95%CI=4.47-28.65, p=0.001).
Conclusion: In conclusion, we found an association between LGALS3 rs4652 polymorphism and the risk of RA in a sample of Iranian population. our data showed that LGALS3 (+292 A>C) polymorphism is a genetic factor for susceptibility to RA. In agreement with our findings, one study found a positive association between LGALS3 rs4652 (+292 A>C) polymorphism and susceptibility to RA.

P5.03.04
Molecular Analysis of Interleukin-17A Gene Polymorphisms in Patients with Multiple Sclerosis

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Introduction: Multiple sclerosis (MS) is a complex autoimmune disease that damages central nervous system. Both environmental and genetic factors are involved in pathogenesis of this disorder. Th17 cells and their related cytokines, most importantly IL-17, have crucial role in immunopathology of MS.
Methods: In this study, gene sequences of exons 2 and 3 of IL-17A gene were analyzed in forty individuals who suffered from MS disease and forty-four normal subjects as controls. Genomic DNA was obtained from all MS patients and controls. Two exons of the IL-17A gene and their flanking intronic sequences were amplified from the extracted genomic DNA samples. After genotyping by direct sequencing method, distribution of polymorphisms detected in the IL-17A gene.
Results: In exon 3, 25% of MS patients had 3566G>A, 7.5% had 3419A>T, and 7.5% had E125V SNPs. In normal controls, 40% had 3566G>A, and 2.5% had 3480A>G SNPs. There was a significant difference in frequency of the these SNPs in both MS patients and healthy controls in exon 3 of IL-17 gene (p = 0.041 <0.05). In Exon 2 : only 15% of MS patients had SNPs : 7.5% had 1509A>T, 2.5% had R29Q, 2.5% had 1429A>T, and 2.5% had 1587A>G SNPs. 85% of MS cases had not any SNPs in Exon 2. Normal group had not had any SNPs in exon 2. The difference in frequency of SNPs in exon 2 of MS patients and control group was not significant

P5.03.05
Role of the GULP gene in renalglomerular macrophage phagocytosis in the autoimmune disorder Systemic Lupus Erythromatosus (SLE) model mice MRL/LPR mesangial cells

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SLE is a major autoimmune disorder affecting a vast majority of people,very little is known about the etiology of the disease or any effective therapeutic advances have taken place.90% of the SLE patients die as a complication of renal failure.Several earlier reports have suggested that there is a defect in macrophage phagocytosis in SLE patients.In this study we investigated the role of the GULP gene in phagocytosis capability of the glomerular macrophage mesangial cells isolated from the SLE model MRL/LPR mice. The protein encoded by this gene is an adapter protein necessary for the engulfment of apoptotic cells by phagocytes.Our initial phagocytosis assays showed decreased phagocytosis by the SLE derived renal macrophage mesangial cells when compared to normal RAW 264.7 cells (a mouse leukaemic monocyte macrophage cell line).We analyzed the GULP gene expression in MRL/LPR mesangial cells in comparison to the normal macrophages, RAW cells by Real Time PCR and Western Blot.Our results showed both in the transcriptional level and the translational level there is a decreased expression of the GULP gene.We further transfected the MRL/LPR mesangial cells with GFP fused pCMV-GULP construct .We tested the GULP transgenic cells for increased GULP expression by Real Time PCR and GFP expression by fluroscence microscopy.The GULP transgenic MRL/LPR glomerular mesangial cells showed increased phagocytosis capabilities when compared with nontransfected cells.Thus, our results show a decreased phagocytosis in the diseased SLE model mouse glomerular mesangial cells and a role for the GULP gene in this process.

P5.03.06
HLA DRB1-genotype in families with T1DM patients

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We have previously shown in population studies that the high risk of T1DM was associated with HLA-DRB1 genotypes, including two any variants of the following: DRB1*01,*03,*04,*08,*09,*10 - «T1DM associated DRB1-genotype» (T1DAG). Now we examined 357 members of the families with type T1DM: 160 parents without T1DM, 117 children with T1DM and 80 children without signs of T1DM at the time of the study. 300 blood donors were the control group. HLA-DRB1 SSP typing at low resolution was done. The highest proportion of T1DAG was found in children with T1DM (72%), without gender differences. The proportion of T1DAG was 39% in children with no symptoms of T1DM at the time of the study. Differences in proportions T1DAG between T1DM children and their brothers and sisters, not sick at the time of the study were highly significant (p <0,0000). 40% mothers and 49% fathers have T1DAG, so they have the same proportion of T1DAG as their children without T1DM, but they have significantly lower proportion of T1DAG than that of their children with type 1 diabetes (p <0,01). The proportion of T1DAG in the control blood donors (13%) was significantly lower than in families with T1DM: in parents (p <0,000) and in children without T1DM (p <0,000) and in children with T1DM (p <0,000). More significant proportion of T1DAG in parents and children without T1DM compared to control, suggesting that for the implementation of HLA-induced genetic predisposition to disease, there must be additional, yet unidentified factors that require further investigation.

P5.03.07
Epigenetic regulation of HLA class II genes and their role in autoimmune diseases

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Background: Type 1 diabetes (T1D) is a multifactorial autoimmune disease. The highest T1D risk is associated with HLA (human leukocyte antigen) class II genes. HLA class II expression is controlled by regulatory module that is situated 150 - 300 base pairs upstream of the transcription initiation site in all HLA class II genes. There were identified several promoter alleles (named QAP) in the *HLA DQA1* gene promoter region. Most of the polymorphisms appear to be conserved within haplotype.
Aims: Our aim was to define methylation profile of *HLA DQA1* promoters and determine the mRNA expression of individual alleles of *HLA DQA1* gene in T1D patients.
Methods: 30 diabetic patients (age range 21 to 76 years), were included in this pilot study.
The genotyping of *HLA DRB1, HLA DQA1* and *HLA DQB1* was performed using PCR with sequence specific primers. Genomic DNA was treated with sodium bisulfite, promoter region of *HLA DQA1* gene was amplified and cloned into the *E. coli*, strain DH5α. Positive transformants were selected on agar plates containing ampicillin X-Gal, and IPTG. Positive transformation was confirmed by colony PCR. Sequencing of individual clones was performed.
Results: We found statistically significant differences in total QAP methylation and in methylation at nucleotide position -311. The most significant result in total QAP methylation was found between 2.1 and 1.3 alleles (P = 0.0280). While 2.1 QAP allele was completely methylated at position -311, 1.4 allele was not methylated at all (P = 0.0064).

P5.03.08
Pristane-induced arthritis loci interact with *Slc11a1* gene to determine susceptibility in mice selected for high inflammation

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AIRmax (maximal inflammation) and AIRmin (minimal inflammation) mice show distinct susceptibility to pristane-induced arthritis (PIA). *Slc11a1*, which regulates macrophage and neutrophil activity, is involved in this disease. AIRmax^{SS} mice homozygous for the non-functional *Slc11a1 S* (gly169asp) allele are more susceptible than the other lines. The aim of this work was to identify genes in acute inflammatory reaction loci that interact with *Slc11a1* alleles to modulate PIA. Mice received two ip injections of 0.5 mL pristane with 60 days of interval. Global gene expression analysis was performed on Affymetrix mouse 1.0 ST bioarrays (27k genes) using RNA (n=4) from arthritic or control paws. In parallel, genome wide linkage studies were performed to search for arthritis QTL in an F2 (AIRmax x AIRmin, n=290) population. Significant (LODscore > 4) arthritis QTL on chromosomes 5 and 8, and suggestive QTL on chromosomes 7, 17 and 19 were detected. Global gene expression analysis demonstrated significantly (P<0.001) over-represented genes related to inflammatory response and chemotaxis in AIRmax^{RR} and AIRmax^{SS} mice, as well as in the AIRmax heterozygous group. Higher up-regulation of chemokine genes *Cxcl1*, *Cxcl9*, *Cxcl5*, *Cxcl13* on chromosome 5 was observed in AIRmax^{SS} than in the other lines. In chromosome 8, *macrophage scavenger receptor 1* and *heme oxigenase (decycling) 1* genes were also more expressed in AIRmax^{SS} mice. RT-qPCR experiments validated microarray analyses. Results revealed that the gene expression profile of two arthritis QTL (on chromosomes 5 and 8) correlates with *Slc11a1* alleles, resulting in enhanced AIRmax^{SS} mice susceptibility to PIA.

P5.03.09
Clinical relevance of typing HLA-DR7-DQ2 haplotype in celiac disease

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Celiac disease is a systemic immune-mediated disease that affects genetically predisposed individuals when they are in contact with a gluten diet. It has a strong association with HLA-DQ2 and HLA-DQ8 haplotypes. These haplotypes have the ability to present gliadin peptides to reactive T cells in gut. More than 95% of patients present HLA-DQ2 heterodimer in *cis* (HLA-DR3-DQA1*05:01-DQB1*02:01) or in *trans* (HLA-DR7-DQA1*02:01-DQB1*02:02; DR11-DQA*05:05-DQB1*03:01). The rest presents HLA-DQ8 heterodimer (DR4-DQA1*03:01-DQB1*03:02).
The aim of this study was to compare clinical, histological and analytical characteristics of celiac patients carrying HLA-DR7-DQ2 *versus* HLA-DR3-DQ2 haplotypes. Since 2010 we have typed the HLA-DR7-DQ2 together with HLA-DR3-DQ2 for testing HLA-DQ2.
A total of 85 celiac disease patients were studied, 96.5% were HLA-DQ2 and 4.7% were HLA-DQ8. 16.5% of patients carried only HLA-DR7-DQ2 haplotype, 63.5% carried only HLA-DR3-DQ2 and 16.5% carried HLA-DR3-DQ2 together with HLA-DR7-DQ2. We did not find significant differences between HLA-DR7-DQ2 and HLA-DR3-DQ2 patients with respect to histological damage and anti-tissue transglutaminase and anti-endomysium autoantibody levels. We also compared clinical characteristics and we did not observe significant differences between these haplotypes except for irritability. This characteristic is more frequently associated with HLA-DR7-DQ2 than HLA-DR3-DQ2 patients (14.3% vs. 0.0%, p=0.041).
In contrast to what has been published by some groups, celiac patients with HLA-DR7-DQ2 haplotype have the same clinical behaviour than HLA-DR3-DQ2 patients. Moreover there is a stronger association with irritability in HLA-DR7-DQ2 patients.
For a correct typing HLA-DQ2, HLA-DR7-DQ2 haplotype has to be performed due to its frequency and clinical presentation, at least in our geographical population.

P5.03.10
Association of BANK-1 and Cytokine gene polymorphisms with Type 1 diabetes: A family-based and case-control study

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Type 1 diabetes (T1D) is an autoimmune disease (AID) with both genetic and environmental components. We aimed to investigate the genetic association of polymorphisms in genes previously linked with other AIDs, namely the BANK1, IL15, IL21 and IL2 genes. Twelve polymorphisms in the BANK1, IL15, IL21 and IL2 genes were genotyped in cases (76 T1D patients), unrelated controls (n = 162), and multicase families (59 nuclear families; 86 T1D cases and 169 first degree relatives) from Southern Tunisia.
In the BANK1 gene, G allele and the GG genotype of rs3733197 and TT genotype of rs17266594 were significantly increased in the T1D patients group compared to healthy controls. Furthermore, one IL15 SNP (rs13117878) exhibited a marginal association with the T1D, whereas the family study revealed a significant association of the rs2221903 in IL-21 gene with the disease.
Interestingly, the IL-15/ IL-2: G-C-A-T and A-T-T-T haplotypes exhibited a significant effect on T1D susceptibility. In addition, a significant gene-gene interaction between the BANK1/IL-15/IL-21 (TGGT) significantly increases in T1D patients as compared to controls.
These results suggest that common variants of the BANK1 gene are involved in the genetic component conferring general susceptibility to T1D, whereas IL-15 and IL-21 appears to play a minor role in the pathogenesis of the diseases. This work provides motivation for studies aiming to replicate these findings in larger populations.

P5.03.11
Causes of Multiple Sclerosis: a functional genomics approach

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Multiple Sclerosis (MS) is the most common disabling neurological disease affecting young adults in Western Society. To date, 55 strongly associated single nucleotide polymorphisms have been discovered. We now need to identify causal genes. While T-cells as targets for therapeutic intervention have rarely proven useful, there is strong clinical and in-vitro data identifying NK cell deficiencies in patients, and key roles for monocytes in myelin and axon destruction and autoantigen presentation. RNA extracted from magnetic bead sorted monocytes and NK cells, of healthy controls (HC) and untreated patients with relapsing remitting MS (RRMS), was labelled and hybridised to Affymetrix Human Gene 1.0 ST arrays. Expression values were standardized across chips using RMA and quantile normalization as implemented in GenePattern. Genes were ranked by expression difference significance by Mann Whitney U test and ANOVA. To date, we have analysed monocytes of 30 patients and 39 HC, and NK cells from 25 patients and 32 HC. Expression differences of those genes adjacent to MS associated risk SNPs lying between 110kb upstream and 40kb downstream of a candidate gene were considered. We have identified three genes worthy of further analysis on this basis: RGS1, HHEX and THEMIS. To test the relevance of these candidates to central nervous system (CNS) autoimmunity, we aim to mimic phenotypes associated with these expression quantitative trait loci (eQTL) in in-vitro cultures of purified NK cells and monocytes, and in-vivo in a mouse model of MS - experimental autoimmune encephalomyelitis (EAE).

P5.03.12
Significance of single framework mutations for DNA-binding and hydrolyzing activity of antibodies from mice with autoimmune disorders

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Anti-DNA autoantibodies are known as important factors of tissue injury in autoimmune diseases and may reveal DNA-hydrolyzing activity.
DNA-binding and catalytic activity of BV04-01 antibody from (NZBxNZW) F1 mice and its recombinant scFv (SCA04-01) derivative produced in *E. coli* suggested that significant conformation changes in antibody-DNA complex are necessary for activation and cleavage of the phosphodiester bond, and subsequent product release.
Autoantibody MRL-4 from mrl/lpr mice contained VH domain that differed from VH of BV04-01 by only few residues. Recombinant MRL-4 scFv, produced in *E. coli,* displayed no DNA-hydrolyzing activity, while replacement of BV04-01 VL by MRL-4 VL in SCA04-01 yielded antibody with DNA-hydrolyzing activity indistinguishable from SCA04-01.
Site-directed mutagenesis of MRL-4 and BV04-01 has shown that reversion of the P23 in the MRL4 scFv to A23, encoded by germline gene, resulted in significant loss of DNA-binding capacity of this antibody. Both DNA-binding and DNA-hydrolyzing activity of A23P SCA04-01 were decreased due to A23P mutation in SCA04-01 VH. SPR data on DNA-binding capacity of mutant and wild-type variants of MRL-4 scFv and SCA04-01 indicated that the biggest decrease in DNA binding efficiency was found in SCA04-01 VH A23P mutant.
Dramatic changes in DNA-binding and DNA-hydrolyzing activity due to single proline mutation permit hypothesizing that some DNA-binding Abs, possessing intrinsic low affinity to DNA, can first evolve via classic antigen-driven process using a (self)antigen of proteinaceous or other (LPS, viral capsid) origin, and then acquire high affinity to DNA by some mutation causing major conformational rearrangement in the mature antibody molecule.

P5.03.13
CTLA4 +49G allele in combination with HLA-DR3 associates with type 1 diabetes as well as its onset in North Indians

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Type 1 diabetes is a complex autoimmune disease with a strong genetic influence. We have investigated the role of the CTLA4 (Cytotoxic T-lymphocyte associated antigen -4) exon 1 A49G polymorphism and its role as a risk factor for T1D in the North Indian population. We studied 232 type 1 diabetic patients and 200 healthy controls by PCR amplification followed by restriction enzyme digestion with FnU4HI. Allele G at +49 position in codon1 of CTLA 4 gene was significantly increased in type 1 diabetic patients as compared to that in healthy controls (37.3% in patients vs 29% in controls, p= 0.012, OR=1.46, 95%CI= 1.09-1.94). Further, the frequency of homozygous GG genotype was significantly increased among patients than controls (15.5% vs 5.0%, p=0.001, OR=3.49 (95% CI=1.7-7.13)). The patients with younger age at onset of disease (<14 years) had a significantly high frequency of G positive genotypes (A/G or G/G) than those with an older age at onset of disease ≥(14 years) (66% vs 47.1%, p=0.007, OR=2.18 (95%CI=1.27-3.76). Further analysis involving the HLA data revealed that the CTLA4 +49G allele is not in linkage disequilibrium with the reported susceptible HLA-DR3 allele, however the susceptibility conferred by +49G allele as well as its effect on the disease onset was observed to be dependent on the presence of HLA-DR3 allele. These results suggest that CTLA4 +49G allele in the presence of HLA-DR3 allele not only confers susceptibility to T1D and but is a strong marker for acceleration of disease development

P5.03.14
TRIM39R, but not TRIM39B regulates type I interferon

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Behçet's disease (BD) is a chronic systemic inflammatory disease characterized by recurrent oral and genital ulcers, skin legions, uveitis and arthritis and its pathogenesis is not fully elucidated. However, the onset of BD is biased among regions and human races, thus, both genetic factor such as HLA-B*51 and environmental factor such as infection of some kind of bacteria or virus are thought to be important for the pathogenesis. Previously, we identified two SNPs are associated with BD. One is l is located on tripartite motif 39 (TRIM39) coding exon and another is also located on ribonuclease P/mitochondrial RNA processing 21 kDa subunit (Rpp21).
Recent studies suggest that a few member of family play an important role for regulation of innate immune pathway including type I interferon and defenses of viral infection. Moreover, TRIM39 fused Rpp21 (TRIM39R) probably generated by intergenic splicing, which is expressed in human normal tissues although conventional TRIM39 (TRIM39B) is also expressed. We hypothesized that TRIM39 may be involved in immune responses such as anti-virus response and type I interferon pathway and the pathogenesis of BD. We found TRIM39B, TRIM39R and RPP21 protein is expressed in human T and B cells. However, molecular function of TRIM39 including both TRIM39B and TRIM39R remains to be unidentified.
In this study, in order to identify the molecular function, we thus established gain of function models using CMV promoter-mediated overexpression and performed microarray analysis. Our results indicate that TRIM39R, but not TRIM39B, regulates type I interferon pathway.

P5.03.15
A "candidate-interactome" analysis of genome-wide association data in multiple sclerosis

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The study of gene-environment interactions in multifactorial diseases is crucial for interpreting the relevance of non-heritable factors and prevents from overlooking genetic associations with small but measurable effects. We propose a "candidate interactome" (i.e. a group of genes whose products are known to physically interact with environmental factors that may be relevant for disease pathogenesis) analysis of genome-wide association data in multiple sclerosis. We looked for statistical enrichment of associations among interactomes that may be representative of gene-environment interactions of potential, uncertain or unlikely relevance for multiple sclerosis pathogenesis: Epstein-Barr virus, human immunodeficiency virus, hepatitis B virus, hepatitis C virus, cytomegalovirus, HHV8-Kaposi sarcoma, H1N1-influenza, JC virus, human innate immunity interactome for type I interferon, autoimmune regulator, vitamin D receptor and a panel of proteins targeted by 70 innate immune-modulating viral open reading frames from 30 viral species. Interactomes were either obtained from the literature or were manually curated. The P values of all single nucleotide polymorphism mapping to a given interactome were obtained from the last genome-wide association study of the International Multiple Sclerosis Genetics Consortium & the Wellcome Trust Case Control Consortium,2. The interaction between genotype and Epstein Barr virus emerges as relevant for multiple sclerosis etiology. However, in line with recent data on the coexistence of common and unique strategies used by viruses to perturb the human molecular system, also other viruses have a similar potential, though probably less relevant in epidemiological terms.

P5.03.16
Immunological relations between autoimmune diseases in Thyroid Screening with Vitiligo

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Objectives: Vitiligo is a well-recognized pigmentary disorder known as an autoimmune disease. Autoimmune plays an important role in the pathogenesis of Vitiligo. Autoimmune thyroid disease is also a common autoimmune disease, and it is related to Vitiligo. The objectives of our study were to find the relationship of between autoimmunity, autoimmune thyroid disease and Vitiligo.
Methods: Our study was planned to evaluate Immunological the thyroid function test (Free T3, Free T4 and TSH) and to detect the presence of anti-TPO in patients with Vitiligo in our population.
Results: According to our study the rate of anti-TPO positivity was found to be 22.44% which shows statistically significant in Vitiligo patients compared with control. The difference in the prevalence of anti-TPO was significant in male cases compared with female. There was also a significant difference between the means of FT3, FT4 and TSH between patients and control group. Serum levels of FT4 and TSH was lower and higher respectively in Vitiligo patients as compared with controls.
Conclusions: Cellular immunity plays an important part in the pathogenesis of Vitiligo. Vitiligo cases are shown to be more at risk of evolution toward subclinical thyroid disease particularly; hypothyroidism and Thyroid dysfunction may result in the progress of Vitiligo.

P5.03.17
Alteration of promiscuous gene expression in the thymus is associated to susceptibility to autoimmune type 1 diabetes mellitus in mice

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Thymic central T cell self tolerance is a process dependent of promiscuous gene expression (PGE), which is characterized by ectopic expression of peripheral tissue antigens (PTAs) in medullary thymic epithelial cells (mTECs) a phenomenon partially controlled by Aire. Autoimmune type 1 diabetes (T1D) results from the breakdown of T cell self tolerance to pancreatic β cells autoantigens. Based on these evidences we hypothesized that alterations of PGE in mTECs might contribute to T1D susceptibility. To test this, we compared such expression of mTECs from two mouse strains: NOD (autoimmune, which develops T1D) and BALB/c (non-autoimmune). The expression profiles of PTA genes were evaluated in mTECs cells from 5-6 week female animals by the microarray method. Hierarchical clustering of microarray expression profiles showed that expression of Aire was not changed between strains. Nevertheless, the major known diabetes autoantigens such as Ins1, Ins2, Gad1, Gad2, and Ica1 were comparatively down regulated in NOD mice whereas IA-2 was up regulated. Interestingly, the qRT-PCR analyses for Aire showed that in NOD mice and during T1D development, the expression of this gene is higher in the pre diabetic period while T1D autoantigen genes are decreased. Taking on account the recent evidence that Aire also controls the expression of microRNAs, the opposite correlation between the expression of this gene and T1D autoantigens might be due to post-transcriptional effect of Aire-dependent microRNAs. These results contribute to a better understanding on transcriptional and post-transcriptional control of PGE and central tolerance.

P5.03.18
Analysis of B-cell activating factor (BAFF) gene polymorphisms (-2841 T/C, -2704 T/C, -2701 T/A, -871 C/T) and soluble BAFF levels in primary Sjögren´s syndrome

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B cell activating factor (BAFF) has been found over expressed in primary Sjögren´s syndrome (pSS) patients. BAFF may be responsible of an exacerbated survival, recruitment and differentiation of autoreactive B cells in salivary gland of pSS patients. Haplotypes inferred from polymorphisms -2841T>C, -2704T>C, -2701T>A, and -871C>T in BAFF gene, may be associated with over expression of this cytokine. We included 104 pSS patients and 115 healthy subjects. SSDAI/SSDDI activity and damage indexes were applied. Genotypes were identified by PCR-RFLP technique. Linkage disequilibrium and haplotype inference were performed using SNPAnalyzer v2.0 and Arlequin v.3.5.1.3. Soluble BAFF levels (sBAFF) were quantified by ELISA test. Statistical analysis was performed using PASWv.18.The C allele carriers of -2704T>C polymorphism, showed increased damage disease (p=0.038). The A allele carriers of the -2701 T>A polymorphism showed high levels of rheumatoid factor (p=0.024). Polymorphisms were in linkage disequilibrium; twelve haplotype was inferred. TTTC haplotype was associated with susceptibility to pSS (p=0.023), but only 2.3% of patients were carriers. Elevated sBAFF levels were found in pSS (p=0.042). Association between sBAFF levels and SSDDI index was found (p=0.001). In patients, no significant differences for sBAFF levels, according to alleles, genotypes or haplotypes were found. Our findings suggest an association of -2704T>C and -2701T>A polymorphisms with an increased severity of pSS. Despite high sBAFF levels in pSS patients and the association of TTTC haplotype with susceptibility to pSS, given its low frequency is not feasible to use it as a genetic marker for susceptibility to the pSS in our population.

P5.03.19
Interleukin-10 gene polymorphisms (-1082 A/G, -819 C/T, -592 C/A) in primary Sjögren's syndrome

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Introduction: Primary Sjögren´s syndrome (pSS) is an autoimmune disease of unknown etiology affecting primarily salivary and lacrimal exocrine glands. It is characterized by a progressive lymphocytic infiltration where B cells produce a great variety of antibodies, including those against ribonucleoproteins Ro and La. IL-10 plays an important role in pSS by promoting the differentiation of B cells and inducing antibody production. Three polymorphic sites located at positions -1082 (A/G), -819 (C/T) and -592 (C/A) in the IL-10 promoter show a linkage disequilibrium and form haplotypes that may influence the IL-10 production and consequently an increased activity of the disease. Objective: To determine the frequency of haplotypes in the IL-10 promoter gene in patients with pSS. Methodology: The study included 113 Mexican mestizo patients with pSS, classified according to the American-European Consensus criteria, from the Department of Rheumatology (Hospital General de Occidente, SSJ). As control group, 154 clinically healthy subjects were included. The polymorphisms were genotyped by PCR/RFLP technique. Statistical analysis was carried out with the Stata 9.0 and EmHapFre software. Results: Polymorphisms were in Hardy-Weinberg equilibrium (-1082 A/G p=0.8377, -819 C/T p= 0.1174, -592 C/A p=0.7953) and showed high linkage disequilibrium (100%, pc=1.42 x 10-34).The most frequent haplotypes were: ACC (41-61%), ATT (21.38%), GTA (14.86%), and GCC (11.18%). GTC haplotype was associated with a 4.35-fold less probability to develop pSS [OR; 95%CI: 0.23 (0.064-

0.792) p= 0.01]. Conclusion: Our results suggest that carriers of the GTC haplotype have a protective status for the development of pSS.

P5.03.20
Association between cytokine genes' polymorphism and susceptibility to and severity of mixed connective tissue disease (MCTD)

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Introduction. Mixed connective tissue disease (MCTD) is a relatively rare systemic autoimmune disease (about 10 cases/100 000). The altered pattern of cytokines or/and immune cells may contribute to the pathogenesis of MCTD and their balance determinates disease activity. In our study we analyzed the potential association of the cytokine gene variants encoding IL-10, IL-12 and IL-17F with disease susceptibility and clinical phenotype in MCTD patients.
Methods. A study group consisted of 68 patients with MCTD and of 106 healthy individuals. The SNPs in the IL- 10, IL-12B and IL-17F genes were investigated by PCR-RFLP approach. Results were correlated to clinical and laboratory parameters.
Results. The frequency of the IL-10-592CA, -592AA, -1082GA and -1082AA genotypes were higher in MCTD patients compared with the control group (p=0,0001, p=0,0419, p=0,0004 and p=0,0336, respectively). The homozygous wild genotype for IL-17F (+7488AA genotype) was observed in 80% of patients with MCTD and in 92% of controls (p=0,0334). In addition the -1082G/A IL-10 gene polymorphism was associated with puffy hands, esophageal involvement, ESR/CRP elevation and with anti-U1-A and -C antibodies positivity in our group. The IL-17 +7488A/G variant showed correlation with esophageal involvement anti-SmB and anti-DNA antibodies presence, while the IL-17F +7383A/G variant was associated with Sjögren syndrome. The IL -12 SNP +1188A/C correlated with sclerodactyly in MCTD patients.
Conclusion. Our results for the first time showed the relationship between IL-10, IL-12B and IL-17F gene polymorphisms and susceptibility to and severity of MCTD pinpointing gene variants that may be considered as risk factors in that entity.

P5.03.21
Dysfunction of ABIN1 as genetic basis for lupus nephritis

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It is estimated at least five million people worldwide have a form of systemic lupus erythematosis (SLE).Lupus nephritis (LN) occurs in about 50% of patients with SLE, and it is a major cause of morbidity and mortality. Recent studies implicate NF-κB and MAPK activity in the pathogenesis of LN. We previously reported that a knock-in mouse with native A20 binding inhibitor of NF-κB 1 (ABIN1) replaced by ABIN1[D485N] demonstrated enhanced activation of NF-κB and MAPKs in immune cells in response to TLR agonists and developed lupus-like autoimmune disease. In the current study we show that ABIN1[D485N] mice develop progressive glomerulonephritis similar to the most common and severe form of human LN. To investigate a clinical relevance for ABIN1 dysfunction, we genotyped 5 single nucleotide polymorphisms in the ABIN1 gene TNIP1 previously associated with systemic sclerosis, psoriasis, and SLE in European-ancestry, African American, Asian, Gullah and Hispanic populations. Comparing SLE cases with nephritis versus SLE cases without nephritis showed strong associations with LN at rs7708392 in European Americans and rs4958881 in African Americans. Our data suggest that polymorphisms in the TNIP1 gene participate in the pathogenesis of LN, via aberrant ABIN1 regulation of NF-κB and MAPK activity. Further, this mouse model closely replicates the human disease and, therefore, potentially serves as an effective tool to study diagnostic and therapeutic strategies for SLE and LN.

P5.03.22
Neurotransmitter GABA-A MAY regulate immune system functions as detected by gene networks of peripheral blood TCD4$^+$, TCD8$^+$ and CD14$^+$ cells of recently diagnosed Type 1 Diabetes Mellitus

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Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter primarily produced by neurons and pancreatic beta cells. GABA functional receptors (GABRA) are expressed in brain, pancreas and peripheral blood lymphomononuclear cells (PBMC). After evaluating the differential gene expression in PBMC of recently diagnosed type 1 diabetes mellitus patients (T1D), three GABRA networks were observed, modulating the expression of genes of the immune system. The study was conducted on T CD4$^+$, T CD8$^+$ and CD14$^+$ cells obtained from 20 pre-pubertal patients and 20 age and sex-matched healthy controls, using cDNA microarrays. Significant and differentially expressed genes, revealed using the SAM program, were studied for gene networks. Three different GABRA subunit class genes (alpha1, alpha6 and beta1) up-regulated the expression of the genes related to cell cycle (CNNB1 cyclin M1, STOM stomatin), apoptosis control (FAS, TNF receptor superfamily, BAT3, HLA-B associated transcript 3), cellular receptor (IL1RAP, interleukin 1 receptor accessory protein, BCAP29, B cell receptor, IK cytokine, down-regulator of HLA II), complement control (CD93, C1q receptor), glucose metabolism (PFKFB2, 6-phosphofructo-2-kinase), insulin receptor (INSR), ubiquitins (UBC, ubiquitin C, UBE2Z ubiquitin-conjugating enzyme E2Z, UBR4 ubiquitin protein ligase E3 component recognin 4, and UBAP, ubiquitin associated protein 1), growth factor (COL6A2, collagen, FGF13, fibroblast growth factor 13, FGF9, Fibroblast growth factor 9). Although the role of GABA and GABRA in the pathogenesis of diabetes has not been established, some studies have shown that GABRA down regulates many immune system functions. This study further supports the role of these neurotransmitters on the pathogenesis of T1D.

P5.03.23
The PRL -1149 G/T polymorphism is associated with rheumatoid arthritis and anti-CCP antibodies in Mexican population

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Background. Prolactin (PRL) is a hormone-cytokine that has been involved in autoimmunity due to its immunoregulatory and lymphoproliferative effects. It is produced by various extrapituitary sites including immune cells, under control of an alternative promoter that contains a single nucleotide polymorphism -1149 G/T and could influence PRL mRNA expression.
Objective. To associate the *PRL* -1149 G/T polymorphism with mRNA expression and clinical activity in rheumatoid arthritis (RA) patients from Mexico.
Methods. We enrolled 258 RA patients and 333 control subjects (CS). The clinical indexes (DAS-28, Spanish HAQ-DI) and laboratory assessments (ESR, CRP, RF, anti-CCP, PRL) were performed. The genotypes were identified using the PCR-RFLP method and the *PRL* mRNA expression was determined by qPCR.
Results. According to the dominant model of inheritance, we found an association between the T allele (GT+TT) and decreased RA susceptibility (OR 0.64, 95% CI 0.45-0.92; *p*=0.011). Furthermore, the carriers of the T allele had lower titers of anti-CCP antibodies in comparison to the carriers of the G allele [66 U/mL (10-171) *vs* 125 U/mL (24-222); *p*= 0.03]. The PRL mRNA expression was as follows: GG>GT>TT in both groups (RA: 1>0.72>0.19; HS: 1>0.54>0.28). 5.7% of the RA patients had hyperprolactinaemia, however, the PRL serum levels were similar in both groups (RA: 8.6±8.5 ng/mL; HS: 7.4±5.2 ng/mL).

Conclusions. The *PRL* -1149 T allele is a genetic marker for decreased RA susceptibility and is associated with lowers titers of anti-CCP antibodies in Mexican population. We suggest influence of the genotype upon PRL mRNA expression.

P5.03.24
Combinatorial control of Th17 and Th1 cell function by genetic variation at genes associated with the IL-23 signaling pathway in spondyloarthritis

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Genome-wide association studies have revealed numerous genetic associations between specific single nucleotide polymorphisms (SNPs) and immune-mediated inflammatory diseases, pointing to new directions in the study of disease mechanisms. The current challenge is to correlate the genetic variants with the effector mechanisms implicated in pathogenesis, to allow translation of the genetic data into novel diagnostics and treatment strategies. We have investigated the link between genetic variation at loci associated with spondyloarthritis (SpA) and the effector function of $CD4^+$ T lymphocyte subsets involved in chronic inflammatory disease.
We found that the effector functions of Th17 and Th1 cells in SpA patients are under combinatorial control by multiple SNPs at genes associated with the IL-23/Th17 pathway. SpA patients carrying risk-associated alleles of genes in this pathway expressed the highest levels of genes involved in the differentiation and function of Th17 and Th1 cells, whereas the presence of protective alleles was associated with low-level expression of these genes. In contrast, variation at loci genetically linked to SpA, but not associated with the IL-23 pathway, did not affect the expression of Th17 and Th1 genes, suggesting that these SNPs may contribute to SpA pathogenesis through distinct cellular mechanisms. Our results show that genetic variation at genes associated with the IL-23 signaling pathway affects the effector functions of Th17 and Th1 cells in SpA patients and provide a framework to delineate the mechanisms by which genetic variants contribute to pathology.

P5.03.25
Increased systemic autoimmunity in men with sex chromosome aneuploidy (47,XXY) reveals a X-chromosome dosage effect

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Most autoimmune diseases are more common in women than men. Studies in animal models suggest a role of sex chromosome genotype in modulating autoimmune susceptibility. Here, we begin to translate these murine findings onto humans. Our hypothesis is that X chromosome dosage, rather than the female sex itself, imparts susceptibility to autoimmune diseases. Results show that levels of IgG anti-chromatin, anti-nucleosome and anti-histone (H2A, H2B and H3) autoantibodies were significantly higher in men with sex chromosome aneuploidy (47,XXY) compared to 46,XY men. XXY men, however, did not have non-specific B cell hyper-reactivity, as the levels of anti-thyroid peroxidase antibody that is associated with autoimmune thyroiditis were similar between the two groups. A preliminary analysis of clinical questionnaire revealed an increase in autoimmune conditions when compared with known population prevalence of these diseases. Immune phenotyping of blood cells conducted thus far showed a higher frequency of natural killer T-cells in 47,XXY men than in 46,XY men. These data suggest a role of X chromosome dosage in inducing autoimmunity in humans. Since animals and humans with autoimmune diseases have reduced numbers and/or functions of natural killer T-cells, our observation of reduced natural killer T-cells in 47,XXY men may be related to increased autoimmunity in these men.
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P5.03.26
Study of programmed cell death 1 (PDCD1) genepolymorphims in Iranian patients with Ankylosing Spondylitis

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Ankylosing spondylitis (AS) is a chronic inflammatory disease, characterized by axial arthritis in which the genetic-environmental factors seem to be involved in the pathogenesis of the disease. This study was performed to investigate the role of polymorphisms of the programmed cell death 1 (PDCD1) gene on susceptibility to AS. In this study, 161 Iranian patients with AS and 208 normal controls were enrolled; two single-nucleotide polymorphisms (SNPs) of the PDCD1 gene PD-1.3 (G, A) in nucleotide position +7146 of intron 4 and PD-1.9 (C, T) in nucleotide +7625 of exon 5 were studied. Analysis of PD-1.3 revealed that 82% of patients and 79% of controls had GG genotype, while GA and AA genotypes were detected in 17% and 0.6% of patients, respectively, and 20% and 1.4% of controls, respectively. Moreover, the genotype CC (PD-1.9) was present in 92% of patients and 97% of controls. Although these differences were not statistically significant between patients and controls, comparisons of genotypes frequencies in the AS patients, based on human leukocyte antigen (HLA)-B27, revealed that all patients who had CT genotype (PD-1.9) were HLA-B27 positive, whereas 30% of patients with CC genotype were HLA-B27 negative.There was no evidence of association for PDCD1 SNPs with AS in our study, but CT genotype (PD-1.9) seems to be associated with HLA-B27 positivity in the patients with AS.

P5.03.27
Endocrine orgam-specific autoimmunity in myasthenia gravis with thymoma related to defective function of the autoimmune regulator (*AIRE*)

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There are unexplained parallels between patients with the monogenic auto-immune polyendocrine syndrome type I (APS-I), caused by *AIRE* gene mutations, and with thymoma. The parallels include autoantibodies neutralizing type I interferons and IL-17/ IL-22. To test our hypothesis that these parallels extend beyond cytokine antibodies, we screened 239 MG patients with or without thymoma for the seven endocrine autoantibodies we routinely test in our APS-I patients. Conversely, we also tested 38 APS-I patients for autoantibodies against acetylcholine receptor and titin. Forty-four per cent of MG/thymoma patients had antibodies against at least one of the APS-I-type autoantigens, and 8.3% of the MG/thymoma-patients had clinical manifestations typical of APS-I, but they did not always co-occur in the same individuals: both were rare in other MG subgroups. By contrast, no APS-I patient had antibodies against the muscle antigens tested, nor did any of them show the muscle weakness characteristic of MG. The obvious connection between these autoimmune syndromes is thymopoiesis in the absence of AIRE. We demonstrate that thymoma expression of AIRE correlates with thymoma expression of some APS-I antigens although not with others. Antibody presence against APS-I targets did not correlate to either AIRE expression nor to the corresponding autoantigen for the panel we analysed. Notably, large individual differences were seen.
These results indicate a one-way clinical and serological overlap between thymoma and APS-I patients. We propose that the AIRE-deficiencies in the thymic epithelial cells in each syndrome create a hostile microenvironment against selected available AIRE-independent tissue-specific antigens, including abundant cytokines.

P5.03.28
Behçet's Syndrome in Iranian Azari People

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Behçet's syndrome (BS) is a chronic recurrent multisystemic inflammatory disorder characterized by oral and genital ulcers, ocular inflammation. Behçet's syndrome has a complex genetic etiology. However, epidemiological studies recommend that genetic factors have a significant influence to its pathogenesis, alike to other autoinflammatory disorders. Epidemiological statistics, clinical records and Human Leukocyte Antigen (HLA) typing were studied in Iranian Azari patients with Behçet's syndrome. This investigation is considered HLA associations with BS, and HLA with certain clinical characteristics, age and sex in the (Tabriz) Iran, which has an ethnically homogeneous population. HLA-A and HLA-B typing was performed in 290 BS patients, conforming to International Study Group criteria and in 300 blood donors, as controls. Patient records were retrospectively reviewed and patients reassessed clinically. HLA-B5, HLA-B35, HLA-51, HLA-B52, and HLA-CW4 presented significantly high frequencies in all patients. No other HLA type was associated. There was a significant HLA link with male sex in BS patients and Mean age (34 ± 1.1) was determined. We present the frequency and correlation between Iranian Azari patients with Behçet's syndrome and particular HLA antigens. All patients had mouth ulceration, 64% genital ulceration, 72% skin lesions, and 52% ocular involvement. This study supports HLA-B5, HLA-B35, HLA-51, HLA-B52, and HLA-CW4 immunogenetic predisposition in an ethnically homogeneous (Iranian Azari) population.

P5.05 Environmental factors in autoimmunity and allergy

P5.05.01
Nutrition has relation with lifetime asthma diagnosis in middle-aged

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Background: There is abundant research relevant to genetic and environmental influences on asthma and hayfever, but little is known about dietary risk factors in Australian adults. This study's purpose was to identify dietary factors associated with lifetime asthma (AS) and asthma or hayfever (AS/HF) diagnosis in Australian middle-aged and older adults.
Methods: From The 45 and Up Study baseline self-report data, this study included 156,035 adult men and women. Participants were sampled from the general population of New South Wales, Australia in 2006-2009. About 12% of participants reported ever receiving an AS diagnosis (men 10%; women 14%) grains/alcohol) or five factors for women (meats; fruits/vegetables.
Results: For men, the meats/cheese factor was positively associated with AS (AOR = adjusted odds ratio for highest versus lowest quintile = 1.18, 95%CI = 1.08, 1.28; P_{trend} = 0.001) and AS/HF (AOR for highest versus lowest quintile = 1.22, 95%CI = 1.14, 1.29; P_{trend} < 0.001). Poultry/seafood was also associated with AS/HF in men (AOR for highest versus lowest quintile = 1.11, 95%CI = 1.04, 1.17; P_{trend} = 0.002). For women, significant risk factors for AS/HF included meats (AOR for highest versus lowest quintile = 1.25, 95%CI = 1.19, 1.31; P_{trend} = 0.001P_{trend} = 0.011). In contrast, the cheese/
Conclusions: Generally, diets marked by greater intakes of meats, poultry, and seafood were associated with diagnosed AS and AS/HF. Taken together, these findings suggest that adherence to a more meat-based diet may pose risk for AS and AS/HF in Australian adults.

P5.05.02
High psychological stress in children may alter the immune response

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Psychological stress is a public-health issue in children and has been associated with a number of immunological diseases. The aim of this study was to examine the relationship between psychological stress and immune response in healthy children, with special focus on the insulin-producing beta-cells.
Psychological stress was based on a composite measure of stress in the family across the domains: 1) serious life events; 2) parenting stress; 3) lack of social support and 4) parental worries. Peripheral blood mononuclear cells (PBMC), collected from five-year-old high-stressed children (n=26) and from five-year-old children without high stress within the family (n=52), from the All Babies In Southeast Sweden (ABIS)-cohort, were stimulated with antigens (tetanus toxoid and beta-lactoglobulin) and diabetes-related autoantigens (GAD_{65}, insulin, HSP60 and IA-2). Immune markers; cytokines and chemokines, clinical parameters; C-peptide, pro-insulin and glucose; cortisol, in serum and hair, as an indicator of stress, were analysed.
Results: Children from families with high psychological stress showed a low spontaneous immune activity (IL-5, -10, -13, -17, MIP-1α, MCP-1 and IP-10 ($p<0.01$)) but an increased immune response to tetanus toxoid and beta-lactoglobulin and the autoantigens GAD65,HSP60 and IA-2 (IL-5, -6, -10, -13, -17, IFN-γ, TNF-α, MIP-1α, MCP-1 and IP-10 ($p<0.05$)). Children within the high-stress group showed higher levels of cortisol, pro-insulin and C-peptide compared to the control group ($p<0.05$).
Conclusion: Psychological stress may contribute to an imbalance in the immune response but also to a pathological effect on the insulin-producing beta-cells.

P5.05.03
Infection with the respiratory pathogen *Bordetella pertussis* modulates the course of experimental autoimmune encephalomyelitis

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Epidemiological studies have described an association between infection with certain bacteria or viruses and development of autoimmune diseases, such as multiple sclerosis (MS). Conversely, infection with helminth parasites has been associated with a reduced incidence and severity of autoimmunity. Whooping cough is a re-emerging vaccine-preventable infectious disease caused by the bacteria *Bordetella pertussis*. Clearance of the bacteria is associated with the induction of Th1 and Th17 cells. However, the infection is persistent and is also associated with the induction of and recruitment of Treg cells to the lungs during the acute stage of disease. In this study we have used a murine model of MS, experimental autoimmune encephalomyelitis (EAE), to examine the effect of infection on development of autoimmunity. Our data reveal that concurrent infection with *B. pertussis* significantly reduced the clinical scores and weight loss in mice with EAE. This reflected a significant reduction in the frequency of infiltrating IL-17$^+$, IFN-γ$^+$ and IFN-γ$^+$IL-17$^+$ CD4 and γδ T cells into the central nervous system (CNS) of *B. pertussis* infected compared with uninfected mice with EAE. An examination of antigen-specific T cell responses in lymph nodes and spleen revealed that systemic IL-17A and IFN-γ production was significantly enhanced in infected mice with EAE. Our findings demonstrate that a bacterial infection can attenuate the course of EAE in mice and that this may reflect the suppressive effect of bacteria-induced Treg cells, which appear to be capable of preventing Th1 and Th17 cells from entering the CNS.

P5.05.04
Bisphenol A suppresses interferon-gamma production and related biochemical pathways in human PBMC

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Bisphenol A (BPA) is one of the most widely distributed chemicals. Humans are exposed to it mainly via polycarbonate plastics that are used for food packaging and plastic bottles and contain BPA as a softener. There is accumulating evidence of a carcinogenic and allergy-promoting effect of BPA. *In vitro* we investigated the impact of BPA on freshly isolated human peripheral blood mononuclear cells (PBMC) from healthy donors. PBMC were incubated with increasing doses of BPA, and after 30 min either left unstimulated or stimulated with the mitogen phytohemagglutinin (PHA) and cell viability was controlled.
After 48 h, formation of interferon-γ (IFN-γ), macrophage product neopterin as well as tryptophan breakdown by indoleamine 2,3-dioxygenase (IDO), expressed as the kynurenine to tryptophan ratio (Kyn/Trp), were monitored. All these immunobiochemical pathways are characteristic for cell-mediated (= Th1-type) immune response. Upon PHA stimulation, neopterin production and tryptophan breakdown increased significantly compared to unstimulated cells. BPA exerted a dose-dependent suppressive effect in unstimulated and in PHA-stimulated PBMC at 12.5 - 200 µM concentrations. Due to the cross-regulation of Th1- and Th2-type immunity, the suppressive effects of BPA on Th1-type immunity could relate to the allergy-promoting properties of the compound. Moreover, the inhibition of IFN-γ production and its related antiproliferative effects could be of relevance for the tumor-promoting effect of this chemical. On the other side, the antioxidant compound could also have beneficial effects, possibly delaying or even preventing Th1-type immunopathologies.

P5.05.05
Epicutaneous exposure to nanoparticles is potential initiator of IgE-mediated hypersensitivity induced by IgG reduction

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It is widely known that exposure to ambient particulate matter (PM), such as diesel exhaust or crystalline silica, exacerbate allergic disorders. Because lung-related disorders were often observed after PM exposure and PM might not penetrate the skin barrier, we have believed the importance of inhaled PM for PM-related allergic disorders. On the other hand, recent studies suggest the involvement of sensitization of skin with airborne antigens in the pathogenesis to asthma. We further revealed that nano-sized particle (≦100 nm) can penetrate the skin barrier. Considering the recent reports that PM contains a large nano-sized particles fraction together, it is possible that PM-related allergic disorders might be induced not only by inhaled exposure, but also epicutaneous exposure to PM. Here we investigated the effect of epicutaneous exposure to silica nanoparticles (nSP) on epicutaneous sensitization in NC/Nga mice. Repeated epicutaneous applications of mixture of mite extract antigen (Dp) with nSP did not change Dp-specific IgE levels compared with Dp-alone. In contrast, the Dp-specific IgG levels were significantly reduced by epicutaneous exposure to nSP30. It is known that allergen-specific IgG work as blocking antibody to inhibit IgE-mediated allergic responses and we showed nSP-mediated reduction of IgG induced IgE-mediated hypersensitivity in Dp-mediated anaphylaxis model. We also showed that the changes of antigen dynamics by the interaction between nSP and antigen might be critical determinant to induce IgE-mediated hypersensitivity. Our results suggested that epicutaneous exposure to PM including nano-sized particles potentially contributed to induction of PM-related allergic disorders.

P5.05.06
Serum levels of pro-inflammatory cytokines in rats exposed to car exhaust and cigarette smokin

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Background: The immune system mechanisms are changed due to the smoking and airborne pollutant but it is not completely understood to what extent such changing are happening. The current study was conducted to evaluate the smoking exposure and resulted cytokine variations in rat. Materials and methods: Pathogen-free male Wistar rats 4 to 5 weeks old were obtained from the Pasteur Institute of Iran. The rats were placed in three groups each of 8 rats; cigarette smokers, car smokers and the control groups. Rats were placed in some designed racks during smoking so that the smoke can reach them similar to what happens in heavy traffic and what a cigarette smoker does daily. After one month of exposure the blood samples were extracted from the rats and cytokine profiles were measured by ELISA using related diagnostic kit. Data were analyzed using Post Hoc analyzing method and LSD test to compare the mean of cytokine levels within the groups. Results: Car smoke suppressed the production of IL-2, IFN-γ and TNF-α by more than 50 %.amongst all the rat in cases groups while the control group had no changing in their cytokine profile. In contrast, cigarette smoke inhibited production of TNF-α and increased production of the IL-2 and IFN-γ.
Conclusion: This study indicated that cigarette and exhaust smoke contains potent inhibitors and/or suppressor of cytokine production even in a short term exposure time.

P5.05.07
Autoimmune pulmonary alveolar proteinosis and exposure to industrial spray paint

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Pulmonary aleveolar proteinosis is a rare disease characterized by accumulation of surfactant lipids and proteins in the alveolar spaces resulting in impaired gas exchange (1). We report the case of a 52 year-old man employed as a spray painter in an automobile shop for 20 years who presented with increasing dyspnea and weight loss for 3 months. CT chest showed diffuse bilateral consolidations with ground glass opacities, interlobular and intralobular septal thickening suggestive of a crazy paving pattern. Subsequent bronchoscopy and right lung lavage showed eosinophilic proteinaceous material, consistent with alveolar proteinosis. Blood tests revealed elevated GM-CSF autoantibodies. PAP is generally classified as autoimmune, acquired, or congenital (2). The autoimmune type, which makes up the majority of reported cases is caused by autoantibodies against GM-CSF (granulocyte-macrophage colony stimulating factor). Environmental exposure to airborne dusts such as aluminum and silicone dioxide has been associated with acquired PAP (3). The clinical presentation may vary greatly from asymptomatic disease to life threatening pulmonary complications (4). Industrial spray painters are known to be exposed to isocyanates and chromium compounds, both of which are linked to asthma. Furthermore, PAP is associated with occupational exposures including silica dust, titanium, aluminum, cement, and tin. Our patient had elevated GM-CSF antibodies, which suggests autoimmune PAP. However, his long-term exposure to industrial spray paint may have served as an environmental trigger to his underlying autoimmune condition. PAP should thus be considered in a patient presenting with respiratory symptoms with occupational exposure to industrial spray paint.

P5.05.08
The investigation of prevalence of allergic rhinitis and single nucleotide polymorphisms of STAT6, IL-4R in Xinjiang of China

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Objective To investigate the prevalence of allergic rhinitis (AR)and the effect factors in North and South Xinjiang.And study the relationship of polymorphism of STAT6(rs167769,3024974,304015,703817), IL-

4R (rs6498011) and IL-4(rs2243283) between allergic rhinitis.Methods Cross-sectional epidemiological study was carried out,and the questionnaires,nasal examination and nasal mucous membrane pathological examination were used to diagnose the prevalence of allergic rhinitis.The relevant factors were analyzed by using the multivariate Logistic regression analysis.Results Among the investigated 4254 cases,644 cases of allergic rhinitis were diagnosed,the total prevalence rate was 15.1%,and the prevalence rate in Uigur population was12.8%.which was less than that in Han (26.1%,P<0.01).The differences of distribution frequencies of genotypes and allelic genes(STAT6 rs703817, IL-4R rs6498011) between AR group and control group were statistically significant (P< 0.05).Conclusion The prevalence rate of allergic rhinitis in Han is much more popular and there is a significant ethnic distribution difference in Xinjiang of China.The STAT6 rs703817 A/G and IL-4R rs6498011A/G may be associated with allergic rhinitis in Uygur.

P5.05.09
Immunological analysis of allergic reactions to the carbohydrate α-Gal

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Purpose. Galα1-3Galβ1-4GlcNAc-R (α-Gal) is a carbohydrate expressed by non-primate mammalians and has recently been described as an important allergenic structure in meat allergy. Since the role of carbohydrates in allergy is still controversial, we sought to investigate the relevance of α-Gal in meat allergy.
Methods. We included 20 patients who had experienced generalized urticaria after consuming meat and showed IgE-reactivity (>0,35 kUA/l) to beef or pork in ImmunoCAP. Additionally, 20 non-meat-allergic patients with birch pollen-allergy and 20 non-allergic individuals were included. α-Gal-specific IgE and IgG1-4 was analysed by ELISA. Protein extracts produced from beef were used in immunoblots to test IgE-reactivity to individual meat proteins.
Results. 19/20 meat-allergic patients showed α-Gal-specific IgE. In immunoblots, 13/19 α-Gal sensitized patients showed IgE-reactivity with a protein at approximately 160 kDa, equivalent to bovine gamma globulin (BGG). IgE-reactivity to BGG was completely abolished after pre-incubation of sera with α-Gal. All individuals displayed α-Gal-specific IgG. Meat-allergic patients showed significantly higher levels of α-Gal-specific IgG, IgG1 and IgG3 than birch pollen-allergic and non-allergic individuals. α-Gal-specific antibodies were mainly IgG1 whereas IgG4 dominated the response to the major birch pollen-allergen Bet v 1 in birch pollen-allergic patients. Addition of sera with high levels of α-Gal-specific IgG to sera of meat-allergic, birch pollen-allergic and non-allergic individuals did not inhibit IgE-binding to α-Gal.
Conclusion. Our data indicate that IgE-reactivity to α-Gal on BGG is important in meat allergy and that it is directed exclusively against the sugar-epitope. The high levels of α-Gal-specific IgG antibodies have no IgE-inhibiting activity.

P5.05.10
Both reactive oxygen species and reactive nitrogen species participate in the redox-regulation of toluene diisocyanate-induced lung inflammation

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Toluene diisocyanate (TDI) is one of the oxidizing chemicals which induce occupational asthma. Workplace exposure to TDI leads to airway inflammation with distinct leukocyte infiltration. Our previous research showed that leukocyte NADPH oxidase is essential for inducing pulmonary inflammation with TDI. To clarify the role of the redox regulation in TDI-induced pulmonary inflammation, we investigated the redox regulation in wild type (WT) mice, NADPH oxidase subunit p47phox single knockout mice (*Ncf1*$^{-/-}$), iNOS single knockout mice (*iNOS*$^{-/-}$), and Ncf1 and iNOS double knockout mice (*Ncf1*$^{-/-}$*iNOS*$^{-/-}$). We found that TDI induce stronger airway hyperresponsiveness (AHR) in WT than in *Ncf1*$^{-/-}$ and *iNOS*$^{-/-}$ mice. Immunochemical staining revealed a marked increase of inflammatory cells in the bronchovascular bundle of WT mice and *Ncf1*$^{-/-}$*iNOS*$^{-/-}$ mice after TDI stimulation. Different from the strong tissue inflammation found in WT mice and *Ncf1*$^{-/-}$*iNOS*$^{-/-}$ mice, the lung tissue from *Ncf1*$^{-/-}$ mice and *iNOS*$^{-/-}$ mice showed milder cell infiltration. In addition, the major cell type of infiltrated cell in *Ncf1*$^{-/-}$ *iNOS*$^{-/-}$ mice was B cell. The levels of IFN-γ and IL-17A in WT mice and *Ncf1*$^{-/-}$*iNOS*$^{-/-}$ mice were significantly higher than that in *Ncf1*$^{-/-}$ mice and *iNOS*$^{-/-}$ mice. Although there was no significant difference in the level of IL-10 in WT, *Ncf1*$^{-/-}$ and *iNOS*$^{-/-}$ mice, the level of IL-10 in *Ncf1*$^{-/-}$*iNOS*$^{-/-}$ mice was significantly higher than that in other groups. We hence conclude that both ROS and RNS participate in the redox-regulation of TDI-induced lung inflammation and the balance between NADPH oxidase and iNOS determines TDI-induced lung inflammation.

P5.05.11
Screening for celiac disease in patients with Hashimoto's thyroiditis

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Aim. To perform a serological screening for celiac disease (CD) in patients with Hashimoto's thyroiditis (HT).
Patients and methods: Sera from 156 patients with HT were collected and stored at -20° C between January 2010 and June 2012. The control group consisted of 2500 blood donors. All sera were tested for anti-endomysium antibodies (EmA) using indirect immunofluorescence on cryostat sections of human umbilical cord.
Results: The mean age of our patients was 42 years 10 months and the sex ratio F/M was 6,1. EmA were identified in two patients out of 156 (1,28%) and they were present in seven subjects in the control group (0,28%). The frequency of EmA was higher in male patients with HT (4,54%, 1/22) than in females (0,74%, 1/134) but the difference was not statistically significant. In men, the frequency of EmA found in patients with HT was higher than that found in control group but reaching a borderline significance (4,54% vs. 0,23%, p = 0.06).
Conclusion: In this study, the frequency of CD in patients with HT was 1.28%. However, the small size of our sample may represent a limit to our study. Prospective studies involving a larger number of patients are needed.

P5.05.12
The influence of Professional Contact to Fungal Allergens on developing Respiratory Allergy Disorders in workers of bakeries in Georgia

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Introduction: The inducing role of sensitization to fungal Allergens of RA (Respiratory Allergosis) originated from exposure to wheat flour environmental dust - has not been studied in Georgia.The aim of the research is to study the specifics of the spread, epidemiological issues of RA induced by professional contact with Fungal Allergens. According to the Global Strategy for Asthma Management and Prevention (NHLBI/WHO), one of the main reasons of developing RA in adults is occupational sensitizing agents.

Materials and Methods: We studied 390 people working in bakeries. They were divided into 2 groups: (1)workers with symptoms of RA [AR(Allergic Rhinitis), BA(Bronchial Asthma) and SEAA(Syndrome of Extrinsic Allergic Alveolitis)] ; (2)workers with no symptoms. There were selected 2 control groupswithout contact with the professional environment : (1)with RA symptoms; (2)healthy individuals.
There was used the unified three-stage respiratory questionnaire (National Heart, Lung and Blood Institute,USA). For the skin tests were used the fungal allergens AF(*Aspergillus fumigatus),* MR(*Mucor racemosus*), AT(*Alternaria tenius),* PN*(Penicillium notatum),* RN*(Rhizopus nigricans)* .
Results: In workers with AR and BA symptomes the sensitization was similary: the highest to AF, high to all three fungal allergens MR, PN and RN (66,7%), The workers with BA were mainly sensitized to the AF(80%) and the sensitization to the MR and PN were revealed to be the least of all. In subjects with SEAA, the highest sensitization was to AF(77,8%).
Conclusion: The professional contact to grain dust frequently induces RA and the reciprocal influence of fungal sensitization significantly increases the risk of RA development.

P5.05.13
Lack of association between serotonin transporter gene polymorphisms and asthma

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Background: Asthma is a worldwide disease which genetic factors play a major role in its pathogenesis. Serotonin (5-hydroxytryptamine, 5-HT) likely has effect on pathophysiology of asthma because higher levels of free serotonin in plasma of asthmatic patients. Serotonin clearance is mediated by a specific protein called the serotonin reuptake transporter (SERT or 5-HTT). Polymorphisms in the promoter region and intron 2 of this gene have effects on transcriptional activity, resulting in altered serotonin reuptake efficiency. The aim of this study was to investigate the relationship of SERT genetic polymorphisms and asthma.
Methods: The 5-HTT LPR, rs25531 and STin2 VNTRs polymorphisms of serotonin transporter gene were assessed by PCR and PCR-RFLP in 100 patients with asthma and 100 healthy controls.
Results: Serotonin reuptake transporter polymorphisms were found to be similar in asthmatic patients and healthy controls and there was no significant difference in allele, genotype, and haplotype frequencies between patients and controls.
Conclusion: Our findings suggest that genetic polymorphisms in the gene encoding for the serotonin transporter is not associated with asthma. While the interaction between environmental factors and predisposing genetic factors are important in the pathophysiology of asthma, further genetic and epigenetic investigations may provide novel insights of the mechanisms contributing in asthma.

P5.05.14
Effect of the indoor air pollutant 2-ethyl-hexanol on immune cells

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The polyvinyl chloride floor coverings in damp office buildings emit the 2-ethyl-hexanol (2-EH). 2-EH is related to asthma symptoms by epidemiological investigation, and it is suggested that 2-EH is a causative agent of the sick building syndrome (SBS). However, biological effects of 2-EH have not been fully elucidated. The present study investigated the effects of 2-EH on immune cells from C57BL/6 mice using splenocytes, bone marrow-derived dendritic cells (BMDC) and bone marrow-derived macrophage (BMDM). After the 2-EH was administered intranasally to mice, the mice were sacrificed, and then splenocytes and bone marrow cells were isolated. We examined the splenocytes proliferative response to mitogen-stimulation (e.g., IL-2 and LPS) and the expression of surface makers (e.g., T cells, B cells, macrophages, dendritic cells, and costimulatory molecules) on splenocytes by flow cytometry. Furthermore, BMDC and BMDM were differentiated by culture with granulocyte macrophage-colony stimulating factor (GM-CSF), and then its function was analyzed by the measurement of cytokine secretion, the expression of costimulatory molecules and MHC class II molecules.
As a result, the mitogen-induced proliferation of splenocytes and cytokine productions of the BMDC and BMDM were enhanced in 2-EH administrated mice. This may suggest that 2-EH causes the disorder to the primary and secondary lymphoid tissues, and also may be considered as one of the evidence that 2-EH is associated to the immune disorders.

P5.05.15
Therapeutic potential of Allium cepa L. and the flavonoid quercetin in an experimental model of respiratory allergy

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Asthma is characterized by chronic pulmonary inflammation. It is stimulated by environmental allergens. Corticosteroids are the most commonly used drugs, because they act in controlling symptoms, however producing multiple side effects. Some plants, such as species Allium cepa L., are popularly used for the treatment of asthma. The objective of this study was to assess the therapeutic potential of the methanol extract of Allium cepa L. (AcE) and its flavonoid quercetin (Qt) in vivo. These experiments, AJ mice were sensitized (100µg per animal - sc) and challenged (10mg per animal - in) with extract of mite Blomia tropicalis (BtE). The sensitized animals were treated or not with AcE (100 or 1000mg/Kg) or Qt (30mg/Kg) to analyze the following parameters: BAL, EPO in the lung; total serum IgE levels, levels of IL-4, IL-5, IL-13 in BAL, and histopathological changes in the lung. In vivo, there was a reduction in the total number of BAL cells in sensitized animals that were treated with AcE and Qt when compared to untreated allergic animals. EPO, in lung, also suffered deletion in the treatment with AcE (100 or 1000mg/Kg).Treatment with AcE (1000mg/Kg) or Qt (30mg/Kg) was able to reduce IL-4 and IL-5 in BAL. However, there was no significant reduction in serum IgE levels and levels of IL-13. The results of this research suggest that Allium cepa L. and Qt have significant potential as drugs antiasthma with immunomodulatory properties and may be future candidates for drugs to make up the therapeutic arsenal of this pathology.

P5.05.16
Characteristics of effector and regulatory T cell subsets in the lungs of smoking and non-smoking healthy individuals

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Smoking influences the immune system in many ways and it has also been linked to risk of disease in a number of important inflammatory and autoimmune disorders, such as rheumatoid arthritis and multiple sclerosis. Recently, the lung has been implicated in the induction of these diseases. T cells are of central importance for the pathogenesis of autoimmune diseases, but the precise effects of smoking on pulmonary effector and regulatory T cells have not been well characterized. The aim of this ongoing study was to investigate frequencies and characteristics of lung and blood CD4+ and CD8+ T cell subsets such as T helper 1 (Th1) cells, Th17 cells and regulatory T cells (T reg). Samples were obtained by bronchoalveolar lavage (BAL) and from the peripheral blood of healthy smokers (1-5 pack years smoking history) and non-smokers. Cells were stimulated in vitro by anti-CD3/anti-CD28 and intracellular cytokines and select lineage-specific transcription factors were analyzed by flow cytometry. Our data indicate that smokers have higher frequencies of T cells expressing IFN-gamma as well as IL-10 in their lungs, and also that

they have elevated numbers of the lung IFN-gamma/IL-10 and IFN-gamma/IL-17 double-producing cells. Our results indicate that smoking is associated with distinct changes in the cytokine profiles of pulmonary T cells. The altered frequencies of “hybrid” phenotypes such as Th1/Th17 cells may indicate an effect of smoking on the plasticity of T cells subsets in the lung, which could be of importance for the induction or maintenance of smoking-associated inflammatory or autoimmune diseases.

P5.05.17
Immune markers in oral discomfort patients before and after elimination of oral galvanism

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An enhanced release of metals in the mouth due to galvanic cell formation is considered to be one of the causes of oral discomfort. The aim of this study was to investigate the influence of galvanic cell on salivary immune defense factors. The levels of IgA1, IgA2, secretory IgA, lysozyme and antiIgA/HSP60 were evaluated in representative samples from 159 patients with galvanism, from 177 patients without galvanism and in two control groups. All the participants underwent personal history taking, clinical examination, galvanic currents measurement and saliva collection. Electro active dental materials were removed in 30 patients. There was a significant increase in IgA2 level, a significant decrease in antiIgA/HSP60 levels and an increase in IgA1, secretory IgA and lysozyme levels found after the removal of electro active restorations. Morphological symptoms disappeared in 70 % of the treated patients. The study confirmed that pathologic galvanic phenomena influences the immune defense reactions in the oral cavity and thus deteriorates the symptoms of oral discomfort. A measurement of the galvanism and a subsequent removal of electro active restorations should become a common therapeutic procedure in the patients with oral discomfort.
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P5.05.18
Prolonged exposure to type-I IFN inhibits IL-10 signaling in memory and regulatory T cells: a new mechanism contributing to the development of autoimmune diseases

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Interleukin 10 (IL-10) is a fundamental immunoregulatory cytokine. Mice with memory T cells (Tmem)- or regulatory T cells (Treg)-specific depletion of the IL-10 receptor (IL-10R) develop fatal autoimmune diseases. However, no correlation is known between pathological conditions and altered IL-10 signaling in T cells.
We investigated the influence of prolonged exposure to the supernatant of mouse maturing dendritic cells, exposed to LPS, on the integrity of the IL-10 signaling pathway in Tmem and Treg. Following 36-48h incubation, a block in phosphorylation of STAT3 in response to IL-10, but not in response to IL-6, was evident. This IL-10-specific unresponsiveness was not associated with reduction in surface IL-10R expression, or with reduced STAT3 cytoplasmic availability. Additionally, this inhibition was not induced by IL-6 or IL-1. Instead, incubation with IFN-β impaired IL-10 signaling in both Tmem and Treg. This effect was not blocked by AKT inhibition, suggesting a role for Jak/STAT or other IFN-β-initiated signaling pathways. In NOD mice, a model of human type-1 diabetes (T1D), the spontaneous development of diabetes is linked to high levels of type-I interferons in pancreatic lymph nodes (PNC-LN). We analyzed the IL-10 response in T cells from spleen, PNC-LN, other LNs, and pancreata of NOD mice and discovered that around 4 weeks of age, Tmem and Treg in PNC-LN and pancreata display suppressed responses to IL-10, while the same subsets in all other tissues remain unaltered.
Overall, these data reveal a new molecular mechanism that controls the early development of autoimmunity and the pathogenesis of T1D.

P5.05.19
Novel immunoregulatory function of the carcinogen Acrolein: inhibition of allergic sensitization and promotion of tumor growth in BALB/c mice

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Background: Since allergic sensitization has been linked to smoking, we investigated the contribution of acrolein, a compound generated in large during smoking, during nasal sensitization and - based on the surprising intermediate results - on tumor growth.
Methods: BALB/c mice were nasally sensitized 5 times in biweekly intervals with KLH alone or in conjunction with acrolein. Airway hyperreactivity as well as KLH-specific anaphylactic reaction was monitored. Levels of specific antibodies as well as cytokine profile of KLH-stimulated splenocytes were analyzed. Further, D2F2-tumor was grafted to the flanks and tumor growth monitored in mice previously exposed to acrolein or buffer.
Results: Nasal application of KLH as model antigen induced specific IgG1-, IgG2a-, IgA- and IgE-levels. The same mice secreted elevated levels of IL5, IL13, IL10 and IFN-γ φρομ τηειρ σπλενοχυτεσ. They showed increased airway-hyperreactivity and had a significant drop in body temperature upon allergen challenge. Pointing towards tolerance, and against our expectations, presence of acrolein in the KLH-antigen significantly reduced specific antibody-titers, resulted in lower splenocyte cytokine production and prevented anaphylaxis. The impaired immune response also led to a significantly higher tumor growth in mice exposed to acrolein than in the control group.
Conclusion: Acrolein in smoke - best known for its carcinogenic effect - decreases the risk of sensitization towards a specific antigen by inhibiting immune activation. Our data further suggest that Acrolein via the same mechanism acts tumor promoting in smokers.

P5.05.20
Effect of petrochemical pollutants on *Avicennia marina* pollens allergenicity

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Allergic diseases due to the pollens are increasing. The reason of this phenomenon is not clear but probably air pollution intensifies allergic reactions. *Avicennia marina* in Bushehr province of Iran grows in two regions: Assaluyeh and Bordekhoon. Contrary to Bordekhoon, Assaluyeh is industrial city with petrochemical factories. We tried to compare pollen allergenicity of this plant in Bordekhoon and Assaluyeh in order to investigate the effects of petrochemical pollution on pollen allergenicity. Pollens were collected from the two regions and extracted in PBS. BALB/c mice were divided into four groups. The first group was treated with Bordekhoon pollen extract plus Alum and the second group was treated with Assaluyeh pollen extract plus Alum. The third and fourth groups served as controls and received PBS and Alum respectively. Intradermal skin test showed no differences in wheal diameter between the second group and first group. IgE level and rate of eosinophils increased in second group compared to the first group but this increase was significant only for IgE level. SDS-PAGE revealed that pollen proteins patterns were different in two regions. The immunoblotting analysis showed two bands in each region that reacted with IgE antibodies induced in treated mice. Results of this research showed that pollen exposure to petrochemical pollutants in Assaluyeh region intensified some allergic characteristics of *Avicennia marina* pollens.

P5.05.21
Treatment of patients with severe Pneumonia and Pneumonia associated with Diabetes

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The purpose of this work was the development of combined lymphotropic immunotherapy (CLIT) for patients with severe pneumonia (P) and diabetes (D). 121 patients aged from 15 to 65 years were enrolled: 45 patients with P, 27 patients with P+D, and 11 patients with D (control group). Patients were subdivided into groups. Group I (22 patients with P) and group II (12 patients with P+D), received only TT (antibacterial, pathogenetic, physiotherapy and symptomatic treatment indications). Group III (23 patients with P) and group IV (15 patients with P+D) received CLIT and TT for 10 days. Patients in groups III and IV showed normalization of phagocytosis, reliable reduction of cytotoxic immune complexes, restoration of imbalances in antibody and CD4/CD8 cell levels. Patients receiving CLIT, compared with patients receiving TT only, showed also an improvement in general conditions, reduction of symptoms, and over 30% decreased hospitalization time . Patients in groups I and II, receiving only TT, did not experience T-cell recovery of to the control group level. Thus, the inclusion of CLIT in an integrated therapy program in patients with severe P and P+D significantly increases the effectiveness of treatment and the quality of life.

P5.05.22
From gut immune regulation to self-tolerance: preventing autoimmune diabetes in the gut mucosa

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Type 1 Diabetes (T1D) results from the autoimmune destruction of pancreatic beta cells. Environmental factors acting at the intestinal level such as diet and microbiota modulate the pathogenesis of T1D in humans and pre-clinical models (NOD mice). We hypothesize that those factors affect T1D by altering the gut immune system. Many immune regulatory mechanisms that originate in the gut "educate" the immune system to maintain immune tolerance towards self-antigens outside the gut. These mechanisms include extrathymic development and/or expansion of regulatory T cells, driven by tolerogenic dendritic cells (DCs). We recently reported a defect of FoxP3+ Treg cells in the intestinal mucosa of T1D patients compared with healthy subjects, which correlated with the impaired capacity of gut DCs to elicit their conversion in vitro (Badami E et al Diabetes 2011). We are testing the hypothesis that alteration of diet and microbiota alterations affect T1D in NOD mice by shaping gut immunity. We found that NOD mice carry a significantly reduced numbers of tolerogenic DCs and have an increased Th17/Treg cell ratio in the gut mucosa compared to non-autoimmune mice. A gluten-free diet rescued the number and tolerogenic function of intestinal DCs and restored a normal Th17/Treg cell ratio in the gut and pancreatic lymph nodes. This was associated with protection from clinical diabetes and reduced percentages of diabetogenic effector T cells. We are testing the hypothesis that altered gut immunitty in NOD mice and improved tolerogenic mechanisms promoted by gluten-free diet correlate with modulation of microbiota species and genes.

P5.05.23
Thymic Atrophy Induced by Malaria Infection Aggravates Experimental Autoimmune Encephalomyelitis (EAE)

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Background: Malaria is the most prevalent infectious disease in the world causing one million deaths per year. We showed that malaria infection in mice causes thymic atrophy (Ta) by apoptosis and release of immature lymphocytes to the peripheral immune system. The presence in the periphery of cells that failed to experience negative selection may alter the severity and susceptibility to autoimmune diseases, such as Autoimmune Encephalomyelitis (EAE). In this context, we aimed to evaluate whether thymic atrophy previously induced by malaria infection modulates EAE.
Methods/Principal findings: Ta was induced in C57BL/6 mice by infection with Plasmodium berghei NK65. Treatment with chloroquine (CQ, 5mg/kg for five consecutive days) was initiated at the peak of Ta (7dpi) to eradicate parasites. Three days after the last dose of CQ, mice were immunized with MOG35-55 peptide and Pertussis toxin to induce EAE. Our results show that mice previously infected with P.berghei developed aggravated EAE with higher clinical scores, and infiltration of inflammatory cells in the Central Nervous System when compared to the control groups (CQ+EAE and only EAE). Also, the frequency of regulatory T cells was reduced in the periphery of previously P.berghei infected-EAE mice while INF-γ- and IL-17-producing cells were higher in this group than on the other two control groups.
Conclusion: Collectively, our results strongly demonstrate that thymic atrophy induced by malaria infection aggravates experimental autoimmune disease.
Support: This work was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (#2011/13191-3, #2011/17965-3).

P5.05.24
Natural tolerance towards peanuts in African allergic patients

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Background: Peanut allergy is known to cause severe and life-threatening allergic reactions.
Objective: To investigate allergic patients from Africa who show IgE reactivity to peanuts but regularly eat them without experiencing allergic reactions.
Methods: Sera from 54 African allergic patients with IgE sensitization to peanut were analyzed regarding total IgE levels and IgE antibody levels to peanut allergen extracts using quantitative ImmunoCAP measurements. A detailed analysis of the IgE and IgG reactivity profiles towards more than 145 purified natural and recombinant allergen molecules including 6 peanut allergens (Ara h 1, 2, 3, 6, 8, 9) was done using an allergen microarray.
Results: Each of the patients showed IgE reactivity to peanut extract. Notably, 44% of the patients showed IgE reactivity towards at least one of the peanut allergens (Ara h 1, 2, 3, 6 and 9) which reportedly cause severe anaphylactic reactions. These patients also had elevated allergen-specific IgG. One patient displayed IgE reactivity to the less allergenic Ara h 8. Forty-eight percent of the patients showed IgE reactivity only carbohydrate epitopes (MUXF3, nCup a 1, nCry j 1, nCyn d 1, nPhl p 4), but not to the peanut proteins.
Conclusion: In approximately half of the patients the lack of peanut symptoms can be explained by exclusive IgE reactivity to non-allergenic peanut epitopes. In the other patients natural clinical tolerance to peanuts may be caused by the development of allergen-specific IgG due to continuous peanut uptake.
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P5.05.25
Resveratrol modulates collagen-induced arthritis by inhibiting Th17 and B-cell function

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Rheumatoid arthritis (RA) is an inflammatory disease in which T and B cells play pathogenic roles. Resveratrol, a safe, plant-derived compound, possesses anti-inflammation and immune-regulatory properties. We therefore evaluate the therapeutic effect of resveratrol on collagen-induced arthritis (CIA) and its putative immune modulation in mice.
CIA was induced in DBA1 mice by immunisation with collagen II. Different doses of resveratrol were administered before or after the development of CIA. Either prophylactic or therapeutic administration of resveratrol attenuated clinical parameters and bone erosion in CIA mice. The arthritis-protective effects were associated with markedly reduced serum levels of pro-inflammatory cytokines and collagen-specific, but not total, IgG, and with reduced numbers of Th17 cells and the production of IL-17 in DLN.
Thus, resveratrol may offer a novel, effective and safe pathway whereby novel agents could be developed to treat RA.
This work was supported by Arthritis Research UK and the Medical Research Council UK.

P5.06 Regulatory T cells in autoimmunity and allergy

P5.06.01
CD4+ T cells limit their own-pathogenicity by activating regulatory T cells by different mechanisms in different inflammatory conditions

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The CD4+CD25+Foxp3+ regulatory T cells (Tregs) play a major role in peripheral tolerance. The suppressive effect of Tregs on CD4+ effector T cells (Teffs) has been extensively studied; however, the reverse in vivo impact of Teff activation on Tregs is poorly known. Under condition of autoimmune diabetes, we show that the activation of pancreatic islet antigen-specific Teffs strongly boosts the expansion and suppressive activity of Tregs with similar antigen-specificity in the pancreas and its draining lymph nodes. This Treg boost was partially dependent on TNFα and OX40 but not on IL-2. Interestingly, plasmacytoid dendritic are also involved in this Teff-dependent Treg boost. In another condition, we observed another Teff-dependent Treg boost that has a different mechanism. In a context of strong inflammation in draining lymph nodes of mice injected with complete Freund adjuvant and cognate peptides for Teffs and Tregs, the presence of activated Teffs increased Treg activation that depend on IL-2 but not on TNFα. Altogether, this data show that Teff activation increase activation of Tregs with different mechanisms that depend on the inflammatory condition. A better understanding of this feedback regulatory loop between Teffs and Tregs may be crucial to limit the development of autoimmune diseases and other immune responses.

P5.06.02
Role of Follicular Regulatory T cells in Multiple Sclerosis

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Both autoreactive T cells and autoantibodies are identified as key players in the immunopathogenesis of multiple sclerosis (MS). These overt autoimmune responses may results from disturbances in regulatory T cells ($CD4^+CD25^+FoxP3^+$ T cells) important for the maintenance of peripheral tolerance. Recently a new subset of regulatory T cells was discovered in mice. These follicular regulatory T cells (T_{FR}, $CD4^+CXCR5^{hi}PD\text{-}1^{hi}FoxP3^+$) participate in germinal responses by controlling the maturation of B cells and the production of (auto)antibodies.
We recently identified a similar Treg subset in human blood. The goal of this study is to investigate the functionality of T_{FR} in MS patients. Therefore, the frequency, phenotype and function of T_{FR} in the blood of MS patients and healthy controls are compared. In addition, the effect of current treatment regimens on this cell population is taken into account.
A significant difference (p=0.0401) could be found in the percentage of T_{FR} between MS patients without treatment (0.1252 ± 0.01052 % of T_{reg}, n=74) compared to healthy controls (0.1631 ± 0.01509% of T_{reg}, n=38). Furthermore, we have shown that this human subset is able to suppress $CD4^+CD25^-$ responder T cells *in vitro.* We are currently investigating T_{FR} functionality in MS patients.
Unravelling the functionality of T_{FR} and its association with B-cell alternations will help elucidate the involvement of this subset in autoimmune disease.

P5.06.03
Monocytes drive the induction of CD8+FOXP3+ regulatory T cells via the production of membrane TNF-alpha

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Limiting the severity and the eventual resolution of an inflammatory response is vital for the protection of host tissues from the damaging effects of chronic inflammation. The induction of $CD8^+$ regulatory T cells (Treg) expressing FOXP3 has been described within the context of chronic inflammation in cancer, infection and autoimmunity. For instance, we have shown that a small population of $CD8^+FOXP3^+$ Treg exists in the periphery of patients with inflammatory arthritis, which is not found in healthy individuals. The aim of our study was to dissect the mechanism of FOXP3 induction in CD8+ T cells after T cell receptor signalling, using CD3-specific antibodies. Our results found that anti-CD3 antibodies variably induce, not expand, a potent $CD8^+FOXP3^+$ Treg population. These Treg suppress $CD4^+$ T cell proliferation, IL-17 and IFN-γ production in vitro and express TGF-β and CTLA-4. Investigation into the derivation of these cells determined that monocytes are crucial for the induction of FOXP3 expression following CD3 activation. In particular, monocytes provide co-stimulation through CD86 and membrane TNF-α. In vitro, these signals could be substituted with agonist antibodies to CD28 and TNFR2. Artificial expression of membrane TNF-α on monocytes was found to drive FOXP3 expression in a proportion of $CD8^+$ T cells initially unresponsive to anti-CD3 stimulation. Our data identify the signalling pathways required for FOXP3 induction by $CD8^+$ T cells and that these pathways are amenable to manipulation to augment regulatory capacity during autoimmunity or limit their effects in cancer and infection.

P5.06.04
Diverse T-cell profiles in children with type 1 diabetes and/or celiac disease

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Type 1 diabetes (T1D) and celiac disease (CD) are both characterized by an autoimmune outcome. As T1D and CD share the same risk genes, patients have risk of developing the other disease subsequently. This study aimed to investigate the expression of T-helper (Th)-, T-cytotoxic (Tc)- and T-regulatory (Treg) cells in children with T1D and/or CD in comparison to healthy children.
Subgroups of T-cells (CD4+ or CD8+); naïve (CD27+CD28+CD45RA+CCR7+), central memory (CD27+CD28+CD45RA-CCR7+), effector memory (early

differentiated;CD27+CD28+CD45RA-CCR7- and late differentiated;CD27-CD28-CD45RA-CCR7-), terminally differentiated effector cells (TEMRA;CD27-CD28-CD45RA+CCR7-) and Treg (CD4+CD25+FOXP3+CD127-) cells, and their expression of CD39, CD45RA, CD101 and CD129, were studied by flow cytometry in children with T1D and/or CD or without any of these diseases (references).
Children with exclusively T1D had higher MFI of FOXP3 ($p<0.05$) but lower percentage of CD39+ and CD45RA+ within the Treg population (CD4+CD25+FOXP3+CD127-) ($p<0.05$). Children with exclusively CD had a higher MFI of CD101 ($p<0.01$) as well as a higher percentage of CD129+ ($p<0.05$), in the CD4+CD25hi lymphocyte population, compared to references. Children diagnosed with both T1D and CD showed higher percentage of terminally differentiated (TEMRA) CD4+ cells ($p<0.05$), but lower percentages of effector, both early and late, memory CD8+ cells ($p<0.05$), compared to references.
In conclusion, children with combined T1D and CD have higher percentage of differentiated Th-cells, compared to Tc-cells. T1D children show signs of low CD39+ Treg cells that may indicate loss of suppressive function. On the contrary, CD children show signs of CD101+ Treg cells that may indicate suppressor activity.

P5.06.05
$^{+}$ Foxp3 $^{+}$ Treg and its ICOS $^{+}$ Subsets in Patients with Myocardial Infarction

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Atherosclerosis is a multifactorial disorder with chronic inflammatory conditions in which immune cells play a significant role in its pathogenic process. Regulatory T cells (Treg), as a part of immune system, are involved in controlling autoimmune and inflammatory diseases. Quantitative and/or functional alteration of Tregs has been shown to play an atheroprotective role and may also promote plaque stabilization. To assess if inducible costimulatory molecule (ICOS) expression on one subtype of Treg cells with high suppressive potential correlates with the pathogenesis of atherosclerosis. Methods: Patients with myocardial infarction (MI) and/or stable angina (SA), diagnosed as atherosclerosis by angiography, and a group of individuals with normal coronary angiography (NCA) were recruited for the present study. Peripheral blood mononuclear cells (PBMCs) were prepared and the expression of ICOS, Foxp3 and CD4 molecules was tested by flowcytometry. The percentage of CD+Foxp3+Treg cells was reduced in MI group compared to NCA and SA groups ($p<0.005$). Evaluation of the two Treg subsets according to ICOS expression showed a decreased ICOS+/ICOS- Treg ratio in MI and SA groups compared to NCA individuals ($p=0.002$ and $p=0.048$, respectively). The present data indicate that Tregs and its ICOS+ subsets are decreased in patients with MI or SA, suggesting a potential role for Treg in atherosclerosis progression or onset of acute coronary syndrome.

P5.06.06
Association between T cell immunoglobulin domain and mucin domain-1 gene polymorphisms with Allergic Rhinitis patients

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Allergic rhinitis is one of the most common chronic inflammatory disease of nasal airways induced by IgE-mediated type I hypersensitivity that is caused by the interaction of multiple genetic and environmental factors. The aim of present study is to analyze the association between T cell immunoglobulin domain and mucin domain (TIM-1) gene polymorphisms with susceptibility to allergic rhinitis.
In a case-control study, 155 allergic rhinitis patients and 163 allergy-free controls were analyzed. Two TIM-1 promoter single nucleotide polymorphisms (SNPs), −416G/C and -1454G/A were investigated by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). X^2 test and Logistic regression analyses were used for calculating the risk associated with individual alleles and genotypes between case and control groups by using SPSS 18 software.
We found that there was statistically significant difference in TIM-1 −416 C allele and -1454 A allele (OR = 1.64, P = 0.006; OR = 1.55, P = 0.041, respectively) with susceptibility of allergic rhinitis. In addition, the total serum IgE and peripheral blood eosinophil number in the subgroup with homozygous genotype of -416 CC variation site were significantly higher than the subgroup without this SNP (p= 0.001, p= 0.02 respectively).

P5.06.07
Glycogen synthase kinase-3 regulates IL-10 production in Th1 cells

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The ubiquitous serine/ threonine kinase glycogen synthase kinase-3 (GSK3) is a point of convergence of several signaling pathways. In the immune system GSK3 has been shown to have an important role in the balance of production of pro- and anti-inflammatory cytokines. GSK3 inhibition has been shown to have an anti-inflammatory effect in several disease models and in monocytes, macrophages and human memory T cells has been shown to increase production of the anti-inflammatory cytokine IL-10. We have used inhibitors of GSK3 to examine its role in the production of IL-10 by $CD4^{+}$ T cells from myelin basic protein (MBP)-specific TCR transgenic (Tg4) mice. Treatment with GSK3 inhibitors of naive cells from Tg4 mice does not affect their production of IL-10. However, in differentiated Th1 cells, culture in the presence of the GSK3 inhibitors CHIR99021, SB216763 or SB627772 leads to a dramatic increase in the production of IL-10. These GSK3 inhibitor treated cells caused less severe disease than control treated cells in adoptive transfer experiments where experimental autoimmune encephalomyelitis (EAE) was induced. Inhibition of GSK3 in Th1 cells can therefore enhance the differentiation of IL10-Tregs and redirect the fate of pathogenic Th1 cells to a regulatory cell type. These findings are particularly important in the context of Th1-mediated autoimmune disease where it may be possible to reprogram disease-causing cells by GSK3 inhibition to become regulatory, disease-suppressing cells.

P5.06.08
Enhanced regulatory T cell function and attenuated DSS-induced colitis in the mice deficient with GPx1 and catalase

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Reactive oxygen species (ROS) are highly reactive and are likely to induce cellular damage and tissue destruction. Enhanced autoimmunity was observed in the mice in which ROS production was reduced, such as Ncf1 or Nox2 deficiency, as well as in human disease, such as chronic granulomatous disease. On the contrary, inflammatory response was attenuated in the mice in which ROS level was elevated, such as glutathione peroxidase 1 (GPx-1) deficiency. The recent observations bring about an innovative concept on the protective role of ROS in inflammation and tissue damage. Meanwhile, the underlying cellular mechanism is not yet sufficient. Regulatory T cell (Treg) function was studied only in the mice in which ROS level was reduced. To address this issue, in the present study, we investigated the function of $CD4^{+}CD25^{+}$ Tregs in the GPx-1 and catalase knockout mice in which ROS level was elevated. We also investigated on the colitis symptoms induced by 3% dextran sodium sulfate (DSS). The results showed enhanced Treg function and attenuated DSS-colitis symptoms in the $GPx1^{-/-}$ x $Cat^{-/-}$ mice. N-acetyl cysteine (NAC) or catalase decreased Treg function in vitro and in vivo, while DSS-colitis symptoms were aggravated in the $GPx1^{-/-}$ x $Cat^{-/-}$ mice as well as in the WT mice. Taken together, the results suggest that ROS level is pivotal to the functional activity of Tregs that play a key role in the regulation of immune responsiveness in vivo.

P5.06.09
The extrinsic factors blocking regulatory T cell function in the pathogenesis of SLE

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Systemic lupus erythematosus (SLE) is an intractable autoimmune disease. The pathogenesis of SLE still remains to be unknown. Recently, many investigators have studied on the role of regulatory T cells (Tregs) in the pathogenesis of SLE. Meanwhile, both the results of quantitative and functional studies are controversial. One of the possible reasons is the unreliable conventional in vitro functional assays for Tregs. However, we have established an accurate quantitative assay for the suppressive function of Tregs. Employing our quantitative assay, we analyzed that the suppressive function of Tregs from MRL/lpr mice were not decreased, rather enhanced, suggesting that intrinsic functional defect was not critical and other extrinsic factors might be more important in the pathogenesis of SLE.
Moreover, we established the mouse model of induced SLE by using pristane in C57BL/6 WT and $GPx1^{-/-}$ x $Cat^{-/-}$ mice which have stronger suppressive activity of Tregs. Then, we confirmed lipogranuloma formation in their peritoneum and the suppressive function of Tregs from induced SLE animal model was consistent with that of MRL/lpr mice. In addition, interstitial fibrosis was observed in their kidney and follicles were induced in lung. On the other hand, decreased IL-2 and increased IL-4 and IL-6 are consistently observed in the serum from C57BL/6 WT and $GPx1^{-/-}$ x $Cat^{-/-}$ mouse. IL-2 enhances, whereas IL-6 inhibits, the development and function of Tregs. Accordingly, we hypothesized that the decreased IL-2 and/or increased IL-6 may contribute to the pathogenesis of SLE, extrinsically inhibiting development or function of Tregs and induce differentiation to Th2.

P5.06.10
Targeting PAI-1 signaling results in the enhancement of iTreg and reduction of mortality in a mouse model of septic shock

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Mycoplasma arthritidis causes arthritis and septic shock in natural infections of rodents. Here we show that the level of type 1 plasminogen activator inhibitor (PAI-1), the primary inhibitor of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), is markedly increased in septic shock-susceptible mouse strains injected with live *M. arthritidis*. We also show that *M. arthritidis*-infected mice exhibit severe septic shock with elevated levels of inflammatory cytokines TNFα, IL-1β but lower level of TGFβ production. Repeated injections of animals with anti-PAI-1 blocking antibody effectively prevent mice from lethal toxicity and suppress production of inflammatory cytokines. Importantly, anti-PAI-1 treatment results in increased level of TGFβ and IL-10 expression in livers as well as in lymphoid organs. Parallel results demonstrate that, the expression of Foxp3, a marker of regulatory CD4+ T cells, is significantly increased in anti-PAI-1-injected mice with *M. arthritidis* infection. Furthermore, the treatment of disease animals with anti-PAI-1 antibody induces tolerogenic DCs that promotes the suppressive function of inducible Treg (iTreg) contributing to the alleviation of mortality and morbidity in septic shock animals. Thus, these results reveal a novel function of PAI-1 in developing septic shock in *M. arthritidis*-infected mice and suggest a potential therapeutic target for the treatment of inflammatory diseases.

P5.06.11
General control nonrepressed 2 (GCN2) kinase participation in the remission phase of experimental autoimmune encephalomyelitis (EAE) in mice

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Experimental autoimmune encephalomyelitis (EAE) is an animal model widely employed to study immune mechanisms involved in multiple sclerosis. Although evidences suggest that the remission phase in EAE is associated with a decrease in infiltrating Th1 and Th17 lymphocytes and an increase in regulatory T cells within the CNS, the mechanisms governing this process are poorly understood. Recent researches demonstrated that chemically induced amino-acid starvation response (AAR) might suppress CNS immune activity. However, the exact mechanisms by which it occurs and if AAR could be physiologically involved in the modulation of CNS inflammation are still open questions. This study verified the participation of the general control nonrepressed-2 kinase (GCN2), a key regulator of AAR, in EAE course. By immunizing wild type (WT) and GCN2 knock-out mice (GCN2-KO) with MOG35-55, it was noted that GCN2-KO showed higher clinical scores and levels of CNS inflammatory infiltration, specially during the remission phase of the disease. These animals also showed lower levels of regulatory T cells (Treg) compared to the WT group. Higher levels of IDO (indoleamine 2,3-dyoxigenase) and plasmacytoid dendritic cells (pDCs), which seems to present immunosuppressive effects, were found at the peak of the disease in the CNS of WT animals. Moreover, pDCs from WT animals were able to induce higher levels of Treg differentiation in naïve T cells from WT animals. The results obtained suggest that the presence of GCN2 kinase, due to the activation of AAR, contributes to the EAE remission by suppressing CNS inflammation and probably promoting Treg differentiation/expansion.

P5.06.12
Effect of fetal therapy on T-regulatory cells during development of experimental allergic encephalomyelitis

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Recovery of functional activity of T-regulatory cells (Tregs) must be in the base of therapy of autoimmune diseases, including multiple sclerosis (MS). Today fetal neural cells (FNCs) have proved themselves well for MS treatment. However the data on FNCs effect on the state of T-regulatory link of immunity are virtually absent.
The research aim was to investigate the effect of cryopreserved FNCs on formation of natural and induced Tregs in development dynamics of experimental allergic encephalomyelitis (EAE).
The studies were performed in white outbred rats with induced EAE. FNCs of the rats of 11 gestation day were cryopreserved and intraperitoneally introduced to the animals to the 14th day of EAE development. Native FNCs and adult animals' neural cells served as the control. CD4+CD25+Foxp3+ and dendritic cells (DCs) were examined every 7 days within a month with the method of flow cytometry in thymus and peripheral blood.
It has been found that on the peak of pathology development the content of nTregs significantly increased (in 3.5±0.13 times) and the one of iTregs decreased almost in 30 times versus the control. This is related to the thymus disordered export function at EAE. The content of DCs in thymus of the animals with EAE was one-way changed with the number of nTregs. This fact testifies to a direct participation of thymic DCs in the formation of nTregs. Introduction of cryopreserved FNCs contributed to the recovery of thymus export function. Possible mechanisms of FNCs effect on T-regulatory link of immunity are under consideration.

P5.06.13
Regulatory T cell depletion reveals a large polyclonal population of self-reactive T cells in the normal peripheral T cell repertoire

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The ability to actively suppress immune responses makes Treg cells important elements of peripheral tolerance. In fact, acute depletion of Treg cells in otherwise normal adult mice results in systemic and catastrophic autoimmunity.
We have found that 48 hr after acute depletion of Treg cells, there is a significant increase in the number of both CD4+ and CD8+ T cells expressing markers of recent activation. In addition, a significant accumulation of differentiated/effector cells occurs in the secondary lymphoid organs beginning 4 d post-depletion. Simultaneous adoptive transfer of polyclonal and monoclonal TCR-transgenic T cells confirmed that this T cell activation/differentiation is due to specific TCR stimulation and not just a response to generic factors, such as space or cytokines.
To more precisely examine the TCR, we sorted recently activated (48 hr) and effector CD4+ T cells (7 d) following Treg depletion and determined TCR rearrangements by deep sequencing. We found that recently activated as well as 7 d effector T cells were highly diverse.
Analysis of T cell proliferation suggests that 4.5% of the initially transferred polyclonal T cells responded following Treg depletion. Additionally, examination of T cells from Nur77 reporter mice following depletion further suggested that specific TCR stimulation occurs in approximately 5% of the T cell population.
Taken together these data suggest that normal adult mice harbor a highly diverse and high frequency of self-reactive T cells. Together, these findings raise many questions about negative selection of self-reactive T cells in the thymus.

P5.06.14
IL-10 and NOx are associated with CD4(+)FoxP3(+) regulatory T-cell subsets in the Systemic Lupus Erythematosus patients

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The Systemic Lupus Erythematosus (SLE) is an inflammatory chronic disease characterized by cytokine and natural regulatory T cells (Treg) imbalance. Many data point to defective aspects, both quantitative and qualitative, are involved in SLE pathogenesis and flares of activity. Based on this, it is tempting to correlate the frequency of T cell population and inflammatory molecules with disease activity. This study aims to quantify and compare the inflammatory profile and the frequency of CD4+FoxP3+ Treg subsets (CD4+CD25highFoxP3+, CD4+CD25lowFoxP3+ and CD4+CD25-FoxP3+). In this work we considered the activity of disease.Thirty-six female SLE diagnosed patients, including 23 with active (SLEDAI>6) and 13 with inactive disease (SLEDAI<6) were enrolled in this study, together with 15 healthy volunteers. Circulating IL-2, IL-10, IL-17, TNF and IFN-g were unchanged in SLE patients. Only IL-1, IL-6 cytokines and Nitric Oxide (NOx) showed statistic differences when compared with control individuals or activity disease. When compared with control group the T CD4+ CD25Low FoxP3 + T cells subset showed elevated in PBMC from SLE patients. Independent of Treg subsets, the active SLE patients presented statistic differences compared to inactive or control group. The CD4+CD25HighFoxP3+ and CD4+CD25LowFoxP3+ showed negative correlation with SLEDAI score and CD4+CD25negFoxP3+ showed positive correlation. Only circulating IL-10 is correlated with T CD4+CD25LowFoxP3+ regulatory-T cells subset. Together, the results suggest that IL-10 and NOx in association with the CD25high and CD25low Treg subsets may be considered SLE activity markers. However, further longitudinal investigations need to be executed to confirm this hypothesis.

P5.06.15
Activin-A-induced human regulatory T cells: novel controllers of allergic asthma

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Regulatory T cells (Tregs) are essential for the maintenance of airway tolerance. Our previous studies have uncovered activin-A as an inducer of mouse Tregs that restrain Th2 responses and protect from experimental asthma. Still, whether activin-A can drive the generation of human Tregs remains elusive. Our data reveal that activin-A inhibits naïve $CD4^+$ T cell proliferation, IL-5 and IL-13 release during stimulation with allergen *in vitro*. Activin-A-treated $CD4^+$ T cells remain hyporesponsive upon secondary allergen stimulation. Importantly, activin-A-treated $CD4^+$ T cells suppress allergen-driven responses by human naïve T responders, pointing to the generation of a Treg population (act-A-iTregs). Act-A-iTregs are $CD4^+ICOS^+$, do not express Th2 cytokines but produce significantly increased IL-10. Notably, a major induction in the mRNA levels of the transcription factor Aryl hydrocarbon Receptor (AhR) and its gene target, CYP1A1, is observed. In fact, addition of an AhR antagonist reverses activin-A-mediated induction of $ICOS^+IL\text{-}10^+$ Tregs, indicating a novel link between activin-A and AhR in the generation of human IL-10-secreting Tregs. Using a humanized mouse model of asthma, we show that act-A-iTregs remain hyporesponsive upon transfer *in vivo* and do not induce allergic airway inflammation. Furthermore, adoptive co-transfer of act-A-iTregs with Th effectors ameliorates cardinal asthma features *in vivo* and allergen-specific Th2 responses. Of clinical relevance, act-A-iTregs restrain robust Th2 responses in asthmatics *ex vivo*. Collectively, our findings introduce activin-A as a novel inducer of functional human Tregs and may pave the way for the use of act-A-iTregs in adoptive-transfer cell therapy regimes aiming at re-establishing airway tolerance.

P5.06.16
Immune regulation in lupus nephritis: role of CD4+CD25high FOXP3+ T regulatory cells and related cytokines (IL-6, IL-10, TGFβ)

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Background: Platelet-derived microparticles (PDMPs) represent small vesicles that promote coagulation by exposure of negatively charged phospholipids. Aim of this study was the assessment of PDMPs in systemic lupus erythematosus (SLE) patients and their association with disease activity, presence of antiphospholipid antibodies (aPL) and previous thrombosis.
Patients and Methods: Forty consecutive SLE patients (38 females, 2 males, mean age 50±16.4 years, mean disease duration 110.7±26.4 months) were included. PDMPs were isolated from citrate-treated plasma and characterized by flow cytometry using Annexin-V and CD41 antibodies to platelet surface markers.
Results: SLE patients had significantly increased concentrations of PDMPs (1800±293MP/UI, normal range <1000MP/UI), irrespectively to disease activity, as PDMPs did not differ between active and inactive disease (1879±186 vs. 1805±343MP/UI, p=NS). Interestingly, PDMPs were detected in higher levels (although non significantly) in patients with a previous history of arterial or venous thrombosis (1855±219 vs. 1781±396MP/UI). In accordance, they were marginally elevated in patients with antiphospholipid syndrome (APS) (1892±233 vs. 1776±360MP/UI). Moreover, PDMPs were found in higher levels in patients with positive anti-dsDNA antibodies (1907±317 vs. 1762±303MP/UI) and low levels of C3/C4d complement fragments (1982±334 vs. 1721±256MP/UI, $p<0.05$).
Conclusions: PDMPs were found in higher levels in lupus patients with a previous history of thrombosis or concomitant APS. Elevated PDMPs in patients with positive anti-dsDNA antibodies and low complement levels suggest that these microparticles may further

induce thrombosis via the induction of immune complexes formation. These results warrant further characterization of PDMPs in SLE to elucidate their role in disease pathogenesis.

P5.06.17
Intravenous immunoglobulin selectively enhances $CD4^+CD25^+FOXP3^+$ Treg function in patients

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High-dose intravenous immunoglobulins (IVIg) is used as anti-inflammatory therapy for auto-immune diseases and protect against acute transplant rejection. In mice, we observed that prevention of skin allograft rejection by IVIg is mediated by $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs). To determine whether IVIg stimulates Tregs in humans *in vivo*, we studied Treg activation and suppressive function in patients treated with either low- (≤0.6g/kg, n=12) or high-dose (>0.6g/kg, n=15) IVIg monotherapy (indications: hypogammaglobulinaemia or autoimmune diseases). Blood was collected before, immediately after, and 7 days after IVIg infusion. Treg numbers and activation status were measured by flowcytometry, while suppressive capacity of $CD4^+CD25^+CD127^-$ Tregs purified from blood was determined in an *ex vivo* assay using IFN-γ secretion by autologous PHA-stimulated $CD4^+CD25^-CD127^+$ T-helper cells as read out. High-dose, but not low-dose, IVIg treatment enhanced the activation status of circulating Tregs, as shown by increased HLA-DR (+31% at day 7, $p<0.01$) and FOXP3 (+30% at day 7; $p<0.01$) expression, while numbers of circulating Tregs remained unchanged. The suppressive capacity of purified Tregs was increased by 42% at day 7 after IVIg treatment ($p=0.001$). Interestingly, the activation status of Tconv was not affected by IVIg. In conclusion, we show that IVIg treatment can selectively activate and enhance the suppressive capacity of Tregs in humans *in vivo*. We propose that Treg stimulation may be one of the mechanisms by which IVIg controls autoimmune and systemic inflammatory disorders.

P5.06.18
Deficient differentiation of Treg cells in a murine model of Sjögren's syndrome

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Foxp3+ regulatory T (Treg) cells play key roles in the maintenance of immunologic self-tolerance. Disruption in the development or function of Treg cells is a primary cause of autoimmune. Sjögren's syndrome (SS) is an autoimmune disorder characterized by lymphocytic infiltrates and destruction of the salivary and lacrimal glands. However, how autoimmune lesions develop in SS remains unclear. In this study, we examined the roles of Treg cells in pathological immune response of SS.
We first examined whether Treg cells suppress proliferation of effecter T cells using a SS-model, NFS/sld mutant mouse thymectomized at 3 days after birth. Treg cells from SS-model mice expressed similar level of CTLA4, a functional marker Treg cell, to that of control Treg cells and were capable of suppressing in vitro proliferation of effecter T cells comparable to control Treg cells. In addition, the number of Treg cells in SS-model mice was significantly reduced compared with that in control mice. In vitro differentiation of Treg cells from naïve CD4+ T cells of SS-model mice using TGF-β was considerably inhibited compared with that of control mice.
These data suggest that deficient differentiation of the Treg cells in SS-model mice may influence the pathogenesis of SS-model mice. Moreover, to understand phenotypic differences or functions of Treg cells between control and SS-model mice, we are trying to perform DNA microarray analysis.

P5.07 Animal models of autoimmunity

P5.07.01
Biochemical studies on glyoxal mediated glycated histones; Implications for presence of serum antibodies against the glycated histones in patients with type 1 diabetes mellitus

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Reactive carbonyl species (RCS) reacts non-enzymatically with amino groups of lysine and arginine to form advanced glycation end products (AGEs). In the present study role of glyoxal mediated glycoxidation of histone in type 1 diabetes was undertaken. Histone was modified with glyoxal and it was characterized by polyacrylamide gel electrophoresis, UV and fluorescence spectrophotometry. RCS-induced modification was confirmed by quenching study involving penicillamine and aminoguanidine as carbonyl scavengers. Presence of antibodies against glycoxidated histone in sera of type 1 diabetes patients was evaluated by solid phase enzyme immunoassay. Spectral analysis and electrophoresis of the protein clearly demonstrate structural perturbation in the histone by glyoxal. Glyoxal produce AGE specific absorbance and fluorescence in the protein together with cross-linking of histone leading to aggregation. The findings indicate that as a result of structural perturbation in histone by glyoxal, the modified histone was involved in production of serum antibodies in the diabetes patients.
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P5.07.02
Effect of IL-7 on T cell differentiation during autoimmunity

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Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease of the central nervous system mediated by autoreactive CD4+ T cells. IL-17-producing CD4 T (Th17) cells represent a major pathogenic T cell subset in multiple sclerosis and EAE. These cells, which are generated by the combination of IL-6 and TGF-β, require IL-23 receptor-mediated signaling for their maintenance and effector functions. IL-23R- and IL-23p19-deficient mice are resistant to the development of EAE, demonstrating the importance of Th17 and IL-23 in CNS autoimmunity. IL-7 has been proposed to serve as a growth factor for Th17 cells, and IL-23R-deficient T cells were shown to have diminished IL-7Rα expression, preventing sustained IL-7 signaling. Using a transgenic mouse system that constitutively expresses the IL-7R alpha chain (IL-7RαTg), we determined whether sustained expression of IL-7Rα could rescue Th17 expansion and EAE development in IL-23R-deficient (IL-23R KO) mice. Similarly to IL-23R KO mice, IL-7RαTg/IL-23R KO mice were resistant to the development of EAE, indicating that IL-7 itself cannot promote Th17 expansion in the absence of IL-23. Furthermore, IL-7RTg/IL-23R KO cells did not display a proliferative or survival advantage over the IL-23R KO cells upon immunization for EAE. Instead, we observed an inhibitory effect of IL-7 on the differentiation of Th17 cells and the promotion of IFN-γ and GM-CSF secretion by activated T cells. These results address the contribution of IL-7 and IL-23 for Th17 development and maintenance in CNS autoimmunity.

P5.07.03
Systemic autoimmunity caused by a chronic innate response to intracellular nucleic acids

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Chronic type I interferon (IFN) production is a hallmark of the systemic autoimmune disease Lupus erythematosus (LE) and the rare hereditary condition Aicardi-Goutières Syndrome (AGS), a monogenic variant of LE. Although both conditions were associated with spontaneous production of the antiviral type I IFN, a disease-triggering virus could not be detected. Mutations in the genes encoding one of the four intracellular enzymes Trex1, RNase H2, SAMHD1 or ADAR1 have been shown cause AGS. All proteins are involved in nucleic acid metabolism. We generated RNase H2- or SAMHD1-deficient mice. RNase H2 deficiency triggered a spontaneous DNA damage response that lead to early embryonic lethality, caused by defective removal of misincorporated ribonucleotides from genomic DNA. It is presently unclear whether in mice RNase H2 deficiency can also lead to a spontaneous IFN response. In SAMHD1-/- mice, however, we found spontaneous IFN production. Thus, these mice recapitulate an important feature of LE and AGS pathogenesis. The nature of the IFN-inducing nucleic acids in cells defective for one of the AGS-associated enzymes is unclear. All four enzymes were demonstrated to affect retroviral replication. Trex1 deficiency was associated with enhanced retrotransposition of endogenous retroelements. These findings suggest that defects of AGS-associated enzymes could lead to uncontrolled activity of endogenous retroelements. Sensing of their replication intermediates by cellular nucleic acid sensors could activate the IFN response and trigger autoimmunity.

P5.07.04
Lack of T-bet does not inhibit dendritic cell ability to initiate an immune response in a dendritic cell driven model of experimental eutoimmune encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) is a commonly used model of the demyelinating central nervous system (CNS) disease multiple sclerosis (MS). EAE is primarily a CD4 T-cell driven disease, requiring interactions between the innate and adaptive immune cells to initiate disease. Previous studies showed that although CD4 T helper cells with a Th1 phenotype (IFN-ɣ producing) are enriched in EAE, knocking-out or neutralising IFN-ɣ exacerbates disease. Subsequently, the master Th1 transcription factor T-box 21 in T cells (T-bet), has been shown to be required in EAE. T-bet is also present in other leukocytes including dendritic cells (DC), therefore may have a role at multiple stages of disease. In studies using T-bet KO mice and our DC driven EAE model, we have been able to study the interactions between DCs and CD4 T-cells. This has allowed us to independently study the role of T-bet in initiating an immune response in DC, and the resulting inflammatory response to antigen in CD4 T cells. Our data suggest that when provided with activating TLR signals and antigen, T-bet KO DC are able to up-regulate co-stimulatory molecules and induce proliferation in TCR transgenic CD4 cells *in-vitro*. *In-vivo,* these DC are able to provide appropriate stimulation to CD4 T cells, allowing for the induction of EAE. This model provides evidence that T-bet is not required in the innate elements of the EAE immune response, and that the role of T-bet may be restricted to the adaptive elements of the immune response in EAE.

P5.07.05
Genistein derivatives down-modulates pro-inflammatory cytokines in activated J774A.1 cells and reverses clinical signs of experimental autoimmune encephalomyelitis

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Genistein down modulate pro-inflammatory cytokine by inhibiting the NF-κB signaling pathway. Although genistein has some potential in clinical application, it has some disadvantages related to its chemical structure, such as rapid in vivo metabolism and a low concentration in serum after oral administration. In this report, the immunomodulatory effect of genistein derivatives on the production of pro-inflammatory cytokines by activated macrophages and their applications in EAE model were investigated. J774A.1 macrophages were incubated in the presence of genistein or genistein derivatives. Supernatants were collected for cytokine, NO and MTT assay. C57BL/6 mice were immunized with MOG35-55 and treated or not with genistein or genistein derivative. Mice were sacrificed on the 21st dpi and brains were removed for histological, cytokine production and cell markers analysis. In J774A.1 macrophages the genistein has inhibited both IL-12 and TNF-α, the lipophilic derivatives showed high capacity to inhibit IL-12 without altering TNF-α, indicating a more specific mechanism in the modulation of IL-12 and the glycosylated derivative AAP37A showed elevated inhibition of NO (100.0±0.5), IL-1β (100.0±12.9) and IL-6 (94.1±5.9). Treatment with O-tetradecanoyl-genistein derivative improved prognosis of EAE by reduction of inflammatory infiltrate, decrease of pro-inflammatory cytokines and reduction of cells producing IL-17 (0.6±0.03 vs. 3.5±0.6), and increasing of cells producing IL-10 (0.6±0.1 vs. 0.1±0.07) and expressing FOXP3 (1.4±0.2 vs. 0.3±0.1) and CTLA-4 (4.8±0.4 vs. 1.0±0.04) in the central nervous system. Thus, these derivatives showed potential to be used in the treatment of inflammatory diseases and deserve further investigations.
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P5.07.06
Genistein modulates TLR3, TLR4 and TLR9 in antigen presenting cells and delayed clinical signs of experimental autoimmune encephalomyelitis

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Genistein is an isoflavonoid derived from soy that can affect many different cellular mechanisms such as the inhibition of NF-κB activation factor, known to play a critical role in inflammation, immune modulation and cell proliferation. In this preliminary report, the immunomodulatory effect of genistein in innate immune response in EAE model, by analyzing the expression of Toll-like receptors were investigated. C57BL/6 mice were treated or not with genistein 7 days before the induction. The EAE were induced by immunization with MOG35-55 emulsified in complete Freund's adjuvant supplemented with 400µg of attenuated *Mycobacterium tuberculosis* H37RA. Pertussis toxin, 300ng/animal, was injected intraperitoneally on the day of immunization and 48h later. Animals were monitored daily until 21st dpi and neurological impairment was quantified. Mice were sacrificed on the 7th dpi and spinal cords were removed for analyses of TLR3, TLR4 and TLR9 by F4/80+ or CD11c+ cells. Genistein treatment delayed clinical signs in the 12th dpi in comparison to group not treated (0.3±0.1 vs. 2.2±0.6). The group treated with genistein showed an elevation of mean fluorescence intensity (MFI) of cells F4/80+ expressing TLR3 (2,232.0±35.1 vs. 1,105.0±86.8), TLR4 (1,219.0±21.9 vs. 1,095.0±39.4) and TLR9 (1,803.0±35.5 vs. 942.7±29.4) in relation to EAE not treated. CD11c+ cells showed increased MFI of TLR3 (1,486.0±53.5 vs. 631.1±76.3) and TLR9 (1,508.0±62.7 vs. 918.7±65.2) in relation to EAE not treated and reduced MFI of TLR4 (596.7±27.1 vs. 743.4±50.9). The results suggest that alterations in the TLR expression could have important roles for the evolution of EAE.
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P5.07.07
Prolactin levels correlate with abnormal B cell maturation in MRL and MRL/lpr mouse models of systemic lupus erythematosus-like disease

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Prolactin (PRL) has an important role in modulating the immune response. PRL is secreted by the pituitary gland and many other organs and cells, among them lymphocytes. In B cells PRL enhances antibody production, including those with self-specificity; as such PRL has been associated with B cell triggered autoimmune diseases, such as *systemic lupus* erythematosus (SLE). In this study, our aims were to determine the expression of PRL-receptor during bone marrow B cell development and whether the presence of high PRL serum concentration influences receptor expression, absolute numbers of developing populations and disease outcome in lupus-prone murine models. Using real time-PCR and flow cytometry, we observed that the PRL-receptor is expressed in bone marrow early B cells (pro-B, pre-B, immature); in lupus prone mice the highest level of expression was found in pro-Bs and immature cells, an expression pattern that differed with the one of wild type early B cells. In hyperprolactinemia, pro-B cells from lupus prone strains further down-regulated the expression of the PRL-receptor correlating with a decrease in the absolute numbers of both pro-Bs and immature B cells. In this latter population the anti-apoptotic BIRC5 gene was increased in hyperprolactinemia, also coinciding with a higher activity of the disease. These results support an important role of PRL in the early stages of the B cell maturation process, thus helping to clarify its relevance in the development of SLE.

P5.07.08
Vitamin D does not boost MOG therapeutic effect in experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) associated with a destructive immune response directed against CNS antigens. Most MS treatments cause general immunosuppression that determines severe side effects. Therefore, induction of antigen-specific tolerance is highly desirable as a disease treatment. The active form of vitamin D3 (vitD3) has immunomodulatory properties as induction of tolerogenic dendritic cells and regulatory T cells. We studied the therapeutic potential of myelin oligodendrocyte glycoprotein (MOG) alone or associated with vitD3 supplementation in experimental autoimmune encephalomyelitis (EAE) that is a MS model. Female C57BL/6 mice were submitted to EAE induction by immunization with MOG emulsified in CFA plus BCG and also injected with two intraperitoneal doses of *Bordetella pertussis* toxin. One day after immunization, mice were treated with MOG or MOG associated with vitD3. Animal were injected every other day for 15 days (on days 1, 3, 5, 7, 9, 11, 13 and 15) with vitD3 (0, 1µg). On days 3 and 11, MOG was coinjected with vit D3 or alone. Both therapeutic schedules were similarly protective. They determined significant reduction in EAE incidence and also in clinical scores. MOG, but not MOG/vitD3 treatment, avoided the characteristic weight loss during the acute phase of the disease. The MOG treated group also produced high levels of IL-10 in response to *in vitro* MOG stimulation. These findings indicate that MOG by intraperitoneal route was highly tolerogenic. They also show that addition of vitD3 did not boost this effect.

P5.07.09
Galectin-9 contributes to enhanced experimental autoimmune encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) is a typical autoimmune disease which is an inflammatory and demyelinating disease in murine central nervous system (CNS). It is used as an animal model of human multiple sclerosis (MS) because its symptoms are similar to MS. Meanwhile, galectins are a family of glycan-binding proteins and divided into subtypes based on their structure. Some members of galectin family such as galectin-1, galectin-3, and galectin-9 (Gal-9) have been reported to play an important role in the development of innate and adaptive immune responses. Especially, Gal-9 is reported to induce cell death of terminally differentiated Th17 cells via interaction with Tim-3 while it suppresses Th17 development in an IL-2 dependent but Tim-3 independent manner in EAE. In contrast, the role of Gal-9 in the autoimmune disease remains uncertain and its function has not been fully elucidated in EAE. In this study, we examined the association between Gal-9 expression and EAE progression. We observed an incremental rise in Gal-9 level in C57BL/6 mice as EAE progressed, with a sharp rise by peak of disease. This suggests possible biological connection between Gal-9 and EAE warranting further exploration of its function in EAE. It also suggests that the identification of relationship between Gal-9 and EAE progression is critical for better understanding Gal-9 biology. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2012011645).

P5.07.10
Neutrolphils infiltration, increased IL-1 production and SAPHO syndrome-like phenotype in PSTPIP2 deficiency mice

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Proline-serine-threonine-phosphatase-interacting protein 2 (PSTPIP2) belongs to the Pombe Cdc15 homology (PCH) family of proteins involved in actin-based cytoskeletal functions, including membrane ruffing, filopodia formation, motility and adhesion, mainly expressed in macrophages. Previous studies have been reported that mice with missense mutation in *Pstpip2* gene developed an autoinflammatory disorder presenting multiple sterile inflammatory bone lesions. In this report, we generated PSTPIP2 knockout (*Pstpip2*$^{-/-}$) murine model. All affected mice developed paw swelling, synovitis, hyperostosis, osteitis, resembling SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis, osteitis), an inflammatory disorder of the bone, skin, and joints. Histological examinations of *Pstpip2*$^{-/-}$ mice revealed multifocal osteomyelitis in the inflamed paws with increased macrophage and marked neutrophil infiltrations in the bone, joint, and skin. Micro-computed tomography revealed profound osteolytic lesions with marked osteoporosis in the paws and limbs. Spenomegaly and enlarged lymph nodes with increased neutrophils also developed. Neutrophil attracting chemokines (CXCL1, CXCL2, CXCL5, MIP-1α and IL-8) and inflammatory cytokines (TNFα, IL-6, and IL-1β) were elevated from macrophages of the inflamed tissues. These studies suggest that PSTPIP2 may play a role in innate immunity and the development of autoinflammatory bone disorders.

P5.07.11
Induction of SLE-like syndrome in Balb/c mice by immunization with active chromatin

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Introduction: Systemic lupus erythematosus is an autoimmune disease, which is characterized by production of autoantibodies against self- antigens specially DNA. Disease is multifactorial and the main cause of it is still unknown. Thus animal models of SLE can be usefull for studying the cellular and molecular mechanisms which are involved in the disease pathogenesis.
This study aim to stablish a murine model for SLE-like syndrome by immunization with active chromatin for assessment of vitamin D effects on Th17 cells and related cytokines in SLE.
Methods and Materials: Normal female Balb/c mice (6-8 weeks) were immunized subcutaneously with 50 µg active , and non- active chromatin, derivated from spleen lymphocyte cells. Proteinuria was measured by coomassie brilliant blue asssay and anti-ds DNA antibodies determined in peripheral blood samples by using enzyme-linked immunosorbent assay kit. Kidney sections viwed by a pathologist for detection of immune complexes deposition. Blood and urine samples of the mice were collected every two weeks after final immunization for 8 weeks
Results: The mice immunized with active chromatin had higher levels of proteinuria with pick value in the last week in comparison to the mice immunized with non-active chromatin. This project in under study, and the results demonstrating the effects of Vitamin D on lupus like mice will be presented in the the congress.
Conclusion:Active chromatin can induce SLE-like syndrom in normal mice. This modle can help us for studying the pathogenesis of SLE.

P5.07.12
X-ray irradiation suppresses SLE-like morbidity in NZBWF1 mice

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CD180 molecule is a homologue of TLR-4 and expresses on mature B cells. The molecule shows a protective effect against radiation- or dexamethasone-induced apoptosis *in vitro*. CD180 is a key regulator of cell proliferation and death. We previously showed CD180-negative spleen B cells exhibited sensitivity for radiation-induced apoptosis compared with CD180-positive cells *in vivo*. In SLE patients, the number of CD180-negative B cells increased in peripheral blood, and appeared to be associated with the activity of SLE. In addition, such negative cells collected from SLE patients produced autoantibodies *in vitro*. We previously reported CD180-negative B cells might lead to development of SLE morbidity in SLE-model mouse, NZBWF1. In the present study, we attempted to examine the effects of X-ray irradiation on CD180-negative B cells in NZBWF1 female mice. After irradiation, the mean life span of the irradiated mouse group was significantly extended compared with that of the sham-irradiated one. In addition, the latency to the development of grade three-proteinuria in irradiated mouse is significantly longer than in sham-irradiated one. The amount of complement C3 in peripheral blood of the irradiated mouse was sustained at normal level, while that of sham-irradiated mouse was markedly decreased at 3 weeks after sham-irradiation. These findings suggest that radiation could effectively eliminate CD180-negative B cells, leading to reduction of autoantibody production, and thus relieve SLE symptoms. The findings also suggest a possibility that the radiotherapy into autoimmune diseases associated with the expression of CD180-negative cells might be a novel practical application.

P5.07.13
Autoantibody repertoire and inflammatory myeloid cell expansion drive the transition from autoreactivity to autoimmunity in a novel mouse model of systemic lupus erythematosus

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The mechanisms regulating the transition from autoreactivity (loss of tolerance and autoantibody production) to autoimmunity (immune-mediated end organ damage) are incompletely understood. CD45E613R mice express a point mutation in juxtamembrane wedge that results in dysregulated phosphatase activity. Despite similar hyperactivation of ITAM-mediated signaling pathways in multiple cell lineages, the phenotypic consequences of this mutation vary with genetic background. CD45E613R BALB/c mice develop high titer anti-dsDNA antibodies but no end organ disease while C57Bl/6 (B6)-129 F1 mice develop anti-dsDNA antibodies and severe glomerulonephritis. The genetic separation of autoreactivity from autoimmunity makes this an ideal model system for identifying risk factors and novel targets in lupus nephritis. Interestingly, both B6-129 F1 and BALB/c CD45E613R mice have extensive IgG/immune complex and C3 deposition in their kidneys. However, comparison of the autoantibody repertoire in B6-129 F1 and BALB/c CD45E613R mice indicate that CD45E613R B6-129 F1 mice have a broader repertoire of autoantibodies, particularly of the more pathogenic IgG2a subtype. Using flow cytometric and immunohistochemical approaches to compare infiltrating and resident hematopoietic cells in the kidneys reveals an expansion of monocytes and skewing towards an M1 macrophage phenotype in the B6-129 F1 background. Consistent with this, IL-1α, IL-6, TNF-α and MCP-1 are elevated in B6-129 F1 but not BALB/c CD45E613R mice. Taken together, these data support a model in which an expanded antibody repertoire combined with altered kidney immune cell subsets and increased inflammatory cytokines mediate the transition from autoreactivity to autoimmunity.

P5.07.14
Specificity of antinuclear antibodies in Swiss Webster mice following exposure to gold salts and mercury

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Exposure with gold in genetically susceptible inbred mouse strain, A.SW (H-2s), indicated that gold caused autoimmunity characterized by lymphoproliferation, development of anti-nuclear antibodies targeting the nucleolar protein fibrillarin, hypergammaglobulinemia and development of immune-complex deposits. In this study we investigated the effect of gold on the immune system of the outbred Swiss Webster (SW), and the congenic A.SW strain derived from A/WySn and Swiss Webster.
Mice were given either weekly intramuscular injections of gold in the form of natrium aurothiomaleate (AuTM) for 15 weeks and ontrols received equimolar amounts of PBS.
Using indirect immunofluorescence detection of serum antibodies in response to AuTM, all SW mice developed anti-nuclear antibodies while A.SW mice produced significant amounts of anti-nucleolar antibodies. Further investigation of the autoantibodies specificity by line immunoassay (LIA) and Western blotting indicated that gold-induced autoantibodies in SW mice targeting mainly nucleosomes, histone H1 and H2A whereas; autoantibodies developed in A.SW were targeted fibrillarin. The effects of gold on lymphocyte subpopulations were determined by flow cytometry indicating lymphoproliferation of T- and B-cells, proliferation of T-helper cells and particularly proliferation of CD8 cytotoxic T-cells in SW mice.
Based on these observations we conclude that i) in these mice the interaction between gold and the immune system may differ by generation of autoantibodies targeting different nuclear- and nucleolar proteins, and ii) presence of particular and known susceptible genes play a key role in development of murine autoimmune response.

P5.07.15
Effect of mercury (Hg) and silver (Ag) in mice model of autoimmunity- Re-challenging of mice by additional heavy metal exposure leads to acceleration of the autoimmune response

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The effect of mercury (Hg) and silver (Ag) in genetically susceptible A.SW mice is characterized by anti-nucleolar antibodies (ANoA), lymphoproliferation and systemic immune-complex (IC) deposits. We used A.SW mice to see whether the autoimmune response is stronger and faster following a secondary exposure, implying a memory in the immune system.
The mice receiving mercury, silver or drinking water during 3 weeks (phase-1). The following thirteen weeks the mice groups were given only tap water (phase-2) followed by exposure with mercury in all groups for further 5 weeks (phase-3). Controls were given drinking water until phase-3. Analyses were consisted of ANA, IgG concentration, immune-complex depositions in kidney and spleen, and accumulation of mercury in the whole body retention (WBR) and organs using a gamma counter.
Regardless of the phase-1 exposure, mice that were exposed with either mercury or silver induced a higher and faster increase of fraction of ANoA IgG subclasses as well as the deposition of splenic and lymphatic nodes of Hg compared with control mice at phase-3. The level of IgG1 and IgG2a accelerated faster in phase-3 when mice were exposed to two different metals. Renal immune-complex deposits were more marked when mice treated twice with heavy metals.
We conclude that for certain parameters i.e. ANA and tissue- and WBR mercury deposits, an B-cell restricted immunological memory plays important role while increased amount of IgG1 and IgG2a is particularly controlled by sensitization of mice twice with at heavy metals.

P5.07.16
Human parvovirus B19 NS1 protein aggravates hepatic fibrosis in NZB/W F1 mice

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Human parvovirus B19 (B19) has been associated with a variety of diseases. However, the effects of B19 viral proteins on liver in SLE are still obscure. Therefore, we aimed to investigate the effects of B19 on liver in NZB/W F1 mice by injecting subcutaneously with PBS, recombinant B19 nonstructural protein (NS1), VP1 unique region (VP1u) or VP2, respectively. Our results revealed that B19 NS1 protein significantly enhanced the transforming growth factor β (TGF-β)/Smad fibrotic signaling by increasing the expressions of TGF-β, Smad2/3, phosphorylated Smad2/3, Smad4 and Sp1. The consequent fibrosis-related proteins, PAI-1 and α-smooth muscle actin (SMA), were also significantly induced in livers of NZB/W F1 mice receiving B19 NS1 protein. In addition, markedly increased collagen deposition was also observed in livers of NZB/W F1 mice receiving B19 NS1 protein. Meanwhile, no significant difference was observed in livers of NZB/W F1 mice receiving B19 VP1u or VP2 as compared to the controls. These findings indicate that B19 NS1 plays a crucial role in exacerbating liver fibrosis in NZB/W F1 mice through enhancing the TGF-β/Smad fibrotic signaling.

P5.07.17
RORγt transgenic mice developed spontaneous sialadenitis like Sjögren's syndrome

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[Backgrounds] The aim of this study was to clarify the pathological role of RORγ in sialadenitis using RORγt transgenic (Tg) mice under the promoter of hCD2 which spontaneously developed sialadenitis like SS.
[Methods] 1) Histological analysis of salivary glands (SG) was determined and saliva flow was measured. 2) Infiltrating cells of SG were analyzed by fluorescent immunostaining and flow cytometry. 3) Analysis of CD4+ T cells in spleen, cervical lymph node (cLN) and SG were performed by quantitative PCR. 4) Splenic CD4+ or CD4- cells were transferred to Rag2-/- mice (CD4+or CD4-→Rag2-/-) and histological analysis was examined.
[Results] 1) RORγt Tg mice developed the severe sialadenitis like SS. Saliva flow of RORγt Tg mice was significantly decreased than that of C57BL/6 mice. 2) Majority of infiltrating cells was CD4+ T cells at early phase of sialadenitis, and B cells were gradually increased at late phase. 3) CD4+ T cells from spleen, cLN and SG retained effecter memory phenotype. Expression of T-bet, GATA3 and Bcl6 in CD4+ T cells were significantly higher than in SG than in spleen and cLN. Subset of Th17 (IL-17, IL-23r), Th1 (IFN-γ, T-bet), Th2 (IL-4, GATA3) and Tfh (IL-21, Bcl-6) related molecules were increased in salivary glands. 4) In CD4+↔Rag2 -/-, sialadenitis was observed, but not in CD4-→Rag2-/-.
[Conclusion] These results suggested that the overexpression of RORγt on CD4+ T cells play a crucial role in the development of spontaneous sialadenitis like SS.

P5.07.18
Early local IL-12p40/p(40)2 production and intensity of peritoneal inflammation correlate with susceptibility to pristane induced arthritis.

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Genetically selected HIII (high antibody producer) mice are resistant, while LIII (low antibody producer) are susceptible to pristane-induced arthritis (PIA). The peritoneum (PerC), site of PIA induction, is rich in myeloid and lymphoid cells and might be the site where the genetic divergence in susceptibility of HIII/LIII mice would translate into functional differences, affecting the early inflammatory cell response and cytokine balance. Mice were injected with 0.5mL pristane i.p. and after 48h, 4, 7, 15 or 30 days, the PerC was washed with RPMI1640+10%FBS. The supernatant was used for cytokine ELISA and cells were counted in hemocytometric chambers and phenotyped by FACS. Inflammatory cell infiltration was highest in LIII mice at 7 days, with increased numbers of most cell populations. Leukocyte infiltration in HIII mice, consisting mostly of neutrophils, increased slowly until d30, while monocytes/macrophages and lymphocytes were reduced. Dendritic cells and T cells increased only in pristane treated LIII mice. Both lines produced similar amounts of CCL-2, IL-1beta, IL-6, IL-4, and low levels of IL-12p70 after pristane injection, while IFN-gama was not detected. However, IL-12p40/p$(40)_2$ was produced only in LIII mice, increasing from d4 to d30. Our results suggest that the divergent susceptibility of HIII/LIII mice might be linked to their distinct peritoneal response to pristane, and that IL-12p40/p$(40)_2$, which is mainly produced by dendritic cells, might be involved by promoting dendritic cell recruitment and migration to lymph nodes, thereby increasing the potential of autoreactive T cell activation in susceptible LIII mice.
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P5.07.19
Immune regulation of monophasic experimental autoimmune uveitis influencing relapsing disease

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Rat experimental autoimmune uveitis (EAU) can be induced with S-Ag peptide PDSAg, (monophasic EAU) or IRBP peptide R14 (relapsing EAU). We have previously shown different dynamics of intraocular T cell populations during the two types of EAU, but the exact mechanisms behind the disease courses remain elusive. Here we used different combinations of the two antigens for immunization (either administered separately at contralateral sides or as a mixture of both) to investigate the mutual influence on the disease course and the immune response. Clinical uveitis was determined daily and cytokine pattern (IFN-γ, IL-17, IL-10) and Foxp3-expression of intraocular cells was determined at onset, peak and resolution of disease. Data of the combined immunizations were compared with that from the conventional PDSAg and R14 immunizations. While in R14-induced EAU 75% of the eyes developed relapses, none of the PDSAg- or PDSAg/R14-mixture immunized rats had recurrences. However, contralateral administration of both antigens allowed relapses in 12.5% of eyes. The cytokine pattern of intraocular cells looked similar in those animals immunized with both antigens, but differed from the pattern of the rats which were immunized with PDSAg or R14 only. Rats immunized with both antigens showed an R14-like cytokine pattern at onset of EAU and a PDSAg-like cytokine expression at resolution. Disease course and cytokine pattern of intraocular cells confirmed a dominant role of the monophasic, PDSAg-specific immune response. These data indicate a strong peripheral (mixed immunization: no relapse) and a slightly less stringent intraocular regulation (contralateral immunization) of the autoimmune response.

P5.07.20
Restriction of TCR diversity in NOD mice inhibits the development of Type I Diabetes but not Sjögren's syndrome

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The NOD mice serve as a well-established model of independently developing autoimmune diseases, Type 1 Diabetes (T1D) and Sjogren's syndrome (SjS). One of the striking differences in their development is that T1D depends on exclusive presence of I-Ag7 molecules, whereas SjS develops regardless of the MHC haplotype, suggesting different sensitivity to diversity of self MHC/peptide complexes. So far it has been shown that restriction of TCR precursor frequency in TCRβ transgenic NOD mice doesn't prevent development of T1D. It suggests that, regardless of the initial TCR diversity it's the inherent defect in the thymic selection that allows for escape of pathogenic T cells.
Surprisingly, when we generated transgenic mice using TCRVβ14 chain originally selected on I-Ab molecule we didn't observe development of T1D but SjS developed normally. This lack of T1D development is associated with absence of CD4 cells specific to islets antigens in the periphery, as we didn't detect CD4 cells specific for INSp9-23 or GAD65p524 peptides and co-transfer of wild type CD8 cell didn't help in initiation of the disease. This suggests that reduction of TCR precursor frequency in NOD mice allows for efficient thymic selection that prevents escape of T1D-pathogenic T cells. To further reduce TCR diversity, we generated NOD.mini mice, where the same TCRβ chain is co-expressed with TCRα chains from TCRα.mini locus. This greater restriction of TCR repertoire still allows for SjS development indicating that onset of the disease for SjS is less dependent on a particular antigen for CD4 cells than onset of T1D.

P5.07.21
Spatial learning and memory impairment in an acute experimental autoimmune encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) could be associated with cognitive disturbances, so the aim of the study was investigation of spatial learning in mice with EAE. EAE was induced in eight-week-old C57BL/6 female mice by subcutaneous immunization (MOG35-55 in complete Freund's adjuvant) and pertussis vaccine intraperitoneally. Animals were scored daily for clinical signs of EAE. Morris water maze test (MWM), consisting of a circular tank filled with water and a submerged platform in a fixed spot was performed before and after EAE induction. The path, time, and motor funcion during MWM test were observed. Hematoxylin-eosine staining of brain tissue was used for histological examination. After two trials mice mostly swam in the quadrant with the platform and after 8 training days all the mice swam directly to the platform. 50 % of mice with EAE showed partial loss of tail tonicity approximately 13 days post immunization. In all mice with EAE hind limb weakness after swimming was observed 18 days post immunization. Significant difference ($p<0.05$) in the time for finding the platform was found between mice with mild clinical signs of EAE in comparison with other mice (EAE without symptoms and non-treated). Interindividual behavioural difference within mice with EAE signs were observed, in 40 % there were no changes while in 60 % signs of disorientation and anxiety-like behaviour was noted. Histopathological examination showed lesions in the brain parenchyma, especially in the hippocampal region of EAE mice. Hippocampal tissue destruction in EAE mice indicates the cognitive learning and memory impairment detected.

P5.07.22
Altered Lyn activity leads to exaggerated Th2-immunity or multiple chronic obstructive pulmonary disease-like changes in mouse

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Lyn tyrosine kinase is intricately important in both signaling initiation and down modulation as there are multiple implications with altered Lyn's activity. Lyn knock out (Lyn(-/-)) mice develop lupus-like autoimmune disease and has allergic traits. The Th1/Th2 paradigm explain autoimmunity and allergy/atopic disease manifestations as caused by extremes of two opposing immune responses which creates an apparent contradiction of coexisting autoimmune disease and atopy in the Lyn(-/-) mouse model. To investigate this balance between Th1 versus Th2-immune responses, the regulator of Th2 immunity, STAT6 was deleted. Atopy and spontaneous peritoneal eosinophilia, characteristic of Lyn(-/-) mice, were lost in young Lyn(-/-)STAT6(-/-) mice; however, autoimmune disease was markedly exacerbated. Furthermore, aged Lyn(-/-)STAT6(-/-) mice showed dramatically increased levels of serum IgE but minimal IgG1, suggesting that class-switching to IgE can occur in the absence of an IgG1 intermediate. The results show that Lyn-deficient mice can overcome the effects of disabling Th2 immunity, highlighting the importance of Lyn in down modulating Th2 immune responses. On the other hand, deregulated signaling initiation by Lyn was implicated in myeloid leukemia and several types of epithelial cancer. The Lyn knock in (Lyn(up/up)) mutant mouse was created to investigate putative proto-oncogenic roles of Lyn. Lyn(up/up) mice develops chronic lung inflammation, emphysema at young age and some eventually had lung tumors. The Lyn(up/up) mouse will provide an excellent model to investigate the co-determinant that leads to both airspace tissue destruction and lung cancer which deceivably seems like two opposite outcomes of lung disease that co-develops in COPD patient.

P5.07.23

Assessment of vitamin D effects in lupus- like syndrome induced mouse model

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Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by auto-reactiveT cells and serological presence of anti-double-stranded DNA and lethal glomerulonephritis. The etiology of the SLE is not clear yet, but is considered combination of multiple genetic and environmental factors involved in the pathogenesis of disease. Vitamin D is one of the main environmental factors that have immunomedulatory effects on immune system. The majority of SLE patients are reported to have insufficient or deficient serum levels of vitamin D. In this study we aim to assay Vitamin D effects on SLE in lupus-like syndrome induced mice models.
Materials and Methods : Normal female Balb/c mice (6-8 weeks) were fed 50 ng/day active form of vitamin D from two weeks before immunization with 50 µg active DNA and some of mice fed vitamin D at the same time of immunization for 8 weeks. Urine and blood samples were collected every two weeks after final immunization and examined for protein and anti-ds DNA respectively.
Results: Proteinuria decreased in mice which received vitamin D compared with control mice and didn't show any clinical aspects of SLE disease. This project in under study, and the results demonstrating the effects of Vitamin D on lupus like mice will be presented at the congress.
Conclusions: Studies on vitamin D effects on autoimmune diseases can provide new therapeutic strategies in the treatment of SLE.

P5.07.24

B lymphocyte-induced maturation protein 1 (BLIMP-1) attenuates autoimmune diabetes and encephalomyelitis by suppressing Th1 and Th17 cells

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Recently, Blimp-1 expands its control over T cells and is associated with susceptibility to colitis in mice with T cell-specific Blimp-1 deficiency. However, its role in regulating other autoimmune diseases is unclear. We demonstrate that transgenic expression of Blimp-1 in T cells significantly decreases the incidence of diabetes and MOG_{35-55}-induced encephalomyelitis in NOD mice. In contrast, mice lacking Blimp-1 in T cells developed markedly increased Th1 and Th17 cells and exacerbated encephalomyelitis. Our results reveal that Blimp-1 orchestrates a T cell-specific modulation on autoimmunity by affecting the cell proliferation, Th1 and Th17 cell differentiation, and Treg cell function. We also demonstrate that Blimp-1 suppresses IL-23R and GM-CSF which are essential for Th17 pathogenicity and that Blimp-1-mediated Th17 downregulation is through a RORα-RORγt-dependent pathway. Strikingly, overexpansion of Th1 and Th17 cells in CKO mice is significantly restored by introducing a Blimp-1 transgene, reinforcing the emerging role of Blimp-1 in autoimmunity and providing a basis for developing therapeutic strategies.

P5.07.25

Dominancy of encephalitogenic peptide itself directs sustainable remission of a model of multiple sclerosis, through later expansion of 'armoured' effector regulatory T cells: possibility of vaccination for autoimmune disease

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Autoimmune diseases occur as dysregulation of host defense caused by imbalance of pathogenic and protective cells. The mechanism of their chronicity is still unveiled and even most effective therapy cannot completely inhibit their activity.
Focusing on experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS), which represents relapsing-remitting initially and progressing course later, we found out that two correlated non-genetic factors can shut down the chronicity, from the phenomena that immunizing peptide itself determines the difference between monophasic-EAE and relapsing-EAE.
One is kinetics and component of $CD4^+CD25^+$ regulatory T cells (Treg), which are characterized by their expansion after emerging of encephalitogenic T cells and by their subset expressing both CD69 and CD103 (=DP-Treg). DP-Treg is the most efficacious subset showing highest Treg-dominant signatures and exerting stability even under inflammatory milieu due to continuously lowest expression of IL-6R, nevertheless with activated effector phenotype. DP-Treg also possesses hybrid Tregs like $Foxp3^+RORgt^+$ and $Foxp3^+Tbet^+$ cells, concordant to Th17 and Th1. Therefore we term DP-Treg for "armoured" Treg.
The other is hierarchy of encephalitogenic peptide itself, which means that the more dominant peptide can develop acute EAE, the less relapse and re-induction of EAE occurs. The dominancy of peptide is characterized by specificity to itself, that is, it can suppress response to another peptides broadly and lead to permanent remission, unlike peptide-specific tolerance.
Taken together, efficient induction of "armoured" Treg is correlated with dominancy of self-peptide itself, suggesting 'immunological homunculus'. This can be applied for treatment such as tissue-specific vaccination for autoimmune diseases.

P5.07.26

Aged IRF-4-deficient lupus-prone MRL/*lpr* mice show peripheral B-cell deficiency with elevated serum IFN-γ levels and increased expression of IFN-γ-receptor-1 on B cells

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The transcription factor Interferon Regulatory Factor-4 (IRF-4) is required for early B-cell development, and is also influential at these later stages of B-cell development and function in antibody somatic hypermutation, class-switch recombination and secretion. IRF-4 is also required for T-cell development in differentiation into Th2/Th9/Th17 cells. At the 14th ICI, we reported that *Irf4*$^{-/-}$ lupus-prone MRL/*lpr* mice developed granulomatous lesions in multiple organs with significantly increased numbers of IFN-γ-producing Th1 cells compared to wild-type littermates. In the present study, we further investigated the role of IRF-4 in B-cell development in MRL/*lpr* mice.
At 6 weeks of age, there was no statistically significant difference of splenic $CD19^+IgM^+$ B-cell numbers between *Irf4*$^{-/-}$ and wild-type C57BL/6 or MRL/*lpr* mice. Unexpectedly, *Irf4*$^{-/-}$ MRL/*lpr* mice showed significant loss of splenic $CD19^+IgM^+$ B cells (<1/20 of wild-type littermates) after 12 weeks of age. Similar splenic B-cell loss was observed in age-matched *Irf4*$^{-/-}$ MRL-*Fas*$^{+/+}$ mice but not in *Irf4*$^{-/-}$ C57BL/6 mice. Multiplex cytokine analysis revealed significant increase in serum IFN-γ levels in *Irf4*$^{-/-}$ MRL/*lpr* mice compared to wild-type littermates and *Irf4*$^{-/-}$ C57BL/6 mice. FACS analysis showed abundant expression of IFN-γ-receptor-1 (IFNgR1) on splenic B cells of MRL/*lpr* and MRL-*Fas*$^{+/+}$ mice, while minimal IFNgR1-expression was observed on those of C57BL/6 mice. *Irf4*$^{-/-}$ *IFNgR1*$^{-/-}$ MRL/*lpr*

mice showed full restoration of splenic $CD19^{+}IgM^{+}$ B cells even after 12 weeks of age.
Our results suggest that lack of IRF-4 has a significant impact on serum IFN-γ levels and peripheral B-cell survival/maintenance likely via IFNgR1 in lupus-prone MRL background.

P5.07.27
Temporal lineage tracing of Aire-expressing cells reveals a requirement of Aire for their maturation program

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Understanding the cellular dynamics of Aire-expressing lineage(s) among medullary thymic epithelial cells (AEL-mTECs) is essential to gain insight into the roles of Aire for establishing self-tolerance. We sought to monitor the maturation program of AEL-mTECs by a temporal lineage tracing in which bacterial artificial chromosome transgenic mice expressing tamoxifen-inducible Cre recombinase under the control of the Aire regulatory element were crossed with reporter strains. We estimated the half-life of AEL-mTECs subsequent to Aire expression approximately 7~8 days, which was much longer than previously reported due to the existence of post-Aire stage. We found that loss of Aire did not alter the overall lifespan of AEL-mTECs, inconsistent with the previous notion that Aire expression in mTECs might result in their apoptosis for efficient cross presentation of self-Ags expressed by AEL-mTECs. In contrast, Aire was required for the full maturation program of AEL-mTECs as exemplified by the lack of physiological down-regulation of CD80 during the post-Aire stage in Aire-deficient mice, which accounts for the increased CD80high mTECs abnormally seen in Aire-deficient mice. The results further enforce the roles of Aire in the differentiation program of AEL-mTECs.

P5.07.28
Natural killer cells promote induction of experimental autoimmune encephalomyelitis but are protective at the acute stage of disease

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Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model of multiple sclerosis and its induction is associated with the infiltration of encephalitogenic T cells into the CNS. Autoantigen-specific IL-17-producing Th17 cells and IFN-γ-producing Th1 cells are central to the pathogenesis of EAE. However, cells of the innate immune system, including γδ T cells and natural killer (NK) cells are also involved. Here we examined the role of NK cells in the induction and effector stages of EAE. Depletion of NK cells with anti-asialo GM1 at the induction of EAE led to a delayed onset of clinical signs with reduced disease severity. This correlated with a reduction in the numbers of Th1, Th17 and NKT cells infiltrating the CNS as well as a marked reduction in microglia in the CNS. In contrast, depletion of NK cells at onset of symptoms increased disease severity, while depletion at induction and throughout the course of EAE delayed the onset of clinical signs but enhanced disease severity once symptoms developed. We observed a similar pattern of delayed onset but more severe disease following induction of EAE in IFN-γ-/- mice (without NK cell depletion). These findings suggest that IFN-γ production by NK cells is involved in the induction of pathogenic immune responses that mediated EAE but is protective at the acute stage of disease, most likely by suppressing pathogenic Th17 cells.

P5.07.29
Expression of CXCR3 on specific T cells is essential for homing to the prostate gland in an experimental model of autoimmune prostatitis

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Experimental Autoimmune Prostatitis (EAP) is considered a valid a model for the human disease Chronic Prostatitis/Chronic Pelvic Pain Syndrome. In the present report, we analyzed phenotypic characteristics of T cells that gain access to the prostate and induce leukocyte recruitment in mice with different susceptibility to EAP. After EAP induction, NOD mice developed a specific cellular response characterized by a mixed Th1/Th17 pattern with specific T cells mainly expressing CXCR3 that infiltrated and damaged the prostate. In contrast, NOD-IFNg-/- mice, exhibited only Th17 cells mainly expressing CCR6 that were not capable of infiltrating the prostate gland. Adoptive transfer experiments of T cells from NOD or NOD-IFNg-/- mice to NOD-SCID recipients showed that only T cells from NOD mice were detected in the prostate gland. However, after “in vitro” or “in vivo” treatment with rIFNg T cells from NOD-IFNg-/- mice became capable of homing to the prostate and induced leukocyte recruitment. Chemokine levels in prostate tissue from NOD mice showed increased expression of CXCR3 ligands. Additional experiments using adoptive transfer of sorted CXCR3+CD3+ or CXCR3-CD3+ T cells or administrating a CXCR3 antagonist treatment confirmed these previous findings. Results demonstrated that the functional chemokine receptor was required for homing to prostate. Altogether, our results demonstrate that the expression of CXCR3 on effectors T cells is essential for their homing to the prostate gland in EAP model. CXCR3 and their ligands, emerge as a potential therapeutic target to control in an inflammation processes in the prostate gland.

P5.07.30
Analysis of the immune response and the prostate-reactive T cell repertoire in a mouse model of experimental autoimmune prostatitis in NOD mice

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Autoimmunity has been proposed as an etiology of the prevalent disease Chronic Pelvic Pain Syndrome. Experimental Autoimmune Prostatitis (EAP) model has proved useful for the study of CPPS because it has mirrored most findings in patients. Herein, we studied the immune response and the prostate-reactive T cell repertoire in EAP in NOD mice.
Six- to 8-week-old NOD/LtJ mice were immunized with a prostate protein extract (PAg group), purified Prostatein (PSBP group), or vehicle (Control group) at days 0 and 15. Animals were euthanized on days 10 and 24 and the specific immune response was evaluated. CDR3-lenght spectratyping analysis of the prostate-reactive T cell repertoire from spleen, prostate-infiltrating cells and from a PSBP-specific short term cell line was performed.
T-cell-mediated response showed a specific response characterized by enhanced proliferation and high levels of IFN-gamma, IL-2 and IL-17 secretion that could be detected in spleen and draining LN cells of mice from groups PAg and PSBP. Conversely, low-to-null levels of IL-4 were detected. Prostate infiltration and tissue damage accompanied that response. Also, high levels of IFN-gamma secretion were observed in a PSBP-specific cell line from autoimmune animals that transferred disease to naïve recipients. Clonotypes bearing TCR Vβ5.1, Vβ7, Vβ10 and Vβ15 were detected in spleen and prostate infiltrating cells from diseased animals and also in the PSBP-specific cell line.
A strong Th1 response and the public clonotypes Vβ5.1, Vβ7, Vβ10 and Vβ15 are induced after immunization in EAP. These clonotypes secrete high levels of IFN-gamma and infiltrate the target organ causing disease.

P5.07.31
Establishment of collagen-induced arthritis in cynomolgus monkeys, a model for rheumatoid Arthritis

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Pharmaceutical compounds of new technologies are mostly human-specific, such as biological proteins and sequence-based oligonucleotides or aptamers, etc. Since these compounds frequently cross-react with the corresponding drug targets in non-human primates (NHP), it is apparently a more efficient approach to evaluate them in monkeys for their potential efficacy and pharmaceutical profile in a proper disease models. Collagen-induced arthritis (CIA) in both Rhesus and Cynomolgus monkeys has been reported, however, it is insufficient for characteristically modeling rheumatoid arthritis (RA) due to the hampering of lower incidence and inconsistent disease. To establish a reliable RA model for drug evaluation, we characterized the disease progression following immunization of female Cynomolgus with bovine type II collagen. In our studies, the overall incidence of individual arthritis reached 80% and the average incidence of proximal interphalangeal (PIP) joint arthritis reached near 70%, significantly higher than those reported previously. The PIP joints swelled approximately by 25% from the basal level in average in correspondence to the increase of arthritic scores starting from the third week after first immunization. The pathological changes in joint bone and cartilage were well supportive for the RA disease examined by radiopathology and bone histopathology. The biomarker analysis of C-reactive protein (CRP) and ALP levels in peripheral blood showed a close correlation to the arthritic incidence and severity along with the disease progress. The CIA monkeys also presented anemic symptoms, a solid evidence for the similarity to RA with anemia of chronic disease (ACD) in the patients.

P5.07.32
Activation of T cells around the blood brain-barrier illuminated by a fluorescent NFAT marker

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We previously showed that auto-reactive T cells infiltrating the central nervous system (CNS) undergo highly dynamic interactions with different structures of the blood brain-barrier (BBB), which may result in intracellular signaling and subsequent T cell activation. We now introduced a truncated nuclear factor of activated T cells (NFAT) fused to GFP (ΔNFAT -GFP), as a fluorescent real-time activation indicator, and used it to elucidate sequential activation events in auto-reactive T cells interacting with different elements of the BBB. Intravital two-photon imaging ΔNFAT -GFP expressing encephalitogenic T cells, documented that perivascular antigen presenting cells (APCs), and not endothelial cells induce cytosol-nuclear translocation of the marker. Activation correlated with reduction of T cell motility, but did not necessarily imply their long-lasting arrest, as activated T cells continued to visit other APCs. In addition, we found that individual APCs in the CNS differ in their ability to present endogenous auto antigen, with some being much more potent than the others. Our data suggest that perivascular APCs in the CNS, presenting endogenous auto antigens, provide stimuli to infiltrating T cells that enable them to migrate further into the CNS parenchyma.

P5.07.33
A mouse model for paraneoplastic neurological syndrome: when efficient anti-tumoral immunity leads to autoimmunity in the CNS

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The Hu syndrome, a paraneoplastic neurological disorder, develops in patients with cancer in whom an effective anti-tumoral immune response against antigens shared by tumor cells and normal neurons leads to neurodegenerative disease.
Growing but indirect evidence suggests a role for T cells in the Hu syndrome. However, their contribution still remains to be determined as well as the identification of CD4 and/or CD8 subsets involved. Development of immunotherapeutic approaches to limit neurological insult is also greatly needed.
To address these issues we developed a mouse model for Hu syndrome. To mimic the paraneoplastic situation, we use a tumor cell line expressing the hemagglutinin (HA) of influenza virus to activate a cross-reactive anti-tumoral response in CamK-HA mice expressing HA in most CNS neurons. To promote a T cell response against the HA antigen shared by neurons and tumor cells, naïve HA-specific CD8 T cells alone or in combination with naïve HA-specific CD4 T cells originating from TCR-transgenic animals were transferred into CamK-HA mice bearing HA-expressing tumor.
We showed that HA-expressing tumor cells induce in vivo activation, proliferation and differentiation of naïve HA-specific T cells into effector cells. Both HA-specific CD8 and CD4 T cells were needed to control tumor growth and induce CNS inflammation. Control groups in which either the tumor cells or the recipient mice did not express HA failed to promote neurological disease.
This mouse model increases our knowledge on how successful anti-tumor immunity triggers a cancer-associated autoimmune disease, and helps develop new therapeutic strategies against this disabling condition.

P5.07.34
Induction of systemic lupus erythematosus-like syndrome in BALB/c mice leads to disturbance in peripheral T cell subpopulations

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Mechanisms underlying the pathogenesis of Systemic Lupus Erythematosus (SLE) have not yet been elucidated. The T cell role in development of disease was intensively studied. Animal models of SLE are valuable tools in the study of pathogenic mechanisms involved in SLE.
We immunized normal BALB/c mice subcutaneously with 50µgr DNA extracted from cells cultured in different conditions: splenocytes+ polyamines, splenocytes+ Con A, splenocytes+ polyamines+ Con A and splenocytes only (control). Anti- ds DNA antibodies were assessed by ELISA, proteinuria was assessed by Bradford method and other SLE associated auto antibodies were determined by immunofluoroscence ANA. Intracellular cytokines and transcription factors of T helper subsets were examined by flow cytometry and Real-Time PCR respectively.
The results showed that serum level of Anti-dsDNA antibody in mice immunized with DNA extracted from ConA+ Polyamine was higher compared to other groups. ANA titer was increased in ConA and then in ConA+ polyamine stimulated groups and level of proteinuria in polyamine and ConA+ polyamine stimulated groups was higher than in control group ($p<0.01$), The ratio of T-bet/GATA3 and T-bet/Foxp3 in mice immunized with DNA from ConA stimulated were insignificantly increased compared with control group. The ratio of IFN-γ/IL-4, IFNγ/Foxp3 and IL-17/Foxp3 of the CD4+ splenocytes in polyamine stimulated group was increased significantly compared with control group. ($p<0.05$, $p<0.01$ and $p<0.05$, respectively).
Our results show that the mice immunized with DNA extracted from splenocytes+ polyamines+ Con A manifested more obvious signs of the disease and increased ratio of Th1/Th2.

P5.07.35
TLR8-deficiency accelerates autoimmunity in a spontaneous lupus mouse model

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Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disorder characterized by increased B, T and dendritic cells (DCs) activation, by the formation of autoantibodies and subsequent development of immune complex glomerulonephritis (GN). The role of endosomal TLR7 and TLR9 in the development of anti-nuclear autoantibodies, because of their respective recognition of RNA and DNA, respectively, has been extensively studied. TLR7 plays a critical role in SLE development. Little is known on the TLR8 activation *in vivo* in mice, and its interaction with TLR7, we therefore conducted a study on its implication in autoimmune responses in mice. For that purpose, we introduced the TLR8 null mutation into C57BL/6-Nba2.*Yaa* (Nba2.*Yaa*) mouse model and followed the development of SLE, *i.e.* Ig production, GN, mortality, splenomegaly, B, T cells and DCs activations, monocytosis and *in vitro* or *in vivo* cell-response to TLR7-ligation. Nba2.TLR8$^{-/-}$.*Yaa* developed an accelerated mortality (47% vs. 8% at 8 months of age, p=0.001), increased splenomegaly and displayed higher IgM and IgG levels in the sera, compared to Nba2.*Yaa*. TLR8-deficiency resulted in an increased monocytosis which was further enhanced after *in vivo* TLR7 stimulation. TLR7 and MHCII expression were upregulated on Nba2.TLR8$^{-/-}$.*Yaa* APCs. DC capacity to present allogenic antigens to T cells was also improved in TLR8-deficient mice after TLR7 stimulation. In conclusion, TLR8-deficiency in lupus-prone mice induces acceleration of the pathology. We demonstrated an enhanced TLR7-dependent activation of DCs, due to functionally upregulated expression of TLR7, thus providing an explanation for the accelerated autoimmune responses in TLR8 deficient lupus-prone mice.

P5.07.36
Conditional mouse model to study the tissue- and time specific effects of NADPH oxidase 2 -derived reactive oxygen species during arthritis

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Neutrophil cytosolic factor 1 (NCF1) is the key regulatory component of the phagocytic NADPH oxidase 2 (NOX2) complex, and mutations in this gene lead to lowered production of reactive oxygen species (ROS). *NCF1* has been linked to rheumatoid arthritis and mutations in this gene increase arthritis severity in rodents. Interestingly, ROS production protected rodents from autoimmunity implying a regulatory role for ROS in inflammation.
We aimed at generating targeted *Ncf1* knock-in mice with inducible *Ncf1* expression and EGFP reporter to identify the cell type and the time window protecting the mice from arthritis. The mice were successfully generated on a pure C57BL/6N genetic background. As expected, *Ncf1* expression was induced *in vivo* by Cre or FLP recombinase, and the gene activation was followed by EGFP reporter. Furthermore, the targeting silenced the *Ncf1* gene as intended. After activating the targeted knock-in gene by recombination, using universally expressed Cre or FLP recombinase, the expression pattern of NCF1 was similar to that of the wild type mice, mostly showing expression in granulocytes and other cells known to make oxidative burst. After crossed with B10.Q mice, *Ncf1* knock-ins were susceptible to collagen induced arthritis. Granulocyte oxidative burst could be restored by tamoxifen in mice carrying an allele of the inducible ROSA-CreER recombinase. In conclusion, ROS production can be controlled in the *Ncf1* knock-in mice by the Cre or FLP recombinase expression specificity. The model can, thus, be utilized to study the spatiotemporal effects of NOX2 complex derived ROS during experimental arthritis or other conditions.

P5.07.37
Lack of IRF-1 accelerates development of tubulointerstitial nephritis and pulmonary granulomas with predominant Th2 polarity in lupus-prone MRL/*lpr* mice

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The transcription factors Interferon Regulatory Factor-4 (IRF-4) and IRF-1 are involved in the differentiation of naïve CD4$^+$ T cells into Th2/Th9/Th17 and Th1 cells, respectively. At the 14th ICI, we reported that *Irf4*$^{-/-}$ lupus-prone MRL/*lpr* mice lacked serum autoantibodies and Th17 cells but developed proliferative glomerulonephritis (GN) and granulomas containing Langhans-type multinucleated giant cells (MGCs) in multiple organs with significant increase in serum IFN-γ and IFN-γ-producing CD4$^+$ T cells; suggesting autoreactive Th1 cell-mediated mechanism for their pathogenesis. To further investigate the roles of autoreactive CD4$^+$ T cells in murine lupus, we analyzed disease phenotype of *Irf1*$^{-/-}$ MRL/*lpr* mice in the present study. By 18 weeks of age, unlike wild-type MRL/*lpr* mice, all *Irf1*$^{-/-}$ MRL/*lpr* mice showed minimal to no GN but developed severe diffuse tubulointerstitial nephritis characterized by predominant infiltration of CD4$^+$ T cells. They also developed pulmonary granulomas with severe infiltrations of CD68$^+$ macrophages/epithelioid cells and frequent formation of foreign-body-type MGCs. Intracellular cytokine staining showed significantly increased numbers of IL-4-producing CD4$^+$ T cells in the spleens, peritoneal lymph nodes, and kidneys of 18-week-old *Irf1*$^{-/-}$ MRL/*lpr* compared to wild-type littermates. Age-matched *Irf1*$^{-/-}$ C57BL/6 mice also showed significantly increased numbers of IL-4-producing splenic CD4$^+$ T cells; however none of *Irf1*$^{-/-}$ C57BL/6 mice developed renal or pulmonary disease.
In conclusion, these results indicate that IRF-1 plays a role in the regulation of Th2 polarity in MRL/*lpr* and C57BL/6 mice. Our results suggest that their pathogenesis, in *Irf1*$^{-/-}$ MRL/*lpr* mice, is most likely caused by autoreactive Th2 cell-mediated mechanisms.

P5.07.38
Novel anthraquinone derivatives reduce inflammatory cytokines *in vitro* and ameliorate clinical signs of experimental autoimmune encephalomyelitis

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Multiple sclerosis is a non-traumatic demyelinating disease of the central nervous system. Mitoxantrone is an anthraquinone derivative approved for treatment of multiple sclerosis. This study investigated the effect of three classes of anthraquinone derivatives on macrophages and their potential to treat EAE. Macrophages were cultured for 48h with serial dilutions of anthraquinone derivatives. Supernatant were collected to evaluate the NO, TNF-α and IL-1β inhibition. C57BL/6 mice were immunized with MOG35-55 and treated with chosen compounds. The lipophilic compounds **3**, **4**, **5** and **6** (62.4%, 92.6%, 73.4% and 58.4%) and the halogenated compounds **7** and **8** (57.8% and 53.4%) showed reduction in NO production without cytotoxicity. Other compounds with heteroatom's addition were not effective. For the compounds with addition of amino alcohols chains the compounds **4**, **5** and **6** showed inhibition of NO (40.2%, 49.7% and 62.5%) in the 5µg/ml concentration. For the TNF-α production, only the compounds **8** and **9** showed inhibitory effects (62.4% and 52.9%). For the IL-1β production the compounds **4**, **5**, **6**, **8**, and **9** showed inhibitory effects (70.8%, 67.1%, 74.3%, 63.4% and 51.2%). The compounds **3**, **10** and **12** showed properties similar to mitoxantrone with high toxicity on cells. The halogenated compound **8** and the amino chains inserted compound **6** were tested *in vivo* for EAE and showed reduction of the clinical score of disease (1.4±0.9 and 1.2±0.3 vs. 3.6±0.2, respectively). In conclusion, anthraquinone derivatives were prepared in a good yields and showed promissory anti-inflammatory properties.
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P5.07.39
The up-regulation of innate immune response can be observed by expression of TLR3, TLR4 and TLR9 by antigen-presenting cells in EAE model

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Multiple sclerosis is a chronic inflammatory demyelinating disorder of the central nervous system. EAE is a model for the study of MS. The objective of this study was evaluating the innate immune response including Toll-like receptors in EAE model development. The EAE mice were immunized with MOG35-55, emulsified v/v in complete Freund's adjuvant (CFA) supplemented with 400µg of attenuated *Mycobacterium tuberculosis* and Pertussis toxin (300ng/animal) was injected intraperitoneally on the day of immunization and 48h later. The MOG negative group only received supplemented CFA and Pertussis toxin. The animals were euthanized at 2nd, 4th and 7th days post-immunization. The spinal cords were removed and analyzed. Only EAE group developed clinical signs. In comparison to the MOG negative group the level of CCL5 and CCL20 in homogenized spinal cord was higher in the EAE group at 4th (1,983.0±237.2 vs. 1,245.0±110.5 and 4,546.0±569.8 vs. 1,992.0±343.0) and 7th (1,459.0±97.3 vs. 717.6±110.0 and 3,262.0±331.4 vs. 1,762.0±259.6) days post-immunization. The levels of IL-10 (8,828.0±1,652.0 vs. 2,063.0±447.4) and TGF-β (128,139.0±55,265.0 vs. 14,311.0±5,461.0) were also higher in EAE group, which showed higher number of antigen-presenting cells expressing TLR3 (34,131.0±3,068.0 vs. 6,620.0±316.8), TLR4 (29,466.0±1,864.0 vs. 6,650.0±589.3) and TLR9 (104,346.0±4,407.0 vs. 21,626.0±1,434.0). The presence of MOG increases the release of chemokine in the spinal cord and up-regulation of TLRs expression by APC. Studies seeking the understanding of EAE may be important for the development of agents that modulate innate immune responses as signaling pathways TLRs may be effective in treating EAE and MS.
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P5.07.40
Mouse SCID Models of Systemic Lupus Erythematosus

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Objectives: Self-specific B cells play a main role in pathogenesis of Systemic lupus erythematosus (SLE) and are a logical target for a selected therapy. SCID mice, which lack both T and B lymphocytes and accept xenogenic cells have been used for cell transfer for evaluating the pathogenesis of SLE.
It has been shown that complement receptor type 1 (CR1) on human and FcγRIIb on mouse B-lymphocytes has suppressive activity. The co-crosslinking of these receptors with BCR inhibits B cell activation and it could be an attractive target for therapeutic intervention.
We hypothesize that it may be possible to establish mouse and humanized models of lupus and to eliminate selectively the pathogenic B cells by protein engineered molecules, which co-crosslink the immunoglobulin receptors and CR1/FcγRIIb delivering a strong suppressive signal.
Methods: The protein chimeric molecules were constructed by coupling an DNA-mimotope peptide to a monoclonal anti-CR1 or anti-CD32 (FcγRIIb) antibodies.
We established autoimmune models by transferring splenocytes from MRL/lpr mice or PBMC from SLE patients to SCID mice and tested the effects of the chimeric molecules.
Results: Reconstituted SCID mice showed presence of auto-antibodies, as well as immunoglobulin deposition in the renal glomeruli. Treatment of the transferred SCID mice with anti-human or anti-mouse DNA-like chimera prevented appearance of anti-DNA antibodies and proteinuria, while the PBS-injected animals had high levels after the transfer.
Conclusions: This study demonstrates the possibility to silence selectively pathogenic B cells in transferred or humanized SCID mice and to delay the progression of autoimmune disease using chimeric molecules.

P5.07.41
Reduced disease severity in an APRIL-deficient lupus mouse model

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Systemic Lupus erythematosus (SLE) pathogenesis is complex, but it is widely accepted that autoantibodies play a key role in the autoimmune process. A proliferation inducing ligand (APRIL), a member of the TNF superfamily, is crucial for the control of plasma-cell survival. We previously showed that *in vivo* APRIL blockade delays the disease in lupus-prone mice. To confirm the role of APRIL in the pathology, we investigated the effect of APRIL-deficiency in our lupus mouse model.
We introduced the *APRIL*-null mutation into C57BL/6-Nba2.*Yaa* (Nba2.*Yaa*) mouse model and followed the development of the disease manifestations such as mortality due to glomerulonephritis (GN), autoantibody production, splenic cells frequencies and Ig producing plasma cells levels in Nba2.APRIL$^{-/-}$.*Yaa* compared to control mice.
Nba2.APRIL$^{-/-}$.*Yaa* developed a reduced mortality due to GN (13% vs. 40% at 11 months of age, p=0.04) compared to Nba2.*Yaa* control mice. APRIL-deficient mice displayed less IgG and IgM deposits in the glomeruli and lower levels of IgG, IgM, anti-dsDNA and anti-chromatin autoantibodies in the sera. Ig-producing cells were decreased in spleen from APRIL-knockout mice whereas no changes in B and T cells frequencies were observed compared to controls.
As observed with the use of an anti-APRIL blocking antibody, our data indicate that APRIL deficiency reduces lupus severity in mice by decreasing the survival of plasma cells leading to lower circulation of immune complexes. APRIL would then become an attractive molecule to target in human SLE since it preserves B-cell homeostasis contrary to other B cell-depleting therapies.

P5.07.42
Effects of Cystamine on CD4+/CD25+ regulatory T cells in lupus-prone mice

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Elevated oxidative stress and attenuated expression of regulatory T cells have been strongly associated with the pathogenesis of systemic lupus erythematosus (SLE). Recently, cystamine is indicated to be beneficial on SLE. To examine the effects of cystamine on anti-oxidant activities and regular T cells in SLE, Western blot, flow cytometry and various assays such as Glutathione (GSH), 1,1-dipheny l-2- picryl-hydrazyl (DPPH) and malondialdehyde thiobarbituric acid (MDA) were performed to investigate the effects of cystamine on NZB/W F1 mice. In the present study, significantly reduced MDA and increased GSH and DPPH were observed in NZB/W F1 mice receiving cystamine compared to those mice receiving PBS. Meanwhile, significantly increased CD4+/CD25+ regulatory T cells were also detected in NZB/W F1 mice receiving cystamine as compare to those mice receiving PBS, accompanying with significantly reduced IL-6/phosphorylated STAT-3 expression. These findings suggested the beneficial effects of cystamine on increasing antioxidant activities and CD4+/CD25+ regulatory T cells in lupus-prone mice via suppressing IL-6/STAT3 signaling.

P5.07.43

Autoantibody induction by DNA-containing immune complexes requires HMGB1 with TLR2/miR-155 pathway

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Anti-dsDNA autoantibody has been reported as a central pathogenic factor of SLE. However, the mechanisms of anti-dsDNA antibody production still remain unclear. Recent evidence indicates that circulating DNA-containing immune complexes, one of the hallmarks of SLE, might be involved in the autoantibody production. Here we mainly explored the potential role and underlying mechanisms of DNA-containing immune complexes in the anti-dsDNA antibody production. We found that these immune complexes primarily provoked anti-dsDNA antibody production through their HMGB1 component, whose level was highly and positively correlated with the anti-dsDNA antibody level in SLE patients. Furthermore, the HMGB1-mediated autoantibody production was in a TLR2/MyD88 dependent pattern, and modulated by the miR-155 via targeting Ets-1. Our results firstly showed the crucial role of HMGB1 component of DNA-containing immune complexes in the autoantibody production, which was mediated and regulated by TLR2/MyD88/miR-155/Ets-1 pathways. These findings may provide novel mechanistic insight into the persistent anti-dsDNA antibody production and new clues for the development of therapeutic strategies for SLE.

P5.07.44

Differential Modulation of Effector and Regulatory T Cells Leads to Attenuation of Autoimmune Diabetes in Ptpn22 Transgenic Nonobese Diabetic Mice

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Ptpn22 encodes PEST domain-enriched tyrosine phosphatase (Pep), which negatively regulates TCR proximal signaling and is strongly associated with a variety of autoimmune diseases in humans. The net effect of Pep on the balance of immunity and tolerance is uncertain because of the simultaneous inhibition of TCR-mediated signaling of counterregulatory effector and regulatory T cells (T_{regs}). In this study, we generated transgenic NOD mice that overexpressed Pep in T cells. The transgenic mice had a significantly lower incidence of spontaneous autoimmune diabetes which was accompanied by fewer IFN-r-producing T cells, and an increased ratio of $CD4^+Foxp3^+$ T_{regs} to $CD4^+IFN\text{-}r^+$ or to $CD8^+IFN\text{-}r^+$ T cells, respectively, in pancreatic islets. Transgenic T cells showed markedly decreased TCR-mediated effector cell responses such as proliferation and Th1 differentiation. By contrast, the inhibitory effect of transgenic Pep on TCR signaling did not affect the differentiation of T_{regs} or their suppressive activity. Adoptive transfer experiments showed that transgenic splenocytes exhibited attenuated diabetogenic ability. To examine further the pathogenic features of transgenic T cells, we generated *Ptpn22*/BDC2.5 doubly transgenic mice and found reduced proliferation and Th1 differentiation in $CD4^+$ T lymphocytes with additional Pep in pancreatic lymph nodes but not in inguinal lymph nodes of NOD/SCID recipients. This finding indicates that transgenic Pep attenuates T cell functions in an islet antigen-driven manner. Taken together, our results demonstrate that Pep overexpression in T cells attenuates autoimmune diabetes in NOD mice by preferentially modulating TCR signaling-mediated functions in diabetogenic T cells but not in T_{regs}.

P5.07.45

Adoptive cell transfer from multiple low dose streptozotocin diabetic donors can induce diabetes in healthy young C57/BL6 mice

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Multiple low dose of streptozoticin (MLD-STZ) diabetes in C57/BL6 mice is the result of T cell-dependent process directed against the pancreatic beta cells. However, diabetes induction after adoptive transfer of splenocytes and pancreatic lymph node cells (pLNc) has not been established. Therefore, we investigated whether splenocytes (1x108 viable cells) and pLNc (5x106 viable cells) from diabetic donors can induce diabetes in young (6-wk-old) C57/BL6 mice. Our work shows that i.p. transfer of splenocytes and pLNc from MLD-STZ mice altered i.p. glucose tolerance, stimulated the onset of glycosuria and intra-insulitis in young recipients, which was accompanied by the increased levels of TNF-α, IFN-γ, IL-17, and decreased level of IL-10 in the sera. The precise role of different types of mononuclear cells in diabetes induction has been determined by FACS analysis. The number of CD4+TNF-α+, CD4+IFN-γ+, CD4+IL-17+, CD3+CD8+, CD19+, F4/80+IL-12+ cells was significantly increased ($p<0.001$), while the number of CD3-CD8-NK1.1+ cells was decreased ($p<0.05$) in pancreatic lymph nodes and pancreas of recipients in comparison to healthy mice. In contrast with pancreatic lymph nodes, the number of CD19+ B cells in pancreas was similar in the recipients and healthy mice, suggesting that effector Th1, Th17, CD3+CD8+ T cells and M1 macrophages generated infiltrates in the islets of Langerhans in young C57/BL6 recipients. Our study provided the first evidence that splenocytes and pLNc of MLD-STZ diabetic male C57/BL6 mice can transfer diabetes to young syngeneic mice and identifies a new strategy for the examination of autoimmune diabetes.

P5.07.46

Downmodulation of peripheral MOG-specific immunity by pVAXhsp65 treatment during EAE do not reach the CNS

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Multiple sclerosis (MS) is the most common inflammatory and demyelinating disease of the central nervous system (CNS). Most of the therapeutic strategies to control this disease are directed to immune modulation and inflammation control. Heat shock proteins, especially hsp65, are described as targets for regulatory T cells due to their enhanced expression in inflamed tissues. In addition, there is strong evidence that they are able to induce anti-inflammatory immunoregulatory T cells. In this context, the objective of this work was to investigate the therapeutic effect of a genetic vaccine containing the mycobacterial hsp65 gene (pVAXhsp65) on EAE development. C57BL/6 mice were submitted to EAE induction by immunization with MOG in Complete Freund's Adjuvant and 5 days later we initiate the treatment with 3 or 4 pVAXhsp65 doses. Although pVAXhsp65 was immunogenic for mice with EAE and was also able to downmodulate the specific induction of cytokines by MOG, this vaccine was not able to decrease the clinical severity. Previously immunized mice presented weight loss and clinical scores comparable to non-immunized animals. Vaccination also did not decrease the intensity of inflammation in the CNS neither the cytokine production by cells isolated from brain and spinal cord in response to *in vitro* stimulation with MOG or hsp65. Immunization with pVAXhsp65 did not increase the frequency of CD4+CD25+Foxp3+ cells in spleen and CNS. These results indicate that hsp65 administered as a genetic vaccine is able to downmodulate immunity against MOG but this effect is not associated with any therapeutic activity against EAE development.

P5.08 Atherosclerosis and cardiovascular diseases

P5.08.01
Elevated vascular adhesion protein-1 (VAP-1) levels in cardiovascular disease events

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Inflammation in the vasculature can progress to atherosclerosis, which in turn leads the way to more severe cardiovascular diseases. Normally, the arterial endothelial cells can resist adhesion of passing leukocytes, but any harmful stimulus (e.g. oxidized LDL, modified oligosaccharides, smoking, and diabetes) can activate the endothelial cells, which then start to express different adhesion molecules, enhancing the local inflammation.
One of the adhesion molecules involved in leukocyte migration from the blood into sites of inflammation is vascular adhesion protein-1 (VAP-1). VAP-1's ligands, Siglec-9 and Siglec-10, are found on the surface of monocytes and B-lymphocytes. In addition to its adhesive function, VAP-1 is also a semicarbazide sensitive amine oxidase, an enzyme capable of producing hydrogen peroxide and aldehydes, both of which are damaging for vascular endothelium. Hence, VAP-1 can help the leukocytes to extravasate the vascular wall and aggravate the inflammation by producing harmful agents. As most endothelial adhesion molecules, VAP-1 is also found as a soluble form (sVAP-1) in plasma.
We have shown that in young Finns (n=2200) sVAP-1 correlates with cardiovascular risk factors and early atherosclerotic manifestations. In this study, we have measured the sVAP-1 levels of 2775 Finns aged over 55 years (the Finrisk 2002 -study) with an in-house ELISA assay. The plasma was collected in 2002, and the subjects have been followed up through the national registers thereafter. Since 2002 there has been over 500 cardiovascular disease related events. The correlations and predictive value of sVAP-1 will be shown.

P5.08.02
ROS and RNS signaling in cardiovascular diseases: Could antioxidants be useful?

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There is not too much success in the antioxidant treatment of heart diseases in humans. However a new approach suggests that depending on their structures and concentrations antioxidants can exhibit a complex activity in many pathological disorders. It is well established that physiological free radicals superoxide and nitric oxide together with their derivatives hydrogen peroxide and peroxynitrite, collectively reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an important role in heart diseases through their signaling functions. This presentation will focus on damaging signaling by ROS and RNS in various heart and vascular disorders: heart failure (congestive heart failure or CHF), left ventricular hypertrophy (LVH), coronary heart disease, cardiac arrhythmias, and so forth. It will be demonstrated that ROS overproduction (oxidative stress) is a main inducer of the transformation of normal physiological signaling processes into the damaging ones. Furthermore, the favorable effects of low/moderate oxidative stress through preconditioning mechanisms in ischemia/reperfusion will be considered. Then we will discuss the possibility of efficient application of antioxidants and enzyme/gene inhibitors for the regulation of damaging ROS signaling in heart disorders.

P5.08.03
Serum amyloid A stimulates macrophage foam cell formation via formyl peptide receptor 2

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Elevated levels of serum amyloid A (SAA) is a risk factor for cardiovascular diseases, however, the role of SAA in the pathophysiology of atherosclerosis remains unclear. Here we show that SAA induced macrophage foam cell formation. SAA-stimulated foam cell formation was mediated by c-jun N-terminal kinase (JNK) signaling. Moreover, both SAA and SAA-conjugated high density lipoprotein stimulated the expression of the important scavenger receptor lectin-like oxidized low-density lipoprotein receptor 1 (*LOX1*) via nuclear factor-κB (NF-κB). A LOX1 antagonist carrageenan significantly blocked SAA-induced foam cell formation, indicating that SAA promotes foam cell formation via LOX1 expression. We also show that SAA induced-macrophage foam cell formation was inhibited by formyl peptide receptor 2 (FPR2) antagonist WRW^4, as well as by FPR2-targeted siRNA knockdown. In addition, FPR2 is upregulated in peripheral blood mononuclear cells from patients with atherosclerosis. Our findings therefore suggest that SAA stimulates foam cell formation via FPR2 signaling, and thus likely contributes to atherogenesis.

P5.08.04
Association of monoctye expression of Toll-like receptor 4 with coronary luminal stenosis

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Background and objectives: Toll-like receptors are well-defined barriers against various infections. Among them hTLR4 on the surface of monocytes, plays a critical role in the formation of atherosclerotic plaques, plaque instability and arterial remodeling. This study was designed to examine the association of hTLR4monocyte expression and response with coronary luminal stenosis in patients with stable angina (SA).
Materials and methods: Blood samples were obtained from 39 patients with SA who were scheduled for a coronary angiography and from 28 healthy volunteers. The samples were collected before the procedure. Expression of hTLR4 on CD14+ monocytes and serum levels of TNF-α and IL-1β were measured using flowcytometry and ELISA techniques respectively. hTLR4 monocyte expression and response were examined with angiographic coronary stenosis. The degree of stenosis was measured by comparing the area of stenosis to an adjacent normal segment of the vessel.
Results: Compared to the control group, patients showed upregulation of hTLR4+/CD14+ monocytes. Furthermore, patients with more severe coronary stenosis exhibited enhanced expression of hTLR4+/CD14+ monocytes ($p<0.01$). This was paralleled by elevation in the serum levels of TNF-α ($p<0.05$) and IL-1β. In addition, the increase in monocyte expression of hTLR4 was positively correlated with the degree of coronary stenosis ($r=0.5$).
Conclusion: Monocyte expression of hTLR4 was positively correlated with the degree of coronary stenosis. Moreover, the serum levels of pro-inflammatory cytokines were associated with hTLR4 expression as well as coronary luminal stenosis. These findings suggest that hTLR4 monocyte expression and sensitization may be involved in progression and deterioration of atherosclerosis.

P5.08.05
Elevated CD14++CD16- monocytes predict cardiovascular events

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Background:
Although monocytes in peripheral blood are no longer considered to be a homogeneous population, associations between distinct monocyte subsets and cardiovascular disease have not been highlighted in large epidemiological studies.
Methods and results:
The study included 700 randomly selected subjects from the cardiovascular arm of the Malmö Diet and Cancer study. Among these, 123 subjects experienced ischemic cardiovascular events during the follow-up until December 2008. Mononuclear leukocytes frozen at the baseline investigation in 1991 to 1994 were thawed and analyzed with flow cytometry to enumerate monocyte subsets, based on CD14 and CD16 expression. The percentage and number of classical CD14(++)CD16(-) monocytes were increased in the cardiovascular-event group compared with the event-free subjects (median, 344 [interquartile range 251-419] cells/μL versus 297 [212-384] cells/μL, P=0.003). The hazard ratio was 1.66 for suffering a cardiovascular event in the highest tertile of the number of CD14(++)CD16(-) monocytes compared with the lowest tertile, even after adjustment for common risk factors (HR, 1.66; 95%CI: 1.02-2.72). CD14(++)CD16(-) monocytes did not, however, associate with the extent of atherosclerosis at baseline. In contrast, the percentage of monocytes expressing CD16 was negatively associated to the extent of carotid atherosclerosis measured as intima-media thickness at baseline. The chemokine receptors CCR2, CX3CR1, and CCR5 were not differentially expressed between cases and controls on any of the monocyte subsets, but CCR5 expression on CD14(+)CD16(++) monocytes was negatively associated to carotid intima-media thickness.
Conclusion:
This study shows that classical CD14(++)CD16(-) monocytes can predict future cardiovascular risk independently of other risk factors.

P5.08.06
T-helper 2 immunity is associated with reduced risk of myocardial infarction and stroke

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Objective:
Experimental studies in mice have attributed T-helper (Th) 1 and Th2 cells important roles in atherosclerosis, but the clinical importance of these cells in cardiovascular disease (CVD) remains to be clarified. Here, we investigated associations between Th1 and Th2 cells, carotid intima-media thickness, and cardiovascular risk.
Methods and Results:
Blood drawn at baseline and incident cardiovascular events during 15-year follow-up were assessed in 700 participants. Baseline Th1 (CD3(+)CD4(+)IFN-γ(+)) and Th2 (CD3(+)CD4(+)IL-4(+)) cells were analyzed by flow cytometry, and cytokine-release from activated mononuclear leukocytes was measured by multiplex technology. High numbers of Th2 cells were independently associated with decreased mean common carotid intima-media thickness. High numbers of Th2 cells were also independently associated with a reduced risk of acute myocardial infarction in women (hazard ratio, 0.19; 95% confidence interval, 0.06-0.56; P=0.002 for the highest versus the lowest tertile of Th2 cells). Moreover, release of the Th2 cytokine IL-4 from activated mononuclear leukocytes was independently associated with a reduced risk of CVD. No independent associations between Th1 cells and carotid intima-media thickness or CVD risk were found.
Conclusions:
Our observations provide the first clinical evidence for a protective role of Th2 immunity in CVD. They also suggest this protection is more prominent in women than in men. In spite of convincing evidence from experimental studies, we found no support for a role of Th1 immunity in CVD.

P5.08.07
Identification of NOD like receptors (NLRs) in human atheroma lesions

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Activation of innate immune receptors in atheroma lesions is involved in initiation and disease progression. Recently, a group of innate receptors, Nod Like Receptors (NLRs), was described and associated with recognition of several PAMPs/DAMPs. Members of the NLRs can assemble multimolecular complexes, inflammasomes, promoting activation of inflammatory caspases and maturation of IL-1beta and IL-18. The aim of this study was to investigate whether components of the NLRs family are present in atheroma lesions and involved in the activation of inflammasomes by atheroma related antigens. 44 patients with critical stenosis of carotid submitted to endarterectomy were evaluated. Carotid specimens were analyzed for mRNA and protein expression by real time RT-PCR and immunohistochemistry. Blood monocytes-derived macrophages of 4 healthy donors were cultured in the presence or absence of LPS or cholesterol crystals or both and production of IL-1beta and TNF-alpha was analyzed by ELISA. Our data showed expression of mRNA for NLRP1, NLRP2, NLRP3, NLRC1, NLRC2, NLRC3, NLRC4, NLRC5, Naip, ASC, caspase-1, AIM2 and IL-1beta in all carotid lesions. Immunoreactivity for NLRP1, NLRP3, NLRC2, NLRC3, NLRC5 and caspase-1 in macrophages and foam cells was also detected. Macrophages stimulated with LPS induced small production of IL-1 beta while the LPS + with cholesterol crystals promoted high levels of IL-1beta, while TNF-alpha production was unaffected. Altogether our results showed expression of several components of NLRs family in macrophages and foam cells of human atherosclerotic lesions. In addition, the production of IL-1beta but not TNF-alpha by macrophages after stimulation with cholesterol crystals suggests inflammasome activation.

P5.08.08
Oral anti-CD3 antibody treatment ameliorates mHSP65-induced atherosclerosis

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Aim-Accumulating evidence suggests that activated T-cells are the first invaders of the arterial intima in early human atherosclerosis lesions (1). Early atherosclerosis can also be reduced in animal models after T-cell depletion and after oral anti-CD3 antibody feeding. However, no study has shown the effect of oral anti-CD3 antibody treatment after immunizations with potentially atherogenic antigens.
Methods-Mice received 4 immunizations with mycobacterial heat shock protein 65 (mHSP65) (50μg/dose). Non-immunized mice served as controls. To test if we could reduce atherosclerosis in ApoE-/- mice we gave 8 oral feedings with anti-CD3 antibody or with the control IgG (5μg/feeding). The oral feedings were given both to non-immunized and mHSP65-immunized mice. Concomitantly with the first immunization, mice were either kept on normal chow diet (CD) or given high-cholesterol diet (HCD). All animals were sacrificed at week 22 and the aorta was surgically removed for Oil-Red staining to determine total plaque size. Blood was collected for determination of autoantibodies against eukaryotic (human) HSP60 (hHSP60) and prokaryotic mHSP65. Spleen and lymph nodes were collected for CD4+CD25+Foxp3+ T regulatory cell (Treg) determination.
Results-Oral administration of anti-CD3 antibody significantly reduced atherosclerotic lesion formation both in non-immunized and in mHSP65-immunized mice compared with controls. We observed an increase in Tregs in anti-CD3 antibody treated mice. mHSP65-immunized mice showed increased autoantibody titers to hHSP60/mHSP65. These titers were unchanged after oral treatment.
Conclusion-Our findings indicate that oral anti-CD3 antibody treatment has an atheroprotective effects both, in non-immunized and mHSP65-immunized mice. This effect might go via the induction of Tregs.

P5.08.09
Study of endothelial progenitor cells as a non-invasive strategy to detect endothelial alterations in patients with idiopathic thromboembolism

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BACKGROUND: Thromboembolism is defined idiopathic (ITE) when occurring without triggering circumstances. Inflamed and dysfunctional endothelium may play a pathogenic role in the disease. Endothelial progenitor cells (EPCs) are newly identified bone-marrow-derived cells crucial to endothelial homeostasis. Yet, their analysis in the blood may represent a novel, non-invasive strategy to study the endothelial compartment. In this study, we investigated possible alterations of circulating EPCs that may reflect endothelial perturbation in ITE patients.
METHODS: EPCs were isolated and expanded ex-vivo as endothelial colony-forming cells (ECFCs), using a method recently optimized in our lab. ECFCs were cultured from peripheral blood mononuclear cells (PBMCs) obtained from 13 ITE patients and 20 matched controls. Cultures were analyzed for efficiency of ECFC colony isolation, cell viability, morphology, immunophenotype, cytokine production, migration, in-vitro vasculogenesis.
RESULTS: ECFC colonies were isolated from ITE patients with the same efficiency as controls, as assessed as number of colonies and time of colony appearance starting from PBMC seeding. ECFC colonies from ITE patients were smaller and characterized by a high rate of early mortality, with cultures undergoing cell senescence within the first passage in a higher proportion of cases in ITE than controls ($p < 0.05$).
CONCLUSIONS: The preliminary results of this study indicate alterations in the growth of EPCs isolated from ITE patients that may indeed contribute to impairment of endothelial integrity in these patients. A full characterization of expanded ECFCs, still in progress, may further reveal functional defects of these cells that may possibly hinder the physiologic antithrombotic function of endothelium.

P5.08.10
A murine model of atherosclerosis, frequency and phenotype of the B cell subsets involved

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Atherosclerosis is a chronic inflammatory disease of the artery wall. Several studies have described a role for B cells in atherosclerosis. Adoptive transfer of follicular B cells promoted atheromas, whereas B1a cells protected atherosclerosis onset. Currently, the mechanisms responsible for this modulation are unknown. To characterize the B cell subsets involved in a murine model of atherosclerosis, C57BL/6 wild-type and atherogenic apolipoprotein-E knockout (*Apoe*$^{-/-}$) mice were fed with high fat (HFD) or regular diet for 12 weeks. At that time, *Apoe*$^{-/-}$ mice developed overwhelming lesions in the aortic sinus, compared with wild-type ones. The study of splenic cells according CD19, CD21, CD23, and CD5 expression, showed not differences in the frequency of follicular, marginal zone and transitional 2 (T2) B cells between wild-type and *Apoe*$^{-/-}$ mice, before and after the pro-atherogenic diet. In *Apoe*$^{-/-}$ mice with HFD a significant reduction of CD19 expression was observed in total B cells that was more evident in T2 cells. Also, in this group of mice the percentage of CD5^{+} T2 cells was decreased, and in this subset CD21 expression was decreased while CD40 was increased. There were not differences in IgM, IgD, CD80 and CD86 expression in B subsets among the groups. Since, CD19, CD5 and CD40 are associated with the regulatory function of B cells, the next step in this line of investigation will be the evaluation of the regulatory function of these T2 cells in mice with established atherosclerosis.
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P5.08.11
Specific oligoclonal T-cell recruitment within epicardial adipose tissue of patients with acute coronary syndrome: evidence for a local, immune-mediated, pathogenetic mechanism

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Immune-driven T-lymphocyte recruitment within epicardial adipose tissue EAT is possibly implicated in acute coronary syndrome (ACS).We examined the T-cell receptor (TCR) repertoire using CDR3 BV-BC spectratyping both in EAT samples (obtained during coronary artery bypass grafting) and in PBMC of patients with either ACS (27) or chronic stable angina (SA; 26). Patients undergoing cardiac surgery for mitral insufficiency, with angiographically normal coronary arteries, served as control group (10). The TCR repertoire enriched in EAT samples of ACS patients was restricted, involving 24% TCR-BV families. In particular, we observed a disproportionately high expression of TCR-BV10 and BV6.2 that were found in 44% and 26% EAT samples, respectively. Intriguingly, TCR-BV10 was strongly associated with the first clinical manifestation of ACS (61% patients at first manifestation vs 11% patients with previous events ($P=0.019$)). Although the size of the repertoire used by SA and control was comparable to that of ACS patients, 62% SA patients expressed preferentially TCR-BV3 in EAT samples, while BV10 and BV6.2 (both 8%) were less frequent ($P<0.01$ vs ACS). Control samples were enriched in BV3 (30%) and BV9 (20%), but none had BV10 or BV6.2 ($P<0.01$ vs ACS). We thus observed that instability of coronary plaque in ACS is associated with specific T-cell recruitment also in the surrounding adipose tissue, indicating that T-cells bearing selected TCRs might be involved in the pathogenesis of ACS.

P5.08.12
Kinins promote aortic aneurysm through stimulation of neutrophil activation via the B2 receptor

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Abdominal aortic aneurysm (AAA) is a chronic disease associated with inflammation of the aortic wall involving cells of the innate and acquired immune systems. Aneurysm rupture is responsible for ~1000 deaths annually in Australia. In the absence of effective medication, AAA treatment is restricted to surgical repair. The neutrophil, which plays a central role in inflammation, harbours the complete system for kinin release. This project investigates the effect of kinin receptor agonist (B9972), antagonist (B9430), kinin receptor deficiency and neutrophil depletion on AAA development in a mouse model. Aneurysm formation was monitored by ultrasound at 7-day intervals with aortic diameter determined at day 28 using morphometric analysis. Kinin receptor blockade and activation inhibited and promoted aortic dilatation respectively compared to a saline control ($p<0.0001$). In AngII-infused kinin receptor deficient mice (ApoE-/-B1B2-/-), aortic dilatation was lower compared to ApoE-/- controls ($p=0.0011$). Neutrophil depletion abrogated the ability of the kinin agonist to promote AAA development. Analysis of plasma myeloperoxidase (MPO) levels using ELISA indicated a decrease in MPO in kinin deficient mice compared to ApoE-/- controls ($p=0.0006$). In vitro studies using purified murine neutrophils treated with the kinin receptor agonist or antagonist indicated higher MPO activity with kinin promotion and lower MPO activity with kinin blockade ($p< 0.0001$). In summary, kinin receptor activation promotes AAA development, while kinin receptor blockade, deficiency, or neutrophil depletion, inhibits AAA development. Blocking the kinin pathway could be a novel intervention for treatment of AAA.

P5.08.13
Increased levels of MDA-modified collagen type IV in symptomatic lesions - a possible marker of unstable lesions

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Objective: Subendothelial LDL-aggregation and its subsequent oxidation are considered as key events in the development of atherosclerotic lesions. During oxidation of LDL reactive aldehydes, such as malondialdehyde (MDA) are formed, which modify amino groups of the associated apolipoproteinB100. However, the possibility that these reactive aldehydes also could leak out of the LDL-particle and modify surrounding extracellular matrix proteins has been largely unexplored.
Methods and results: The amount of MDA-modified collagen type IV and native collagen type IV were determined by in-house ELISAs in homogenates from 155 carotid artery lesions, removed by endarterectomy from patients with or without symptoms. Oxidized LDL was determined by ELISA. MDA-collagen type IV, but not native collagen type IV, showed a strongly significant association to oxidized LDL (r=0.31, *P*<0.001) and lipoprotein-associated phospholipase A2 (Lp-PLA2) (r=0.44, *P*<0.001). MDA-collagen type IV was also associated to inflammatory chemokines and cytokines. The amount of MDA-collagen type IV was higher in symptomatic lesions than in asymptomatic lesions. MDA-modification of collagen type IV decreased endothelial cell attachment *in vitro*. In addition, there was an association between autoantibodies against MDA-collagen type IV in plasma and the amount of MDA-collagen type IV in lesions.
Conclusion: Our data supports the hypothesis that LDL-oxidation induces modifications of nearby extracellular matrix proteins, which could contribute to more vulnerable lesions and cardiovascular events.

P5.08.14
Myd88 signalling in CD4+ T cells promotes atherosclerosis and Th17 responses

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Background
MyD88 is a transducer of TLRs and IL-1R signalling and crucial for responses against pathogen associated molecular patterns. Myd88 deficiency reduces atherosclerosis, an effect that primarily has been attributed to inhibition of macrophage activation. However, recent studies in autoimmune diseases have highlighted the importance of MyD88 signalling in T-helper cells. Here, we tested whether MyD88-dependent signalling in CD4+ T-cells affects atherosclerosis.
Methods
Apoe-/-Myd88-/- or Apoe-/-Myd88+/+ CD4+ T cells were transferred to lymphocyte deficient Apoe-/-Rag1-/- mice. The mice were fed high-fat diet for 7 weeks and atherosclerosis at the aortic root quantified by Oil Red-O staining and immunohistochemistry. Flow cytometry and multiplex analysis of cytokine-release was done to assess T cell function.
Results
Apoe-/-Rag1-/- mice receiving MyD88-deficient CD4+ T cells developed significantly smaller aortic root lesions than to mice receiving MyD88-competent CD4+ cells (108 000 ± 14 000 µm2 vs 157 000 ± 17 000 µm2, p<0.05). Total plaque area for lipids and collagen were lower mice receiving MyD88-deficient CD4+ T cells and there was also a trend towards less macrophage accumulation. However, the relative amounts of lipids, macrophages and collagen did not differ between the genotypes. Moreover, we observed lower levels of IL-17+ Th17 cells and double-positive IFNγ+-IL-17+ cells in spleens from mice receiving CD4+ T cells lacking MyD88. There were no differences in IL-10+ or IFNγ+ CD4+ T cells comparing genotypes.
Conclusion
This study demonstrates that MyD88 signalling in T cells contributes to development of atherosclerotic plaques and suggests that this involves activation of IL-17.

P5.08.15
Loss of B-cell function in MRas- knock-out mouse and reduced macrophage infiltration in atherosclerotic plaques at the aortic root

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Recently, we identified by genome-wide association study the MRas gene locus on human chromosome 3q22 to be associated with coronary artery disease. M-Ras is a member of the small GTPases family.
We crossbred the MRas-KO mice onto the ApoE-KO background and fed these mice with western diet. In parallel, we performed adhesion and migration assays with leukocytes to study the influence of MRas on adhesion and migration as a potential pathomechanism of atherosclerosis.
The MRas/ApoE-KO mouse showed no difference in plaque size at the aortic root in comparison to the ApoE-KO mouse after 10 weeks of high fat diet, but there were significant less macrophages in the plaque (n=19/10/15; ~10%; p=0.002). In addition, the ratio between macrophage and collagen in the plaque at the aortic root was significant higher in the MRas/ApoE-KO than in the ApoE-KO under high fat diet (n=19/9/15; p=0.002).
We observed a 15% reduction of CD19+ B-cells in lymphnodes in MRas-KO mice (p<0.05), but not in bone marrow, spleen and blood. Moreover, we noticed a 20% reduction of leukocyte adhesion in in vitro assays with an endothelial cell line (p<0.05). Again, especially B-cell adhesion was reduced in MRas-KO mice in comparison to WT mice. In vitro migration assays show also a reduced migration of B-cells towards the chemokine CxCl12 (p<0.01).
Our data suggests that MRas-KO has no effect on plaque formation at the aortic root but on the plaque stability by less macrophage infiltration. This could be linked to the reduction of adhesion and migration by B-cells.

P5.08.16
Human carotid atherosclerotic plaques with high Interleukin 16 mRNA levels are associated with less clinical symptoms

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Background and purpose. Interleukin (IL-16), a proposed ligand of CD4, has previously been attributed a pro-inflammatory role in allergy and rheumatoid arthritis. CD4 expressing T-helper cells are involved in the pathogenesis of atherosclerosis. The aim of this study was to determine whether CD4 and IL-16 mRNA expression measured in human atherosclerotic plaques was associated with symptoms, plaque inflammation and a vulnerable plaque phenotype.
Methods. Carotid plaques surgically removed from 153 patients were analyzed. Plaques obtained from patients that displayed symptoms within one month before removal (n=86) were compared to plaques from patients without symptoms but more than 80% stenosis of the carotid artery (n=67). Natural lipid, collagen, elastin, smooth muscle cell and macrophage content was evaluated histologically. Cytokines were measured in plaque homogenates using multiplex immunoassay and ELISA. IL-16, as well as CD3 and CD4, mRNA expression was analyzed with quantitative real-time PCR.
Results. The IL-16 mRNA expression was significantly higher in asymptomatic plaques compared with symptomatic plaques (p=0.043). The highest tertile of IL-16 mRNA expression was associated with symptoms with an odds ratio of 0.36 (p=0.026 and C.I. 0.15-0.88) compared to the lowest tertile of IL-16 mRNA expression when adjusting for common risk factors as age, gender, hypertension, CRP and statin use. IL-16 mRNA expression correlated to elastin and plasma triglyceride levels. No correlations were detected with pro-inflammatory cytokines in the plaque.
Conclusion. This study shows that high mRNA expression of IL-16 is associated with less clinical symptoms indicating that IL-16 might have a protective role in human atherosclerosis.

P5.08.17
Regulation of endothelial cell survival by the MEKK3-GAPDH axis

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Endothelial cell death is key to vascular damage, endothelial dysfunction and atherosclerosis. The molecular pathways that mediate endothelial cell death and survival remain poorly understood. While the MEKK3-MEK5-ERK5 module is critically important to maintaining endothelial cell survival, the mechanisms by which this pathway imparts such action remain poorly understood. We now report, in human endothelial cells, the co-immunoprecipitation of ERK5 with GAPDH, a glycolytic enzyme that also signals for cell death. Serum starvation-induced cell death was associated with decreased binding of GAPDH to the MEKK3-MEK5-ERK5 complex and increased levels of nuclear GAPDH. In contrast, halting cell death by serum readdition was associated with enhanced ERK5 activity, increased amount of GAPDH in the complex and normalisation of nuclear GAPDH. Other inducers of cell death (H_2O_2, tumour necrosis factor α + cycloheximide and 30 mM glucose) also caused an increase in nuclear GAPDH. R-(-)-deprenyl, an inhibitor of the nuclear translocation of GAPDH, inhibited cell death caused by H_2O_2. Interestingly, GAPDH bound directly to MEKK3, and not to ERK5 or MEK5. This GAPDH-MEKK3 interaction was enhanced by serum stimulation. Constitutively active MEKK3 not only exhibited enhanced binding to GAPDH but it also directly phosphorylated GAPDH on 3 sites. The data reveal that nuclear translocation of GAPDH leads to endothelial cell death and this is prevented by growth factors which activate MEKK3 and promote GAPDH binding. Our data suggest that phosphorylation of GAPDH by MEKK3 may play a role in preventing GAPDH-induced death. The MEKK3-GAPDH axis may represent a novel target for atheroprotection.

P5.08.18
IgE and eosinophilia levels in peripheral blood of patients with cardiovascular disease

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Background: Recently, several studies have been performed to assess the relationship between the higher eosinophilia and IgE levels with the incidence, progress and severity of cardiovascular disease (CVD) but their exact correlation remains unclear. The current study was designed to measure the levels of IgE and the eosinophilia levels amongst CVD patients .
Materials and Methods: Case group included 59 randomly selected CVD patients and 55 healthy individuals without any history of allergy and parasitic. A questionnaire including age, sex, smoking status was completed by each participant. Subsequently, 7 ml blood was taken from each participant for the CBC measurements and sera were obtained to measure IgE levels by ELISA.
Results: There were significant differences in variables such as family history, diabetes, hyperlipidemia, high blood pressure and physical activity between case and control groups. The mean IgE titers in the case and control group were 95.3 ± 71 and 62.44 ± 49, respectively. The mean eosinophila levels in peripheral blood were 3.95 ± 1.057 in case and 1.53± 0.57 in control group. The differences between the IgE and eosinophilia levels were statistically significant.
Conclusion: We conclude that the higher levels of IgE and the eosinophilia can be considered in the incidence, severity and progression of different types of CVD.

P5.08.19
Immunotherapy with tolerogenic Apolipoprotein B-100-loaded dendritic cells inhibits progression of atherosclerosis in hypercholesterolemic mice

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Objective - Atherosclerosis is a chronic inflammatory disease characterized by a massive intimal accumulation of low-density lipoprotein (LDL) that triggers chronic vascular inflammation with an autoimmune response to LDL components. Treatment of young hypercholesterolemic mice with dendritic cells (DCs) pulsed with the LDL protein apolipoprotein B100 (ApoB100) in combination with the immunosuppressive cytokine interleukin-10 (IL-10) reduces atherosclerotic development.
Methods and Results - To investigate if treatment with DC pulsed with ApoB100 in combination with IL-10 could have an effect on established atherosclerosis, 22 week old huB100tgxLdlr-/- mice (mice transgenic for human apolipoprotein B100 [ApoB100] and deficient for the low-density lipoprotein receptor) fed a high-cholesterol diet were intravenously injected with DC pulsed with ApoB100 in combination IL-10. 8 weeks after treatment atherosclerotic burden in the aorta was measured. Whereas atherosclerotic lesion area showed a 50% increase 8 weeks after treatment with DC only pulsed with ApoB100 and in untreated mice, mice injected with DC pulsed with ApoB100 in combination with IL-10 displayed inhibited progression of atherosclerosis.
Conclusions - Tolerogenic DCs pulsed with ApoB100 inhibits progression of atherosclerosis.

P5.08.20
Apolipoprotein B100 danger-associated signal 1 (ApoBDS-1) is a potent platelet agonist that triggers procoagulant and proinflammatory responses

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Low-density lipoproteins (LDL), occurring in vivo in both their native and oxidative form, modulate platelet function and thereby contribute to atherothrombosis. We recently identified and demonstrated that 'ApoB100 danger-associated signal 1' (ApoBDS-1), a native peptide of Apolipoprotein B-100 (ApoB100), the protein component of LDL, induces inflammatory responses in innate immune cells. Platelets are critically involved in the development as well as in the lethal consequences of atherothrombotic diseases, but whether ApoBDS-1 has also an impact on platelet function is unknown. In this study we examined the impact of ApoBDS-1 on human platelet function and platelet-leukocyte interactions.
By using in vitro models, we observed that ApoBDS-1 is a potent platelet agonist that induced platelet activation, degranulation, adhesion and release of proinflammatory cytokines. ApoBDS-1-stimulated platelets triggered innate immune responses by augmenting leukocyte activation, adhesion and transmigration. The platelet-activating effects were sequence-specific, and stimulation of platelets with ApoBDS-1 activated different intracellular signaling pathways, including PI3K, PLC and MAPK. Moreover, we show that inhibition of the purinergic receptor P2Y1 mitigated ApoBDS-1-induced platelet activation, whereas P2Y12 and COX-1 inhibition had only minimal effects.
ApoBDS-1 is a potent platelet agonist that modulates coagulation and inflammatory processes, and therefore is likely to contribute to the disease-promoting effects of LDL in the pathogenesis of atherothrombosis.

P5.08.21
Study the correlation of haptoglobin phenotypes in acute heart attack patients

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Introduction: AMI is disorders of acute blood and oxygen transfer to the heart cells and part of the heart muscle. Symptoms are differ with pain and burning sensation in the chest and accompanied by nausea and vomiting. Numerous immunological and non immunological factors are involved that can be pointed to the role of inflammatory factors such as haptoglobulin. The aim of this study was to find association of disease with haptoglobulin phenotypes expression and patients demographic factors.
Methodology: 120 patients and healthy control were selected for study. After obtaining serum used for haptoglobulin phenotype distribution by electrophoresis method and results of each phenotype differences in patient and healthy control groups were analysed using the X^2 test and SPSS software.
Results: 50.9% of patients were women and 47.9% men and 47.9% upper 60 years old. High serum haptoglobin Hp2-2 phenotype frequencies were found in 75 patients (62.5%) and in 70 control (58.3%) groups. Comparison of the results between two groups showed no meaningful statistic correlation between these two groups (P Value=0.484) and Hp1-1 phenotype had meaningful statistic correlation with the increased amount of serum acute heart attack indexes, such as hyper lipidemia and Hp2-2 with high blood pressure of patients compared with healthy control (p value =0.01 and 0.04 respectively).
Conclusion; This study revealed that no specific phenotype was related to the disease process whereas some demographic factors were involved in diseases susceptibilities that needs to be investigate more.

P5.08.22
Sublingual vaccination with Porphyromonas gingivalis GroEL elicits protective immunity against atherosclerosis

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Atherosclerosis has proved to be associated with microbial infection, inflammation, and autoimmunity. Periodontal disease is one of the most prevalent chronic inflammation of oral cavity, and has been reported to be associated with atherosclerosis. Accumulating evidence suggests a positive association of anti-heat shock protein 60 (HSP60) autoantibodies and the presence of atherosclerosis in humans. In this study, we assessed the potential of sublingually administered recombinant HSP60 from Porphyromonas gingivalis (rGroEL) for prevention of atherosclerosis accelerated by P. gingivalis. Apolipoprotein E deficient spontaneously hyperlipidemic (Apoeshl) mice were sublingually immunized with GroEL two times a week for two weeks. P. gingivalis 381 was challenged intravenously three times a week for two weeks and animals were euthanized 16 weeks later. Atherosclerotic lesions in proximal aorta of each animal were analyzed histomorphometrically, and the serum concentration of rGroEL-specific antibodies and cytokines were determined. Sublingual immunization with rGroEL induced significant rGroEL specific serum IgG responses in Apoeshl mice. Antigen-specific mononuclear cells isolated form spleen cells produced significantly high levels of IL-10 and IFN-γ. FACS analysis indicated that IL-10 and IFN-γ production was not derive from the Foxp3+ regulatory T cells. Interestingly, however, mice given rGroEL sublingually possessed significantly reduced atherosclerotic plaque accumulation in aortic sinus and lowered the serum levels of CRP, MCP-1 and ox-LDL compared to nonimmunized mice. These results suggest that sublingual immunization with rGroEL could be an effective vaccine for prevention of atheroscleorosis accelerated by P. gingivalis. Collaborator: Kazuhisa Yamazaki (Niigata University)

P5.08.23
IL-25 treatment inhibits atherosclerosis development in ApoE deficient mice

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IL-25 has been implicated in the initiation of type 2 immunity and in controlling the outcome of Th1/Th17 responses suggesting a protective role in autoimmune diseases. The aim of this study was to evaluate the role of IL-25 in the initiation of atherosclerosis development as well as in already established atherosclerosis.
Osmotic pumps containing recombinant mouse IL-25 or control medium (CM) were surgically placed in atherosclerosis prone *apoE*$^{-/-}$ mice either during 10-14 weeks or 20-24 weeks of age, delivering 1µg of IL-25 / day or equal volume of CM. In parallel for the same time periods mice were weekly injected i.p. with 100µg of anti-IL-25 (or control antibody). Upon termination at 25 weeks of age, blood and spleen cells were analyzed with flow cytometry. Oil Red O staining was performed in *en face* preparations of aortas, cytokine levels were determined using Luminex technology and plasma immunoglobulins were assessed with the use of a multi-spot assay and ELISA.
ApoE$^{-/-}$ mice treated with IL-25 during the initiation of atherosclerosis had reduced plaque areas in the aorta while IL-25 blockade led to increased plaque areas in the aortic arch, decreased Th2 cytokines in plasma and increased pro-inflammatory cytokine release from splenocytes. Furthermore, *apoE*$^{-/-}$ mice with established atherosclerosis treated with IL-25 had reduced plaque areas in the aorta while treatment with the IL-25 blocking antibody did not affect plaque development.
In conclusion, IL-25 treatment of atherosclerosis prone mice results in reduced plaque areas indicating a protective role in atherosclerosis.

P5.08.24
Soluble FcγRIIIaMφ levels in plasma correlate with stenosis rate in patients examined with carotid ultrasonography

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Macrophages play a major role in the development of vascular lesions in atherogenesis. The cells express FcγRIIIa (CD16) identical to that in NK cells, but with a cell type-specific glycosylation, and these soluble forms (sFcγRIIIa) are present in plasma. We had measured sFcγRIIIaMφ derived from macrophages in plasma with anti-FcγRIIIaMφ monoclonal antibodies and found that the levels of sFcγRIIIaMφ had been related to the severity of coronary atherosclerosis in patients with coronary artery diseases (CAD). In volunteers, the sFcγRIIIaMφ levels measured at the annual medical chekup had been positively related to both the number of risk factors for atherosclerosis and carotid maximum intima-media thickness (IMT). In the present study, we measured sFcγRIIIaMφ in plasma from patients examined with carotid ultrasonography. The levels of sFcγRIIIaMφ, but not the sFcγRIIIa levels, were significantly increased in those patients compared with age-matched healthy controls. Patients with echogenic/echolucent plaques or mixed with echogenic/echolucent and with calcificated plaques had the highest sFcγRIIIaMφ levels, but patients with only calcificated plaques had levels similar to patients with no plaque or intact carotid artery. The levels of sFcγRIIIaMφ were correlated with the stenosis rates, and the levels of total sFcγRIII were correlated with maximum plaque diameters. Because unstable plaques contain a lot of lipids and macrophages inside, the sFcγRIIIaMφ may serve as a novel risk biomarker for unstable angina or myocardial infarction.

P5.08.25
Atherosclerosis in rats after immunization with human native low-density lipoproteins

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The principal unsolved question of atherogenesis is why and how lipoprotein metabolism becomes unbalanced. The purpose of this research is to test the hypothesis that autoimmune reaction to native LDL (nLDL) is the cause of dyslipoproteinemia and atherogenesis in the vascular wall. In order to check our hypothesis we made an attempt to induce autoimmune reaction to nLDL in rats. In cases of autoimmune reaction we expected dyslipoproteinemia and development of atherosclerosis features. A single immunization of rats with human nLDL leads to self-maintained, self-reinforced anti-nLDL autoantibodies production in most rats. Anti-nLDL autoantibodies response was accompanied by dyslipoproteinemia and caused increased epicardial and perivascular adipose tissue volume, decrease of cholesterol level in adrenal glands, accumulation of inflammatory cells in aorta wall, together with intima destruction and media disorder. The results obtained favor the hypothesis that autoimmunity to native LDL is the cause of atherosclerosis development.

P5.08.26
Susceptibility to ischemic stroke and HPA4/Annexin5 polymorphisms

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Introduction: Some polymorphisms of genes coding for platelet alloantigens systems (HPA:Human Platelet Antigen) or glycoproteins linking membrane phospholipids and feature anti-thrombotic properties, such as annexin5 (AnxA5) can play an important role in ischemic stroke. In this context, polymorphisms (-1C/T) of AnxA5 gene and HPA4a/4b of HPA4 system were determined to find a possible association between these polymorphisms and susceptibility to ischemic stroke in Tunisian patients.
Methods: Case-control study was conducted in 50 patients with ischemic stroke and 249 healthy subjects. (-1C/T) AnxA5 and HPA4 polymorphisms were investigated by PCR-RFLP and PCR-SSP, respectively.
Results: The HPA4b minor allele was significantly higher in patients (0.92) compared to healthy subjects (0.642) (OR: 1.8, 95% CI [1.16-2.78], p=0.007). In contrast, no difference in genotypic and allelic frequencies of C/T mutation of AnxA5 was found between patients and controls (p=0.67). Besides, no significant association was found between studied polymorphisms and age of disease onset, either lesions localization.
Conclusion: If HPA4b allele appears to be a risk factor for ischemic stroke in Tunisian patients, the clinical significance of SNP (-1C/T) AnxA5 is not clearly demonstrated.

P5.08.27
Inhibition of IL-27 attenuates atherosclerosis by inducing regulatory T cells

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Interleukin 27 binds to cytokine receptor WSX-1 to activate cells, resulting in both pro- and anti inflammatory responses. In this study IL-27 was blocked in vivo to study the role in atherosclerosis.
LDLR -/- were injected i.m. with a sWSX-1 encoding lentivirus or an empty virus. Two weeks after the injection the mice were put on a high fat diet for 6 weeks to induce atherosclerosis. The experiments were repeated in combination with a regulatory T cell depletion by αCD25 treatment.
Neutralization of IL-27 via the soluble receptor resulted in a 31% reduction in aortic lesion size. The lesion size negatively correlated with the concentration of the soluble receptor in the serum. The collagen content was not affected, while there was a trend toward increased macrophage content. FACS analysis showed that blockade of IL-27 increased the circulating T helper cell population. Furthermore, CCR2 expression on monocytes was reduced while CD163 expression increased. The expansion of regulatory T cells was proven to be crucial for the reduction in lesion size, as no change was observed in combination with the αCD25 treatment. The anti inflammatory monocyte phenotype was not affected by the regulatory T cells depletion.
Taken together these results indicate that blockade of interleukin 27 resulted in an anti-inflammatory immune response by inducing regulatory T cells and anti-inflammatory monocytes . Astonishingly, the regulatory T cells are indispensible for the reduction in lesion size.

P5.08.28
Splenectomy increases atherosclerotic lesions in apolipoprotein E deficient mice

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Introduction: The spleen plays an important immune function, but its influence in development of atherosclerosis remains unclear. Atherosclerosis is an inflammatory immune disease associated to lipid accumulation in the intima layer of arteries. Evaluation of the role of the spleen in atherosclerosis is justified due to the high incidence of total splenectomies. The objective of this work was to investigate the effect of splenectomy in development of atherosclerosis in apolipoprotein E (ApoE) deficient mice.
Methods and Results: Mice were divided into sham operated control group (CT) and splenectomized group (SP). Thirty days after surgery, animals were fed on the high fat western diet. After 8 weeks, mice were euthanized for blood, heart and aorta analyses. Atherosclerotic lesion areas in the aortic root were stained with hematoxylin-eosin and quantified by morfometry. The atherosclerotic lesions in the thoracic and abdominal portions of aorta were determined by the percentage of the luminal surface area stained by Sudan IV. Total serum cholesterol and anti-oxidized LDL antibodies were measured. Levels of total serum cholesterol did not vary significantly after splenectomy. Anti-oxidized LDL antibodies were also similar between groups. However, compared to control group, lesions in the aortic root were significantly larger in splenectomized mice ($p<0.01$). This data was confirmed by the increase of atherosclerotic area in the thoracic and abdominal portions of aorta in splenectomized mice.
Conclusions: These data indicate that splenectomy increases atherosclerotic lesions in ApoE deficient mice fed an atherogenic diet, suggesting a atheroprotector role of the spleen.
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P5.08.29
IL-22 deficiency induces vascular remodeling and alterations in immune cell populations after carotid injury

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IL-22 is a recently discovered cytokine that has anti-inflammatory properties in several inflammatory diseases. IL-22 is produced by e.g. Th17, NKT and innate lymphoid cells and is believed to act on stromal cells as a way for the immune system to control tissue responses. The role of IL-22 in the vascular wall is not widely studied but given the involvement of both immune and tissue responses in atherosclerosis and vascular remodeling we aimed to study the contribution of IL-22 in vascular responses after carotid injury.
In this study, we used a carotid collar model to induce neointima formation in C57Bl6 and IL-22-/- mice. Preliminary results show no significant difference in neointima size but rather a significant increase in media area in IL-22 deficient mice. This might reflect an effect of IL-22 on vascular smooth muscle cell migration or

proliferation, processes which are now under further investigation in the context of IL-22 deficiency. However, the increased media area is not due to any differences in collagen percentage. Furthermore, carotid injury induced substantial alterations in immune cell populations in IL-22-/- mice having larger lymph nodes accompanied with increased amounts of CD4+ and CD8+ T cells, decreased levels of Tregs and IL-17 producing cells as well as more antigen-presenting cells. Taken together, these preliminary results indicate that IL-22 might play a role in the vascular wall by affecting vascular cells as well as inducing a more inflammatory environment in the draining lymph nodes potentially reflecting an overall increased inflammatory status in the vasculature.

P5.08.30
The role of anti-apolipoprotein A1 autoantibodies in cardiovascular risk stratification

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Autoantibodies to apolipoprotein A1 (anti-ApoA1 IgG) have been shown to be both a marker and a mediator of cardiovascular disease, promoting atherogenesis and unstable atherosclerotic plaque. Previous studies have shown that anti-apoA1 IgGs, assessed by ELISA, are independently associated with major cardiovascular events in patients with myocardial infarction. Our investigations have focused on understanding the pathophysiology underlying the ApoA1 autoantibody response. Autoantibody responses can be multifactorial and it is possible that more than one epitope may exist. In order to better characterize the specific epitopes, we have isolated human ApoA1 from plasma utilizing high resolution purification. Since ApoA1 has no cysteines in its sequence, we used thiophilic interaction chromatography to purify ApoA1 monomer from thiol-containing proteins, generating a fraction containing ApoA1 with purity circa 99%. A high level of immunoreactivity to the purified ApoA1 was detected by ELISA, using samples containing high titers of autoantibodies. Moreover, a short synthetic ApoA1 peptide with proper tertiary structure was shown to compete the endogenous epitope. We determined the diagnostic accuracy of patient immunoreactivity to this peptide for non-ST elevation myocardial infarction (NSTEMI) diagnosis on 132 consecutive patients presenting at the emergency room for acute chest pain. ROC curve analyses demonstrated that synthetic peptide was a significant predictor for NSTEMI diagnosis (AUC:0.64; p=0.01), and risk analyses demonstrated that high levels of immunoreactivity to the peptide was associated with a 7-fold risk of NSTEMI (OR:6.98, p=0.001). Taking together, this study may generate innovative prognostic and therapeutic biomarkers, potentially improving the current patient cardiovascular risk stratification.

P5.08.31
Platelet-derived microparticles may contribute to the thrombotic tendency in systemic lupus erythematosus by both procoagulant and immune-mediated mechanisms

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Background: Platelet-derived microparticles (PDMPs) represent small vesicles that promote coagulation by exposure of negatively charged phospholipids. Aim of this study was the assessment of PDMPs in systemic lupus erythematosus (SLE) patients and their association with disease activity, presence of antiphospholipid antibodies (aPL) and previous thrombosis.
Patients and Methods: Forty consecutive SLE patients (38 females, 2 males, mean age 50±16.4 years, mean disease duration 110.7±26.4 months) were included. PDMPs were isolated from citrate-treated plasma and characterized by flow cytometry using Annexin-V and CD41 antibodies to platelet surface markers.
Results: SLE patients had significantly increased concentrations of PDMPs (1800±293MP/UI, normal range <1000MP/UI), irrespectively to disease activity, as PDMPs did not differ between active and inactive disease (1879±186 vs. 1805±343MP/UI, p=NS). Interestingly, PDMPs were detected in higher levels (although non significantly) in patients with a previous history of arterial or venous thrombosis (1855±219 vs. 1781±396MP/UI). In accordance, they were marginally elevated in patients with antiphospholipid syndrome (APS) (1892±233 vs. 1776±360MP/UI). Moreover, PDMPs were found in higher levels in patients with positive anti-dsDNA antibodies (1907±317 vs. 1762±303MP/UI) and low levels of C3/C4d complement fragments (1982±334 vs. 1721±256MP/UI, p<0.05).
Conclusions: PDMPs were found in higher levels in lupus patients with a previous history of thrombosis or concomitant APS. Elevated PDMPs in patients with positive anti-dsDNA antibodies and low complement levels suggest that these microparticles may further induce thrombosis via the induction of immune complexes formation. These results warrant further characterization of PDMPs in SLE to elucidate their role in disease pathogenesis.

P5.08.32
GDF-15, ST-2 and Galectin-3 plasma levels and their potential relations in patients with coronary artery disease

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Aim: The key process leading to heart failure is cardiac remodelling in response to chronic disease stresses caused by atherosclerosis and evolved into coronary artery disease (CAD). There is a growing interest in using new circulating biomarkers such as GDF-15, ST-2 and Galectin3 (Gal-3) to obtain pathophysiological insight and improve the management of patients with CAD. Our goal was to investigate these markers of heart disease to better understand involvement of these in different pathology such as coronary artery disease and cardiac valvular replacement (VR). Methods: We have studed 3 groups of subjects: the first consisted of 20 patient affected by CAD undergoing coronary artery bypass grafting (CABG); the second of 20 patient with no signs of CAD undergoing VR and the third group of 20 healthy man without apparent pathologies as controls. Blood samples of patients undergoing elective CABG or VR surgery and of control subjects were collected after an overnight fasting to measure GDF-15, ST-2 and Gal-3 levels by immune-enzymatic assay. Results: Our data show a statistically significant increase of plasma levels of GDF-15, ST-2 and Gal-3 in CABG and VR patients compared to controls (p<0.05), but no difference has been observed between the two groups of CVD patients.
Conclusions: Our results it seems that although these two differentgroups of patients had similar increased circulating levels of GDF-15, ST-2 and Gal-3 none of these resulted mainly associated to coronary artery disease (CAD).

P5.08.33
Aggravation of atherosclerosis by MHC class II antigen deficiency is associated with a loss of regulatory T cells but is independent on IL-10

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It has been proposed that atherosclerosis involves a loss of tolerance against modified self-antigens generated in response to hypercholesterolemia and that presentation of such antigens on MHC

class II lead to activation of pro-inflammatory Th1 cells. Furthermore, anti-inflammatory Tregs have been shown to reduce atherosclerosis development indicating that both pro- and anti-inflammatory responses exist. To further study the role of adaptive immunity in atherosclerosis we have used hypercholesterolemic mice lacking MHC class II (ApoE-/-MHCII-/-). ApoE-/-MHCII-/-mice had reduced levels of CD4+ T cells as well as Th1, Th2 and Treg cytokines in plasma. Splenic CD115+ monocyte numbers and plasma levels of pro-inflammatory cytokines were reduced indicating reduced systemic inflammation. Despite this, ApoE-/-MHCII-/-mice had significantly more atherosclerosis as assessed both by *en face* lipid staining of the aorta (4.7±2.9% versus 1.9±1.3%; P<0.01) and cross-sectional area of subvalvular lesions (7.7±2.2x105 versus 4.6±2.8x105 µm2; P<0.05). Moreover, macrophage accumulation in lesions was significantly increased (44.8±8.0% versus 24.8±7.8% MOMA-2 stained area; P<0.001). To test the hypothesis that the aggravated atherosclerosis in ApoE-/-MHCII-/- mice was caused by a decreased release of IL-10 from Tregs we gave weekly injections of IL-10 blocking antibodies to inhibit atherosclerosis in ApoE-/- but not in ApoE-/-MHCII-/- mice. Blocking IL-10 significantly reduced the plasma level of IL-10 in ApoE-/- mice but did not promote the development of atherosclerosis. The present observations unexpectedly show that the net effect of MHC class II-dependent antigen presentation in atherosclerosis is athero-protective and that IL-10 does not play a major protective role at this stage of the disease.

P5.09 Neuroimmunology

P5.09.01
Nanotherapy for drug addiction

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Substance abuse involves activation of the dopaminergic signaling pathway and a 32kDa dopamine- and cAMP-regulated phosphoprotein (DARPP-32) is a phosphoprotein that is critical to the pathogenesis of drug addiction. Since DARPP-32 plays a central role in regulating the efficacy dopaminergic neurotransmission, we hypothesize that sustained knockdown of DARPP-32 may interfere with conditioned responses and promote brain plasticity in patients who abuse recreational drugs. Our study used a highly innovative nanotechnology-gene therapy approach, using gold nanorods (GNR) conjugated to siRNA to silence DARPP-32 gene expression of a critical molecular target of drug addiction, DARPP-32. We propose that suppression of DARPP-32 expression may help break the cycle of addiction and prevent relapse to drug abuse by reducing the rewarding effects of addictive drugs on the brain. Our results showed significant inhibition of DARPP-32 gene expression both in- vitro primary human dopaminergic cells (85%, p<0.001) and in-vivo by stereotaxic injection to the brain VTA region (68%, p<0.01) in a opiate addicted rat model using GNR-DARPP-32 siRNA nanoplexes up to a period of 4 weeks post transfection when compared to untreated and scrambled controls. Gene silencing was determined by real time QPCR analysis. Further, behavioral outcome was determined in the animal model using condition placed preference (CPP) studies, which showed a significant amelioration of addictive behavior in these opiate addicted rats. This innovative nanotherapy using targeted delivery of DARPP-32 siRNA offers a unique opportunity for pharmacological intervention to treat drug addiction.

P5.09.02
Immunofluorescence patterns in mouse cerebellum and kidney tissues revealed by seric antibodies of patients with various neuroinflammatory diseases

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Rationale. The autoimmune neurological disorders are severe and disabling conditions. A specific autoantibody associated to Devic's syndrome (or neuromyelitis optica) is NMO-IgG, which recognize acquaporine-4. Routine determination of this Ab by our group has revealed non-classic or nonspecific immunofluorescence patterns (IFP). In this study we analyzed the relation of IFP with the diagnosis of patients with various neuroinflammatory diseases.
Methods. IFP in mouse tissues (cerebellum and kidney), revealed by seric antibodies from 16 patients with: neuromyelitis optica, NMO (n = 1); Devic's syndrome, DS (n = 3); optic neuritis, ON (n = 5); o transverse myelitis, TM (n = 7) were determined. Some of these patients also had diabetes mellitus type 2 (DM2), systemic lupus erythematosus (SLE) or myasthenia gravis (MG).
Results. Three types of IFP were detected: 1) A NMO classic IFP, characterized by staining of blood vases in cerebellum and kidney; 2) An nonspecific IFP, characterized by staining of the nuclei from cerebellum and kidney; and 3) A mixed IFP, in which IFP 1) and 2) coexisted. It is worth to mention that the IFP 2) was found in patients with a neuroinflammatory condition associated to DM2, SLE or MG; and the IFP 3) was seen in a patient with ON and prostate cancer.
Conclusions. Nonspecific IFP in mouse tissues revealed by patients with neuroinflammatory diseases is associated with the presence of an autoimmune disease and the coexistence of NMO-IgG and nonspecific IFP might be a marker of an additional severe disease.

P5.09.03
Importance of the vagus nerve in immunodepression following stroke

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Impaired immunefunction due to overactivation of neuro-humoral stress pathways, in particular of the sympathetic nervous system, has been proposed as risk factor for thehigh incidence of pneumonia after stroke. However, changes in pulmonary immunity and the role of the parasympathetic vagus nerve in stroke-induced suppression of immune responses are poorly understood so far.
We hypothesize that after stroke pulmonary macrophage and epithelial function is impaired due to release of acetylcholine resulting in reduced lung anti-bacterial responses. This anti-inflammatory effect mediated by the vagus nerve is dependent on nicotinergic α7 acytylcholine receptor (α7nAChR).
Inhibition of vagus nerve activity by vagotomy ameliorated development of spontaneous bacterial infections after stroke in wt mice and significantly increased release of pro-inflammatory cytokines upon *ex vivo* stimulation of lung immune cells with TLR-ligands. To determine the role of the α7nAChR onpulmonary immune vs. parenchymal lung cells we used bone marrow chimeras reconstituted with $\alpha7nAChR^{-/-}$ and WT cells. Interestingly, our mixed bone marrow chimera experiments indicate that α7nAChR expression on alveolar epithelial cells is important for impaired pulmonary anti-bacterial responses mediated by increased vagus nerve activity after stroke.
We could show that the vagus nerve and the α7AchR play a crucial role in suppression of lung immunity after stroke. Inhibition of parasympathetic activity by vagotomy or α7AchR deficiency reduces susceptibility to bacterial lung infection after acute CNS damage. Expression of α7AchR on both lung macrophages and alveolar epithelial cells seem to be important to the immunosuppressive effect of increased vagus nerve activity after stroke.

P5.09.04
Expression of cytokines during the innate immune response in a model of hepatic amoebiasis in sympatectomized hamsters

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The sympathetic nervous system (SNS) release noradrenaline that activate the α1 adrenergic receptors expressed on cells of the innate immune response, thus release pro-inflammatory cytokines during an inflammatory process. On the other hand, During the formation of amebic liver abscess (ALA) an inflammatory process occurs. We analyze the role of SNS in modulating the inflammatory process in a ALA model. 3 groups were used: intact, Sham and sympatectomized with 6-OHDA for 15 days hamsters, then were inoculated 300,000 trophozoites via intrahepatic and sacrificed at different times (6, 12, 24h, 2, 4 and 7d) post-inoculation. In the samples of liver ALA were identified tyrosine hydroxylase positive cells, 220+ kDa amebic lectin, IL-1β+, IL-6+, IL-8+, INF-γ+ and IL-10+ cells by immunohistochemistry. The sympatectomized ALA showed no thyroxine hydroxylase + cells in the hepatic portal triad, demonstrating an effective sympathectomy. Were quantified lectin 220 Kda + trophozoites and we found that at 24h was a decrease of trophozoites (3 per field) with respect to control, concurrently with increased IL8+ cells and decline IL1+ and IL10+ cells. On the other hand, during late times of ALA was observed a significant increase of 220 kDa lectin+ trophozoites (5.1 per field) and decreased IL-1β+, IL-6+, IL-8+ cells, and IL-10+ cells increased. These results suggest that increased IL10 and decreased pro-inflammatory cytokines are crucial to the viability of the parasite. The sympatectomy in acute inflammation of ALA induces a variable behavior of anti and pro-inflammatory cytokines, however, it is maintaining an anti-inflammatory profile during chronic inflammation.

P5.09.05
Detection of ANCA (Anti Neutrophil Cytoplasmic Antibodies) by indirect immunofluorescence / ELISA and related neurological diseases: preliminary results on a Moroccan series

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Introduction: ANCA are associated with different pathologies among other vasculitis. Their diagnostic value, prognostic, specificity and their place as a marker of activity deserve to be clarified. Objective: To identify and develop the methodology for the detection of these auto-antibodies through the use of two reference techniques: indirect immunofluorescence (IIF) and ELISA as well as establish possible correlations with neurological diseases. Materials and methods: We tested sera from 47 patients of Neurology Department, Ibn Rochd University Hospital of Casablanca, for whom ANCA were sought in the etiological. The presence of ANCA was investigated by IIF on slides of neutrophils cytocentrifuged and fixed in ethanol and ELISA (anti-proteinase 3 (PR3) and anti-myeloperoxidase (MPO) kits). The fluorescence is split in two aspects: cytoplasmic (c-ANCA) or perinuclear (p-ANCA). The main target of c-ANCA type autoantibodies is PR3, while that of p-ANCA type is MPO. Results: The presence of ANCA by IIF was established in 48.93% cases in two profiles: cytoplasmic fluorescence (c-ANCA) (73.91%) and perinuclear fluorescence (p-ANCA) (26.08%). These autoantibodies with or without PR3 and / or MPO antigenic targets correspond to various neurological diseases: with infectious etiology, associated with vasculitis but also connective tissue disorders (Gougerot Sjogren syndrome) and amyotrophic lateral sclerosis. Conclusion: In case of strong suspicion of vasculitis, it should look for ANCA by IIF and ELISA. In addition to PR3 and MPO, identify other antigenic targets of ANCA. The association of ANCA with amyotrophic lateral sclerosis has never been reported and should be investigated.

P5.09.06
Expression of the galanin system in immune cells: hypothetical key players of the immune response

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Neuropeptides participate in innate immunity and tolerance. Galanin is a neuropeptide with a widespread distribution in the central and peripheral nervous systems. Several in vivo studies indicate that galanin plays a role in inflammation. The aim of the present study was to identify immune cells expressing components of the galanin system including, not only the peptide but also its three receptors (GalR1-3).
Quantitative RT-PCR analysis was performed on mRNA isolated from different types of human primary immune cells as well as human immune cell lines. We observed the expression of galanin in keratinocytes, endothelial colony-forming cells (ECFC), NK cells, skin mast cells, monocyte and macrophages. Accordingly, galanin expression was detected in the human mast cell line LDA2 and human monocyte cell line THP1.
The analysis of GalR1, GalR2 and GalR3 revealed variable expression, which did not correlate with each other. GalR1 was only expressed by skin mast cells and THP1. GalR2 was detected in keratinocytes, polymorphonuclear cells (PMNs), monocytes, macrophages, skin mast cells, ECFC, LDA2 and THP1. GalR3mRNA expression was observed in ECFC, monocytes, LDA2 and THP1. Moreover, flow cytometric analyses (FACS) revealed expression of GalR3 on T cells, monocytes, B cells and a minority of PMNs.
Our results show the presence of galanin and GalRs in several types of immune cells. Experiments on different types of immune cells are under way to elucidate the functional role of galanin on immune cells expressing GalRs.
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P5.09.07
Blocking Glutamate Carboxypeptidase II Inhibited Glutamate Excitotoxicity and Regulated Immune Responses in Experimental Autoimmune Encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) is a demyelinating autoimmune disease murine model which presents clinical similarity to human multiple sclerosis (MS). Although glutamate-induced excitotoxicity has been implicated in the disease progression of MS patient, glutamate carboxipeptidase II (GCPII), an enzyme that produces glutamate and affects the pathogenesis of various neurological disorders, has not received proper attention in MS/EAE.
In this study, we show that GCPII is a potent therapeutic target for MS/EAE. First, the expression of GCPII was significantly elevated on astrocytes of EAE mice proportional to the severity of EAE clinical sign. Furthermore, treatment with a specific GCPII inhibitor, 2-Phosphonomethylpentanedioicacid (2-PMPA), resulted in significantly ameliorated paralysis with ensuing attenuation of EAE-associated neuropathic features. Upon antigen restimulation, lack of GCPII led to marked reduction in myelin-reactive T cell responses and T effector cell polarization in periphery. Moreover, GCPII inhibition favorably altered the expression of inducible metabotropic glutamate receptor 1 (mGluR1) on CD4+ T cells. These data suggest previously unreported interaction between astrocytic GCPII expression and critical EAE-associated features including neuroinflammation and highlight mGluR1 as one of key modulator of glutamate-induced T cell responses during EAE. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2012011645)

P5.09.08

A new concept to address inflammatory immune activation in neurological disorders

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The involvement of immune pathological pathways in the manifestation of complex neuroinflammatory diseases is difficult to address in humans. Schizophrenia is associated with systemic inflammation, trauma, and a higher prevalence of antibody formation. An altered immune response in the central nervous system also plays a role in multiple sclerosis, with evidence for virus infections, and viral reactivation during relapse. Recent evidence from our group supports the concept of altered immune activation in major Depression as well as other psychiatric diseases. These results are based on a newly developed investigative concept: Primary analyses are measurements of inflammatory cytokines, followed by electron microscopy of dendritic cells with phagocytic properties (phDC) in the cerebrospinal fluid (CSF). The third component addresses the contents of miRNA species in fluid as well as membrane particle fractions of the CSF as compared to plasma isolated by ultracentrifugation. We repeatedly found phDC in the CSF of patients with major depression and a high content of microparticles, ectosomes and exosomes. These particles were successfully used to prepare miRNA isolates. Structural analyses suggest that the phDCs with extensive membrane protrusions gave rise to microparticles. Variations in the miRNA species identified, could explain altered gene expression patterns of meningeal cells in the brain. The protocol developed, is feasible to study larger patient populations and compare the miRNA patterns in microparticles, ectosomes and exosomes of CSF as compared to plasma. Results based on this concept may contribute to our understanding the complex etiology underlying these diseases.

P5.09.09

Astrocytes play a key role in EAE pathophysiology by facilitating CNS immune cell infiltration and suppressing remyelination

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Astrocytes respond to insult with a process of cellular activation known as reactive astrogliosis. One of the key signals regulating this phenomenon is the transcription factor NF-kB, which is responsible for modulating inflammation, cell survival and cell death. In astrocytes, following trauma or disease, the expression of NF-kB-dependent genes is highly activated. We previously demonstrated that inactivation of astroglial NF-kB in vivo (GFAP-IkBalpha-dn mice) leads to improved functional outcome in EAE, and this is accompanied by reduction of pro-inflammatory gene expression in the CNS. Here we show that recovery from EAE in GFAP-IkBalpha-dn mice is associated with reduction of peripheral immune cell infiltration into the CNS at chronic EAE. This is not due to an effect on blood-brain barrier permeability, rather to reduced immune cell mobilization from the spleen. Furthermore, the ability of infiltrating T cells to produce pro-inflammatory cytokines is significantly diminished. In parallel, we also show that the number of total and activated microglial cells is reduced, suggesting that functional improvement in GFAP-IkBalpha-dn mice is dependent upon inhibition of the inflammatory response within the CNS sustained by both resident and infiltrating cells. This results in preservation of myelin compaction as well as enhanced remyelination, as shown by electron microscopy analysis of the spinal cord. Collectively our data indicate that astrocytes are key players in driving CNS inflammation and are directly implicated in the pathophysiology of EAE, since blocking their pro-inflammatory capability results in protection from the disease.

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P5.09.10

A single dose of Pirfenidone attenuates neuronal cell death after excitotoxic kainic acid-induced in rat hippocampus through inhibition of microglial activation, but not TNF-α and IL-1β production

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Excitotoxicity plays an important role in a variety of neurological diseases such as stroke or Alzheimer's disease. It has been known that pivotal pathways of these neuronal disorders are strongly linked to neuroinflammation. In this sense excitotoxicity KA-induced is a robust model that provides an important tool for pharmacology strategies. The pharmacological inhibition of inflammation in animal model seems to be neuroprotective. In this context, the aim of the current study was to demonstrate whether Pirfenidone (PFD, 5-methyl-1-phenyl-2-(1H)-pyridone) which has a well-known anti-inflammatory properties, protect to the hippocampal neurons from excitotoxic neuronal damage KA-induced in a juvenile 5- to 6-week old rats. We determined the neuroprotective effect of 325 mg/Kg p.g. PFD 90 min after 12 mg/Kg of KA i.p. injection in the rats. Neuronal loss and degenerated cell in CA3c hippocampal areas under Hematoxylin-Eosin and with Fluoro-Jade B stain respectively was reduced at 72 h after treatment($p<0.05$). We demonstrated that the neuroprotective effect was attributed to the inhibition of microglial activation quantifying two markers by tissue immunochemistry of MHCII and Isolectine-B_4 ($p<0.05$). There is no inhibition of mRNA TNF-α and IL-1β measured by real-time PCR in hippocampal brain homogenates 6h after treatment. Further investigations needs to perform in order to elucidate the mechanics of the neuronal damage amelioration, but we infer that neuroprotection observed in our study could be attributed a partial anti-inflammatory effects of PFD. The results support that the uses of PFD in excitotoxic and neuroinflammatory diseases could be explored.

P5.09.11

Increased expression of Toll-like receptors 7 and 9 in Epstein-Barr virus-infected myasthenia gravis thymus

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Considerable data support thymic involvement in myasthenia gravis (MG), an autoimmune disease affecting neuromuscular junction. We recently demonstrated an active Epstein-Barr virus (EBV) infection in the thymus of MG patients, suggesting that EBV might promote onset or maintenance of the autoimmune response within MG thymus, because of its ability to immortalize B-cells. EBV can elicit and modulate Toll-like receptor (TLR) 7 and 9 signalling, known to impair B-cell function and favour autoimmunity.

The aim of this study was to analyse TLR7 and 9 expression in MG thymuses. By real-time PCR, we found that TLR7 and 9 transcripts were significantly up-regulated in EBV-positive MG compared with EBV-negative control thymuses. By confocal microscopy, TLR7 and 9 were detected in B- and plasma cells of MG thymic germinal centers (GCs) and lymphoid infiltrates, where they co-localized with EBV antigens. Proliferating EBV-positive B-cells expressing TLR7 and 9 were detected in MG thymuses, suggesting that TLR7/9 stimulation contributes, along with EBV, to abnormal B-cell activation and proliferation in MG thymus. Both MG GCs and GCs-free thymic sections expressed TLR7 and 9 at higher levels than control thymuses, indicating that, along with GC-cells, other MG thymic populations over-expressed TLR7 and 9. Double immunofluorescence revealed enhanced TLR7 and 9 expression in MG thymic epithelium and plasmacytoid dendritic cells, suggesting TLR7/9 involvement in the inflammatory/interferon signature characterizing MG patients' thymus.

These findings suggest that EBV-driven innate immune responses may contribute to immunological alterations promoting or maintaining autoimmunity in MG thymus.
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P5.09.12
Contact Hypersensitivity To Oxazolone Provokes Vulvar Mechanical Hyperalgesia In Mice

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Vulvodynia can affect ~20% of women of child-bearing age. Activities such as contact with tight clothing or sexual intercourse provoke burning pain sensations whose etiology and underlying mechanisms are poorly understood. Epidemiological evidence suggests a link between a history of environmental allergies and the risk of developing vulvodynia. Clinicians find increased mast cell numbers, hyperinnervation and up-regulation of inflammatory cytokines in biopsies from vulvodynia patients vs. controls. An allergy-based mast cell focused mouse model of vulvodynia is needed to elucidate the mechanisms underlying this pathology. We show that challenge with contact hypersensitivity allergen oxazolone produces acute mechanical hyperalgesia in the vulvar region of previously sensitized female mice. Acute pain is localized to the challenge site, lasts up to 24 hours post-challenge and is accompanied by influx of neutrophils into the labiar tissue and up-regulation of several inflammatory cytokine genes including IL-6, Cxcl-1 and Cxcl-2. Pre-challenge administration of H1R-antagonist pyrilamine, tricyclic antidepressant amitryptiline, and sodium cromoglycate all abrogate the hyperalgesic response. Our findings provide the first evidence of the induction of measurable pain following an allergic response in the labiar/vulvar tissue in mice thus establishing a model that can be adapted to chronic challenges and long-term assessment of pain with and without ongoing inflammation.

P5.09.13
Expression of dopamine receptor D3 on CD4$^+$ T-cells favors Th1-mediated immunity

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Inappropriate CD4$^+$ T-cell differentiation may lead to infection susceptibility, tumor development or autoimmunity, thereby this process requires strict regulation. Since T-cells express dopamine receptors (DRs), dopamine may modulate T-cell function. Among five known DRs, D3R displays the highest affinity for dopamine and its stimulation enhances IFN-γ production on human CD4$^+$ T-cells *in vitro*. Thus, the aim of this work was to analyze the role of D3R on effector CD4$^+$ T-cell differentiation and function. Our results show that Th1, but not Th17, -polarizing CD4$^+$ T-cells transiently express D3R transcript and that stimulation of D3R potentiates IFN-γ production by CD4$^+$ T-cells under Th1 conditions. Moreover, whereas D3R-deficient CD4$^+$ T-cells show normal differentiation towards Th17, they display impaired Th1 polarization, which is evidenced by reduced *Tbx21* and *Ifng* transcript expression and limited IFN-γ production. D3R was also shown to be important for Th1 polarization *in vivo*, since D3R-deficient CD4$^+$ T-cells showed attenuated IFN-γ production in response to Th1-immunogen. Importantly, D3R also contributes to CD4$^+$ T-cell activation, as pharmacologic stimulation of D3R potentiates IL-2 secretion and D3R-deficiency on CD4$^+$ T-cells results in reduced IL-2 production and proliferation both *in vitro* and *in vivo.* Furthermore, D3R-deficient CD4$^+$ T-cells were unable to induce weight loss in a colitis model. In line with this, a reduced frequency of IFN-γ-producing CD4$^+$ T-cells was found in colonic lamina propria of mice receiving D3R-deficient CD4$^+$ T-cells. These findings support a relevant regulatory mechanism of CD4$^+$ T-cell function in which D3R expression contributes efficiently to CD4$^+$ T-cell activation and Th1 differentiation.

P5.09.14
Prolactin is dispensable for the development of experimental autoimmune encephalomyelitis

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In recent years several pieces of evidence have suggested that hormones might influence the development of both multiple sclerosis (MS) and the animal model for this disease, experimental autoimmune encephalomyelitis (EAE). In the present work we investigated the role of the hormone prolactin (PRL) in EAE by studying disease expression in PRL receptor (PRLR)- or PRL-deficient mice. *Prlr*$^{-/-}$ and *Prl*$^{-/-}$ mice developed EAE with a slightly delayed onset but with full clinical severity compared to controls. In line with clinical outcome, lymph node cells (LNCs) from *Prlr*$^{-/-}$ mice exhibited reduced proliferation to myelin peptide and decreased production of pro-inflammatory cytokines IFN-, IL-17A and IL-6 during disease priming (7 days p.i.), but not later (day 10 p.i.). Serum titers of anti-MOG$_{35-55}$ IgG antibodies were not affected by PRLR deficiency. Similar findings were obtained in *Prl*$^{-/-}$ mice. LNCs and CD4$^+$ T cells isolated from naïve C57BL/6 mice expressed *Prlr* transcript. Levels of *Prlr* were reduced in LNCs and CD4$^+$ T cells obtained from mice 7 days p.i., as well as in CD4$^+$ T cells stimulated *in vitro* with anti-CD3/CD28 Abs, suggesting that this receptor is down-regulated in stimulated T cells. We did not detect PRL either at transcript level in CD4$^+$ T cells or at protein level in stimulated CD4$^+$ T cell supernatants. These data suggest that PRL plays a redundant role in the development of EAE and of an optimal Th1/Th17 autoreactive T cell response against myelin.

P5.09.15
The immunogenicity of amyloid beta oligomers

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Background: The central molecule in the pathogenesis of Alzheimer's disease (AD) is believed to be a small-sized polypeptide – beta amyloid (Aβ) which has an ability to assemble spontaneously into oligomers. Various studies concerning therapeutic and prophylactic approaches for AD are based on the immunotherapy using antibodies against Aβ. However, knowledge on the mechanisms of Aβ-induced immune response is rather limited. Previous research on Aβ1-42 oligomers in rat brain cultures showed that the neurotoxicity of these oligomers considerably depends on their size. In the current study, we evaluated the dependence of immunogenicity of Aβ1-42 oligomers on the size of oligomeric particles and identified the immunodominant epitopes of the oligomers. **Results:** Mice were immunized with various Aβ1-42 oligomers. The analysis of serum antibodies revealed that 1-2 nm Aβ1-42 oligomers are highly immunogenic. They induced predominantly IgG2b and IgG2a responses. In contrast, larger Aβ1-42 oligomers and monomers induced a weak IgG response in immunized mice. Monoclonal antibody against 1-2 nm Aβ1-42 oligomers was generated and used for the antigenic characterization of Aβ1-42 oligomers. Epitope mapping of both monoclonal and polyclonal antibodies demonstrated that the main immunodominant region of the 1-2 nm Aβ1-42 oligomers is located at its amino-terminus, between amino acids 1 and 19. **Conclusions:** Small Aβ1-42 oligomers of size 1-2 nm induce the strongest immune response in mice. The amino-terminus of Aβ1-42 oligomers represents an immunodominant epitope which indicates its surface localization. The results of the current study may be important for further development of Aβ-based vaccination and immunotherapy strategies.

P5.09.16
Enhanced susceptibility to pulmonary infections after experimental stroke

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Bacterial infection is a relevant complication in patients with acute CNS injury and associated with worse neurological recovery and high mortality. Clinical and experimental evidence indicate that immunodepression is an important factor for the high incidence of pneumonia. Previous studies analysed stroke-induced suppression of immune responses in blood and lymphatic organs. Here, we analyse changes in cellularity and function of lung immune and epithelial cells by FACS and *ex-vivo* stimulation with different TLR-ligands and T-cell mitogens at different time points after cerebral ischemia induced by temporary occlusion of the middle cerebral artery in mice. In addition, we monitored pulmonary host responses *in-vivo* during the course of pneumococcal pneumonia by analyzing cellular changes and cytokine/chemokine levels in lungs of stroke mice and sham controls. Quantitative FACS analysis of cellularity at different time points after stroke shows decreased numbers of lymphocytes, dendritic cells and macrophages in lung. Functional analysis of immune cells indicates an impaired cytokine production in lung cells after *ex-vivo* TLR stimulation. In a model of induced pneumonia we analysed the *in-vivo* host response to *Streptococcus pneumoniae*. Preliminary data paradoxically suggest hyper-inflammatory responses in the early phase with an increase in lung cytokine levels in stroke mice.The correlation between lymphopenia and development of infectious complications is still incompletely understood. It seems that the mechanisms of immunodepression in lung are similar to those in the periphery. Defects in bacterial defences after stroke needs to be further investigated and may lead to new therapeutic strategies to prevent infections and improve outcome.

P5.09.17
Regulatory T cells delay disease progression in a murine model of Alzheimer's disease

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory and cognitive functions. Accumulation of Aβ peptide is considered the initiating cause of pathogenic lesions, and immunotherapy strategies targeting Aβ represent promising therapeutic approaches. Vaccination against Aβ provided encouraging results in experimental mouse models and in a clinical trial (AN1792). Although the latter had to be interrupted due to meningoencephalitis attributed to pro-inflammatory T cell responses. Several reports suggest that Aβ-specific $CD4^+$ T cells may be implicated in the course of AD and could have a strong therapeutic potential, pointing out the need for better understanding the role and regulation of T cell responses to Aβ. We previously showed that regulatory T cells control the magnitude of Aβ-specific $CD4^+$ T cell responses in physiological and pathological settings in response to Aβ vaccination. To define the role of Tregs in disease progression, we analyzed the impact of Treg depletion in a mouse model of AD. Depletion of Treg cells accelerated the onset of cognitive deficits in APPPS1 mice. Alteration in spatial memory was detectable in PC61-treated animals, while PBS-treated APPPS1 mice were not yet cognitively impaired as compared to wt. Early cognitive impairment in Treg-depleted APPPS1 mice was associated with alterations in neuropathological features of the disease.
Our data indicate that Treg cells play a beneficial role in the pathophysiology of AD and delay disease progression in APPPS1 mice. These results open new perspectives in the development of tolerogenic Treg-based vaccine immunotherapy strategies for the treatment of AD.

P5.09.18
Possible role of nerve-glial antigen 2(NG2) in the development of experimental autoimmune encephalomyelitis

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Nerve/glial antigen 2 (NG2) is expressed by oligodendrocyte progenitor cells (OPCs), essential for remyelination, and pericytes, crucial for blood-brain-barrier (BBB) integrity. We used NG2KO mice to investigate if NG2 is involved in development of experimental autoimmune encephalomyelitis (EAE), animal model for multiple sclerosis associated with remyelination failure and BBB leakage. EAE was less severe in NG2KO mice, with little demyelination and a lower number of macrophages/microglia in CNS, but no difference in infiltrated T-cell numbers. While NG2KO and wild-type (WT) mice not induced for EAE had the same number of OPCs, the sharp decrease in EAE-affected WT mice at late-disease did not occur in EAE-affected NG2KO mice. As the difference in infiltrates between NG2KO and WT mice could be related to differences in BBB integrity, we studied the expression of BBB-relevant proteins, claudin-5 and occludin. In WT mice, the expression pattern of these proteins was altered in EAE as expected, changing from a linear cellular contour to a punctuated one. In NG2KO mice, an opposite pattern was observed, with a punctuated expression in non-EAE mice reverting to a linear one in disease. In WT mice, NG2 was expressed on macrophages, dendritic, and T cells; the lack of NG2 on NG2KO T cells was associated mostly with an increase in IL-4, but also IFNγ, and IL-17a, and with lower proliferation. These results suggest that NG2-bearing cells likely play a relevant role in CNS homeostasis. In addition, NG2 expression on immune cells could impact on effector functions possibly related to CNS autoimmunity.

P5.09.19
Dopamine regulates the induction of Cytotoxic T-Lymphocyte by stimulation of dopamine receptor D3 expressed on CD8+ T-cells and on dendritic cells

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Dopamine receptor D3 (D3R) is expressed in many cellular components of the immune system, including dendritic cells (DCs) and CD8+ T-cells. Here, we aimed to understand how D3R may regulate the effector function of CD8+ cytotoxic T-lymphocytes (CTLs). Since the relationship between DCs and CD8+ T lymphocyte is pivotal for the efficient induction of cytotoxic response, we study the contribution of D3R expressed on DCs and on CD8+ T-cells in the regulation of CTL response. Results show that loss of D3R in CD8+ T-cells impairs both secretion of cytotoxic vesicles and IFN-γ secretion in vitro. Similarly, lack of D3R in OT-I cells impaired their cytotoxic capability and IFN-γ production in vivo in response to target cells loaded with ovalbumin(OVA)-derived peptide (pOVA), In contrast, when DCs were loaded with OVA and then transferred into wild-type recipients and CTL function was evaluated, D3R-deficiency on DCs resulted in an increased IFN-γ production by CTLs. In agreement with this, both genetic and pharmacologic approaches showed that D3R expressed on DCs attenuates CD8+ T-cell activation without affecting CD4+ T-cell activation and differentiation in vitro. Furthermore, in vitro experiments showed an increased surface expression of class-I MHC loaded with pOVA on DCs when treated with D3R-specific antagonist, an effect lost in D3R-defficient DCs. Thus our findings indicate that D3R expressed in DCs and

CD8+ T-cells may modulate the CTL response in opposite ways. Thereby, depending on the cellular target, the D3R could be considered as a molecular target for the treatment of cancer.

P5.09.20
ATP-mediated interaction between astrocytes and T cells

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Astrocytes represent a major part of glial cells in CNS and constitute important structural component of the blood brain barrier (BBB). We investigated the biological response evoked in astrocytes and T cells upon their reciprocal interaction, clarifying the role of ATP in the intercellular signaling between these two cell types. T cell costimulation through purinergic P2X receptors, including P2X4 and P2X7 subtype, upon TCR triggering, sustains MAPK pathway activation, whereas blockade of purinergic costimulation results in T cell anergy. Conversely, P2X7 stimulation in the absence of TCR activation determines T cell apoptosis. Thus, extracellular ATP and purinergic signaling play a crucial role in conditioning T cell fate. Real time PCR data revealed that astrocytes inhibit CD4 cells transcriptional program toward effector phenotypes. These results suggest a general anergyzing effect of astrocytes on T cells. We determined extracellular ATP levels by the luciferin/luciferase assay in the supernatants of astrocytes-T cells co-cultures: our data revealed a reduced concentration of extracellular ATP in the presence of T cells, suggesting that T cells may partially inhibit ATP release and/or hydrolyze ATP released from astrocytes. Consistent with the latter possibility, FACS analysis indicated increased expression of the ectonucleotidases CD39 and CD73 in T cells co-cultured with astrocytes. In addition, CD39 knock out T cells did not significantly affect extracellular ATP levels, confirming that ectonucleotidase activity in T cells mediate extracellular ATP hydrolysis. These data suggest that lack of purinergic costimulation in activated T cells upon contact with astrocytes promotes T cell anergy

P5.09.21
Experimental studies of narcolepsy immunopathogenesis in HLA-DQB1*0602 transgenic mice

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Background: A strong association of narcolepsy/cataplexy (N/C) with the HLA class II allele DQB1*0602 (>90%) suggests that this chronic brain disorder is immune-mediated. The use of AS03-adjuvanted influenza A (H1N1) pandemic vaccine (Pandemrix®) in 2009/2010 was associated with an increased risk of N/C in children and adolescents in several European countries.
Objectives: To understand the mechanisms of N/C immunopathogenesis, and to develop a mouse model of immune-mediated N/C.
Methods: Groups of HLA-DQB1*0602, huCD4 transgenic Ab0 NOD ("HLA-DQ6 mice") or HLA-DQ8 control mice were challenged with Pandemrix® (or PBS control) i.m., mouse hypothalamus homogenate in AS03 i.m., or hypocretin (1+2)-KLH in CFA i.d. One group of HLA-DQ6 mice was injected into the left lateral cerebral ventricle (i.c.v.) with Pandemrix®. Mouse N/C was screened for by videomonitoring of behavior. Mice were sacrificed after 5-12 weeks.
Results: The groups of HLA-DQ6 or HLA-DQ8 mice challenged with Pandemrix® i.m. did not show any changes in body weight, behavior, histology or anti-viral serum antibody titers. No unique serum reactivity was observed with rat brain (immunofluorescence). No differences were noted in hypothalamus sections stained for hypocretin (immunohistochemistry), or in hypocretin precursor mRNA levels (RT-PCR). Mice challenged with hypocretin 1+2, but no other mice, developed serum anti-hypocretin peptide IgG antibodies. Further studies are ongoing.
Conclusions: HLA-DQ6 mice did not develop N/C in response to Pandemrix® i.m. challenge. Serum responses did not differ between HLA-DQ6 mice and HLA-DQ8 controls. In addition, no evidence for crossreactivity between vaccine components and brain antigens could be found to date.

P5.09.22
Schizophrenia is associated with over-expression of the interleukin-2 receptor gamma gene

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Altered immune response, including low-grade inflammatory processes, are involved in the pathogenesis of schizophrenia - a chronic neuropsychiatric disorder with complex etiology. Distinct gene variants and a number of regulatory, pro-inflammatory and chemotactic cytokines together with their receptors have been implicated in this disorder. Interleukin-2 receptor gamma (IL-2RG) represents a component of several cytokine receptors and so far, no data have been reported about this receptor in schizophrenia, including its functional state. The aim of this study was, therefore, to investigate mRNA expression of the IL2RG gene in schizophrenia patients in comparison with healthy control subjects.
Total RNA was isolated from peripheral blood of 66 schizophrenia patients and 99 healthy subjects from Armenian population. The mRNA expression was determined by quantitative real-time polymerase chain reaction (RT-PCR) using PSMB2 as housekeeping gene.
IL2RG mRNA expression was upregulated in peripheral blood of patients in comparison with controls (patients vs. controls, mean±SEM: 3.06±0.47 vs. 0.52±0.06, $p<0.0001$). In conclusion, our findings suggest that over-expression of the IL2RG gene may be implicated in altered immune response in schizophrenia and thus associated with pathomechanisms of this disorder. Further investigations are required to extend this observation and relate it to disease clinical phenotypes.
We thank the administration and medical staff of the clinics of Psychiatric and Erebouni Medical Centers of the Ministry of Health of the Republic of Armenia for selection of patients and healthy subjects. The study was supported by the International Visegrad fund scholarship (HG_51200594), NAS RA, IGA_PU_LF_2013_009, and CZ.1.05/2.1.00/01.0030 funds.

P5.09.23
Schizophrenia is not associated with changes in blood expression levels of complement factors B, H and I

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Our previous studies indicated systemic hyperactivation of the alternative complement cascade in schizophrenia. However, molecular pathomechanisms of detected alterations are not clear yet. In the present study we evaluated the expression levels of factor B, H and I in peripheral blood of patients with schizophrenia (n=66) compared to healthy controls (n=99). Factor B is an essential component of the alternative pathway. Factors H and I are negative regulators of this pathway. Methodological design includes quantitative real-time polymerase chain reaction (RT-PCR) of mRNA samples isolated from the peripheral blood cells of the study subjects. Our results showed no significant changes in the selected proteins mRNA expression were detected in patients compared to controls ($p>0.05$). In summary we concluded that hyperactivation of the complement alternative pathway is most probably not conditioned by changes in the expression levels of factor B, factor H and factor I in the peruipheral blood.
We thank the administration and medical staff of the clinics of Psychiatric and Erebouni Medical Centers of the Ministry of Health of

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P5.09.24
IL-18 and IL-18BP in Amyotrophic Lateral Sclerosis

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The role of inflammation in neurodegenerative diseases, included ALS, is increasingly recognized. Activation of caspase-1 has been demonstrated in the spinal cord of ALS mice and humans. To investigate if inflammasome hyperactivation is pathogenic, promoting neuroinflammation and contributing to ALS progression, we analyzed the levels of IL-18 and IL-18BP in serum and CSF from ALS patients.
Serum and CSF samples were collected from 103 patients with sALS. A control group consisted of 40 healthy serum donors; a second group included 69 CSF donors, either healthy or with different kinds of non-infectious non-inflammatory diseases. Cytokine levels were measured by ELISA. The levels of free IL-18 were calculated by the law of mass action.
Levels of IL-18 (21.99±14.03 pM), IL-18BP (471.54±230.50 pM) and free IL-18 (9.92±5.21 pM) were significantly higher in ALS serum than in healthy controls (IL-18: 12.12±7.24 pM, $p<0.0001$; IL-18BP: 268.83±165.85 pM, $p<0.0001$; free IL-18: 7.35±4.02 pM, $p=0.0006$). No correlation between cytokine levels and disease stage was found. IL-18 was undetectable in CSF, while IL-18BP was found at significant and comparable levels in both ALS patients and controls.
High circulating IL-18 concentrations may suggest the involvement of inflammasome-dependent inflammation in the disease, but the lack of correlation between IL-18 levels and disease stage suggests that the cytokine is not involved in triggering the disease exacerbation. The abundant presence of IL-18BP in the CSF in the absence of its cytokine target may imply the physiological need of maintaining homeostasis in a fundamental organ like the brain.

P5.09.25
Using the sodium salt of native DNA as an immunomodulator in the treatment of different diseases

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We used a solution of the sodium salts of native deoxyribonucleic acid in 0.9% sodium chloride solution (15 mg/ml). Deoxyribonucleinat was obtained from sturgeon milt. It is known that nuclease activates when nucleic acid is injected into a cell; and cytosis, bacteriosis, enzymatic processes of the cell directly depend on the nuclease. During therapy with deoxyribonucleinat we observed an improvement in the elimination of the inflammatory response and menstrual disorders, improvement of the functions of related organs, reduction of psychoemotional disorders at women of reproductive age with chronic salpingo-oophoritis. After treatment with deoxyribonucleinat patients had permanent positive effect, manifested by a significant decrease in pain in the projection of the uterus. We showed that with the inclusion of deoxyribonucleinat in the therapeutic complex there were more rapid relief of inflammation (2-3 days), decrease of gingival hemorrhage, as well as reduction of average treatment duration, significant relief of inflammation in the periodontium, improvement of oral hygiene and the general condition of sick children with functional diseases of the nervous system and musculoskeletal system. The results of investigation indicate significant efficacy of deoxyribonucleinat in the complex therapy of different forms of neurodermatitis. Clinical recovery and significant improvement were observed in 85% of patients. A noticeable improvement of skin status was observed earlier than in the standard therapy.

P5.09.26
Prevalence and evolution of Autoimmune Diseases in patients with Cushing's Syndrome compared to other Pituitary Tumors: a Multicenter Italian Study

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New onset of autoimmune diseases (AID) is reported after remission of Cushing's syndrome (CS), likely due to immune system reactivation after suppression induced by hypercortisolism. We provide results of the largest study comparing prevalence and type of AID in CS during active disease vs remission, and vs other pituitary adenomas (PAs). Methods: 116 patients (F=95) with endogenous CS treated with surgery/drugs/RT, with active disease or after remission; 116 patients with other types of PAs matched for sex and age at diagnosis from 3 Italian Centers. Results: 35 patients (28 F) with CS presented AID, single in 30 cases, multiple in 5. AID appeared before CS in 12 cases: 3 resolved before CS's onset, 5 persisted during active phase and CS' remission, 2 improved during active phase but worsened after CS' remission, 2 resolved after CS' remission. In 10 cases AID appeared in the active phase of CS: 9 persisted, 1 resolved after CS' remission. In 17 cases AID appeared only after CS' remission. 40 patients with PA (26 F) suffered from single AID, whose evolution did not correlate with hormonal status. AID's prevalence was similar in ACTH-dependent and independent (33 vs 30.4%) CS and in pituitary CS vs other type of PA. Relative prevalence was significantly higher in males (21/42 vs 54/190); thyroiditis and psoriasis were the most common diseases. Hypercortisolism, but not the levels of the other pituitary hormones, influenced AID's evolution as the great majority of new cases/exacerbation of AID were registered after CS's remission, confirming previous data.

P5.09.27
Measuring cerebrospinal fluid (CSF) immunoglobulins in neurological conditions

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Background: CSF analysis of immunoglobulins can be done using qualitative [oligoclonal band (OCB) patterns Type 1-5) and quantitative [Albumin quotient (QAlb) and IgG index] techniques to evaluate blood-brain barrier dysfunction and detect either intrathecal synthesis or passive transfer of IgG into the brain.
Method: 189 children who underwent CSF investigation for their neurological condition had CSF and serum testing to i) qualitatively identify oligoclonal patterns Type 1-5 ii) quantitatively measure the IgG index (IgG Index=IgGCSF/IgGSERUM)/(AlbCSF/AlbSERUM) and QAlb (QAlb=AlbCSF/AlbSERUM). Patient case notes were reviewed and patients were grouped to either have an inflammatory (n= 104) or non-inflammatory (n=85) aetiology.
Results: Patients with inflammatory condition were significantly more likely to have abnormal results (PValue = 0.0019). CSF restricted OCB were found in 19% of inflammatory group compared with 5% of non-inflammatory patients (PValue = 0.0036). Mirrored OCB were found in 12.5% of the inflammatory group, compared with 6% of the non-inflammatory group (PValue=0.14)., IgG index and QAlb are significantly higher in patients with an inflammatory aetiology. However, a raised IgG and QAlb were seen in both groups with QAlb abnormalities seen more frequently in the inflammatory group (PValue 0.0028). The qualitative and quantitative evaluation collectively revealed additional positive results than when done in isolation, but did not distinguish between both aetiologies. Overall, the

likelihood ratio of a positive test to help differentiate inflammatory from non-inflammatory causes was 3.1 (95%CI 1.6- 6.0).
Conclusion: Both qualitative and quantitative evaluations of CSF immunoglobulins are useful in identifying inflammatory mechanisms in a range of neurological conditions

P5.09.28
Expression of selected Th1/Th2 transcription factors and immune-response genes in cerebral ischemia and senescence

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T helper (Th) subsets have been implicated in ischemic stroke. This study, therefore, aimed to assess functional state of Th1/Th2 cells by measuring expression of genes encoding GATA3 and TBX21, markers of Th2 and Th1, respectively, in stroke-affected and healthy control subjects. 54 patients with ischemic stroke (mean age±SE: 71.91±1.47 years) on 1st day after stroke onset, 28 aged (66.71±1.5) and 46 middle-aged (37.09±1.04) healthy controls of Armenian ancestry were enrolled with ethical clearance. mRNA expression in peripheral blood cells was determined by quantitive real-time polymerase chain reaction (RT-PCR) with PSMB2 as housekeeping gene. There was no difference in GATA3 mRNA expression between the investigated groups ($p>0.05$). However, decreased TBX21 mRNA expression was observed in ischemic stroke patients and in aged controls (3.1 and 2.9-fold, respectively; $p<0.05$) indicating that these changes may be age-dependent. Aging process is a major risk factor for cerebral ischemia and numbers&function of Th1 cells decrease while those of Th2 cells increase during aging. Thus, in further experiments we analysed peripheral blood expression levels of IL2 and IL2RG. Both IL2 and IL2RG mRNA transcripts were downregulated in patients and aged controls compared to middle-aged controls. Moreover, there was a positive correlation between the expression of TBX21, IL2 and IL2RG. In summary, GATA3 expression levels were not altered by ischemic stroke condition or aging processes, whereas TBX21, IL2 and IL2RG were upregulated in ischemic stroke.
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P5.09.29
Activation of protease-activated receptor 2-mediated signaling by mast cell tryptase modulates cytokines production in primary cultured astrocytes

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Background: Since protease-activated receptors-2 (PAR-2), which is abundantly expressed in astrocytes, is known to mediate signaling events in CNS in physiological and pathological processes, the accurate mechanism by which it plays pivotal roles in the astrocytic modulation remains to be elucidated.
Methods: Viability and proliferation of astrocytes was assessed by CCK8 analysis. Intracellular ROS was determined by 2', 7'-dichlorodihydrofluorescein oxidation. IL-6 and TNF-α were measured with commercial ELISA kits. Phosphorylation of MAPKs or AKT and production of TGF-β or CNTF were evaluated by Western blotting.
Results: Tryptase at low concentrations modestly inhibited intracellular ROS levels and proinflammatory TNF-α secretion while significantly increased IL-6 and TNF-α secretion at higher concentrations without affecting astrocytic viability and proliferation, which were both alleviated by specific PAR-2 antagonist FSLLRY-NH2. PI3K/AKT inhibitor LY294002 reversed the effect of tryptase on IL-6 production while inhibitors specific for p38, JNK and ERK1/2 abolished the effect of tryptase on TNF-α production, suggesting distinct pathway responsible for each cytokines production in astrocytes. The enhanced activation of MAPKs and AKT by tryptase administration was eliminated by FSLLRY-NH2, strengthening the evidence of two potential signaling pathways downstream from PAR-2. In addition, the expression of TGF-β and CNTF in astrocytes was as well increased by tryptase treatment.
Conclusions: The present findings first suggest that tryptase is able to regulate cytokines release from astrocytes via PAR-2-MAPK or PAR-2-PI3K/AKT signaling pathway, and reveals a novel profile of PAR-2 as a new target in the regulation of astrocytic function for neuroprotection.

P5.09.30
Detection of novel nitric oxide mediated neuro-inflammatory mechanism against environmental toxins/agents

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Exposure to environmental toxins/agents is associated with neuroinflammation and oxidative stress which are the primary mechanisms for the pathogenesis of chronic neurodegenerative diseases. Recently, it has been proposed that microglia, the innate immune cells of the mammalian brain, become hyperactivated and deregulated in response to exposure to environmental toxins/agents. The mechanisms for neuroinflammatory responses that exaggerate neurotoxicity are poorly understood. Moreover, the studies to date that investigate neuroinflammatory mechanisms have utilized primarily in vitro approaches. We have established a Drosophila model based on exposure to toxic volatile organic compound (VOC), 1-octen-3-ol which recapitulates most behavioral and pathophysiological features of Parkinson's disease (PD). Using this model, we have discovered that 1-octen-3-ol exposure induces nitric oxide synthase (NOS) and a corresponding elevation of nitric oxide (NO) production in the adult Drosophila brain. NOS is recognized as a major marker for neuroinflammation and shown to be produced by activated microglia in mammalian model. Therefore, the observation of NOS induction during Drosophila neurodegeneration parallels the mammalian process and presents a novel discovery of in vivo model to further dissect the genetic components of neuroinflammatory process which are not possible to perform in mammalian or in vitro models. The discovery of a NOS-dependent inflammatory response in flies provides the foundation for future work to explore cellular and molecular mechanisms associated with neuroinflammatory process in efficient and inexpensive manner.

P5.09.31
Regulation of microglia activation mechanisms following chemical or mechanical stimulation

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Aim: Deregulation of microglial activation plays a critical role in neurodegeneration, which has been highly correlated with expression of MHC-II molecules. Using BV-2 cells as an experimental model of migroglia, the present study aimed to define the membrane and intracellular expression of H-2A as well as intracellular H-2M, H-2O and CD74 at different activation states, using flat or 3D-micro laser-textured Si scaffolds.
Methods: Flow cytometry and confocal microscopy analysis defined H2-A, -O, and -M expression, while RT-PCR was used to evaluate CD74 in BV-2 cells in the presence or not of LPS, IFN-γ and IL-4. Secretion of H2-A was evaluated by ELISA. Scanning electron microscopy analysis defined the morphological features of BV-2 cells grown on Si scaffolds with gradient 3D micro-geometries.
Results: BV-2 cells expressed all necessary components for post-translational regulation and transport of MHC-II molecules to the cell membrane. Although LPS did not affect MHC-II expression, it induced TNF-a secretion while promoting cytophagy. IFN-γ reduced MHC-II expression but increased secretion of H2-A molecules, whereas IL-4 reduced H2-A while inducing H2-O expression. Roughness of culture substrate was shown to affect BV-2 morphology. At low-roughness scaffolds, BV-2 cells displayed resting microglia morphology showing

elongated bipolar cell bodies with spine-like processes carrying short branches, while at high-roughness cells exhibited an ameboid-like shape with short processes suggesting microglia activation.
Conclusion: Depending on the chemical or mechanical stimulatory signal, BV-2 cells follow different activation pathways, ranging from neuroprotection to neurodegeneration. Understanding the mechanisms governing microglial activation could dictate new therapeutic strategies for neurodegenerative diseases.

P5.09.32
Macrophage migration inhibitory factor has an essential role in age-related hearing loss

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Objective: The hearing loss related with aging is the most common sensory disorder among elderly persons. Macrophage migration inhibitory factor is the multi-functional molecule. The aim of this study is to identify the role of macrophage migration inhibitory factor in inner ear.
Study Design: Animal experiment
Methods: The macrophage migration inhibitory factor deficient mice on BALB/c background and wild type mice were used in this study. The expression of macrophage migration inhibitory factor protein in inner ear was examined by immunohistochemistry in wild type mice. The hearing threshold was determined both in macrophage migration inhibitory factor deficient mice and wild type mice by click-evoked auditory brainstem response at the age of 1, 3, 6, 9, 12, and 18 months.
Results: The expression of macrophage migration inhibitory factor was observed in spiral ligament, stria vascularis, Reissner's membrane, spiral ganglion cells, saccular macula, and membranous labyrinth. The macrophage migration inhibitory factor deficient mice had significant hearing loss as compared with wild type mice at the age of 9, 12, and 18 months.
Conclusion: The macrophage migration inhibitory factor was strongly expressed in mouse inner ear. The aged macrophage migration inhibitory factor deficient mice showed the accelerated age-related hearing loss. These findings suggest that macrophage migration inhibitory has an important role in inner ear in mice.

P5.09.33
Evaluation of multiple sclerosis (MS) based on Antibody to Aquaporine-4(AQP-4) in urine and serum compared to total IgG antibody

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Introduction- Multiple sclerosis (MS) is a disease in which the myelin is damaged due to inflammation. As AQP-4 is important water chanel protein expressing in MS the current study aimed to measure the antibody titer against AQP-4 either in urine and the sera of MS patients compared to the total IgG to evaluate the diagnosis of the disease this way.
Materials and Methods- 21 MS patients together with 21 healthy people were included in this study. A urine and blood sample was taken and after RBC and WBC count, Hb measurement, MCV, MCHC and RDW, the total antibody titer (IgG class, IgA and IgM) was measured using Nephlometry. Anti-AQP-4 antibody was measured using ELISA. Data were extracted and analyzed using ANOVA and Pearson correlation test.
Results- The total IgM was similar in both case and control group but there was a higher titer of IgG and IgA in case compared to the control group. The mean anti-AQP-4 IgG antibody was 17.4 and 17.8 in urine and serum respectively while in mild MS was 16.1 and moderate and severe ones it was 19.3.
Conclusion- As the antibody titer to AQP-4 in either serum and urine is similar, but it is higher in urine of severe MS patients, urine levels of this antibody could represent a noninvasive method for diagnosis of the MS with a high reliability. Anti-AQP-4 antibody also can be used a good indicator for analyzing the severity of MS..

P5.09.34
The inner ear findings in macrophage migration inhibitory factor knockout mice

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Objective: The hearing loss related with aging is the most common sensory disorder among elderly persons. Macrophage migration inhibitory factor is the multi-functional molecule. We showed that the macrophage migration inhibitory factor deficient mice had significant hearing loss as compared with wild type mice. The aim of this study is to show the inner ear findings in macrophage migration inhibitory factor deficient mice.
Study Design: Animal experiment
Methods: The macrophage migration inhibitory factor deficient mice on BALB/c background and wild type mice were used in this study. We examined the morphological findings of cochlea including cochlear hair cells and spiral ganglion cells both in macrophage migration inhibitory factor deficient mice and wild type mice using scanning electron microscopy and light microscopy.
Results: The scanning electron microscopy showed affected outer cochlear hair cells in macrophage migration inhibitory factor deficient mice. The inner cochlear hair cells were relatively preserved. The number of spiral ganglion cells in inner ear was decreased in macrophage migration inhibitory factor deficient mice. No significant finding was observed in stria vascularis and spiral ligament in the cochlea of macrophage migration inhibitory factor deficient mice.
Conclusion: The cochlear hair cells and spiral ganglion cells are the important components in the auditory system. The macrophage migration inhibitory factor deficient mice had the morphological inner ear abnormalities. These findings suggest that macrophage migration inhibitory has a principal role in neural systems in inner ear.

P5.09.35
Participation of cold sensitive TRPM8 ion channel in modulation of metabolism and immune response

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The response of living organism to any stimulus is a combination of the responses of various systems. The combination is determined by the nature of the afferent information providing constant and precise interaction of the systems. Relationship of thermoregulatory and immune systems is an important aspect of unsolved problems, because the widespread influence of temperature on the body. Currently, as the molecular basis of thermoreception discusses TRP ion channels. Cold sensitive TRPM8 ion channel is mostly interesting because it works in the physiological region of temperatures (28-8°C). *In thermoneutral conditions* a preliminary activation of TRPM8 ion channel by its agonist menthol: 1) caused an increase of metabolism due to enhanced fat oxidation; 2) enhanced the antigen binding; 3) inhibited the antibody production in the spleen; 4) significantly reduced the amount of IgG in blood; 5) increased the level of IL-6 and IL-1β in blood that was very close to changes of these cytokines at cooling. *At cooling* additional activation of TRPM8 ion channel by menthol results in initiation of cold defense Thermoregulatory responses at less cooling and leads also to improved maintenance of core temperature in the cold. More over pharmacological activation of TRPM8 attenuated the suppressive effect of deep cooling on immune response. The low expression of *TRPM8* gene in brain structures, and the expression of this ion channel in sensory skin afferents allow us to suggest that the observed effects of TRPM8 activation on thermoregulatory and immune processes are mostly due to its peripheral actions.

P5.09.36
Chronic co-administration of risperidone (RIS) and fluoxetine (FLU) modulates behavior of mice in forced swimming test (FST) and cytokine production by splenocytes

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Combined treatment with antidepressants and the drugs of different clinical profiles is an option for more efficient therapy. Fluoxetine (FLU) is commonly used as first-line treatment for depression. Risperidone (RIS), an atypical antipsychotic, is being increasingly used for potentiation of therapeutic action of antidepressants. Because antidepressive treatments also regulate immune functions, we investigated whether the chronic combined administration of FLU and RIS to mice subsequently subjected to a forced swimming test (FST) modified the cytokine production by mitogen-stimulated splenocytes. We found that the administration of FLU (10 mg/kg) for 14 consecutive days produced antidepressive-like effects in the FST when assessed 24 h after last dose. Co-administration of RIS (0.1 mg/kg 30 min. prior to FLU) enhanced the antidepressive-like effect of FLU, whereas RIS was ineffective if administered separately. Pro- and anti-inflammatory cytokines were measured in culture supernatants of mitogen-stimulated splenocytes. We found that FLU administered alone or in combination with RIS increased the level of suppressive cytokine IL-10 and decreased the protein p40 level, a common subunit for pro-inflammatory cytokines IL-12 and IL-23. Co-administration of RIS and FLU suppressed IL-4 in comparison to the group treated with FLU alone. Our results showed that chronic administration of FLU inhibited a predisposition for inflammatory processes in the immune system that occurred concomitantly with the FLU antidepressive-like action. Enhanced antidepressive-like effect of combined treatment may be related to suppression of IL-4, a cytokine engaged in amplification of humoral immune response and depressive disorders. Supported by POIG.01.01.02-12-004/09 co-financed by the European Regional Development Fund.

P5.09.37
High prevalence of anti-neuronal antibodies in Tunisian psychiatric inpatients

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Purpose: Antineuronal autoantibodies are associated with paraneoplastic syndrome. They may target nuclear or cytoplasmic antigens. We aimed in this study to determine the prevalence of anti-neuronal antibodies in Tunisian psychiatric inpatients. Subjects and methods: We enrolled in this prospective study, 103 psychiatric inpatients and 41 healthy, age and sex matched blood donors. Patients and controls had no history of malignancies or neurological disorders. For all subjects, we performed indirect imunofluorescence on primate cerebellum sections. Positive sera by immunofluorescence were also tested by an immonoenzymatic test combining a western blot of primate cerebellum extract and recombinant antigens. Results: Using indirect immunofluorescence, antineuronal nuclear autoantibodies were detected in 17 patients and none of controls (p=0.003) and antibodies reacting with cytoplasm of Purkinje cells in 6 patients and 2 controls. There was no difference of the prevalence of autoantibodies across the 4 groups of patients.The immunoblot confirmed well-characterized antineuronal antibodies only in 5 patients: two had anti-Ri (1 man and 1 woman with schizophrenia) and 3 had anti-Yo antibodies (3 men, 2 with bipolar disorder and 1 with schizophrenia). After a mean follow-up of 5 years, none of these patients developed neurological disorder or malignancy. Conclusion: In our knowledge, our study is the first one assessing the prevalence of antineuronal antibodies in psychiatric patients. The high prevalence of these antibodies in our patients may highlight the possible contribution of neuronal autoantigens in the physiopathology of some psychiatric disorders. Further studies on a larger cohort are needed to confirm this hypothesis.

P5.09.38
Usefulness of pituitary antibodies in the management non-tumoral pituitary diseases in Tunisian adults

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Pituitary antibodies (PAs) are directed against cells of the anterior and the posterior lobes of the pituitary gland. The aim of our study was to assess the usefulness of PAs in the management of non-tumoral pituitary diseases in Tunisian adults.In this prospective study, we recruited 43 cases of non-tumoral pituitary pathologies. PAs were detected by indirect immunofluoresence on monkey pituitary gland. Patients were 41 women and 2 men, mean age 43.5 ± 12.8 years. APs were positive in 12 patients (28%), 4 APs were directed against the anterior lobe and 10 against the posterior lobe of pituitary gland (2 patients had APs against anterior and posterior lobe at the same time). Twenty one women had a postpartum hypopituitarism suggestive of a Sheehan syndrome, 7 among them had PAs. Fifteen patients had a hypopituitarism without Sheehan syndrome and without malignancy (according to the MRI of the pituitary gland), 3 of them were APs positive. Seven patients had autoimmune polyendocrinopathy and only 3 of them had hypopituitarism. In this last group APs were positive in 4 cases. APs were more frequently detected in patients with autoimmune polyendocrinopathy than others but the difference was not statistically significant. Despite the low number of our patients, we reported a high prevalence of APs in non-tumoral pituitary diseases even in a context of a non autoimmune hypophysitis confirming the poor specificity of these antibodies. Additional studies are needed to identify more reliable markers for the diagnosis of lymphocytic hypophysitis.

P5.09.39
Idiopathic hypertrophic cranial pachymeningitis: a rare inflammatory cause of chronic daily headache and progressive cranial neuropathies: a case report

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INTRODUCTION: We report a rare case of a 75-year-old male with chronic daily headache for more than one year and later on developed deafness, dysphagia, ophthalmoplegia, and loss of vision. On physical examination, there was ptosis and left gaze palsy, pale optic disc, light perception only on both eyes, weak gag reflex and gross hearing loss. METHODS AND RESULTS: Cranial MRI showed abnormal pachymeningeal enhancement in the posterior fossa, cavernous sinus and orbital apex and bilateral mastoiditis. CSF analyses were normal except for elevated protein, negative results for viral, fungal and bacterial antigen panel and culture, and no malignant cells. ANCA, ANA and RF were negative. Search for occult malignancies was negative. There was elevated ESR consistent with an inflammatory process. Having ruled out infectious and non infectious causes, Idiopathic hypertrophic pachymeningitis was considered, a rare chronic fibrosing inflammatory disease characterized by marked diffuse thickening of the cranial dura causing progressive neurological deficits by compression of anatomic structures by the meninx, thickened by inflammation. After initiation of pulse corticosteroid therapy, methotrexate and maintenance oral prednisone, there was remarkable improvement on hearing, swallowing, and ocular muscle movement, less headaches and marked regression of pachymeningeal enhancement on repeated MRI. However, profound vision loss remained unchanged. CONCLUSION: A high index of suspicion of this rare inflammatory disease is important because early institution and long-term maintenance of steroid may result to complete or partial remission of the neurologic deficits and may help prevent irreversible neurologic sequelae, especially blindness.

P5.09.40
Immune signatures to predict the development and progression of Alzheimer's disease: lymphocyte profiles

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A large body of evidence links inflammation and immune dysfunctions to Alzheimer's disease (AD). Alterations of the immune system may play a major role during the asymptomatic period of AD. To identify immune parameters that may be used to predict AD initiation and/or progression, we analyzed lymphocyte subsets of amnestic mild cognitive impairment (aMCI) and mild to moderate AD patients. The role of innate and adaptative immunity was addresed by analyzing T, NK, CD4+ helper and CD8+ cytotoxic T cells by cytofluorometry. CD107 degranulation assays were performed on CD8+ and NK cells. We found no differences in total numbers of CD4+, CD8+ and NK lymphocytes among the different groups. Analysis of CD4+ and CD8+ T cells revealed an increase in "terminally differenciated" effector memory T cells CD45RA+ (TEMRA) subset and a decrease of naïve T cell subset in AD patients compared to healthy age-matched controls. Functional CD107 degranulation assays revealed that CD8+ and NK lymphocytes of AD patients did not display basal activity. However, treatment with a combination of PMA and ionomycin induced a higher degranulation activity in AD than controls. There were no major changes in the distribution of classical lymphocyte subsets in AD except for some functional changes. Increases in the number of memory subset TEMRA and low threshold of activation of NK and CD8+ T lymphocytes in AD suggested dysfunctions in the immune system of these patients. Our results are an initial step in a global understanding of the dynamic of the immune responses in AD patients.

P5.09.41
Patients with febrile infection-related epilepsy and other syndromes and microtubule-associated tau protein data

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Immune system involvement into epileptogenic process has been postulated in pathogenesis of some partial epilepsies when certain immune-mediated diseases were associated or debuted with seizures. Aim was to evaluate in patients with fever-induced encephalopathy tau which is microtubule-associated phosphor-protein involved in neurotoxic mechanisms found in different neurodegenerative disorders.
Method Controls (n=15; 21-40 years) and patients with febrile infection-related epilepsy (FIRES) and other syndromes (n=8; 18-27 years) were investigated. In anamnesis patients had encephalitis due to immune encephalopathy occurred after onset of acute febrile illness associated with lacking evidence of infectious agent (Mean disease duration 24.7±11.5 years). Tau was measured by immunofluorescent method using monoclonal anti-mouse antitau-2 antibodies also specific for phosho-Tau. Results were expressed as optical density (OD=log10F0/F1) units of FITC-labelled binding sites.
Results Patients had long-standing intractable partial epilepsy, n=6 and primary-generalized epilepsy, n=2, with high seizure frequency and psychiatric complications. Serum tau (Mean±SD) under baseline conditions compare to controls was 0.29±0.412 vs. 0.04±0.016 (P>0.01; t=4.5). Immune status showed presence of serum specific immunoglobulin E in 37.5% patients with a tendency to decreasing of functional T-cells activity. Patients with immunological alterations had chronic drug-resistant course, spike-abnormalities on EEG mainly at occipital, mid temporal lobes, severe cognitive decline; 62.5% had mesial temporal sclerosis identified with MRI; neurological syndromes showed focal symptomatology in 87.5% patients.
Conclusion. FIRES revealed high tau level that could play role in disease progression associated with development of psychiatric complications; changes of humeral immunity may play additional role in pathogenesis of autoimmune-mediated acquired epilepsy.

P5.09.42
The neurotransmitter glutamate is a direct and potent activator of very important functions in normal, cancer and autoimmune human T cells

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Glutamate is the most important excitatory neurotransmitter of the nervous system, critically needed for brain development and function, but should be re-named a 'Neuro-Immuno-transmitter', since recent findings reveal functional glutamate receptors and potent effects on most/all immune cells. Regarding human T cells: glutamate by itself, at low physiological ~10^{-6}-10^{-8}M concentration binds ionotropic and metabotropic glutamate receptors in T cells, and activates key functions including: adhesion to extracellular matrix glycoproteins fibronectin and laminin, chemotactic migration, cytokine secretion, proliferation, inward Ca^{2+} fluxes, outward K^{+} currents via Kv1.3 channels, and others. Glutamate also protects activated T cells of antigen-induced-cell-death (AICD). Glutamate-induced effects on T cells depend dramatically on: its concentration (activation at low/physiological levels, but inhibitory at excess/pathological levels); the T cell resting or activated state; and the T cell subtype. Glutamate receptor subunit AMPA/GluR3 is highly expressed only in resting T cells, since granzyme B cleaves membranal GluR3 after TCR activation.
Glutamate also improves the function of human cancerous T-leukemia and T-lymphoma, by enhancing their adhesion, migration, *in vivo* engraftment into solid organs, and production of the cancer-associated-matrix-metalloproteinase MMP-9, and of the MMP-9 inducer: CD147.
Regarding autoimmune T cells: glutamate was reported to enhance proliferation of autoreactive T cells of multiple sclerosis (MS) patients in response to myelin proteins MBP and MOG; AMPA GluR3 is highly expressed in T cells of MS patients and upregulated during relapse; and mouse encephalitogenic MPB-87-99 T cells respond to glutamate. In conclusion: glutamate, via glutamate receptors, triggers/elevates key functions in normal and pathological human T cells.

P5.09.43
Activation of exploratory behavior in senescence accelerated OXYS rats by stimulation of cell-mediated immune response with BCG vaccine

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Changes in cognitive and emotional spheres are typical of aging. Their prevention is an obligatory condition providing active longevity of elderly people. We demonstrated that senescence-accelerated OXYS rats constitute appropriate model for studying ageing processes. Changes in exploratory activity and the degree of anxiety in OXYS rats are significantly related to activity of cellular immune reactions. The present study included 3-month-old male OXYS and Wistar rats. It was shown that stimulation of the cellular component of the immune response by injection of BCG vaccine significantly modified horizontal motor activity of animals. The effect was dose-dependent and peaked after injection of 600 μg/kg vaccine: in this case horizontal motor activity increased 3-fold and did not differ from that of control Wistar rats. Animals receiving the maximum dose of the vaccine did not differ from Wistar rats also by the parameters of vertical motor activity and number of grooming reactions. So, stimulation of cell-mediated immune response caused a dose-dependent activation of exploratory behavior in senescence accelerated OXYS rats. The possibility of correction of behavioral changes associated with early aging of OXYS rats by stimulation of cellular immune reactions is a direct proof of the involvement of neuroimmune relationships in the process of aging.

P5.09.44
Effects of transplantation of the immune cells treated with psycho modulators on the nervous system functional activity of recipients

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We first demonstrated the possibility of the exploratory behavior direct regulation by the transplantation of the immune cells with definite functional characteristics. Based on the results of our own previous researches in the present study we demonstrated that immune cells, treated with psycho modulators after the transplantation modulated the nervous system functional activity of syngeneic recipients.
I t was shown that in vitro treatment with caffeine or chlorpromazine of splenocytes from (CBAxC57Bl / 6) F1 mice with depressive-like or aggressive-like behavior consequently modified the functional activity of these cells, manifested in changes in spontaneous and antigen induced cytokines production and cells proliferative activity. The intravenous injection of the cells, treated with caffeine to mice with depressive-like behavior were accompanied by the modulation of the recipient's exploratory behavior parameters, the nature of which depends on the dose of the drug. We also demonstrated the possibility of obtaining a neuroleptic effect in mice with aggressive-like behavior by the transplantation of immune cells, treated with chlorpromazine. The modulation of animals behavior after the transplantation of the immune cells treated with psycho modulators were accompanied with definite changes in the synthesis of TNFα, IL-1β, IL-6, INFγ cytokines in brain of mice-recipients.

P5.09.45
Characterization and role of the humoral response after transplantation of porcine neuroblasts in the rat brain

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Cell therapy is a real hope for neurodegenerative diseases. Interesting results were obtained after the transplantation of human fetal neuroblasts into the brain of Parkinsonian patients but tissue availability and ethical concerns limit this approach. Other cellular sources have therefore to be found. Fetal pig neuroblasts are interesting candidates, but intracerebral xenotransplants are systematically rejected, even under systemic immunosuppression. Efforts have been done to inhibit locally the cellular immune reaction but little is known about the necessity of targeting the host humoral response.
We became interested in the humoral response of intracerebral xenograft with porcine neuroblasts in the rat striatum. The antibody production is observed in the sera of rats by immunocytologicals and FACS analysis. We see an increase of IgG production correlated with time post transplant and the stage of rejection. In fact, anti-porcine antibodies appear quite late and maximum of accumulation is observed after the rejection of porcine neurons. Furthermore, a deposit of IgG and complement is found in graft during rejection. Moreover, we demonstrate that IgG recognize neurons, astrocytes and porcine aortic endothelial cells (PAEC).
The question is now to know the balance between humoral response and cellular response in the rejection. For this, the important role of the humoral response in transplant rejection is studied in rats knock-out to immunoglobulin (IgM KO). We observe the presence of a healthy graft at D63 in all IgM KO rats but in WT rats 50% have a graft in rejection or scar and 50% have a healthy graft.

P5.09.46
Neuronal toll-like receptor-4 signalling induces brain endothelial cell activation and neutrophil transmigration

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BACKGROUND: The innate immune response in the brain can be initiated by either pathogen-associated molecular patterns (PAMPS) or danger-associated molecular patterns (DAMPS). These molecules activate members of the Toll-like receptor (TLR) family including TLR4. Although neurons have been reported to express TLR4, the function of TLR4 activation in these cells remains unknown.
METHODS: Using RT-PCR, we assessed the TLR4 mRNA expression in primary mouse glial and neuronal cells. Mouse mixed glial, neuronal or endothelial cell cultures were treated with LPS and the expression of inflammatory mediators was assayed by either cytometric bead array or ELISA. Activation of extracellular-signal regulated kinase 1/2 (ERK1/2), p38, c-Jun-N-terminal kinase (JNK) and c-Jun was assessed by Western blot. The effect of conditioned media of untreated- versus LPS-treated glial or neuronal cultures on endothelial activation was assessed by neutrophil transmigration assay.
RESULTS: LPS induces strong release of the chemokines RANTES and CXCL1 (KC), tumor necrosis factor-α (TNFα) and IL-6 in primary mouse neurons. In contrast, LPS induced release of IL-1α, IL-1β and granulocyte-colony stimulating factor (G-CSF) in mixed glial cells. LPS-mediated neuronal CXCL1 release was completely hampered by VIPER and LPS-mediated activation of JNK in both glial cells and neurons was blocked by SP600125. Moreover, conditioned medium of LPS-treated neuronal cultures induced strong expression of ICAM-1 and VCAM-1 on endothelial cells which induced infiltration of neutrophils across the endothelial monolayer.
CONCLUSION: These data demonstrate for the first time that neurons can play a role as key sensors of inflammation and responder to initiate immune response.

P5.09.47
TLR-2 but not TLR-9 is up-regulated in the Central Nervous System and Lymphoid Organs during Canine Visceral Leishmaniasis

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Visceral leishmaniasis (VL) is an anthropozoonosis caused by the parasitic protozoan *Leishmania chagasi* (= *infantum*). The central nervous system (CNS) undergoes inflammatory alterations during the peripheral infection, and we have previously detected high amount of T lymphocytes, matrix metalloproteinases enzymes and pro-inflammatory cytokines such as IL-1β, IFN-γ and TNF-α within the nervous milieu of dogs with VL. Since toll-like receptors (TLRs) are components of the innate immunity which recognize pathogen-associated molecular patterns, such as the major surface molecule of *Leishmania*, lipophosphoglycan (LPG), we aimed to detect and quantify the gene expression of TLR-2 and TLR-9 in the CNS (brain and choroid plexus) and compare with peripheral lymphoid organs (spleen and popliteal lymph node) from 15 dogs naturally infected with VL and from four uninfected control dogs, using RT-qPCR with specific primers and Taqman probes. G3PDH was used as a reference gene. TLR-2 gene expression was up-regulated in the choroid plexus (3.09-fold more; P=0.0475), spleen (2.60-fold more; P=0.0087) and lymph node (3.48-fold more; P=0.0220) of the infected dogs, and there was a trend to be up-regulated also in the brain (2.69-fold more; P=0.0800). The gene expression of TLR-9 presented no differences between infected and control dogs. The up-regulation of TLR-2 in the lymphoid organs is indicative that this receptor takes part in the immune response against the parasite. Further, the increase of TLR-2 in the CNS suggests that in the nervous milieu there is a response to the peripheral infection by *Leishmania*, which associates TLR-2 to the neuropathogenesis of VL.

P5.09.48
Impact of neurturin on allergic airway inflammation in mice.

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How asthma should be treated and at what stage anti-inflammatory therapy should be introduced is still debated. Furthermore little is known about the relationship between neurogenic and immune airway inflammation. However recent studies suggest that neurotrophins like nerve growth factor and brain-derived neurotrophic factor participate in the pathogenesis of many features and symptoms of asthma.
By using a C57BL/6 mouse model, we studied the influence of the neurotrophic factor neurturin (NTN) as well as its receptors on acute airway inflammation. The absence of NTN expression in bronchopulmonary cells led to increased airway inflammation and AHR and administration of NTN before challenge with OVA partially rescued the phenotype of NTN-/- mice. These results strongly suggest that NTN decreases the allergen-induced characteristics of asthma. However, the underlying mechanisms remain unclear. To determine which cells are involved in these symptoms, DC-T cell assays were performed, showing higher secretion of Th2 cytokines by CD4+T cells from NTN-/- mice. In order to determine if NTN could directly influence immune cells through its receptors, GDNF family receptor alpha 2 (GFRalpha2) and the proto-oncogene RET mRNA expressions were tested by real-time PCR. These receptors were expressed more after inflammation in NTN-/- lung tissues. Taken together, these results suggest that the absence of NTN in our model induces an over-activation of DC and Th2 cells leading to an increased inflammatory response in NTN-/- mice.

P5.09.49
Novel magnetic resonance imaging approaches for monitoring neuroinflammation

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Neuroinflammation during multiple sclerosis involves immune cell infiltration and disruption of the blood-brain barrier which can be visualized by magnetic resonance imaging (MRI), in patients and in the animal model experimental autoimmune encephalomyelitis (EAE). However, discrepancies between conventional MRI results and clinical signs underscore the need for new MRI methods to better monitor CNS inflammation. Here we employed two novel MRI approaches to investigate neuroinflammation. Cerebral magnetic resonance elastography (MRE) measures the viscoelastic properties of brain tissues in vivo. To investigate the relationship between inflammation and brain viscoelasticity, we applied MRE to the EAE model. At peak disease we detected a significant decrease in both the storage and loss modului, indicating that the elasticity and viscosity of the brain are reduced during acute inflammation. Interestingly, these parameters normalized at a later time point corresponding to the clinical recovery phase. Additionally, using very small superparamagnetic iron oxide particles (VSOP), we demonstrated that alterations in the choroid plexus represent an early feature in CNS inflammation. After inducing EAE and administering VSOP prior to onset of clinical signs, VSOP accumulated preferentially in the choroid plexus and spinal cord meninges, in the absence of overt inflammation. Moreover, we observed VSOP in lesions with a perivascular accumulation of immune cells but a preserved glia limitans, in endothelial structures, co-localized with phagocytes, and diffusely disseminated in the parenchyma, suggesting multiple entry mechanisms of VSOP into the CNS. Thus these two novel MR-based approaches emerge as effective tools yielding new insights into mechanisms of neuroinflammatory disease.

P5.09.50
A forebrain-immune cell pathway for the maintenance of CNS immune tolerance

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The ability of the central nervous system (CNS) to maintain immune privilege is well known, but poorly understood. Data from multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), suggests that the cervical lymph nodes (CxLNs) may participate in this process by receiving direct input from the CNS. We therefore wished to ascertain whether there was significant immune cellular traffic from the CNS to the CxLNs. Using dual label IHC, dendritic cells (DCs) were more numerous throughout the rostral migratory stream (RMS), a pathway of neural stem cell migration. Further, DCs migrated from the RMS to the CxLNs in a Fingolimod sensitive manner. Fingolimod.treatment led to selective accumulation of DCs in the RMS and more severe MOG induced EAE. The same treatment of 2D2, MOG T-cell transgenic, but EAE resistant mice, triggered spontaneous EAE with accumulation of CNS antigen specific T-cells in the spinal cord. In these 2D2 mice, spinal cord T-cell infiltration was proportional to the ratio of anti-inflammatory T-regulatory to pro-inflammatory T-effector cells in their CxLNs. Further, RMS Fingolimod treatment compromised CxLN-T-regulatory function suggesting DCs mediated this change. Using a delayed type hypersensitivity model, we demonstrated that the same treatment leads to fewer regulatory DCs in CxLNs. Finally, we show that a CNS DC subset significantly reduce anti-CNS immune responses in the CxLN by modulating T-regulatory activity. Combined, these data suggest that DCs migrate from the CNS to the CxLNs and regulate anti-CNS immunity representing a novel target for the treatment of CNS autoinflammatory disease.

P5.09.51
Immunomodulatory effects of Interleukin-27 on active experimental autoimmune encephalomyelitis

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Interleukin-27 (IL-27), a member of IL-12 cytokine family, has a pleiotropic role in immune responses. IL-27 is known as an inducer of Th1 responses. Several recent studies, however, have reported that this cytokine also has immunosuppressive properties. On the basis of these reports, this study was designed to investigate the immunomodulatory effects of IL-27 on active experimental autoimmune encephalomyelitis (EAE), a murine model of inflammatory demyelinating disease of human multiple sclerosis. 100 microgram of IL-27 coding plasmid (P240-mIL27) or vehicle was injected two times to experimental and control mice at days 15 and 22 post EAE induction, respectively. The results showed that P240-mIL27 markedly suppressed the disease severity and demyelination in association with decreased levels of both IFN-γ and IL-17 in the MOG35-55-induced EAE mice. In contrast, the level of IL-4 and IL-10 increased in experimental group; when compared with control EAE mice. Also the number of both CD4+Foxp3+ and CD4+IL-10+ regulatory T cells significantly increased in EAE mice treated with IL-27. Collectively, these results suggest that IL-27 may be considered as a suitable candidate for the treatment of inflammatory diseases such as MS.

P5.09.52
Synaptic deficits in sepsis: Role of glial cells

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Recent clinical studies have shown that sepsis survivors can develop long-term cognitive impairment. The cellular and molecular mechanisms involved in these events are not yet completely understood. In this study, we investigate the synaptic deficits in sepsis and the involvement of glial cells in this process. Using a clinically relevant model of sepsis (Cecal ligation and puncture/CLP), we observed a memory deficit in the septic animals 9 days after sepsis induction. At the same time, we observed a reduction in structural synapses in hippocampus and cerebral cortex, identified by synaptophysin and PSD-95 co-localization. To define the molecular mechanisms accountable for synaptic loss in sepsis, we used an in vitro approach treating neuronal cultures with conditioned medium from astrocyte (ACM) and microglial (MCM) cultures stimulated with LPS. We observed that the MCM reduced the synapse number and the ACM increased the number of synapsis. Analysis of conditioned medium composition, showed that MCM had increased levels of IL-1β while the ACM had increased levels of TGF-β1, as compared to medium from the non LPS-stimulated cultures. The addition of soluble IL-1β receptor (IL-1 Ra) to the neuronal culture prevented the MCM-induced synapsis loss. Patch-clamp recordings in the MCM- treated neurons showed a reduction in postsynaptic current frequency, while an increase in current amplitudes, suggests a functional synaptic deficit. These findings show, for the first time, a correlation between synaptic deficits and memory dysfunction, suggesting a possible mechanism for cognitive impairment after sepsis as well as a glial-derived molecule mediating synapse reduction.

P5.09.53
Day and Night Alterations of serum melatonin, MIP-1α, MCP-1 and IL-17A levels in multiple sclerosis

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Backround and aims: Multiple sclerosis (MS) is a T cell-mediated autoimmune disease of the human central nervous system (CNS). The pineal hormone melatonin plays a crucial role in immunomodulation, mainly by effecting T cells. The aim of this study was to determine whether serum melatonin concentrations are associated with MS and serum macrophage inflammatory protein 1 alpha (MIP-1), monocyte chemoattractant protein-1 (MCP-1) and interleukin-17A levels.
Materials and methods: Seven patients (4 male, 3 female) newly diagnosed with MS were enrolled into the study. Venous blood samples were drawn from the patients with MS at 03.30 am and 10.00 am for determining the levels of melatonin, MIP-1, MCP-1 and interleukin-17A. Enzyme-linked immunosorbent assay (ELISA) method was used for this purpose.
Results: Serum melatonin and MIP-1 levels at 03.30 am in patients with MS were significantly higher than those serum levels measured at 10.00 am in same patients. In addition, there was a positive correlation between serum MIP-1 and IL-17A levels at 10.00 am.
Conclusion: Although no correlation was found between serum melatonin and MIP-1 levels in patients with MS, simultaneous decline or increase in their serum levels supports their possible role in the pathogenesis of MS through common mechanism.

P5.09.54
Identifying Biomarkers Associated with Pain in Swine using an In Vitro Inflammation Model

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This study sought to determine if biomarkers associated with pain could be measured in an *in vitro* model of inflammation. Success would constitute the first step towards using these biomarkers as surrogate endpoints to help support effectiveness indications for investigational new animal drugs to control pain in swine. Porcine whole blood samples were cultured *in vitro* with *E. coli* lipopolysaccharide (LPS) or without LPS for up to 48 hr. Supernatant from these cultures was collected to determine protein levels. Messenger RNA was also obtained from these cells and quantitative reverse transcription-PCR was used to determine if mRNA levels of genes associated with pain were altered in response to LPS-induced inflammation. Bradykinin protein levels steadily increased over time due to LPS stimulation and returned to 0 hr levels after 6 hr of culture. Corticotrophin-releasing factor protein levels were not affect by LPS. Substance-P protein levels steadily increased over time and returned to 0 hr levels after 4 hr LPS stimulation. Genomically, Chemokine Receptor 4 and Bradykinin Receptor 1 had a 3-fold fold increase at 6 hr after LPS stimulation. Cyclin-Dependent Kinase Inhibitor 1A showed a 4 fold increase at 6 hr and 24 hr after LPS stimulation. An additional 17 genes associated with pain showed no alteration in mRNA levels in this model. These results suggest that 5 biomarkers (2 protein and 3 genomic) may be useful as surrogate endpoints for evaluation of pain. These need to be evaluated *in vivo* to support their usefulness as biomarkers of pain in swine.

P5.09.55
Dopamine receptor D3 expressed on CD4+ T-cells favours neurodegeneration of dopaminergic neurons during Parkinson's disease

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Emerging evidence has demonstrated that CD4+ T-cells infiltrate into the substantia nigra (SN) in Parkinson's disease (PD) patients and in animal models of PD. SN-infiltrated CD4+ T-cells bearing inflammatory phenotypes promote microglial activation and strongly contribute to neurodegeneration of dopaminergic neurons. Importantly, altered expression of dopamine receptor D3 (D3R) in peripheral blood lymphocytes from PD patients has been correlated with disease severity. Moreover, pharmacological evidence has suggested that D3R is involved in IFN-gamma production by human CD4+ T-cells. Here, we studied the role of D3R expressed on CD4+ T-cells in neurodegeneration of dopaminergic neurons in the SN using a mouse-model of PD. Our results show that D3R-deficient mice are strongly protected against loss of dopaminergic neurons and microglial activation during 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD. Notably, D3R-deficient mice become susceptible to MPTP-induced neurodegeneration and microglial activation upon transfer of wild-type (WT) CD4+ T-cells. Furthermore, RAG1 knockout mice, which are devoid of T-cells and are resistant to MPTP-induced neurodegeneration, become susceptible to MPTP-induced loss of dopaminergic neurons when reconstituted with WT CD4+ T-cells but not when transferred with D3R-deficient CD4+ T-cells. In agreement, experiments analyzing activation and differentiation of CD4+ T-cells revealed that D3R favours both T-cell activation and acquisition of the Th1 inflammatory phenotype. These findings indicate that D3R expressed on CD4+ T-cells plays a fundamental role in the physiopathology of MPTP-induced PD in a mouse model.

P5.09.56
Clinical cases of immune-mediated partial epilepsy

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Antibody-mediated pathogenicity has been implicated as immune pathophysiological mechanisms in some epilepsy. The aim of work was to identify best means of immune-mediated seizures by clinical and immunological methods.
Results: 12 patients with partial epilepsy, PE (24-38 ears; mean epilepsy duration: 11.45±8.64 years) and 16 controls were investigated. The etiology was neuron-infection (8 cases) and history of immunization had 4 women. Analysis of immune status showed IgG - 14.65±3.41 mg/ml; IgM - 1.29±0.30 mg/ml; circulating immune complexes < 0.085; and in 8 women the presence of serum specific IgE and lymphocytotoxic autoantibodies, % - 19.3±5.4. All patients have long-standing intractable epilepsy with chronic pharmaco-resistant course, high seizure frequency associated with cognitive decline and behavioral and psychiatric problems and in anamnesis of group the first manifestation of PE was occurred as convulsive status epilepticus - 5 patients (41.6%). Four women (33.3%) were highlighted; their epilepsy was associated with being given diphtheria pertussis tetanus (DPT) vaccine. Now they have intractable PE with onset in early childhood, chronic pharmaco-resistant course, normal CT brain scans and epileptic abnormalities on EEG, predominantly left sided focus mainly at occipital, temporal and central lobes.
Conclusion: The results presented showed women with a subtype of encephalitis who might have had their condition precipitated by DPT-vaccination. Further studies are needed to determine exactly which kinds of seizures are immune-mediated, and whether immune system can guide clinical treatment, or play a direct role in epileptogenesis.

P5.09.57
NMR Metabolomics: a Diagnostic Tool for Multiple Sclerosis

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A definitive diagnostic test for multiple sclerosis (MS) does not exist; instead physicians use a combination of medical history, magnetic resonance imaging, and cerebrospinal fluid analysis (CSF). Significant effort has been employed to identify biomarkers from CSF to facilitate MS diagnosis; however none of the proposed biomarkers have been successful to date. Urine is a proven source of metabolite biomarkers and has the potential to be a rapid, non-invasive, inexpensive, and efficient diagnostic tool for various human diseases. Nevertheless, urinary metabolites have not been extensively explored as a source of biomarkers for MS. Instead, we demonstrate that urinary metabolites have significant promise for monitoring disease-progression, and response to treatment in MS patients. NMR analysis of urine permitted the identification of metabolites that differentiate experimental autoimmune encephalomyelitis (EAE)-mice (disease model for MS), and healthy and EAE-mice treated with fingolimod, a drug recently approved for MS therapy. The potential metabolites include hippurate, fructose, citrate, oxoglutaric acid, taurine and urea. MS is the most common neurological disorder among young adults; and is also a complex disease with multiple manifestations and heterogeneity in its phenotypic expression. Correspondingly, properly diagnosing the disease is challenging and fraught with error. Thus, our proposed development of a non-invasive, rapid, and simple diagnostic tool based on the NMR analysis of urine metabolites has an extraordinary potential to benefit the health and well-being of MS patients.

P5.09.58
Influence of LRRK2 G2019 mutation on peripheral B cells from Parkinson's disease patients

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Mutations in the Leucine Rich Repeat Kinase 2 (LRRK2) gene are associated with the risk of developing Parkinson´s disease (PD). Existing studies show that metabolism of leucocytes of PD and LRRK2-mutant PD patients are different than controls (PMID:19847307), and that B cells on PD patient's blood are decreased (PMID:22910543). However, little is known about how PD and LRRK2-mutations impact on B cells function. Therefore, we collected venous blood from age-matched controls, PD and LRRK2-mutant PD patients. Functional FACS-phenotyping of blood B cells was carried out. Total B cells were isolated by magnetic-beads, and B cell subsets were obtained by cell-sorting. The expression levels of MAPK6 and p38 proteins were evaluated by western-blot for total B cells, and LRRK2 in the B cell subsets. The supernatant of cultured B cells in U13C-glucose-rich medium was analyzed by nuclear magnetic resonance. LRRK2-mutant PD patients had significantly ($p<0.05$) more CD19+BAFF-R+, CD19+CD95+ and CD19+IgD-IgM-CD27+, less CD19+CD40+ cells than controls. Comparing to other PD patients, LRRK2-mutants had significantly ($p<0.05$) less CD19+CD69+ and CD19+CCR1+, and more CD19+BAFF-R+ cells. LRRK2-protein expression was observed in the CD19+IgD+CD27- and CD19+IgD-CD27+ subsets. MAPK6 and p38 expression was significantly decreased in all PD patients, no difference was found regarding B cell lactate production. These data suggest that neurodegeneration in PD is accompanied by functional changes in peripheral B cells, impacting on the MAPK pathway, BAFF-R and CD95 expression, contributing to defective B cell differentiation and proliferation. The G2019S mutation in LRRK2 seems to further increase B cell dysfunction. (Funding: MJFox Foundation, CNPq-Brazil)

P5.09.59
Anti-ganglioside antibodies in Pandemrix®-induced narcolepsy

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Narcolepsy with cataplexy (NC) is a chronic, neurological disease with an unknown etiology. It is caused by a selective loss of hypocretin-producing neurons in hypothalamus. This causes the typical symptoms of the disease: various sleep and metabolic disorders, cataplexy and hallucinations. NC is considered as an autoimmune disease, where genetic and environmental risk factors contribute to its development. NC has a strong association with HLA-DQB1*0602 (DQ6), and recent reports from several European countries have confirmed an association between the adjuvanted H1N1 Pandemrix® vaccine and NC in genetically susceptible children and adolescents. However, the triggering role of the vaccine components and the mechanisms behind the disease remain unsolved. We studied the role of humoral immunity in the pathogenesis of Pandemrix-narcolepsy by screening anti-neuronal antibodies from patients' sera. No antibodies were detected against the neuronal antigens (Hu, Ri, Yo, Tr, MAG, myelin, Ma/Ta, GAD, amphiphysin, aquaporin-4, NMDAR, AMPA-type glutamate receptor, glycine receptor, CASPR2 and LGI1) in patient sera (n=5). However, when sera of Pandemrix-narcolepsy patients (n=20) were screened for 12 anti-ganglioside antibodies (AGA) by line immunoassay a high frequency of (>30%) of AGA (IgG or/and IgM) were found. No AGA were detected in the control sera of healthy unvaccinated children (n=6). The majority of the found AGAs (IgG) were against gangliosides GM4, GM3 and GD3. Our results suggest that humoral immune response against gangliosides is a feature of Pandemrix-associated narcolepsy. AGA could cause at least part of the neuronal damage of the disease.

P5.09.60
Effects of vagotomy during inflammation and cytokine production in hamster amebic liver abscess

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The inflammatory response is modulated by its interactions with the nervous system. The vagus nerve and its neurotransmitter acetylcholine is the major parasympathetic innervation to chest and abdominopelvic viscera, Thus, through the alpha 7 nicotinic receptor on macrophages and other cells, inhibits NF-kB pathway reducing the secretion of pro-inflammatory cytokines. *Entamoeba histolytica* (Eh) in humans causes several diseases including amebic liver abscess (ALA) that is characterized by formation of inflammatory granulomatous lesions. In this context, we studied the role of vagotomy during induction of ALA in hamster, this was done by cutting the ventral vagal branches that innervate the liver and after 30 days was inoculated 350.000 Eh trophozoites (H1-IMSS strain) by intrahepatic via. Animals were sacrificed at 6, 12, 24h, 2, 4 and 7d post-inoculation, then, was analyzed NFKB activation in neutrophils and macrophages, and the presence of pro and anti-inflammatory cytokines+ cells by Immunohistochemical and immunofluorescence techniques. During ALA kinetics in parasimpatectomized hamsters was observed significant NFKB activation in neutrophils (10.43 ± 0.93) and macrophages (7.77 ± 0.15) and an increase of IL-1β+ (8.1 ± 1.0) and TNF-α+ cells (17.02 ± 1.77) compared to Sham and control groups. However, only IL8+ cells significantly increased at 24h post-inoculation in vagotomized animal. No changes were observed in IL10+ cells. Our results demonstrate that vagotomy in ALA model induces a pro-inflammatory response more durable due to increased activation of NFKB and therefore higher release of IL-1β and TNF-α cytokines. The vagus nerve is important in regulation of inflammatory process during ALA.

P5.09.61
Steroids hormones on neurofibromas development

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A descriptive case study was performed on 75 patients with NF1 (neurofibromatosis type 1) from the CNNF (Brazil) database. Serum IgE levels were determined using the IgE radioimmunosorbent test, with the reference values of 75-502 IU mL-1. The patients were divided into groups, with 25 patients presenting plexiform neurofibromas, 25 presenting neurofibromas and 25 presenting no neurofibromas. The purposes of this study were to determine the serum IgE levels of patients with NF1 presenting plexiform neurofibromas, neurofibromas and no neurofibromas, as well as to determine possible correlations between serum IgE levels and the size of plexiform neurofibromas and neurofibromas presented by these patients. Elevated serum IgE levels were observed in all the patient groups. We did not observed a correlation between IgE levels and age in these patients; however, we did observe correlations between IgE levels and neurofibroma and plexiform neurofibroma size. We suggest further studies to confirm these results and to investigate in greater depth the possible role of IgE in the development and growth of neurofibromas and plexiform neurofibromas in NF1.

P5.09.62
Cocaine exposure, abstinence and relapse differentially affect dopaminergic, mu-opioid and CB2 receptors expression on immune cells

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Immune cells express proteins from dopaminergic, opioidergic and endocannabinoid systems, which are key neurobiological targets of cocaine exposure. Therefore, cocaine may also modulate these systems at the immune level. We tested this hypothesis by analyzing the expression of D5 and D3 dopaminergic receptors (DARs), mu-opioid receptors (MOR) and CB2 receptors in splenic T-cells (CD3-FITC+), B-cells (CD45RA-PECy5+) and monocytes/macrophages (CD11b/c-PE+) from male Lewis rats using flow cytometry (FCM). Receptor expression was measured after: 1) cocaine self-administration (1 mg/kg/infusion, i.v.; 21 d); 2) extinction of cocaine self-administration (21 d); and 3) cocaine-induced (10 mg/kg, i.p.) reinstatement of drug-seeking behavior. Cocaine self-administration augmented DARs, MOR and CB2 receptor expression distinctly in T-cells, B-cells and monocytes/macrophages, effects that were not fully reversed during extinction. Reinstatement of cocaine seeking was associated with altered D5 expression in monocytes/macrophages, modifications in MOR+ T-cells percentage and increased T-cell/B-cell ratio compared to animals that did not relapse. Remarkable alterations in relative spleen weight, cellularity, relative and absolute numbers of splenic subpopulations as well as increased CD3 and CD11b/c expression levels were observed following cocaine self-administration, effects that were modulated distinctly by extinction and subsequent re-exposure to cocaine. These findings may help better understand the mechanisms involved in cocaine-mediated immune modulation and aid the development of novel therapeutic strategies to address the behavioral-immunological imbalances associated with cocaine addiction. As FCM allows clinicians to rapidly measure multiple parameters in a single sample, the Ab panel used here may facilitate the peripheral assessment of biological markers modulated by cocaine use.

P5.09.63
Prenatal immunological stress affects the development of reproductive system

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Immune and reproductive systems closely interact in critical periods of early ontogeny. The leading role in programming of reproductive and immune system development and functioning belongs to hypothalamic neuropeptide gonadotropin-releasing hormone (GnRH). GnRH neurons originate in olfactory epithelium and migrate to the forebrain on the surface of olfactory/vomeronasal nerves in prenatal life. Immunomediators are involved in regulatory and morphogenetic processes of GnRH system development during perinatal period. Rats were injected intraperitoneally with lipopolysaccharide (LPS) (18 µg/kg) on the 12th day of pregnancy and GnRH-immunoreactive neuron fractions were count along the migration route on 17th and 19th embryonic days (E). Body weights, the day of vaginal opening was recorded in prenatally LPS-treated and control offspring. Hypothalamic GnRH content was detected by radioimmunoassay on 5th, 14th and 30th postnatal days (P). The receptors to proinflammatory cytokines (IL-6, MCP-1, LIF) in GnRH-neurons were detected in untreated fetuses using double immunohistochemistry. The rate of GnRH neuron migration was decreased in nasal area on E17 after maternal LPS treatment, GnRH neuron distribution wasn't changed on E19. The body weight of postnatal LPS-treated animals was decreased and vaginal opening was also delayed. GnRH content was diminished on 25% in both sexes on P60. GnRH neurons were positive to IL-6R, LIFR and MCP-1 (CCR) in different areas of migration route. Thus, maternal infection on early stages of pregnancy affects GnRH neuron migration in fetuses and as a result GnRH synthesis is suppressed in postpubertal animals. Possible regulators of GnRH neuron migration might be the proinflammatory cytokines.

P5.09.64
Immune response in dorsal root ganglion of mice infected with Herpes Simplex-1 (HSV-1) leads to the development of hyperalgesic behaviour

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Herpes Zoster (HZ) is a disease caused by reactivation of latent herpesvirus Varicella Zoster in the sensory ganglion, characterized by dermal rash and pain. A murine model of HSV-1 infection on the hind paw skin has been used to study HZ, since mice develop HZ-like skin lesions and pain-related responses. There are no data available about the immune response in dorsal root ganglion (DRG) of these mice. Thus, the aim of this study was to evaluate cells and inflammatory mediators present in DRGs and its relationship with hiperalgesia during HSV-1 cutaneous infection. Mice developed hyperalgesia from 3 to 21 days post infection (dpi) only in the ipsilateral (ips) paws. At 12 dpi, all mice recovered from skin lesions. Approximately 50% of mice showed persistent hyperalgesic behavior until 45dpi. A higher viral load was detected in DRGs L4, L5 and L6 of infected mice at 7 dpi. We observed an intense inflammatory infiltrate composed by neutrophils and macrophages in ips DRGs at 7dpi. T lymphocytes (CD4+ and CD8+) infiltration was detected at 15 and 21dpi in ips DRGs. We also observed the activation of satellite cells in ips DRGs (GFAP expression). A higher mRNA expression of COX-2 and TNF-α was detected in ips DRGs of infected mice. Moreover, blockage of TNF-α reduced the development of hyperalgesia. Our results show the presence of an inflammatory infiltrate in DRGs of infected mice, and the early expression of inflammatory mediators that contribute for the induction of herpetic hyperalgesia. Financial support: FAPESP (2010/12309-8), TIMER (HEALTH-F4-2011-281608).

P5.09.65
IFN-ɣ is protective in EAE by activating CNS-resident microglia to phagocytose neurotoxic myelin debris

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The central role of the Th1 cell-derived cytokine IFN-γ as a primary mediator of CNS pathology during EAE has been challenged by the recent characterization of "Th17" cells producing IL-17 and GM-CSF. Additionally, mice deficient in IFN-γ develop more severe disease, implicating its role in EAE and MS is protective. Using a novel quantitative immunofluorescence (qIF) technique to analyze confocal images of antigen-presenting cells (APCs) in the CNS of wild-type (Wt) and IFN-$\gamma^{-/-}$ or IFN-$\gamma R^{-/-}$ C57BL/6 mice, we found that this higher disease severity corresponded to decreased myelin Ag uptake by CNS APCs. qIF further showed that the lack of antigen uptake corresponded to a lack of myelin debris cleanup at CNS lesions. Surprisingly, IFN-$\gamma R^{-/-}$ APCs from EAE mice or *in vitro* cultures with exogenous myelin Ag and 2D2 tg T cells produced more IL-17 and GM-CSF in cytokine ELISPOT assays. Flow cytometry for phagocytosis and antigen presentation markers revealed that $CD11b^{+}CD45^{lo}$ microglia were affected most by the absence of IFN-γ signaling with a dramatic reduction in activation markers, while $CD11b^{+}CD45^{hi}$ infiltrating macrophages were also less activated. However, $CD11c^{+}$ dendritic cells (DCs) only experienced a slight decrease in activation markers. These results illuminate IFN-γ's primary protective role via activation of CNS-resident microglia to promote removal of myelin debris, thus facilitating remyelination. Importantly, the activation of DCs to present myelin Ag to encephalitogenic T cells appeared independent of IFN-γ signaling and thus represents a potential therapeutic target slowing MS progression.

P5.09.66
Influenza split vaccine in regulation of autoimmune processes in experimental autoimmune encephalomyelitis

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Activation of autoantibodies and MHC class I and class II proteins expression represent the main characteristics of experimental autoimmune encephalomyelitis (EAE). Therefore, effects of influenza vaccine on antibody titers, expression of MHC I and II classes in brain tissue and clinical signs were examined. In this study 24 female 8 week-old C57BL/6 mice were divided in four groups: first EAE-influenza vaccinated; second EAE-induced; third influenza vaccinated and fourth non-treated (intact control). Mice were vaccinated intramuscularly with influenza split vaccine (Vaxigrip, Sanofi-Pasteur). EAE groups were immunized subcutaneously with MOG35-55 in complete Freund's adjuvants and pertussis vaccine intraperitoneally injected. The serum antibody titer to MOG35-55 was detected by ELISA test. Significant increase of anti-MOG antibodies ($p<0.01$) was detected in EAE-influenza vaccinated and mice with EAE mice in comparison to both controls (influenza vaccinated and intact). Mild but not significant increase in anti-MOG antibody titer was detected in EAE-influenza vaccinated compared to mice with EAE. There were no differences between both control groups (influenza vaccinated and intact). Strong expression of MHC-II and mild expression MHC-I were detected in EAE-influenza vaccinated and mice with EAE, while no expression was found in both controls. No differences were observed between EAE-influenza vaccinated and mice with EAE in both MHC I and MHC II expression. Results obtained show that influenza vaccine has no significant influence on autoimmune processes and induction of EAE.

P5.09.67
Evaluation of Tumor necrosis factor (TNF-α) cascade in the brain of rat model of collagen-induced arthritis (CIA)

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Tumor necrosis factor α (TNF-α) is an inflammatory cytokine that contributes to the cytokine cascade in collagen-induced arthritis (CIA) and it can cross blood brain barrier via carrier mediated transport. The aim of our work was to study the production of TNF α and its receptors 1 (TNFR-1) and 2 (TNFR-2) and transcription factor NF-kB (nuclear factor kappa B) in brain of CIA rat model with and without methotrexate (MTX) treatment. CIA was induced in Wistar rats by intradermal injection of bovine type II collagen. The rats were divided into normal, CIA and MTX treated groups. Treatment was received the day after the onset of arthritis (day 12) until the day 35^{th}. TNF-α, TNFR-1, TNFR-2, and NF-kB were quantified by enzyme-linked immunosorbent assay (ELISA) in brain homogenates. Our results showed significant increase in CIA rats ($p<0.001$; $p<0.001$ and $p<0.05$ for TNF-α, TNFR-1 and TNFR-2; respectively) compared to normal ones. Remarkable reduction was reported in various mediators (TNF-α, TNFR-1, TNFR-2, and NF-kB)after treatment with MTX ($p<0.001$; $p<0.01$ and $p<0.001$ and $p<0.01$ for TNF-α,TNFR-1, TNF-R2 and NF-kB; respectively). In conclusion,our study revealed a causal link between CIA model and increased expression of cellular TNF-α and its receptors (TNFR-1 and TNF-R2). MTX therapy influences brain immunity in CIA rats, leading to overall amelioration of TNF-α cascade (TNF-α and both receptors 1 and 2 and NF-kB transcription factor), suggesting that anti-rheumatic potential of MTX may extend to affect on brain tissues leading to impairment of TNF-α.

P5.09.68
Microglia induced Foxp3+CD4+Treg through PD-L1 in experimental autoimmune encephalomyelitis

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During experimental autoimmune encephalomyelitis (EAE), microglia has been considered as potential antigen-presenting cells (APC) and had the capability to present antigen. CD4+ Foxp3+ regulatory T cells play a central role in the control of autoimmune responses. However, the role of microglia in CD4+Foxp3+ Treg induction during EAE remains largely unknown. Here, we observed that the number of microglia gradually increased after induction of EAE, and followed by the subsequent reduction during recovery phase. This was closely associated with the change of CD4+Foxp3+ Treg cells. By using real-time PCR and FACS analysis, we found that, at the peak stage of EAE, the expression of MHC II and PD-L1on microglia was significantly up regulated when compared with microglia in control mice. In *ex vivo* co-culture systems, microglia from EAE mice has been found to inhibit the proliferation of antigen-specific CD4+T cell triggered by splenic dendritic cells. Furthermore, microglia from EAE displayed the capacity of induction of Treg, which was diminished by administration of anti-PD-L1 antibody. The suppressive effects of microglia were also inhibited by neutralizing PD-L1 antibody in *ex vivo* co-culture system. Collectively, these data suggest a scenario in which microglia might control of the recovery of CNS autoimmune disease through upregulation of PD-L1 expression and expansion of CD4+ Foxp3+ Treg.

P5.09.69
Gene expression upregulation of TNF superfamily members in temporal lobe epilepsy-associated hippocampal sclerosis

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Temporal lobe epilepsy (TLE) is the most treatment resistant (refractory) form of partial epilepsy. In TLE associated with hippocampal sclerosis (HS) [TLE(HS)], only 10% of patients become seizure free after taking antiepileptic drugs. Recent evidence strongly suggests that inflammation plays a pivotal role in TLE(HS) physiopathology. Neuronal apoptosis in HS can be triggered by excitotoxic damage and specific cytokines. Several TNF superfamily members might be involved in the hippocampal damage caused by seizure precipitation and recurrence. Our goal is to identify critical targets to clarify the molecular physiopathology in TLE-associated HS. We evaluated the relative mRNA expression of fifteen genes, all TNF superfamily receptors or ligands: *FAS, FASLG, NGF, NGFR, TNF*, *TNFRSF10A*, *TNFRSF10B*, *TNFRSF10C*, *TNFRSF10D*, *TNFRSF1A, TNFRSF1B*, *TNFRSF21*, *TNFRSF25*, *TNFRSF6B* and *TNFSF10*. Experiments were carried out in resected hippocampal tissue samples from 12 TLE(HS) patients and compared to four *post mortem* controls. Moreover, immunohistochemistry for TNFRSF1A and TNFRSF1B was performed in patient and control tissues. All target genes were augmented in TLE(HS) patients ($P<0.05$), except *NGF* and *TNFRSF21*. *TNF* showed a marked upregulation ($P<0.01$). Both TNF receptors were clearly activated in patient's tissues. We conclude that the TNF signaling plays a crucial role in TLE hippocampal apoptosis through death receptors. However, TNFRSF1B activation is controversial: it could be related to a survival mechanism or, as it has already been demonstrated, TNFRSF1B may reinforce the TNFRSF1A deleterious action. Our evidence indicates the TNF pathway as an important field for pharmacological studies regarding the benefits of an anti-inflammatory therapy in these patients.

P5.09.70
Prognostic value of autoimmune response in post-stroke hypertensive patients

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Purpose: To define the serum levels of IL-17, NR2-antibody quantity and their prognostic values in post-stroke hypertensives during one-year follow-up.
Methods: 105 patients aged 59±1,5 yrs were prospectively included in this study. Thirty normothensive subjects of equal age were included in control group. Serum levels of IL-17 were detected by immunoassay using eBioscience kits. NR2-antibody quantity were revealed using Gold Dot NR2 Antibody Test, Biotech, INC. Follow-up period was up to 360±20 days. All cases of deaths from any cause and nonfatal stroke and myocardial infarction had been considered as primary clinical end-points.
Results: The mean serum level of IL-17 was up to 32% ($p<0,05$) higher and NR2-antibody quantity was up to 162% ($p<0,05$) higher than in the control group. Patients with multiple brain infarctions had greater amount of NR2-antibodies on 44% ($p<0,05$) compared with subjects with one stroke event in anamnesis. The risk of recurrent stroke and cardiovascular complication was definitely increased in patients with quantity of NR2-antibodies more than 3,97 ng/ml (HR=1,21; 95% CI 1,06-1,37). Autoimmune response was also related to hypertension progression.
Conclusion: Post-stroke bran lesions should be considered as an object of auto-immune activation which has been strongly related to hypertension progression and could increase recurrent stroke risk and risk of adverse cardiovascular events.

P5.09.71
The effect of exogenously administered charged microparticles on the acute phase response

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Circulating microvesicles in the blood are small membrane bound particles of between 50 and 1,000 nanometers (nm). In disease or after injury they are released into the circulation and our taken up in target organs such as the liver where they modify cytokine and chemokine production. We have discovered that the administration of micron sized suspensions of less than 1um reduces basal TNF production by the naïve liver. Here, we sought to discover whether we could manipulate the pathogenesis of the focal cytokine-induced inflammatory lesion in the CNS. We injected 50ng of IL-1b intracerebrally into the brain parenchyma in the presence or absence of intravenously injected positively (NH2) or negatively (COOH) charged particles. The liver, brain, and spleen were collected for cytokine expression profiling. In animals injected with IL-1b in the brain, the presence of circulating microparticles reduced the hepatic expression of cytokines and altered neutrophil recruitment to the brain and liver. This was most marked during the injection of positively charged particles, in which the level of TNF, CXCL10, and CCL2 has been down-regulated in the liver and spleen, while the level of CXCL1 and SAA2 is not changed dramatically. This pilot research supplies charged microparticles as a potential tool for the treatment of neuroinflammation.

P5.09.72
The interaction of polymorphisms of IL-1β and MnSOD was associated with tardive dyskinesia in Chinese Han schizophrenic patients

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Objective: Previous studies have indicated that the immune and antioxidant molecules may be related to the pathogenesis of tardive dyskinesia (TD). Some polymorphisms in immune genes and the Val/9Ala in manganese superoxide dismutase (MnSOD) genes have been associated with TD. The genetic association between the interleukin-1β (IL-1β) -511A/G and TD is unclear. The interaction of two polymorphisms with severity and symptoms of TD has not been reported. This study was to investigate whether these variations and their interaction were associated with clinical phenotypes and TD. Methods: We genotyped the -511A/G and Val/9Ala in schizophrenic patients with TD (n=383) and without TD (NTD; n=430) in a genetically homogeneous northern Chinese Han population. The Abnormal Involuntary Movement Scale (AIMS) and Positive and Negative Syndrome Scale (PANSS) were used to assess the severity of TD and psychopathology of schizophrenia, respectively. Results: The allele and genotype frequencies of -511A/G did not significantly differ between TD and NTD ($p>0.05$), but the genotype frequencies of Val/9Ala showed a significant difference between two groups ($p=0.026$). Interestingly, a significant association was found between -511A/G and total AIMS score in TD patients ($p=0.011$), and the interaction of -511A/G and Val/9Ala showed a trend toward the significant association with the AIMS total score ($p=0.055$). Additionally, the PANSS negative symptom subscore was associated with risk for TD ($p<0.0001$). Conclusion: These findings suggest the role of the Val/9Ala variant in susceptibility to TD. However, the -511A/G and its interaction with Val/9Ala may play a role in the TD severity.

P5.09.73
The function of TREM2 mutants

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TREM2 is a DAP12-coupled immune receptor predominantly expressed in macrophages including microglia. Homozygous inactivating mutations in TREM2 are associated with presenile dementia called Nasu-Hakola disease (NHD) which is a rare autosomal recessive disorder characterized by progressive demyelination in brain. It is hypothesized that the pathogenesis of NHD is due to the inability of TREM2-deficient microglia to remove apoptotic cells and neuronal debris from brain without excess inflammation. Recent studies have demonstrated that TREM2 heterozygous mutations are associated with late onset dementia especially Alzheimer's disease (AD), suggesting an important role of microglial TREM2 in the pathogenesis of AD as well as NHD. In this study, we cloned TREM2 mutants and tested a) their abilities to bind anionic ligands; b) their abilities to activate reporter cell lines. We found that at least one TREM2 mutation impaired binding to anionic ligands and activation of reporter cell lines. We envision that TREM2 enables microglia to clear anionic ligands, such as apoptotic cells in brain, and that impairment of this function results in dysfunction of microglia leading to neurodegeneration.

P5.09.74
Induction of upregulated expression of histamine receptors and increased release of inflammatory mediators from microglia by histamine

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Histamine is a potent mediator of inflammation and a regulator of innate and adaptive immune responses. However, the influence of histamine on microglia, the resident immune cells in the brain, remains uninvestigated. In the present study, we found that microglia can constitutively express all four histamine receptors (H_1R, H_2R, H_3R and H_4R), and the expression of H_1R and H_4R can be selectively upregulated in primary cultured microglia in a dose dependent manner. Histamine can also dose dependently stimulate microglia activation and subsequently production of pro-inflammatory factors TNF-α and IL-6. The antagonists of H_1R and H_4R but not H_2R and H_3R reduced histamine-induced TNF-α and IL-6 production and p38/JNK MAPK pathway activation, and mitochondrial membrane potential loss in microglia, suggesting that the actions of histamine are via H_1R and H_4R. On the other hand, inhibitors of JNK or p38 inhibited histamine-induced TNF-α and IL-6 release from microglia, suggesting that the actions of histamine are through JNK/p38 signaling pathway. In summary, the present study identifies the expression of histamine receptors on microglia. We also demonstrate that histamine induced TNF-α and IL-6 release from microglia are via H_1R and H_4R-JNK/p38 signaling pathway, which will contribute to the development of microglia-mediated inflammation in brain.

P5.10 Multiple sclerosis

P5.10.01
The prokineticin system in experimental autoimmune encephalomyelitis: possible novel targets for immune intervention

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Prokineticins (PK)s are small secreted proteins (8-11kDa), widely expressed in different tissues and involved in a wide spectrum of biological functions. They comprise two proteins, PK-1 and PK-2, signalling through two cognate G-protein-coupled receptors (PK-R1 and PK-R2). Prokineticins and their receptors were reported to modulate immune response. In particular PK-2, highly expressed in inflamed tissues, promotes macrophage activation and migration, and can promote Th1 polarization. However, their role has never been investigated in multiple sclerosis or in its animal model, experimental autoimmune encephalomyelitis (EAE).
During chronic MOG35-55 induced EAE in C57BL/6 mice, we observed a significant increase of mRNA expression of PK-2 in lymph node cells and purified CD4+ T cells. PK-2 protein serum levels were also increased during EAE.
Blocking PK-Rs with the non-peptide-antagonist PC7 reduced the severity of both chronic and relapsing-remitting EAE, when PC7 was used either as a preventive or as a therapeutic treatment. In vitro results showed a dramatic reduction of Th1 cytokines and of IL-17 in antigen-stimulated T cells obtained from PC-7 treated mice, and an increase of the suppressor cytokine IL-10. In vitro treatment with PK-2 reduced IL-4 and IL-10 levels in splenocytes from 2D2 transgenic TCR mice.
These results suggest that PK-2 and its receptors can play an important role in the development of EAE, and might help revealing novel targets of therapy in autoimmune demyelination of the CNS.

P5.10.02
Immunopathological patterns in relapsing-remitting and chronic models of experimental autoimmune enchephalomyelitis and the therapeitc effect of glatiramer acetate

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Multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE) are immune mediated diseases, but the role of inflammatory versus degenerative processes in their pathology is controversial. We analyzed the *in situ* pathological manifestations in relapsing-remitting and chronic EAE models, induced by peptides of the myelin proteins PLP and MOG, respectively, using immunohistochemistry, transmission electron microscopy (TEM) and magnetic resonance imaging. Brain and spinal cord analysis revealed in both models multiple T-cell infiltrations. In particular, Th17-cells were found in sites of myelin damage. Extensive activation of resident immune cells, namely microglia, was also characteristic in all the EAE-induced mice. Notably, different patterns of CNS tissue damages were detected in the two EAE models. Thus, demyelination and remyelination were characteristic to relapsing-remitting EAE, whereas in the chronic model axonal degeneration and neuronal loss was the main manifestation. The effect of glatiramer acetate (GA, Copaxone) was evident by drastic reduction in the prevalence of inflammatory cells such as Th17 and by the elevation of Foxp3-expressing regulatory T-cells. Quantitative TEM analysis provided for the first time evidence for significant augmentation of remyelination as a consequence of GA treatment. In the chronic model, axonal deterioration and motor neurons loss were reduced following GA-treatment. Furthermore, GA augmented the three processes characteristic of neurogenesis - neuronal proliferation, migration and differentiation. All these effects were obtained when GA-treatment was applied by a therapeutic regimen, after disease exacerbation. These results support the notion that immunomodulation can lead to effective neuroprotection and repair consequences in the CNS.

P5.10.03
Association of SNP rs6897932 in CD127 gene in Relapsing Remitting Multiple Sclerosis (RRMS) patients compared to control group

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MS is an inflammatory, demyelination disorder of the CNS. Isfahan is considered as an area with high risk of MS. Recent GWAS have identified a number of SNPs associated with susceptibility to MS. we decided to study the polymorphism of the CD127 gene in RRMS patients.
Materials & Methods: After written consent, blood samples from 200 patients with RRMS (180 females and 20 male; mean age=31.65±8.3) with clinically and MRI definited RRMS who had recuited to Isfahan MS research center , and 203 age and sex matched healthy subjects of blood donors (163 females and 40 male; mean age=31.74±7.75) with no history of neurological disorders were included in the study.DNA was extracted from whole blood using a commercially available kit and stored at -20°C until used for genotyping. SNP analysis was performed using HRM Real Time PCR.Differences in genotype frequencies among the respective groups were evaluated by chi square calculation.
Result: Decreased heterozygosis rate of the genomic C allele was detected in patients with RRMS (p= 0.56) while the CC genotypes were decreased in control (OR: 0.88, confidence interval (0.57–1.36).
Once the effect of the associated *CD127* protective allele is accounted for, as estimated by rs6897932^c, there is no residual evidence of association to MS susceptibility within the *CD127* locus in our region. This result suggests that rs6897932^c is not correlated to MS susceptibility.
Conclusion: The results for the frequency of rs6897932 SNP in CD127 locus was identified no differential allelic expression among patients with RRMS and healthy controls

P5.10.04
Frequency of null allele of HLA-G Locus in East Azarbayjan patients with recurrent miscarriage

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Human leukocyte antigen-G (HLA-G) is a non-classical class I molecule highly expressed by extra villous cytotrophoblast cells . The HLA-G gene contains 15 alleles, including the HLA-G* 0105 N null allele. HLA-G* 0105 N presents a single base pair deletion, which rules out translation of both membrane bound (HLA-G1) and full length soluble isoform (HLA-G5), but other isoforms such as HLA-G2 can compensate their roles. The aim of this study was to demonstrate the frequency of HLA-G* 0105 N null alle le in East Azarbayjan patients with recurrent miscarriage. To investigate the frequency of HLA-G*0105 N null allele in recurrent miscarriage patients, the PCR-RFLP method was used. Genomic DNA was extracted from whole blood of 60 randomly selected patients using the salting-out technique. Then, PCR amplification of the exon 3 of HLA-G gene was performed. The work was continued with the digestion of PCR products with PpuM-I enzyme. Afterwards, the analysis of resulting fragments was carried out using the gel electerophoresis technique. To determine the accuracy of the RFLP method, six samples were selected and sent for sequencing. In this study, digestion of the restriction enzyme showed heterozygous HLA-G*0105 N null allele in 6/6% of the patient population. Data analysis demonstrated that the frequency of heterozygous HLA-G* 0105 N null allele was relatively high in patients with recurrent miscarriage.

P5.10.05
Th1/Th17 imbalance in clinical subtypes of Multiple Sclerosis: association with disability and unresponsiveness to IFN-β therapy in relapsing-remitting disease

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There is an enormous need to understand the immunological processes associated with the different clinical subtypes of Multiple Sclerosis (MS) as well as the identification of immunological parameters associated with disease activity and treatment responsiveness. In this study, ninety-four MS patients diagnosed with Clinical Isolated Syndrome (CIS, n=22), relapsing-remitting (RR) MS (n=44), secondary progressive MS (n=10) and primary progressive MS (n= 18) without treatment and 18 healthy controls (HC) were tested for the plasmatic expression of Th1 (IFN-γ) and Th17 (IL-17A and IL-17F) cytokines by ELISA. We defined the IFN-γ/IL-17F ratio reflecting the Th1/Th17 balance. Th1 and Th17 cell response was determined in PBMC upon CD3/CD28 stimulation by flow cytometry. A group of responders (n=9) and nonresponders (13) RRMS patients to IFN-β therapy was also analyzed. Patients with different MS subtypes exhibited a disturbed and distinctive Th1/Th17 balance skewed towards a Th1 or Th17 disease. Furthermore, a predisposition to develop a Th1 or Th17 cell response was observed in these patients. Remarkably, the Th1/Th17 balance and high levels of IFN-γ and IL-17F were associated with disability (EDSS) in RRMS patients. Most strikingly, the Th1/Th17 balance and high levels of IL-17F distinguished between responder and nonresponder patients to IFN-β therapy. In conclusion, our results provide new evidence to the understanding of immunopathological mechanisms underlying MS subtypes and suggest that the Th1/Th17 balance and plasmatic levels of IFN-γ and IL-17F might have prognostic implications and putative value in monitoring therapy effectiveness. Supported by Fondecyt 1110523.

P5.10.06
The role of STI-571 in the treatment of EAE

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Background: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that leads to an inflammatory demyelination, axonal damage and progressive neurologic disability. STI-571 is a selective protein tyrosine kinase inhibitor that abrogates multiple signal transduction pathways implicated in auto immune diseases.
Method: EAE induction was performed by Hooke Kit. The kit consists of antigen (MOG_{35-55}) in CFA emulsion, and pertussis toxin (PTX) in PBS. The mice were injected subcutaneously on upper back and lower back with 0.1 ml of emulsion respectively. Within 2 hours of injection of the emulsion, the first dose of PTX (0.1 ml per mouse) was injected intraperitoneally. 22-26 hours after injection of the emulsion, the intraperitoneally injection of second dose of PTX into the mice (0.1 ml) were done. The mice were administered orally with STI-571 at the specified dose (60 mg/kg) from day 7 after immunization on six consecutive d per wk for 2 wk. The mice were sacrificed on day 35 post-immunization. Brains, cerebellums and lumbar spinal cords were removed, post-fixed in formalin, embedded in paraffin, sectioned and then stained with Luxol fast blue (LFB) and with eosin and hematoxylin.
Results: Our findings showed that treatment with STI-571 caused a significant delay in the time of onset and a significant reduction in severity of the EAE in treated animals compared with normal groups.
Conclusion: Our results suggest that FDA-approved drug STI-571 has potential therapeutic effects on EAE as an autoimmune demyelinating disease.

P5.10.07
Interleukin-17A and Interleukin-17F mRNA Expressions in Peripheral Blood Mononuclear Cells of Patients with Multiple Sclerosis

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Multiple sclerosis (MS) is a CD4+ T cell-mediated autoimmune disease affecting the central nervous system (CNS). It was previously believed that Th1 cells were pathogenic T cells in experimental autoimmune encephalomyelitis (EAE). How- ever, the functional role of Th1 cells in EAE has been reconsidered upon the discovery of IL-17-producing T cells which are considered as dominant effectors for inducing autoimmune tissue inflammation. Objective: The objective of this study was to assess the role of IL-17A and IL-17F in MS pathogenesis. Methods: We evaluated mRNA expres- sion of IL-17A and IL-17F in thirty-five Iranian patients with relapsing-remitting MS (RRMS) and twenty-five healthy controls by Quantitative Real Time PCR. Results: The results of this study showed a twenty-fold increase in the expression of IL-17A mRNA in MS patients compared to the control group ($p < 0.0001$). IL-17F mRNA expression in MS patients was thirty three-times greater than the control group ($p = 0.0008$). IL-17A mRNA expression in the periphery was positively correlated with the expression of IL-17F transcripts in MS patients and controls ($p < 0.01$ and $p < 0.05$, respectively). Conclusion: These results indicate the critical role of Th17- mediated cytokines in the development of MS which was classically considered as a Th1-mediated disorder. The results of this study showed, for the first time, the importance of IL-17F in MS immunopathogenesis.

P5.10.08
Inflammatory stimuli and oxidative stress alter mitochondrial transport in neurons

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In multiple sclerosis (MS), autoimmune inflammatory processes may lead to neuronal damage. Numerous studies link mitochondrial dysfunction to the pathogenesis of neurodegenerative diseases, and recent reports suggest this may also be the case for MS. Mitochondria are crucial to cell survival: They produce ATP, maintain ion homeostasis and regulate apoptosis. Within the axon, they are delivered to, and remain in areas where metabolic demand is highest. The health of neurons depends critically on their distribution, which raises the possibility that inflammation-induced alteration of mitochondrial dynamics could promote neuropathology. To investigate whether inflammatory stimuli and oxidative stress can directly influence mitochondrial behavior, we used an *ex-vivo* model of peripheral nerves explanted from the murine spinal cord. Axonal tracts and mitochondrial dynamics are preserved for several hours after explantation and can therefore be targeted experimentally. The application of bacterial endotoxin (lipopolysaccharide), a prototypic inflammatory stimulus, induced a significant increase in the number of motile mitochondria. This increase was directional, augmenting the proportion of mitochondria moving towards the cell body. In contrast, incubation with an oxidative stress inducer, buthionine sulfoximine (BSO), produced a substantial decrease in mitochondrial motility. This reduction could be partially prevented by pre-incubating the nerves with the antioxidant (-)-epigallocatechin-3-gallate before BSO addition. Using an *ex-vivo* model we were able to monitor different alteration patterns of mitochondrial trafficking. These results are consistent with a role of inflammation and oxidative stress-induced alterations of mitochondrial dynamics in neurodegeneration, and highlight the utility of simplified models to examine mitochondrial response to pathological stimuli.

P5.10.09
A systems biology approach to identify biomarkers and therapeutic targets in peripheral monocytes of multiple sclerosis patients

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Multiple Sclerosis (MS) lesions are characterized by perivascular infiltration of inflammatory mononuclear cells accompanied by demyelination and axonal injury. Peripheral monocytes serve as a source of the infiltrate and it is assumed that they acquire a unique transcriptional signature after repeated passages through the vascular bed. Because of the unpredictable clinical course, biological surrogate markers are much needed in the field of MS.
This project is aimed to identify gene markers that best discriminate between patients with different disease activity, to achieve an improvement in disease monitoring and in treatment efficacy.
We performed a gene expression and microRNA (miRs) profiling in monocytes from female MS patients in the relapsing remitting (RR; n=13) and primary progressive (PP; n=5) phenotypes, compared to healthy volunteers (Ctr; n=10). We were able to identify significantly regulated miRs in monocytes of MS patients compared to Ctr and between the two different disease phenotypes. Seven down-regulated miRs were validated by Q-PCR, confirming that the miR-130a is down-regulated in RR compared to Ctr while the miR-125a is specifically decreased in RR compared to both Ctr and PP subjects. Current experiments in the laboratory are investigating the role of these molecules in regulating monocytes functions and MS phenotypes. We are also validating our findings on an extended number of MS patients and controls to increase the power of the analysis. In conclusion, our preliminary data suggest that a Systems Biology approach based on gene expression and miRNA profiling is feasible and promising to identify reliable biomarkers in MS

P5.10.10
Micro/nanoparticles platforms for "inverse vaccination" in Experimental Autoimmune Encephalomyelitis (EAE)

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"Inverse vaccination" uses autoantigens in combination with appropriate "adjuvants" to trigger "regulatory" cells specifically inhibiting an autoimmune response. Recently, an inverse DNA vaccine showed a therapeutic effect in patients with multiple sclerosis (MS), but DNA vaccination has potential risks limiting its use in humans. An alternative approach could be use of protein vaccines released from polymeric biodegradable lactic-glycolic acid (PLGA) particles (PLGA-NP), approved by FDA, to sustain release of antigens and regulatory adjuvants for extended periods. Aim of this work was to develop protein-based tolerogenic PLGA-NP vaccines containing myelin autoantingen (MOG35-55) and adjuvants to inverse vaccinate EAE (Experimental Autoimmune Encephalomyelitis) mice. We produced PLGA-NP loaded with either MOG35-55 or rIL-10 used as an adjuvant for regulatory cells. The morphology of these particles was evaluated by Scanning Electron Microscope and showed a mean diameter of 591.7 nm. PLGA-NP were not toxic to cells and did not induce secretion of proinflammatory cytokines (IL-1β and TNF-a) on monocytes. The encapsulated proteins were released in vitro for up to 2 months and the released rIL-10 maintained the ability to inhibit LPS-induced secretion of TNF-α on monocytes. Preliminary experiments showed that subcutaneous treatment with MOG35-55- and rIL-10-loaded PLGA-NP significantly inhibited development of EAE in C57/B6 mice without detectable toxic effects. These data suggest that PLGA-NP-based inverse vaccination may be an effective tool to treat autoimmune diseases.

P5.10.11
In-vitro up-regulation of Regulatory T cells markers with IFN-beta and c-Phycocyanin: pertinence of a combined treatment for Multiple Sclerosis

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Nowadays there is a growing knowledge of the pathogenic process that takes place in Multiple Sclerosis (MS) characterized either by signs of autoimmunity, inflammation and demyelination or primary oligodendrocyte loss. Reactive oxygen species (ROS), have been implicated as mediators of demyelination and axonal damage in MS.
Interferon-beta is an approved therapy for MS, due to the immunomodulatory, antiviral and stimulation of neurotrophic substances and endogenous opioids properties.
C-Phycocyanin is the principal phycobiliprotein of the Spirulina platensis, a blue - green alga, with several reports documenting its pharmacological properties as strongly antioxidant and anti-inflammatory.
After performing the molecular characterization of cellular immune response (TNF-α, IFN-γ, IL-10, MMP-9, TIMP-1) and oxidative stress parameters (MDA, AOPP, Peroxidation Potential, SOD, Catalase, Total Hydroperoxides) we found a profound oxidative stress state and a significant down-regulation in serum levels of the regulatory cytokine, IL-10, in MS patients when compared to controls.
Furthermore, in-vitro stimulation of peripheral blood mononuclear cells from MS patients with IFN-beta or c-Phycocyanin, significantly up-regulated mRNA as well as surface markers of Regulatory T cells.
Most MS approved therapies point only toward the inflammatory and immunological components. Still, there is an increasing need of approaches in response to the more complex physiopathological phenomenon of the disease.
Antioxidant and anti-inflammatory properties of c-Phycocyanin suggest it might be used in the treatment of neurodegenerative diseases like Alzheimer, Parkinson and also MS. Moreover, in MS, our results support the combination of IFN-beta and c-Phycocyanin for a wider spectrum and likely more effective treatment for the disease.

P5.10.12
Complex changes in invariant natural killer T (iNKT) cells in patients with different clinical forms and treatments of multiple sclerosis

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Although associations between Multiple Sclerosis (MS) and defects in invariant natural killer T (iNKT) or in proinflammatory CD8+CD161+ T cells have been reported, a more detailed characterization of these cells is needed to clarify their role in different form of MS.
We analyzed iNKTs subsets and CD8+CD161+ T cells in 66 patients: 52 with a Relapsing Remitting (RR), 9 with a Primary Progressive (PP) and 5 with a Secondary Progressive (SP) MS and 16 healthy controls (CTR).CD3+ T cells were counted with CyFlow Counter (Partec, Germany). PBMCs were stained with different mAbs for the analysis on a 6-color cytometer (Attune, Life Technologies, USA): anti-Va24Ja18 TCR, -CD4, -CD8, -CD161, -CD3, -CD19 and -CD14.
MS RR patients displayed a statistically significant lower number of iNKT cells, compared to CTR. Patients with other MS forms showed a similar trend. Among iNKT cells the CD8+ subset was diminished. The CD4-CD8- subset was significantly lower in the RR form compared to CTR. Among iNKT cells, the population of CD8+CD161+ cells was lower in all MS patients. When compared to CTR, PP and SP patients had a lower number and percentage of CD8+ T cells; CD8+,CD161+ T cells were lower in all SP patients vs. CTR. RR patients taking Fingolimod had a lower number of CD3+ T cells and a lower percentage of iNKT expressing CD4.
Changes in iNKT cell subpopulations we observed in RR patients, along with the trends present in the other patients, suggest a role for these cells in the pathogenesis of MS.

P5.10.13
MBP85-99 specific TCR repertoire in DRB1*15 patients affected by Multiple Sclerosis

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Multiple Sclerosis (MS) is an inflammatory, chronic, disease of the CNS whose main genetic contribution is HLA DRB1*15 (DR2). We used Immunoscope analysis (TRBV-TRBJ spectratyping) to investigate the repertoire of T cells specific for MBP85-99 and MBP111-129 in 18 untreated DR2+ patients and 6 healthy DR2+ subjects. We identified specific MBP85-99TCR repertoire shared among the 18 MS patients that are characteristic of MS disease as they were not founded in HS: one was shared by 90% of MS DR2+ patients at the onset of disease and one present in 50% of CIS and MS patients. Moreover, the TCR repertoire specific for MBP85-99 is apparently broader in CIS than in MS patients. The MBP111-129-specific response used a distinct TCR repertoire with no significant variation in the number of expansions, suggesting that the response to this sub-dominant epitope doesn't play an active role in the disease. Twentyfive % of the investigated TCR rearrangements were used by Th1 cells, whereas one was exclusively used by Th17 cells;

its presence was downregulated following IFNβ therapy that also reduced the overall circulating MBP specific TCR repertoire. Intriguingly, before the relapse the Th17 specific-MBP85-99 TCR repertoire reappeared. Our observations may allow to focus future treatment on these MS-related antigen specific T cells.

P5.10.14
Frequency of Myelin Oligodendrocyte Glycoprotein (MOG) specific B cells in the blood and the CSF of patients with Multiple Sclerosis (MS)

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Multiple Sclerosis is a chronic inflammatory demyelinating disease of the Central Nervous System (CNS) where B cells play a key role. Indeed, recent advances with anti-CD20 therapy underscored involvement of B cells in MS outside their capacity to mature in plasmocytes.
Objectives: We measured the frequency and characterized circulating and intrathecal MOG-specific B cells in MS.
Methods: We used a bead-based method using fluorescent beads coupled to MOG. We first analyzed the frequency of MOG-specific B cells in 30 MS patients and 39 Healthy Volunteers (HV). Then, the anti-MOG B cell phenotype was characterized with CD19, CD27 and IgD staining.
Results: First, we obtained a substantial fraction of B cells binding to MOG coated beads in the blood of both HV and MS patients. We also observed a significant lower frequency of MOG-specific B cells in patients as compared to HV, which was not the case when using negative (albumin) or positive (tetanus toxin) control antigens. No significant difference was observed between MS and HV frequencies of memory and class switched cells in the MOG-specific B cells. Interestingly, preliminary results showed a trend for an increased frequency of MOG-specific B cells in the cerebrospinal Fluid (CSF) of MS patients.
Discussion and perspectives: A decreased frequency of circulating MOG-specific B cells may suggest apoptosis, modified homing and/or migration to CNS, although an increased frequency in CSF may promote a migration to the CNS. Further experiments will compare the transmigration of B cells from MS and HV across a Blood-Brain Barrier model.

P5.10.15
Effector T cell depolarization after glucocorticoid treatment impairs migration and APC conjugate formation

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Glucocorticoids (GCs) are in widespread use for the treatment of acute relapses of multiple sclerosis (MS) patients. Currently, the beneficial effects of GCs are believed to primarily depend on transcriptional repression of T lymphocytes. In this study we investigated modulation of antigen-specific effector T cells within the first few hours after GC application. By use of polarized effector T cells in vitro we identified a novel mechanism by which GCs impact T cell function. Briefly after addition of GCs effector T cells started to depolarize, which impaired their migratory capacity and their ability to interact with APCs. The observed morphological changes were mediated by activation of the cytoskeleton associated ERM-proteins and correlated with an increased rigidity of the cells. The rapid GC effects were dependent on Phospholipase C activity, since its inhibition prevented effector T cell depolarization and ERM activation. In contrast, they were independent of altered gene expression, because inhibition of translation did not affect the observed cytoskeleton rearrangement. Furthermore, we could show that the morphological alterations of effector T cells also occured in vivo. Collectively, we have identified a novel mechanism through which GCs rapidly modulate effector T cell function.

P5.10.16
The atypical antipsychotic agents, clozapine and risperidone, reduce disease severity in the animal model of multiple sclerosis

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Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) whereby immune cells target the myelin sheath surrounding the nerve often leading to severe life-long disability. Current disease modifying medications available are only effective for one of the four MS disease forms and are further limited by treatment efficacy, toxicity, cost, and means of administration; thus, there is much interest in identifying alternative and more effective MS therapies. Recent work with atypical antipsychotic agents (AAPs) has shown that AAPs not only antagonize the dopamine D2 and serotonin 5-HT2α receptors but also have immunomodulatory properties capable of acting within the CNS in schizophrenic patients. We examined ability of the AAPs, clozapine and risperidone, to modify the immune response in MS and found that chronic oral administration reduces disease severity in the animal model, experimental autoimmune encephalomyelitis (EAE). Additionally, treatment with clozapine or risperidone reduces pro-inflammatory cytokine production by LPS-stimulated splenocytes while maintaining the antigen-specific IFN-ɣ response. Furthermore, we have identified synergy with clozapine and the commonly prescribed MS-treatment, glatiramer acetate whereby using suboptimal doses of each reduces the incidence of disease in EAE-immunised mice. Clozapine also demonstrates moderate MS disease protection at human equivalent doses below that prescribed for the treatment of schizophrenia thus circumventing the unwelcome side-effect of weight gain. Together these studies indicate that AAPs are effective immunomodulatory agents with the potential to treat immune-mediated diseases such as MS.

P5.10.17
Development and assessment of multifunctional gold nanoparticle system for dendritic cell targeting in the treatment of EAE

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Experimental autoimmune encephalomyelitis (EAE) is used as an animal model of Multiple sclerosis. Nanocarriers have provided a novel platform for target-specific delivery of therapeutic agents. Gold nanoparticles have recently emerged as an attractive candidate for delivery of various payloads into their targets. Delivering antigen to quiescent DC via DC-205 results antigen specific T cells is deleted and the mice became unresponsive to re-challenge even with adjuvant. Multifunctional gold nanoparticles were prepared by the reduction of aqueous chloroaurate ions with trisodium citrate and conjugate with MOG and Anti-DEC 205. EAE was induced in C57/BL6 mice by subcutaneous injection of MOG and CFA and intraperitoneal injection of pertussis toxin. Treatment group received a daily subcutaneous injection of multi functional MOG-Anti DEC-nanogold for 1 week. Clinical assessment, histological studies, flowcytometry, Brdu assay and Real-time PCR were performed for determine the profile of immune response. Our results showed significant Clinical score decrease in treatment group. Histological studies revealed lower lymphocytic infiltration and demyelination. The percentages of spleen $Foxp3^+$ cell and IL-4 positive cells induced and the percentages of spleen IL-17 and IFN-γ positive cells reduced in treatment group. Expression of transcription factor and cytokines related to Treg and Th2 showed increase in treatment group. It seems that produced multifunctional nanoparticles alleviate disease condition in EAE through reducing inflammatory immune responses. Significant increase in spleen Treg cells might be a sign for suppressed immune responses. Moreover, a significant decrease in Th17 demonstrates reducing inflammatory responses. Consequently, the balance between Treg and Th17 is deviated to Treg.

P5.10.18

1, 25-dihydroxyvitamin D3 ameliorates experimental autoimmune encephalomyelitis in C57BL/6 mice

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Background: The experimental autoimmune encephalomyelitis (EAE) is an animal model for human multiple sclerosis. Vitamin D has several reported immunomodulatory properties including the reduced generation of pro-inflammatory $CD4^+$ Th1 cells and the increase in levels of the anti-inflammatory Th2 subset.
Method: 24 C57BL/6 male mice were divided into 3 groups: 1-Control, 2-EAE without treatment and 3- 1,25-dihydroxyvitamin D3 (Vitamin D3) treated EAE. EAE was induced in Groups2 and 3 by subcutaneous injection of MOG and CFA and intraperitoneal injection of pertussis toxin. Mice in group3 received intraperitoneal injection of 40 ng/kg vitamin D3 daily for 10 days. Clinical and weight assessments were performed daily. On day 25 animals were sacrificed. The brain tissues were stained for histological studies. Spleen cells were analyzed using flowcytometer and Real-time PCR. Brdu assay was used for splenocyte proliferation.
Results: Our results showed significant mean weight increase and Clinical score decrease in group3 comparing with group2. Histological studies revealed lower lymphocytic infiltration and demyelination in group3 compared to group2. Splenocytes proliferation showed significant reduction in group3 in comparison to group2. The percentages of spleen $Foxp3^+$ cell population in $CD4^+$ cells reduced in groups3 compared group2. Expression of transcription factor and cytokines related to Treg and Th2 showed significant increase in group3 in comparison to group2.
Conclusion: It seems that 1,25-dihydroxyvitamin D3 may alleviate disease condition in EAE mice through reducing inflammatory immune responses. Moreover it seems 1,25-dihydroxyvitamin D3 drives the polarization of Th2 and Treg subsets.

P5.10.19

miR-132 suppresses autoimmune encephalomyelitis by inducing cholinergic anti-inflammation: a novel Ahr-based exploration

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MicroRNAs (miRNAs) are a small group of RNAs that is emerging as new pathway able to modulate autoimmune diseases. MiRNAs are involved in pathogenesis of multiple sclerosis (MS). However, the interaction of miRNAs with environmental-responsive transcription factors that play prominent roles in MS is still unexplored. Therefore, identification of novel mechanisms linking these two players in MS development could enable earlier treatment. Activation of aryl hydrocarbon receptor (Ahr) by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alleviates inflammation in Experimental Autoimmune Encephalomyelitis (EAE), the best MS model available; therefore, we predicted that TCDD ameliorates EAE by inducing miRNA(s) targeting inflammatory mediators. Here, we provide evidence about a novel miRNA-based mechanism through which TCDD induces a cholinergic anti-inflammatory response in EAE mice via upregulation of acetylcholiesterase (AChE)-targeting miR-132. $CD4^+CD62L^+$ cells purified from spleens of wild type or $Ahr^{-/-}$ C57BL/6 mice were stimulated with TGF-β alone or combined with IL-6 in the presence of TCDD. EAE was induced by MOG_{35-55} with/without i.p. injection of TCDD or antisense (as)-miR-132. MiR-132 was overexpressed or silenced by oligonucleotide transfection. AChE was quantified by qPCR and immunoblotting. We found that activation of Ahr by TCDD in T cells *in vitro* and *in vivo* induced miR-132 and its cluster partner miR-212,. Treating EAE mice with TCDD alleviated EAE symptoms, manifested by the reciprocal relationship between miR-132, AChE and pro-inflammatory cytokines. In conclusion, TCDD-activated Ahr mitigates autoimmune inflammation by miR-132-mediated cholinergic anti-inflammation. Therefore, our findings may represent a base for future application of miR-132 mimics in the treatment of MS.

P5.10.20

Study of the frequency of different CD8+ T cell subsets in patients with Multiple Sclerosis

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Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS) that affects several million people worldwide and more than 50000 individuals in Iran which is now increasing at an alarming rate. Most prior reports have focused on the role of $CD4^+$ T cells and their subsets in the pathogenesis of the disease, but recent evidence points to an important role of CD8+ T cells comes from their dominance in white matter lesions in the CNS. In addition, like $CD4^+$ T cells, $CD8^+$ T cell subsets with distinct cytokine patterns: Tc1, Tc2 and Tc17, were described in mice and humans. However, the role of these T cell subsets in the pathogenesis of MS has been less investigated.
In this study we analyzed the frequency of CD8+ T cell subsets in Multiple sclerosis patients with different clinical patterns, RRMS (n=18), SPMS (n=10) and PPMS (n=4) using a Multi color flowcytometric assay in comparison with healthy age- and gender-match controls (n=10).
We found that Tc1 cells significantly increased in SPMS patients in comparison to patients in relapse phase, remission phase, PPMS and controls (p=0.04, p=.000, p=0.01 and p=.000 respectively). Our finding also indicated higher frequency of Tc1 cells in relapse patients than healthy controls (p=0.04). Moreover, our data showed significantly increased frequency of Tc17 cells in relapse patients as compared with remission (p=0.04) and healthy controls (p=0.05). These data demonstrate the contribution of different CD8+ T cell subsets in active MS phases.

P5.10.21

Biased Treg/Th17 balance away from regulatory toward inflammatory phenotype in relapsed multiple sclerosis and its correlation with severity of symptoms

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The opposing immune functions of Treg and Th17 lymphocytes despite their common developmental pathway, alongside Treg-Th17 plasticity, has led to a paradigm shift of each subset prevalence and function to their counterbalance and interaction duties in immune homeostasis. We have concomitantly evaluated the frequency of two subsets through surface and intracellular staining and flow-cytometry detection of peripheral blood mononuclear cells obtained from 23 definitively diagnosed relapsing-remitting multiple sclerosis (RR-MS) and 11 clinically isolated syndrome (CIS) patients in documented relapse of disease, and 23 healthy subjects. This study demonstrated a decreased Treg frequency versus an increased Th17 cells frequency and a several fold reduction in Treg/Th17 ratio in relapsed MS (both RR-MS and CIS) against health (all P Values<0.001). Also a reverse correlation between these two lymphocyte subsets was observed in controls but this correlation was lost in patients. Treg frequency and Treg/Th17 ratio were both negatively correlated with severity of symptoms, and Treg frequency had been increased with disease enduring (all P Values<0.05). Our results suggests an important role for Treg/Th17 imbalance in MS relapses which remains to be more studied in details of Treg functionality and Th17 sensitivity to their suppressive effect, also Treg-Th17 conversion and relative rates of IL-17 production by $FOXP3^+$Tcells in this disease. In addition to these clues for basic studies, our data confirms the changes of Treg/TH17 balance as an informative efficacy marker for evaluating or comparing the effectiveness of MS therapies, and highlights Treg-Th17 interaction as a target for new therapeutic approaches.

P5.10.22
Vav1 influences susceptibility to central nervous system autoimmunity

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Genetic studies performed by our group using congenic rats lead us to identify a locus on rat chromosome 9 that controls the size of the Foxp3 regulatory lymphocytes (Treg) and susceptibility to central nervous system inflammation (CNS). Fine mapping of this locus identified a non-synonymous SNP in the Vav1 gene that leads to the substitution of an arginine by a tryptophan. To provide the definitive proof that these Vav1 variants are involved and to understand their mechanisms of action, we generated a knock-in (KI) mouse characterized by an arginine (R) to a tryptophan (W) substitution in the Vav1 protein (Vav1W63 KI). Using this model, we provided the definite demonstration that this Vav1W63 mutation is responsible for the increased proportion of Treg and decreased susceptibility to CNS inflammation. In addition, we showed that autoreactive CD4 T cells from KI mice produce reduced amount of effector cytokines, IFN-γ, IL-17 and GM-CSF. Together, these results demonstrate that alterations in Vav1 signaling are involved in CNS inflammation and this may be related to the effect of Vav1 variants on both regulatory and effectors T cells.

P5.10.23
Microglia are potent producers of IFNβ in the context of autoimmunity and microbial stimulation

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Multiple sclerosis (MS), the most common neurological disease in western countries, is a chronic autoimmune disorder of the CNS. The standard medication of relapsing remitting MS is recombinant IFNβ decreasing frequency of relapses and severity of disease. Genetic deletion of IFNβ in mice also leads to aggravation of disease symptoms in the MS mouse model of MOG35-55 induced experimental autoimmune encephalomyelitis (EAE). The cell type responsible for the endogenous IFNβ production and their protective functions remain unknown.
Time course studies showed that IFNβ mRNA was expressed in the early phase of EAE in secondary lymphoid organs, while in the effector phase highest IFNβ levels were detected in the CNS, especially in the spinal cord. Using an YFP knock-in reporter mouse model for IFNβ we identified resident microglia in the CNS as main producers of IFNβ at the peak of EAE. FACS sorting of intracerebral cell populations according to the expression of CD45 versus CD11b and the analysis of the respective IFNβ mRNA levels confirmed these findings. An adult microglia culture system allowed for further functional analysis of IFNβ/YFP-producing microglia in vitro. Here poly(I:C) stimulation confirmed their high ability to produce IFNβ. In organotypic slice cultures IFNβ/YFP-producing cells showed the morphology of activated microglia and were stained co-positive for Iba-1 in situ.
Taken together, we show for the first time that activated microglia are the major endogenous producers of the protective IFNβ at the peak of EAE with analyses defining the specific functional properties of the IFNβ producers being currently performed.

P5.10.24
Features of Sema4D/CD72 expression in immune system in multiple sclerosis

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Sema4D is a semaphorin family member, mainly of lymphocyte origin, that is exist in membrane-bound or soluble forms and involved both in nerve and immune system regulation. A series of last findings suggests the involving of Sema4D in pathogenesis of multiple sclerosis (MS), namely, the resistance of Sema4D$^{-/-}$ mice to the development of experimental autoimmune encephalomyelitis, the animal MS model, as well as Sema4D-induced apoptosis in oligodendrocytes, the key factors of demyelination. Here, the features of the expression of Sema4D, as well as of their receptor, CD72, by lymphocytes of MS patients have been studied. MS was shown to be associated with the increasing of Sema4D level on the intact T lymphocytes, as well as with more intensive semaphorin shedding from cell membrane in case of lymphocyte activation. Taking into account a negative Sema4D role in nerve fibers myelination, the data obtained suggest its possible involving in MS pathogenesis. Additionally, the decreasing of the expression of Sema4D receptor, CD72, on B lymphocytes has been revealed in MS patients. Since CD72 is a negative B cell regulator, the decreasing of its level in MS may be responsible for hyperactivation of B lymphocytes, a key players in MS, and along with the increasing of Sema4D expression may represent a new mechanism of MS induction and/or progression.

P5.10.25
NK cells are functionally defective in multiple sclerosis

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Multiple Sclerosis (MS) pathogenesis might be linked to impaired function of regulatory subsets of immune cells. In vitro, activation of NK cells with IL-27 or the anti-CD25 monoclonal antibody can induce suppressor function of CD56bright NK (NKbr) cells with mechanisms involving lytic enzymes such as perforin and granzyme K. The pro-inflammatory cytokines, IL-12 and IL-15, activate NK cells and are upregulated in MS patients. Our hypothesis is that under inflammatory conditions, NKbr cells acquire T-cell suppressor function to regulate T-cell activation. We further postulate that such a feedback mechanism is impaired in MS.
Methods: NKbr and CD56dim NK cells (NKdim) isolated from healthy subjects (HS) and MS patients were cultured with activation with IL-12 and IL-15. NK Cells were then co-cultured with autologous CD4+ T cells and T cell proliferation was tested. Cytotoxicity of NK cells towards T cells in co-culture was assessed. Results. Activated NKbr cells from HC, but not MS patients, suppressed autologous T cell proliferation. We found evidence of cytotoxicity of NKbr cells towards T cells in HS. Expression of cytotoxic enzymes was similar in MS patients and HS. In conclusion, we provide evidence that the suppressor function of NKbr cells is induced by inflammatory stimuli in HS. While we found a normal number of circulating NKbr cells in MS patients at early disease stage, their suppressor function appears to be defective. Functional studies are needed in order to dissect the mechanism of such NK immunoregulatory function in HC and the altered response observed in MS.

P5.10.26
Multiple sclerosis mesenchymal stromal cells exhibit a distinct gene profile and decreased immunosuppressive properties in relation to healthy control cells

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Mesenchymal stromal cells (MSCs) have immunosuppressive effects and have been used for the treatment of autoimmune diseases. We evaluated the gene expression profiles and the in vitro immunomodulatory properties of MSCs obtained from multiple sclerosis patients and controls. Bone-marrow MSCs were isolated by plastic adherence. Gene expression profile was evaluated by microarrays using an Agilent platform. For coculture assays, PBMCs were labeled with CFSE, added to patient or control MSCs, and stimulated with PHA. After 5 days, T-cell proliferation was assessed by flow cytometry. IL-10 and TGF-β were measured in supernatants by CBA flex. The results were analyzed by the Limma-T and Mann-Whitney tests. The study was approved by the local Ethics Committee. Compared to controls, 641 differentially expressed genes in patient MSCs ($p<0.01$; fold change≥2), comprising 262 repressed genes, including TGFB1 and HGF and, 379 induced, among them, IL10 and IL6. The inhibitory effect of patients MSCs (52,1 ± 5,6%) on T-cell proliferation was significantly reduced ($p=0.024$) compared with controls (69.4 ± 7.2%). There were no differences in the percentages of CD4+CD25hiFoxp3+ cells in both MSCs cocultures. IL-10 and TGF-β secretion were significantly diminished ($p<0.05$) in supernatants from cocultures with patient MSCs (IL-10: 53.8 ± 49.4; TGF-β: 59.4 ± 24.6 pg/mL) compared with controls (IL-10: 109.9 ± 64.6; TGF-β: 226.6 ± 153.2 pg/mL). Concluding, patient and control MSCs exhibited distinct transcription profiles and patient MSCs secreted low amounts of IL-10 and TGF-β and exhibited decreased ability to inhibit T lymphocyte proliferation. Financial support: FAPESP.

P5.10.27
The regulation synthesis and hydrolysis of cGMP in brain cortex rats during the course of experimental autoimmune encephalomyelitis (EAE)

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cGMP has important functions in the central nervous system. Have a protective effect on oligodendrocytes and microglia modulates macrophage response to inflammatory agents. Mizrachi et al. (2010) observed changes in the levels of phosphodiesterase (PDEs) in multiple sclerosis (MS), which suggests that PDEs may be a potential target for the treatment of MS. The aim of this study was to evaluate the regulation of cGMP concentration in the cerebral cortex of the rat EAE Levis depending on the stage of the disease. To induce EAE, rats were immunized with inoculums containing guinea pig spinal cord in PBS and homogenized emulsion in complete Freund's adjuvant containing 110 mg per 100 ml of the appropriate antigen into the emulsion and the addition of 4 mg / ml Mycobacterium tuberculosis (H37Ra). In the group of rats were sacrificed 5 animals respectively at 0, 4, 8, 12 and 20 days of the experiment. EAE induced a dramatic reduction in the concentration of cGMP in the brain, which corresponded closely to the course of neurological symptoms and weight loss. These changes are accompanied by decreasing soluble guanlylate cyclase, PDE 9 and increase expression of PDE 2. No change was observed PDE5. The greatest changes were observed in 12 and 20 day experiment. Match the severity of the disease in MS relapse. These results suggest that the lower cGMP level by PDE2 might play important role in pathological changes and neuronal dysfunction in EAE.

P5.10.28
Natural killer cells in early multiple sclerosis

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In 85% of young adults with multiple sclerosis (MS), onset is a subacute clinically isolated syndrome (CIS). However, only 30-70% of patients with a CIS develop MS. There are some potential candidates for markers to predict the development of MS in patients with CIS, for example an increase of CD5 positive subset of B lymphocytes in peripheral blood.
The aim of our study was to evaluate other immunological predictors of CIS conversion into MS.
Method: 148 patients with CIS treated with interferon beta have been followed for 3 years. Lymphocyte subsets were analyzed by flow cytometry at baseline and after 6, 12, 24 and 36 months of therapy. 101 patients
remained on the assigned therapy throughout the 3 year follow up(respondents), whereas 47 patients converted to MS (non-respondents).
Results: Higher numbers (16,7%) of natural killer (NK) cells in patients with CIS, compared to patients with MS, were found. A permanent statistically significant very slow reduction of NK cells subpopulation was found in the respondent group after 6,12,24 and 36 months of interferon therapy
(17% to 10% in 3 years). Very significant reduction was found in the non-respondent group (15,7 % to 12% within 6 month) followed by nonsignificant reduction to 11,5% by the end of the study.
As in other autoimmune diseases, NK cells of MS patients naïve to treatment are present in reduced numbers. A relatively high numbers of NK cells and especially their slow reduction in CIS could be good prognostic factor.

P5.10.29
Microvesicles derived from mesenchymal stem cells: potent organelles for induction of tolerogenic signaling

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Generation and maintenance of immunological tolerance is pivotal in autoimmunity. Regulatory molecules of Programmed Death Ligand-1 (PD-L1), galectin-1 and TGF-β are described as key mediators of peripheral tolerance, that actively suppress auto-reactive cells and inhibit their induction of tissue damage. Accordingly, biological intervention in host immune system for induction of peripheral tolerance is pivot to many recent studies. Mesenchymal stem cell-derived microvesicles (MVs) are viewed as potential mediators in peripheral tolerance toward auto-reactive cells via bearing of tolerogenic molecules. Here, MVs were isolated from mesenchymal stem cell (MSC) cultures' conditioned medium. They were explored for the expression of PD-L1, galectin-1 and membrane bound TGF-β by flow cytometery. The immunoregulatory effects of MVs on splenic mononuclear cells (MNCs) derived from experimental autoimmune encephalomyelitis (EAE) affected mice were investigated using MTT assay, ELISA and flow cytometry. MVs derived from MSCs expressed PD-L1, galecin-1 and membrane-bound TGF-β. MVs exhibited the potential to inhibit auto-reactive lymphocyte proliferation and also the potency to promote them to secret anti-inflammatory cytokines of IL-10 and TGF-β. Interestingly, inducing inflammatory setting on MSCs, revealed the enhancing regulatory effects of MVs via increased expression of some regulatory molecules, specifically PD-L1 and TGF-β. Induction of tolerogenic signaling, promotion of CD4+ CD25+ Foxp3+ regulatory T cells generation and apoptotic activity towards activated T cells are shown to be possible mechanisms involved in MV-mediated regulation. A recent study suggests MSC-derived MVs as potent organelles for induction of peripheral tolerance and modulation of immune responses.

P5.10.30
The association of Epstein-Barr virus infection with multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the CNS with as yet unknown cause. Epstein-Barr virus (EBV) infection is widely considered to be a risk factor for multiple sclerosis. Individuals with high levels of antibodies to the EBV viral capsid antigen (VCA) have an increased risk of developing MS.
Objective: The aim of this study was to find out if there is any relationship between the elevated titer of antibody against EBV and the onset of MS.
Method: Sera were collected from fifty patients with primary clinical MS manifestation (10 male subjects, 40 female subjects, mean age 30 years). Samples were prepared from the MS Clinic in Alzahra and Kashani Hospitals in Isfahan. The level of Immunoglobulin G (IgG) against EBV capsid antigen (EBV-CA) was assessed by using commercially available quantitative ELISA.
Results: Our results indicated that in all MS patients' sera higher titer of IgG to EBV-CA with mean of 178.2 IU/ml comparing to individuals normal range ≤ 16 IU/ml were seen in the onset of MS disease.
Conclusion: These finding suggest that this virus plays an important role in the pathogenesis of MS.

P5.10.31
Monomethyl fumarate induces a switch of activated microglia from a pro-inflammatory to a neuroprotective phenotype

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BG-12, a fumaric acid ester with immunomodulatory and neuroprotective potential, is under investigation as treatment for multiple sclerosis. We hypothesize that its beneficial effects are mediated, at least in part, through its action on microglia. This possibility was investigated in vitro using activated microglial line and primary cell cultures in the absence or presence of its bioactive metabolite, monomethyl fumarate (MMF). MMF inhibited microglia activation by lipopolysaccharide (LPS), significantly reducing the production and expression of pro-inflammatory molecules, TNFα, IL1β, and iNOS, as well as inhibiting the expression of PU.1, a transcription factor that is considered a marker of activation phenotype of microglia. MMF also inhibited the proliferation of LPS-activated primary microglia. Conversely, MMF significantly increased the expression of receptors typical of "steady-state microglia", NURR1, CD200R, and CX3CR1. MMF induced functional changes in activated microglia increasing calcium concentration and phagocytosis, itself associated with an increased expression of TREM2, through which signaling in microglia facilitates debris clearance, creating a pro-regenerative environment. These findings suggest that upon exposure to MMF, microglia revert from a pro-inflammatory to a neuroprotective phenotype.
We have postulated that the effects of MMF are mediated, at least in part, through binding to the nicotinic acid receptor HCA2, for which it is an agonist. We have now shown that HCA2 is expressed on microglia and will present results of experiments designed to ascertain if signaling through HCA2 pathway is involved in the mode of action of MMF in microglia, and how this leads to downregulation of the inflammatory phenotype.

P5.10.32
Deficiency of CD8 effector memory T cells impairs response to Epstein-Barr virus in multiple sclerosis

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A large body of evidence indicates that infection with Epstein-Barr virus (EBV) has a role in the pathogenesis of multiple sclerosis (MS) and the other human chronic autoimmune diseases. We have hypothesized that a genetically determined defect in the elimination of EBV-infected B cells by cytotoxic CD8 T cells predisposes to the development of chronic autoimmune diseases by allowing EBV-infected autoreactive B cells to accumulate in the target organ (Pender MP, 2003, Trends Immunol 24:584-588). Using interferon-γ (IFN-γ) enzyme-linked immunospot assays we recently demonstrated that MS patients have a decreased frequency of CD8 T cells reactive to their own EBV-infected B cells (Pender MP et al, 2009, J Neurol Neurosurg Psychiatry 80:498-505). In the present study we used 9-colour flow cytometry and intracellular IFN-γ staining to analyze further the T cell response to EBV-infected autologous lymphoblastoid cell lines and synthetic EBV peptides in EBV-seropositive patients with MS and EBV-seropositive healthy subjects. Our results demonstrate that the decreased CD8 T cell response to EBV in MS is due to a deficiency of effector memory (EM) ($CD45RA^-$ $CCR7^-$) CD8 T cells. We propose that this decreased memory CD8 T cell response allows EBV-infected autoreactive B cells to accumulate in the central nervous system, which leads to the development of MS. These findings have implications for the prevention and treatment of MS and the other human chronic autoimmune diseases by controlling EBV infection.

P5.10.33
Investigating the causes of A20 down-regulation in Multiple Sclerosis: genetic mechanisms and hormonal modulations

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A20 enzyme (codified by TNFAIP3 gene) is a key player in the negative regulation of inflammation and immunity. Recently we demonstrated a significant decrease of A20 expression in peripheral blood of Multiple Sclerosis (MS) patients, with a reversion to normal level during pregnancy.
To investigate the causes underlying A20 expression deregulation in MS, we assessed possible genetic mechanisms and pregnancy-related hormonal factors.
To evaluate a correlation between A20 genotype and gene expression, we performed a haplotype tagging SNPs analysis in A20 coding and regulatory regions. We identified and genotyped 28 tagSNPs in 50 treatment-naïve MS patients and 50 healthy controls (HC). A20 expression resulted significantly lower in MS patients compared to HC. However, no single SNP or haplotype combinations were significantly associated to A20 expression.
In parallel, we evaluated the effects of beta-estradiol, progesterone, hydrocortisone and prolactin, followed or not by LPS/PHA stimulation, on whole blood A20 expression in 10 MS patients and 20 HC. We demonstrated that all hormones are able to modulated A20 expression in both HC and MS patients, but in the latter with a lower magnitude. However, while in HC all hormones increased A20 level, in MS patients progesterone decreases it. This was rescued by LPS/PHA addition but at lower levels compared to HC.
A20 down-regulation in MS patients seems not to depend on genetic mechanisms, but it is partially influenced by sex-hormones. Moreover, we can hypothesize that other mechanisms such as epigenetic ones could have a role in the decreased gene level observed in MS.

P5.10.34
Increased CD8+ T Cell Response to Epstein-Barr Virus Lytic Antigens in the Active Phase of Multiple Sclerosis

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Several evidence support a role for Epstein-Barr virus (EBV) in the aetiology of multiple sclerosis (MS) although the mechanisms are debated. This study was designed to assess the prevalence and magnitude of CD8+ T-cell responses to EBV latent (EBNA-3A, LMP2-A) and lytic (BZLF-1, BMLF-1) antigens in relapsing-remitting MS patients (n=113) and healthy donors (HD) (n=43) and to investigate whether the EBV-specific CD8+ T cell response correlates with disease activity. Using flow cytometry and HLA class I pentamers in MS patients and HD we observed that a significantly lower fraction of inactive MS patients had a detectable CD8+ T cell response to peptides from EBV latent (EBNA-3A, LMP2-A,) and lytic (BZLF-1, BMFL-1) proteins compared to HD and active MS patients. The lower response to EBV in these patients was associated with higher expression of the inhibitory molecule Programmed Death-1 receptor on viral-specific CD8+ T cells. Marked differences in the prevalence of EBV-specific CD8+ T cell responses were observed in patients treated with interferon-β and natalizumab. Most importantly, the frequency of CD8+ T cells specific for EBV lytic antigens was significantly higher in active MS patients compared to HD, and longitudinal monitoring of two patients revealed expansion of CD8+ T cells specific for EBV lytic antigens during active MS. Analysis of post-mortem MS brain samples showed expression of the EBV lytic protein BZLF-1 in inflammatory white matter lesions. We therefore propose that lowered control of EBV infection during inactive MS could set the stage for intracerebral viral reactivation and disease relapse.

P5.10.35
Dysfunction of gene expression in B cells from patients with multiple sclerosis

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Over the past two decades B cells have increasingly moved into the spotlight in multiple sclerosis (MS) research. The primary aim of this project was to analyze the whole B cell-transcriptome (coding and non-coding RNA) in MS context.
We studied peripheral CD19+ B cell subsets obtained from 10 MS patients, 10 healthy donors (HD), and 2 monozygotic twins discordant for MS. All affected individuals were treatment-naïve and all the investigations were performed at least three months after the last steroid therapy. Microarray data was performed using two Affymetrix's GeneChip: Human Exon and miRNA. The statistical analysis was performed by means of two different software packages, whose output results have been compared: Partek Genomic Suite (version 6.6) and our own analysis pipe-line based on EasyExon tools. Ingenuity Pathway Analysis (IPA). The miR Analysis was performed by IPA microRNA Target Filter.
We focused on EasyExon (less permissive) results and we selected 26 genes differentially expressed in MS compared HD ($p<0.03$ and $|\text{fold change}|>=1.5$). The list was then submitted to IPA and performed a pathway analysis that led us to choose seven, among the original 26 genes that were among those differentially expressed MS patients and HD. Performing the miR analysis with IPA, it has emerged that 6 genes from our list are targeted by 9 miR differentially expressed in MS/HD groups. Peripheral B cell compartment seems to be promising for the identification of mRNA and miR signature. We have identified an MS-related pattern, in terms of miR and/or genes.

P5.10.36
The role of Chlamydia pneumonia in Multiple Sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory and relatively common demyelinating disease of central nervous system white matter in humans with as yet unknown cause. *Chlamydia pneumonia* (Cpn) is an obligate intracellular bacterium that infects the respiratory tract of humans. Cpn has been proposed as a possible etiologic agent in multiple sclerosis (MS). Some support for this hypothesis is provided by the detection of higher serum anti-Cpn immunoglobulin G (IgG) titers among Patients with MS compared to controls.
Objective: The aim of this research was to find out the role of *Chlamydia pneumonia* in multiple sclerosis by detecting the level of IgG and IgM in patients with MS.
Method: Sera were collected from fifty patients with primary clinical MS manifestation (10 male subjects, 40 female subjects, mean age 30 years). Samples were prepared from the MS Clinic in Alzahra and Kashani Hospitals in Isfahan. The level IgG and IgM against *Chlamydia pneumoniae* was assessed by using commercially available quantitative and semi quantitative ELISA.
Results: Our results revealed higher titer of IgG in 21out of 50 MS patients' with mean of 87.12 IU/ml comparing to individuals normal range ≥ 22 IU/ml. Detection of IgM showed only one elevated titer in all the patients.
Conclusion: These findings suggest that *Chlamydia pneumonia* plays an important role in the pathogenesis of MS.

P5.10.37
T cell repertoire analyses and comparison between blood, CSF and CNS lesions in patients with Multiple Sclerosis

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Background. Multiple Sclerosis (MS) is an inflammatory autoimmune demyelinating disease of the central nervous system (CNS). Although assumed in several studies, the concordance between the T-cell repertoires in the periphery and in the CNS has never been studied. If proved, T cells implicated in the disease process could be studied directly from the periphery.
Methods. Lesions from the brain and the spinal cord were classified according to their inflammatory and demyelinating status. The TCR Vb repertoire was analyzed by CDR3 spectratyping in 15 lesions, in the CSF and in the blood (purified CD8+ and CD4+ T cells) from three deceased patients with MS. Sequencing was then used to assess clonality.
Results. While some concordance between the blood CD8+ T cell repertoire and the CNS lesions was observed (50% +/- 14%), the TCR Vb repertoire in the CSF was closer to the one in the CNS (80% +/- 7%). Interestingly, several monoclonal Vb families were found in the lesions while appearing polyclonal in the periphery.
Conclusion. Taken together, these results strongly suggest that the CSF is a window on the T cell clones infiltrating the CNS lesions. The presence of monoclonal Vb families exclusively in the CNS lesions may hypothesize a local proliferation of the T cell clones. Additional experiments using laser-capture microdissection on the CNS will be undertaken on the infiltrating CD8+ T cells. This may allow a better understanding of the functional role of these cells, relative to their monoclonal/polyclonal status in the CNS and/or the periphery.

P5.10.38
Expression of Th1/Th17-related genes in IFN-β-treated relapsing-remitting multiple sclerosis patients

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In multiple sclerosis (MS), T helper cells (Th) 1 and Th17 are considered the orchestrators of the myelin destruction. Recently, in animal studies, IFN-γ and IL-17A double-knockout cells were proven enchephalitogenic via GM-CSF production. It is yet unknown how IFN-β treatment, first-line therapy in relapsing-remitting (RR) MS, affects GM-CSF mRNA levels.
RRMS patients (n=45) starting IFN-β1b therapy were considered responders (R, n=20), if without relapses or progression on EDSS during 2 year follow-up and the rest as non-responders (NR, n=25). Gene expression relative to GAPDH was determined by TaqMan qPCR for marker of IFN-β biological response (MX1), Th1/17 polarizing cytokines (IL-12/23p40, IL-12p35, IL-23p19), respective receptors (IL-12Rβ1, IL-12Rβ2, IL-23), transcription factors (Tbet, RoRγt) and effector cytokines (IFN-γ, IL-17A, IL-17F, GM-CSF) in peripheral blood mononuclear cells before start and at 6, 12 and 24 months of IFN-β therapy.
R and NR had similar MX1 mRNA levels. In both groups, IL-12/23p40, IL-12p35, IL-23p19 and GM-CSF mRNA expression decreased, while IL-23R, RoRγt and IFN-γ statistically significantly increased at 6 months. Other mediators did not change statistically significantly during follow-up. However, all altered mRNA levels reverted to baseline at 24 months, except IL-23R, which had statistically significantly higher expression in NR at 24 months. Similarly, GM-CSF had a tendency of higher expression in NR than in R at 24 months.
R and NR did not differ in IFN-β biological response, according to MX1 mRNA levels. The trend towards higher GM-CSF and IL-23R mRNA levels in NR during IFN-β therapy warrants further investigation.

P5.10.39
The vitamin D and expression of IL-10 and NF-KB genes in Iranian multiple sclerosis patients

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Background: Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system characterized by chronic inflammation, demyelination, and axonal damage. Despite decades of research, the etiology and pathogenesis of MS is still unknown, though both genetic and environmental components of the disease seem evident. One potential environmental factor affecting the development of MS may be vitamin D [1,25-(OH)2D3]. A protective effect of Vitamin D on multiple sclerosis is supported by the reduced risk associated with sun exposure and use of Vitamin D supplements. Moreover, high circulating levels of Vitamin D have been associated with lower risk of multiple sclerosis. We hypothesize that 1,25 dihydroxyvitamin D3 may regulate gene expression patterns in a manner that would resolve inflammation.
Methods: To test this hypothesis, Multiple sclerosis patients placed into a vitamin D supplemented group. Blood samples from 10 patients with MS before and 2 months after vitamin D therapy and 10 age and sex-matched controls obtained. Level of the IL-10 mRNA measured using Real Time PCR and the results compared.
Results: We observed expression changes of the IL-10 gene in MS patients after vitamin D therapy.
Conclusion: In summary, this is the first study to report the expression of the IL-10 in MS patients supplemented vitamin D. This study further implicates vitamin D as a strong environmental candidate in MS by demonstrating functional interaction with the effecting genes in MS.

P5.10.40
Alpha-melanocyte-stimulating hormone (α-MSH) inhibits the development and progression of autoimmune encephalomyelitis by generating functional regulatory T cells

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The neuropeptide α-MSH is a potent immunomodulator capable of inducing immunosuppression and tolerance. Using the mouse model of experimental autoimmune encephalomyelitis (EAE) we systemically treated MOG-immunized mice with α-MSH before and after the onset of hind limb paralysis. Whereas control mice showed a significant weight loss and developed severe ascending paralysis, mice preemptively injected with α-MSH were resistant to EAE development. Notably, therapeutic treatment attenuated EAE progression and prevented mice from weight loss. Flow cytometry, immunofluorescence staining and gene expression analyses revealed the absence of pathogenic Th17 and Th1 cells from brain tissue of α-MSH-treated animals. This effect was mediated by up-regulated numbers of $Foxp3^+$ regulatory T cells (Treg) in α-MSH-injected mice versus controls. Since α-MSH has been shown to expand Treg by the induction of tolerogenic dendritic cells (DC) we analyzed the DC phenotype at different stages of disease. DC from α-MSH-treated mice expressed increased levels of PD-L1 or IL-10 and down-regulated maturation markers pointing to the induction of a tolerogenic DC phenotype. Since signaling via melanocortin-1-receptor (MC-1R) mediates the immunomodulatory effects of α-MSH we induced EAE in MC-1R-deficient mice. Interestingly, upon α-MSH injection these mice developed hind limb paralysis similar to PBS treated controls demonstrating that binding to MC-1R is essential for the α-MSH-mediated prevention of EAE. Together, these data indicate that α-MSH induces tolerogenic DC and expands functional Treg *in vivo.* These Treg suppress pathogenic Th1 and Th17 cells during EAE development, suggesting α-MSH as potential therapeutic option for the treatment of patients with moderate multiple sclerosis.

P5.10.41
Regulatory T-cells in relapsing-remitting multiple sclerosis patients: Effect of disease activity and treatment regimens

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Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS), resulting from an imbalance between CNS-specific CD4+ autoreactive T-cells and peripheral suppressive mechanisms, mediated mainly by regulatory T-cells (Tregs). In this study, we collected peripheral blood samples from 83 patients with relapsing-remitting MS (RRMS) and 45 healthy controls (HC), to determine the sizes of their Treg populations, and the effects of disease activity and treatment regimens (methylprednizolone, interferon, natalizumab) thereof. Treg populations studied included CD4+CD25highFoxp3 Tregs (nTregs), CD3+CD4+HLA-G+, CD3+CD56+ (NKT), CD8+CD28- (CD8+ Tregs), and CD56bright. We also cultured isolated PBMC±different peptides, mapping to myelin oligodendrocyte glycoprotein or myelin basic protein epitopes, to determine the responsiveness of Tregs to autoantigens. The results showed decreased levels of nTregs in RRMS patients in the acute phase±methylprednizolone, but HC levels in acute phase patients+interferon; patients in remission(-treatment) had near-to-HC levels, but +natalizumab treatment their nTregs were downregulated to treatment-naive acute phase levels. Patients+interferon had the highest levels of CD3+CD4+HLA-G+ and NKT, and patients in the acute phase±methylprednizolone the lowest. Patients in remission had the highest levels of NKT, and patients in remission+natalizumab, the highest levels of CD56bright cells. nTregs remained functional regardless of disease activity and treatment regimen, and the relevant effector/suppressor ratio changes during culture+autoantigens showed variable degrees of tolerogenicity. In conclusion, in RRMS nTregs are downregulated but

functional during the acute phase, and are restored to normal levels in patients in remission or treated with interferon.

P5.10.42
Plasma biomarkers can discriminate among multiple sclerosis clinical forms

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Multiple sclerosis (MS), the most common cause of neurological disability in young population after trauma, lacks of biomarkers that enable to define which patients are going to evolve to recurrent-remittent (RR-MS) form and to progressive forms (primary and secondary progressive MS: PP-MS and SP-MS), for which there is no effective treatment. These biomarkers are of outmost clinical relevance for the individual patient.
Objective: To carry out a multifaceted evaluation of circulating cytokines, chemokines, soluble receptors, growth/angiogenic factors to identify specific biomarkers and their combinations which might serve as effective tools to differentiate among the different clinical forms in 165 subjects (129 MS patients and 53 healthy controls, HC) in two independent cohorts.
Patients & Methods: The first cohort consisted of 65 MS patients and 16 HC; the second cohort consisted of 64 MS patients and 37 HC. Plasma samples were analyzed by the Human cytokine 30-Plex panel (Invitrogen) with Luminex xMAP technology platform.
Results: A multivariate logistic regression model was obtained (accuracy 82.6%) with combination biomarkers panels, where HGF and Eotaxin were risk factors (positive predictive value, (PPV: 82.5%,) and EGF and MIP-1β were protective factors for developing a progressive MS. FGFb discriminated well the PP-MS form (p=0.01 vs Relapses and vs SP-MS); while VEGF the SP-MS form (p=0.0004 vs RR-MS).
Conclusions: Our findings suggest that specific chemokines and growth/angiogenic factors may differentially account for divergent MS clinical course. Our proposed biomarkers might be useful in the clinical setting.

P5.10.43
Low DPP4 expression and activity in multiple sclerosis patients

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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system. Dipeptidylpeptidase 4 (DPP4) is a multifunctional enzyme with a key immunoregulatory activity. Substrates for DPP4 include several chemokines important in MS pathophysiology.
Objective: To analyze the plasma levels of soluble (s)DPP4, DPP activity and DPP4 surface expression on $CD4^+$ and $CD8^+$ T-cells in 129 MS patients with different clinical forms and 53 healthy controls (HC) in two independent cohorts.
Patients and Methods: The first cohort consisted of 65 MS patients and 16 HC and the second of 64 MS patients and 37 HC. Levels of sDPP4 were measured by ELISA (human DPPIV/CD26 ELISA;R&D Systems) and DPP activity present in plasma was measured using a luciferase-based assay (DPP4-Glo™ protease Assay; Promega). Peripheral blood samples were analyzed by multiparametric flow-cytometry.
Results: sDPP4 levels and DPP activity were significantly diminished in MS patients as compared to controls ($p<0.0001$ and $p<0.01$, respectively). These data were validated in the two independent cohorts. Moreover, DPP activity inversely correlated with clinical disease activity score in MS (EDSS) ($r= -0.315$, $p=0.01$).The lowest DPP activity was found in secondary-progressive MS. The frequency of circulating $CD8^+DPP4^{hi}$ T-cells was significantly increased in MS patients with respect to controls ($p=0.02$).
Conclusions/Discussion: We provide new evidence that sDPP4 levels and DPP activity are significantly diminished in MS patients as compared to controls, which might account in part for the immunoregulatory defect in MS. We hypothesize that DPP4 regulatory activity of chemokines antagonism may be central for MS.

P5.10.44
Andrographolide suppresses chronic and relapsing-remitting experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is an autoimmune and demyelinating disease of the CNS. Despite the several drugs available for MS treatment, a significant percentage of patients do not respond or develop exacerbations to these therapies. Andrographolide is a bicyclic diterpenoid lactone derived from Andrographis paniculata, a plant indigenous to South Asian countries that has been used as an official herbal medicine in China and India. Andrographolide has anti-inflammatory and immunomodulatory action blocking T cell activation and modulating transcriptional activity. We have previously shown that prophylactic andrographolide administration ameliorated experimental autoimmune encephalomyelitis (EAE), an animal model of MS. In this study, we tested the therapeutic effect of andrographolide on the development of chronic and relapsing-remitting (RR) EAE. Control mice were treated with either vehicle or 1 µg/ml of IFN-β1a (Rebif®). Clinical score was recorded daily and spinal cords were collected and fixed for histological analysis. We found that andrographolide (1 to 4 mg/kg) administered intraperitoneally either daily or every other day significantly improved the symptoms of chronic EAE induced by myelin oligodendrocyte glycoprotein in C57BL/6 mice whereas IFN-β1a showed a moderate effect. Remarkably, andrographolide provided long-lasting protection after treatment cessation. Spinal cords from andrographolide treated mice exhibited less cell infiltration, demyelination and microglia activation as compared with vehicle-treated mice. Furthermore, andrographolide prevented relapses in RR-EAE (SJL mice). Our results suggest that andrographolide is more efficacious in mouse EAE as compared with IFN-β and that it might have therapeutic benefits for MS treatment.
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P5.10.45
CD28 individual signals amplify pro-inflammatory cytokine production in relapse-remitting multiple sclerosis T lymphocytes

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CD28 is a crucial costimulatory reseptor that following engagement by its natural ligands, B7.1/CD80 or B7.2/CD86, expressed on the surface of professional APC, lowers TCR activation threshold, thus leading to the enhancement of early signaling events necessary for efficient cytokine production, cell cycle progression, survival and regulation of T cells effector responses. The role of CD28 in multiple sclerosis (MS) has been evaluated as the source of costimulatory signals integrating those delivered by TCR. However, CD28 is also able to act as a unique signaling receptor and to deliver TCR-independent autonomous signals, which account for its critical role in the regulation of pro-inflammatory cytokine/chemokine production. In the present work, we compared the cytokine profiles of CD4+ T cells from relapse-remitting (RRMS) patients and healthy donors (HD) following CD28 individual stimulation. We found that CD28 engagement strongly up-regulates IL-8 transcription and production in

RRMS compared to HD. More interestingly, in RRMS but not in HDs, CD28 stimulation selectively induces the transcription and secretion of IL-6, IL-21 and IL-17A, all cytokines involved in Th17 differentiation. By using specific inhibitory drugs, we also demonstrate that the up-regulation of all pro-inflammatory cytokines tested was dependent on CD28-mediated phosphatydilinositol 3 kinase (PI3K) activation. Our data identify CD28 as a critical regulator of the inflammatory response in MS and suggest the PI3K signaling pathway as a potential therapeutic target.

P5.10.46
Antigen-presenting cells in the central nervous system in EAE

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Experimental autoimmune encephalomyelitis (EAE) is a mouse model for the inflammatory demyelinating disease multiple sclerosis (MS). MS is considered to be mediated by autoreactive myelin-specific T cells. Crucial events in generating autoimmune responses in the central nervous system (CNS) are presentation of self-antigen to naïve T cells in lymph nodes and their subsequent reactivation in the brain by infiltrating or resident antigen-presenting cells (APC). The major APCs in the periphery are dendritic cells (DC). In the steady state they are rarely observed in the CNS parenchyma. However, during CNS inflammation they cross the blood-brain barrier where together with microglia (CNS-resident APC) they can present antigen to infiltrating T cells.
The goal of this study was to compare two subpopulations of microglia: CD11c- and CD11c+ with brain DC in terms of promoting different T cell responses.
Two subpopulations of microglia: (CD11c- CD45dim CD11b+) and (CD11c+ CD45dim CD11b+) as well as (CD11c+ CD45hi) bDC have been sorted from the CNS of C57BL/6 mice subjected to EAE. Sorted cells were used either for ex vivo proliferation and cytokine assays or for qRT-PCR.
Our results show that bDC and CD11c+ microglia are similar with regard to ability to induce proliferative T cell response from both primed and naïve T cells. Nevertheless, they differ in their cytokine profile and ability to promote cytokine release by T cells.
Our findings suggest that different subtypes of APC in the CNS promote different immune responses.

P5.10.47
Transforming growth factor beta and interleukin-10 in multiple sclerosis patients after intravenous infusion of autologous mesenchymal stem cells

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Introduction. Mesenchymal stem cells (MSC) based therapy of multiple sclerosis (MS) is a promising alternative approach. One of the ways via MSC mediate immunosuppression is the production of anti-inflammatory cytokines.
Objective. To investigate in vivo and in vitro dynamics of transforming growth factor beta (TGFβ) and interleukin-10 (IL-10) in serum and cell culture supernatants of MS patients before and after MSC infusion.
Materials and methods. Serum and peripheral blood mononuclear cells (PBMC) were obtained from relapsing-remitting MS patients (n=7) at baseline, 10 days, 1, 3 and 6 months after autologous bone marrow-derived MSC administration. PBMC were stimulated by mitogen or myelin antigen in the presence/absence of MSC. Cytokines concentrations were determined using ELISA kits.
Results. TGFβ, but not IL-10, was detected in serum of MS patients before MSC infusion inversely correlating with EDSS (R=-0,5, p<0,05). In vitro study revealed increased TGFβ and IL-10 under mitogen- but not myelin- stimulation (p<0,05). The addition of MSC to PBMC resulted in significantly elevated TGFβ and IL-10 in all co-cultures irrespectively of antigenic stimuli. After MSC transplantation TGFβ and IL-10 dynamics differed. TGFβ production decreased on day 10 in serum as well as in cell culture in response to mitogen followed by elevation after 3 months of treatment (p<0.05). IL-10 production significantly increased on day 10 in mitogen and myelin stimulated antigen PBMC' supernatants and correlated with the number of transplanted MSC.
Conclusion. MSC transplantation restores insufficient IL-10 production in myelin-specific conditions and promotes TGFβ secretion after a 3-month treatment.

P5.10.48
Characterization of immune cell infiltrates in the lesions of multiple sclerosis patients

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It is generally believed that the onset of multiple sclerosis (MS) is caused by autoreactive pathogenic T cells accessing the central nervous system (CNS). Pathogenic T cells are re-activated by exposure to autoantigen presented by CNS-resident antigen presenting cells (APC). Following reactivation, the pathogenic T cells activate macrophages and microglia (CNS resident macrophages). Subsequently, inflammatory lesions develop, leading to impairment in nerve function.
It is thought that $Foxp3^+$ Regulatory T cells (Treg) play an important role in the control of autoimmune disease. Studies investigating the role of Treg in MS have demonstrated that although the frequency of Treg isolated from the peripheral blood of MS patients is not altered, their function appears to be impaired. Despite these findings, our studies in experimental autoimmune encephalomyelitis (EAE) have shown that the function of Treg in controlling CNS inflammation is most important within the CNS itself. This suggests a potential role for Treg within the human inflamed CNS.
Currently, the presence and location of Treg within the CNS of MS patients is a matter of debate. Using immunohistochemistry we have been able to detect and quantify the number of $CD4^+Foxp3^+$ Treg within different types of MS lesions. Furthermore, the presence of $CD4^+$ T cells, $CD8^+$ T cells and $CD11b^+$ APC were also documented. In order to measure the functionality of CNS Treg, *In situ* hybridization was used to detect the presence of pro- and anti-inflammatory cytokines in close proximity to Treg. Subsequently immunofluorescence was applied to confirm cytokine production on a protein level.

P5.10.49
Activated natural killer T cells recruit myeloid-derived suppressor cells to the central nervous system to suppress multiple sclerosis-like disease

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Background: Invariant Natural Killer T (iNKT) cells are a particular T lymphocyte population that behaves as immunomodulatory cells of the immune system. In a multiple sclerosis (MS) murine model it has been demonstrated that after iNKT activation there is an increase in the myeloid derived suppressor cells (MDSC) percentage in the central nervous system (CNS) and decreases the pathogenic T lymphocytes percentage.
Objectives: 1. Induce an MS-like model in mice. 2. Isolate MDSCs, obtain RNA and cDNA. 3. Evaluate the chemokine receptors expression necessary for MDSC migration to the CNS.
Methodology: C57BL/6 mice were induced with experimental autoimmune encephalomyelitis, a MS-like model. The experimental group was treated with αGalCer; the control group was treated with vehicle. Monocytic and granulocytic MDSCs (CD11b+ Ly6C+ and CD11b+ Ly6G+) from the experimental and the control groups were isolated by FACS. RNA was extracted with Trizol and cDNA was acquired. Real time PCR was performed with 14 pairs of primers for chemokine and integrin receptors. The data obtained was analyzed with Bio-Rad CFX Manager 2.1.
Results: Different chemokine and integrin receptors expression patterns were found in MDSCs after iNKT activation with αGalCer. Chemokine receptor CXCR3 was overexpressed in Ly6C+ MDSCs

whereas chemokine receptor 6 (CCR6) was overexpressed in both MDSCs subpopulations.
Conclusions: iNKT activation induced chemokine receptors CXCR3 and CCR6 overexpression in MDSCs. These chemokine receptors could be responsible for MDSCs migration towards the CNS providing protection against a MS-like model.

P5.10.50
Month of Birth,Vitamin D and Risk of Immune Mediated Disease

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BACKGROUND: A season of birth effect in immune-mediated diseases (ID) such as multiple sclerosis and type 1 diabetes has been consistently reported. We aimed to investigate whether season of birth influences the risk of rheumatoid artritis and immune disease, and to explore the correlation between the risk of ID and vitamin D status during gestation.METHODS: The monthly distribution of births of patients with ID from the Iran (n = 120,172) was compared to that of the general population by Cosinor test. vitamin D status during pregnancy were calculated for each month of birth and correlated with risk of ID using the Spearman's correlation coefficient.RESULTS: The distributions of ID births significantly differed from that of the general population (P = 5e-12) with a peak in April (odds ratio = 1.045) and a trough in October (odds ratio = 0.945). Stratification by disease subtype showed seasonality in all ID but Crohn's disease. The risk of ID was inversely correlated with third trimester vitamin D status (Spearman's rho = -0.54, P = 0.0003).CONCLUSIONS: The risk of different ID in the Iran is significantly influenced by the season of birth, suggesting the presence of a shared seasonal risk factor or factors predisposing to ID. Gestational vitamin D exposure may be implicated in the aetiology of ID.

P5.12 Rheumatoid arthritis and other inflammatory joint diseases

P5.12.01
The investigation of Anti Centromer Antibodies in Autoimmune Diseases

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Objective: Anti-centromere antibodies (ACA) have been determined to be positive in many autoimmune diseases. The purpose of the study was to investigate the relationship of antibody positivity in respect to autoimmune diseases.
Methods: The sera of 10.778 patients with suspected autoimmune diseases sent from different clinics were reviewed retrospectively between January 2012- June2013. ACA was studied by indirect immunofluorescence method (Euroimmun, Lübeck, Germany). Those found positive were evaluated qualitatively.
Results: Female/male ratio of patients was 78/3 and between 16 to 85 years old (average: 58). Of 10.778 patients, 81 (0,76%) were determined to be positive for ACA. ACA positivity was borderline in 12 patients, 1/1000 titer in 12 patients, and 1/320 titer in 53 patients. The autoantibody pattern was found mixed, with 1/1000 titer in two patients and 1/320 titer in two patients. While 19 (23.5%) of ACA positive patients had diseases not associated with autoimmunity, 22 (27%) of patients had CREST Syndrome, 19 (23,5%) had undifferentiated connective tissue disease, 12 (15%) had Sjögren Syndrome, 6 (7,4%) had rheumatoid arthritis, 1 (1,2%) had poliartritis nodosa, 1(1,2%) had iridocyclitis, 1(1,2%) had CREST plus Sjögren Syndrome, respectively.
Conclusions: ACA positivity was found quite often in CREST syndrome, a subtype of systemic sclerosis, as reported in the literature. Although ACA positivity was found rarely in Sjögren's syndrome, it was highly positive in this study. ACA thus represents a valuable laboratory test for the diagnosis and monitoring of many autoimmune diseases.

P5.12.02
Oxidative stress or NK cells: The combination is getting deadlier for rheumatoid arthritis

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Innate immunity has been widely accepted as one of the major cause for the progression of Rheumatoid Arthritis (RA). The supplementation of ROS and altered NK cell immunobiology might be crucial in the disease severity of RA. Hence, the role of oxidative stress and cytolytic impairments of NK cells in the pathogenesis of RA was investigated. The state of oxidative stress in plasma, RBC and PBMC, percentage NK cell and their altered apoptotic signalling pathways involving mitochondrial membrane potential, FAS associated death domain (FADD) mediated pathways, DNA damage and cytolytic impairments were analyzed. Results indicated a state of profound oxidative stress in the peripheral blood of RA patients where percentage of NK and NKT cell diminished. Surprisingly, the balance of NK and NKT cell populations remained unaffected in the disease state. The depolarized mitochondrial membrane potential, FAS, FASL and active caspase-3, perforin and granzyme positive NK and NKT cell subsets were considerably elevated in patients. The DNA damage, assessed as percentage of DNA in comet tail, was significantly elevated. Findings of the present work indicate increased apoptosis of peripheral NK and NKT cells in the diseased condition. PBMC and RBC are the major sites of enhanced oxidative stress. The state of oxidative stress and altered immunobiology of NK and NKT cells strongly correlated with Disease activity score. The present study points towards futuristic therapeutic targets that can be crucial in the treatment strategy of RA patients, especially the balance of NK and NKT cells need to be explored.

P5.12.03
B-cell activating factor versus biopsy in lupus nephritis among Egyptians systemic lupus patients

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Aim: To study the importance of BAFF in lupus nephritis in comparison to renal biopsy in Systemic Lupus Erythematosus patients.
Patients and methods: BAFF levels were measured in 55 lupus patients and 20 healthy volunteers, renal biopsy was obtained from all patients. SLE patients were divided into 2 groups: Group (A) without lupus nephritis (LN) and Group (B) with LN. Disease activity in SLE patients was assessed by SLEDAI score.
Results: Patients with SLE had elevated BAFF as compared to controls and correlated with disease activity ($P<0.001$). Levels of BAFF were higher in patients with LN than those without LN ($P<0.001$). In patients with LN, serum levels of BAFF were significantly correlated with the SLEDAI renal score. BAFF levels varied significantly and there was a significant positive correlation with the activity and chronicity scores of renal biopsies.
Conclusions:BAFF plays an important role in the pathogenesis of LN and its levels parallel the renal involvement assessed by biopsy. The BAFF level could thus be used as a surrogate marker of renal biopsy.

P5.12.04
Carotid intima media thickness, plaques , IL-6 and disease activity in rheumatoid arthritis patients among Egyptians

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Aim: To study the extent of the carotid intima-media thickness (IMT), plaques and the most predicting subclinical atherosclerotic variables (IL-6) in different grades of disease activity in rheumatoid patients .

Patients and methods: 120 RA patients, sex and age matched controls were recruited at rheumatology department Benha university Egypt. They were 98 females and 22 males. Their ages ranged between 25 and 66 years and disease duration from 1 to 26 years with a mean of 9.7± 6.04 .Cardiovascular (CV) risk factors, RA characteristics were recorded. cartoid (IMT) and plaques were assessed in RA patients and controls with high resolution B-mode ultrasound ultrasound (US).
Results: The mean value of IMT in the patients was 1.18± 0.98 mm while in the controls was 0.63± 0.18 mm which show highly statistically significant between two groups(p<0.001). The incidence of carotid plaques in our patients were significantly higher compared to controls. There were statistically significant correlations (P<0.05) between IMT of the CCA ,plaques in RA and disease related variables; articular index, CRP, ESR, BMI,T cholesterol, HDL, TG, hemoglobin leve l,and highly significant correlations (P<0.001) were found in relation to IL-6, LDL, disease duration, Larsen score, and pain severity.
Conclusion: These results support that RA patient have an ultrasonic marker (plaques and intima media thickness) of early atherosclerosis and these findings correlate significantly with disease activity and IL-6 levels, IL-6 being one of the most predictive marker of these findings.

P5.12.05
Anti-Interleukin-6 and anti-TNF-alpha treatment do not have a negative impact on the immune response against CMV, but disease status influences the balance and the cytokine profile in juvenile idiopathic arthritis patients (JIA)

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Th17 cells have been identified as pro-inflammatory cells in several autoimmune conditions, such JIA. This study evaluated the phenotype and the cytokine-production profile of T-cells in JIA showing active disease (group-1,n=17), remission on-medication (group-2,n=39), remission off-medication (group-3,n=10), disease flare after remission (group-4,n=16) compared to healthy donors (HD,n=28) and the CMV-specific immune response using in vitro blockade of Interleukin-6(IL-6) and tumour-necrosis-factor-alpha(TNFalpha) to simulate therapy.
The phenotype characterization and the intracellular production of IFNgamma, TNFalpha, and IL-17 on naïve:CD28+CD45RO-, memory:CD28+CD45RO+, effector:CD28-CD45RO+, terminally differentiated:CD28-CD45RO- and regulatory:Tregs:CD25+CD127-FoxP3+ CD4+ T-cells were investigated by flow cytometry. CMV-specific IFNgamma-production was determined by ELISPOT following stimulation with CMV-antigens:CMVpp65,CMV-IE in combination with monoclonal antibodies against IL-6 and TNFalpha. Results were expressed in spot forming units (SFU)/1,000,000 cells.
IFNgamma-producing CD4+ T-cells(4.9%vs6.9%) and Tregs(2.9%vs3.8%) were significantly lower in JIA than HD. Memory CD4+ T-cells produced lower amounts of IFNgamma in group-4:6.3%, group-1:6.3% and group-2:7.6% compared to HD:11.3%. . However, disease flare group showed higher proportions of IL-17-producing memory-effector T-cells:1.3% than patients in group-3:0.6%. CMV-seropositive JIA patients showed a significant higher number of IFNgamma-producing T-cells:39.03% than CMV-seronegative:16.55%. No differences were found in the IFNgamma-production by CMV-specific T-cells between HD and JIA in the presence of anti-TNFalpha(JIA:27.8%vsHD:27.3%), but a trend to lower IFNgamma-production by anti-IL-6(JIA:13.3%vsHD:35.0%). Memory and effector T-cells in JIA patients have an imbalance in the cytokine production profile. Although the CMV-specific response was not altered, long-term aspects of T-cells chronic stimulation by CMV as known from healthy elderly persons resulting in exhaustion of CD28- T-cells may follow in older age

P5.12.06
IL-1β membrane-bound receptors in health and in rheumatoid arthritis

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Background: IL-1β is involved in the induction and maintenance of chronic inflammation in rheumatoid arthritis (RA). Its activity is regulated by soluble and membrane-bound receptors; herewith the effectiveness of the cytokine depends largely on the number of expressed receptors.
Objective: to investigate the expression of IL-1β membrane-bound receptors (IL-1R1 and IL-1R2): the percentage of cells with receptors and the number of receptors themselves in different subsets of immune cells in RA patients and in healthy donors.
Methods: to determine membrane-bound receptors on T-, B-lymphocytes and monocytes flow cytometry method was used. To determine receptors number on the cells Quantibrite PE Beads(BD) were used.
Results: Among the studied subpopulations the largest percentage of IL-1R1+ cells was estimated for monocytes, besides the percentage in healthy donors was significantly higher than in RA patients. The least number of IL-1R1 was found on healthy donors monocytes, and the number of IL-1R1 on RA patients monocytes was significantly higher in compare with other subsets. The percentage of IL-1R2+ cells in all subsets was higher in RA, and the highest number of receptors was defined on monocytes in the acute stage of RA. At the same time, the number of both types receptors on T-and B-lymphocytes was significantly higher in control group.
Conclusions: Thus, identified changes indicate the active involvement of IL-1R1 high-expressing monocytes in RA inflammation and their participation in the cytokine imbalance development. Decreasing numbers of IL-1R2 on lymphocytes are associated with active shedding of receptors from them in this pathology.

P5.12.07
Anti-C1q antibodies and systemic lupus erythematosus in the Tunisian population

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Objectives. The presence of a wide variety of autoantibodies is a characteristic feature of systemic lupus erythematosus (SLE). Although non-specific, anti-complement C1q (anti-C1q) were shown to correlate with the occurrence of active nephritis. The present study aimed to investigate the prevalence of anti-C1q in Tunisian SLE patients and their association with clinical manifestations, especially renal involvement.
Patients and methods. IgG anti-C1q antibodies were assessed by Elisa in 98 SLE patients, 55 patients with rheumatoid arthritis (RA) and 65 healthy individuals (HI).
Results. Anti-C1q antibodies were found in 53 (54.1%) patients with SLE, three (5%) patients with RA and six (9.3%) HI. Among the 65 patients with renal involvement, anti-C1q antibodies were present in 35 (53.8%) patients. There was no significant association between anti-C1q and renal or extrarenal manifestations. In addition, there was no correlation between anti-C1q titer and SLEDAI index. Anti-C1q were significantly associated with anti-nucleosome (P = 0.001), anti-Sm (P = 0.01) and a low C4 level (P = 0.046). Concomitant presence of anti-C1q and anti-dsDNA antibodies was not associated with renal manifestations.
Conclusion. Our study shows that prevalence of anti-C1q antibodies was comparable with that previously reported in Caucasian populations. These antibodies were associated with a low C4 level. However, there was no association between anti-C1q antibodies and renal involvement or severity of nephritis.

P5.12.08
Performance of anti-CCP3 IgG/IgA (CCP3.1) test compared to anti-CCP2 IgG test

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Introduction: Diagnostic contribution of ACPA and RF finds its place in RA. Improvements have been made in measurement of ACPA (modified citrullinated peptides, simultaneous search of specific IgG and IgA auto-antibodies).
Objective: The aim of this work is to evaluate performance of anti-CCP3 IgG/IgA (CCP 3.1) test compared to anti-CCP2 IgG test.
Methods: Detection and measurement of anti-CCP3 IgG/IgA auto-antibodies were compared to the anti-CCP2 IgG ones using ELISA in sera of 82 patients; RF was measured by nephelometry.
Results: Results are shown in 4 subgroups:
42 patients anti-CCP2 IgG(-) RF(-) : 2/42 (5%) are anti-CCP3 IgG/IgA(+);
15 patients anti-CCP2 IgG(+), RF(-) : 1/15 (6%) are anti-CCP3 IgG/IgA(-);
14 patients anti-CCP2 IgG(-), RF(+) : 8/14 (57%) are anti-CCP3 IgG/IgA(+);
11 patients anti-CCP2 IgG (+), RF (+): 11/11 (100%) are anti-CCP3 IgG/IgA (+).
Discussion: We observe that every patient CCP2 IgG (+) is anti-CCP3 IgG/IgA (+) except one subject (1/26 = 4%) presents RA during 2 years. On the other side, 10 out of 56 subjects (18%) are anti-CCP3 IgG/IgA (+) and anti-CCP2 IgG (-). 8 of these 10 patients RF (+), 5 have known RA. Among the remaining 5 patients with suspected RA disease duration was 1 to 2 years, showing that anti-CCP3 IgG/IgA are the first to become positive, followed by RF, then the other ACPA which was confirmed by Nicoloudi A. in 2007 and Szabo Z. in 2008.
Conclusion: Anti-CCP3IgG/IgA is more sensitive than its competitors in early and proven RA.

P5.12.09
Rheumatoid arthritis: early thymic involution or increased proliferation rate?

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It is often observed impaired thymic function in adults with immunopathology, particularly with rheumatoid arthritis (RA). We determined 2 markers of thymic functional activity (T cell receptor excision circles or TREC number and amount of CD31+ T cells) to establish the mechanisms for replenishment of the peripheral T cell pool in RA.
Absolute and relative TREC number in CD4+ and CD8+ cells was measured in 30 patients with RA (mean age 50±8,2)and 15 healthy age-matched controls using Real-time PCR. Proportion of CD4+CD31+ and CD8+CD31+ cells was quantified in 11 patients with RA (mean age 43±14,3) and 5 healthy donors (mean age 43±5,5).
Relative TREC number was reduced about 2 times in RA compared with control, both in CD4+ and CD8+ cells. Absolute TREC number in CD4+ population did not differ in patients with RA from donors, whereas it was decreased in CD8+ population in RA. To determine whether the reduction of TREC number in RA due to insufficient thymic function or increased proliferation rate, it was quantified proportion of CD31+ T cells. There were no significant differences in the amount of thymic naïve T cells between patients with RA and healthy donors. CD4+31+ value in RA group was 26 (range 21,6-32,2) vs 27,8 (range 24,9-40,1) in control group, and CD8+CD31+ - 62 (49,7-70,8) vs 53,8 (50,8-66,5) respectively.
Obtained data suggest that replenishment of the peripheral T cell pool in adults with RA occurs more due to the cell proliferation, thymic function is maintained within the normal limits.

P5.12.10
TNF receptor function is dispensable for generation of CD23+ CD21-high B cells in inflamed nodes

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We have previously shown that total B cells expand, and a population of CD23+, CD21-high, CD1d-high B cells (B cells in inflamed nodes, or Bin) selectively accumulate in the lymph nodes that drain arthritic joints in the human TNF-alpha transgenic (hTNF) mouse model of inflammatory erosive arthritis. These changes are associated with altered lymph node architecture, lymphatic drainage dysfunction and disease flare in the knee, and can be reversed by B cell depletion therapy. Bin cells also appear in normal lymph nodes in response to other inflammatory stimuli including immunization and antigen-free adjuvants, and display increased antigen capture and processing, and T cell costimulation potential compared to conventional follicular B cells.
Bin cells can rapidly (24-72 hrs) differentiate from adoptively transferred sort-purified normal mature follicular B cells within the milieu of hTNF-transgenic inflamed lymph nodes, raising the question whether their differentiation is directly dependent on TNF-alpha-mediated inflammatory signals. Here we will show: 1) that TNF receptor (TNFR) 1/TNFR2 double deficient B cells give rise to Bin cells when adoptively transferred into hTNF-transgenic mice, and 2) that footpad immunization of TNFR1/TNFR2 double deficient animals also gives rise to total B cell and Bin cell accumulation, as in normal mice. We conclude therefore that TNF receptor function is not required for either the dramatic changes in lymph node B cell composition that accompany lymph node inflammation, or for the specific differentiation of Bin cells in normal and hTNF-transgenic lymph nodes.

P5.12.11
Biomarker of inflammation in juvenile idiopathic Arthritis (JIA)

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Background: Juvenile idiopathic arthritis (JIA) is a relevant autoimmune disease in children. T cells, B cells, and damage-associated molecular patterns (DAMPS) are involved in the pathogenesis of the disease. Biomarkers for JIA and its subtypes are not established. Pro-inflammatory pathways activate enzyme indoleamine 2,3-dioxygenase (IDO) which enhances tryptophan (Trp) conversion to kynurenine (Kyn). Thus, in conditions of chronic immune activation reduced Trp availability and production of Kyn and its down-stream metabolites may inhibit cell proliferation. [1-3].
Methods: In this study, Trp and Kyn metabolism was investigated in children with JIA and compared to serum neopterin concentrations. Fifty-four sera of 25 JIA patients and 10 samples of synovial fluid were examined with HPLC (Trp and Kyn) and Elisa (Neopterin, BRAHMS, Hennigsdorf, Germany). Eighteen sera from 18 children with non-inflammatory diseases were used as controls.
Results: Trp in the sera of patients was mean 57.2 + SD 19.0 µmol/L and Kyn was mean 2.40 + SD 0.81 µmol/L). Serum neopterin was 5.69 + SD 1.72 nmol/L. In the synovial fluid, neopterin was mean 10.5 + SD 7.41 nmol/L), Trp was 36.7 + SD 17.4 µmol/L and Kyn was 2.13 + SD 0.75 µmol/L). In control patients, neopterin was 6.93 + SD 3.10), Trp was 57.6 + SD 14.8) and Kyn was 2.60 + SD 1.60 µmol/L.
Conclusion: Serum Trp concentrations showed no relevant difference in JIA patients vs. controls. IDO activity reduces Trp primarily in the synovial fluid in JIA patients.
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P5.12.12
APRIL, a TNF family member, can be a predictor of resistance to antigen-induced arthritis in mice

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APRIL (A Proliferation-Inducing Ligand) acts as a secreted factor and plays important roles in B cell biology. The reported capacity of APRIL to stimulate B cells *in vitro* raised the hypothesis that APRIL may be a disease promoter in autoimmune diseases such as rheumatoid arthritis. This study was undertaken to examine the ability of APRIL to regulate the B cell-mediated inflammatory response in a model of antigen-induced arthritis (AIA) in mice and to influence cell migration in synovial tissue. AIA was induced by administration of antigen (mBSA) into the knee joints of previously immunized APRIL-transgenic (APRIL-Tg) mice and their littermates. In the acute stage of disease APRIL-Tg mice displayed a decrease in inflammatory response parameters, i.e., joint swelling, disease score and inflammatory infiltrate in articular tissues, as compared with their littermates. In addition, we observed a decrease of cellularity in draining lymph nodes of APRIL-Tg mice. These results were correlated with a milder chronic disease. FACS analysis revealed an increase of IgM+CD5+ cell population in draining lymph nodes in APRIL-Tg mice and increased IL-10 production *in vitro* at the acute phase of the disease. Our results suggest that APRIL and IL-10 producing B cells could reach the inflamed synovia and build an anti-inflammatory environment in arthritis. However, this issue remains to be further elucidated. Our data show that the ectopic APRIL expression can be correlated with higher resistance to arthritis and suggest that this regulatory axis should be investigated in human rheumatoid arthritis.

P5.12.13
Optimization of "in vitro" culture conditions for human arthritis rheumatoid synovial fibroblasts

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In this study we evaluated the use of synovial fluid, obtained from patients with arthritis rheumatoid, as optimal culture condition to perform "in vitro" studies on arthritis rheumatoid synovial fibroblasts. Actually "in vitro" studies are performed on unstimulated fibroblasts or cells cultured in the presence of recombinant cytokines, such as alpha-TNF, but these conditions are not faithful to the real ones, therefore the results obtained might be not corrected. We used a pool of six different arthritis rheumatoid synovial fluids in our "in vitro" study performed with arthritis rheumatoid synovial fibroblasts. Our observations in proliferation studies demonstrate that the synovial fluid was more effective to induce DNA synthesis and proliferation of synovial fibroblasts with respect to alpha-TNF, used at the optimal dose. The morphological aspect of the cells was also modified with synovial fluid. We also evaluated the modulation of expression of some cytokines known to be involved in the pathological process of RA. The cytokines expression was greater in the cells cultured in the presence of synovial fluid than those cultured in the presence of alpha-TNF or in the culture medium. These results emphasize the importance of using the synovial fluid in "in vitro" studies to ensure culture conditions as similar as possible to those of physiopathological environmental characteristic of the rheumatoid arthritis joints. This should ensure the possibility to have more accurate information both to clarify the pathogenesis of rheumatoid arthritis that to evaluate the therapeutic efficacy of new molecules.

P5.12.14
S100A8 plays an anti-inflammatory role in collagen-induced arthritis

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S100A8 and S100A9, cytoplasmic proteins expressed by neutrophils and monocytes, are found in the extracellular milieu during inflammatory episodes. While the pro-inflammatory activity of S100A9 has been clearly established, the functions of S100A8 remain controversial. S100A8 is a chemotactic factor for neutrophils and monocytes and induces a potent inflammatory response in vivo. However, it is easily oxidized and its oxidized form has been shown to inhibit mast cell activation and to suppress eosinophil functions. In this study, S100A8-/- mice were generated to investigate its role in acute and chronic inflammation like rheumatoid arthritis. Leukocytes subpopulations were similar in bone marrow, blood, spleen and thymus between S100A8-/- and WT mice. Interestingly, deletion of S100A8 gene resulted in absence of S100A9 protein in naïve mice, although mRNA for S100A9 was detected, suggesting that S100A8 is necessary to stabilize S100A9 protein. No differences between S100A8-/- and WT mice were detected in the number and subtypes of leukocytes migrating to the air pouch in response to LPS. However, striking differences were observed in the collagen-induced arthritis model of chronic inflammation. S100A8-/- mice showed increased disease incidence, edema, leukocyte infiltration and bone destruction in the LPS-synchronized collagen-induced arthritis model. In conclusion, these data suggest that S100A8 acts as an anti-inflammatory molecule during experimental arthritis and thus contains the disease development.

P5.12.15
ERAP1 gene polymorphisms influence the risk of Spondyloarthritis in HLA-B27 positive Romanians

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Spondyloarthritis (SpA) is a family of rheumatic diseases with common clinical features (inflammatory back pain, asymmetrical oligoarthritis, enthesitis), but also with genetic similarities - such as HLA-B27. ERAP1 gene encodes an endoplasmic reticulum aminopeptidase involved in peptide precursor trimming before loading into HLA class I molecules. Recent studies found associations of ERAP1 variants with the susceptibility to SpA and with ankylosing spondylitis (AS) which is the most common form of SpA. The association of ERAP1 variants with the risk of spondyloarthritis in HLA-B27+ individuals has not been yet investigated.
Our aim was to explore the influence of two coding single nucleotide polymorphisms (SNPs) of ERAP1 gene - rs30187 (Lys528Arg) and rs27044 (Gln730Glu) - on the risk of SpA in HLA-B27+ Romanians.
We genotyped 150 HLA-B27+ SpA patients (105 AS, 31 undifferentiated spondyloarthritis, 14 psoriatic arthritis) and 108 HLA-B27+ healthy controls using TaqMan assays. Statistical analyses were performed with PLINK software.
Both SNPs were associated with the risk of spondyloarthritis (OR 1.80, 95%CI 1.24-2.62, p=0.001 for rs30187, OR 1.58, 95%CI 1.07-2.34, p=0.02 for rs27044). The haplotypic combination of the major alleles of the two SNPs (CC) had protective value for developing SpA (57% in patients versus 70% in controls, p=0.002), while the TG haplotype was a risk factor (33% in patients versus 23% in controls, p=0.01).

Our results show that ERAP1 functional variants influence the risk of spondyloarthritis in HLA-B27+ individuals suggesting that the common pathological pathway of spondyloarthritides implicates not only HLA-B27, but also the ERAP1 molecule.

P5.12.16
TWEAK Promotes Osteoclastogenesis in Rheumatoid Arthritis

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Bone destruction is critical in the functional disability of patients with rheumatoid arthritis (RA). Osteoclasts, specialized bone-resorbing cells regulated by cytokines such as receptor activator of NF-κB ligand (RANKL), are primarily implicated in bone destruction in RA. The aim of the study was to examine whether tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK), a member of the TNF superfamily, has osteoclastogenic activity in RA patients and in animal models including mice with collagen-induced arthritis (CIA mice) and IL-1 receptor antagonist knockout (IL-1RaKO) mice. TWEAK was increased in the synovium, synovial fluid and serum of RA patients and in the synovium of CIA mice and IL-1RaKO mice. TWEAK induced RANKL expression in mixed joint cells and splenocytes from CIA mice, IL-1RaKO mice and in fibroblast-like synoviocytes (FLSs) from RA patients. Both osteoclast precursor cells and osteoclasts express TWEAK receptor fibroblast growth factor-inducible 14 (Fn14). In addition, TWEAK enhanced in vitro osteoclastogenesis without the presence of RANKL-providing cells and by inducing RANKL expression in FLSs. Moreover, treatment with Fn14-Fc inhibited RANKL-induced osteoclastogenesis, indicating endogenous TWEAK also has osteoclastogenic activity. Our data demonstrated that TWEAK promotes osteoclastogenesis in RA, suggesting that therapeutic strategies targeting TWEAK could be effective for treatment of patients with RA, especially in preventing bone destruction.

P5.12.17
CD8+ T cell reactivity against apoptotic self-epitopes in rheumatoid arthritis patients treated with etanercept

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Apoptotic cells play an important role in autoimmune disorders since they represent an important source of self-antigens. We have shown that T cell apoptosis generates a wide array of caspase-cleaved proteins, which can be cross-presented by dendritic cells to a broad repertoire of autoreactive CD8+T cells. This process is involved in establishing chronic immune activation (CIA) in HIV and HCV infections. Here, we evaluated the role of self-apoptotic-specific CD8+T cells in the maintenance of CIA in rheumatoid arthritis (RA) and the clinical impact of these cells on the response to immunosuppressive therapy. Our study has been conducted on RA patients treated with Etanercept at different times. Frequency and function of self-specific CD8+ T cells were analysed by staining with dextramers of HLA-A*0201 molecules complexed to apoptotic epitopes and IFNγ-ELISPOT assay. Our data showed increased frequencies of autoreactive CD8+T cells in patients compared to healthy donors, related to increased frequency of circulating apoptotic T cells. In addition, a large apoptotic epitope repertoire recognized by IFN-γ+CD8+ TEM cells was found in all patients. Interestingly, at baseline a significant difference in autoreactive responses was found in responders compared to non responders. After starting therapy, an early dramatic decrease of self-reactive CD8+T cells was observed only in the group of responders. Altogether these data suggest that chronic inflammation in RA may be sustained by an autoimmune reaction towards apoptotic T cells. The presence of self-reactive T cells, markedly increased in responders patients already at baseline, may be useful to predict the response to therapy.

P5.12.18
The effect of the *Slc11a1* gene on pristane-induced arthritis chronic phase

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Genetic and environmental factors contribute to the development and establishment of rheumatoid arthritis. The *Solute carrier family 11a member 1* (*Slc11a1*) gene is involved in the ion transport at the endosomes in macrophages and neutrophils, interfering in their activation. Mice homozygous for *Slc11a1 S* allele (AIRmax^{SS}) selected from the high inflammatory response AIRmax line are more susceptible than AIRmax^{RR} to pristane-induced arthritis (PIA). In order to study the chronic phase of PIA, AIRmax^{RR} and AIRmax^{SS} mice received two i.p. injections of 0.5 mL pristane at a 60-day interval, and arthritis development of arthritis was assessed for 180 days. Multiplex analysis of serum and peritoneal macrophage culture supernatants showed higher production of IL1β, IL-6, TNFα, IL-10 and MIP-2 by AIRmax^{SS}. We also identified increased H_2O_2 and NO release by AIRMax^{SS} macrophages. Significant differences ($p<0.001$) were found in expression, by AIRMax^{SS} macrophages, of metalloproteinase genes (*Mmp-2* and *Mmp-9)* and their inhibitors (*Timp-1* and *Timp-3)*. Histological analysis of paws of AIRMax^{SS} mice after PIA showed a moderate inflammatory process, characterized by mononuclear cells. These results suggest that *Slc11a1* alleles modulate cytokines profile and proteases contributing for arthritis severity.
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P5.12.19
Role of the expression CCR7+CCR5+ CX3CR1+ CD95 +/- in the peripheral blood lymphocytes in systemic lupus erythematous patients

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SLE is one of the autoimmune diseases characterized by the loss of immune tolerance. Cytokines and chemokines are proteins that play an important role in the pathogenesis of SLE. Previous studies indicated that the expression of CD95 is the determinant for the induction of apoptosis in T-helper lymphocytes, and abnormalities in their expression may lead to ADs. We investigated the expression of CD95 and CCR7, CCR5 and CX3CR1 on freshly isolated lymphocytes from SLE patients. The CD95 and CCR7, CCR5 and CX3CR1 expression on $CD4^+$ lymphocytes were analyzed in 44 SLE patients and 20 controls using multi-parameter flow cytometry. Concentrations of plasma IL-6, IL-12, IL-17, TNF-α and IL-10 were also quantified using ELISA. The percentage of $CD4^+CCR7^+CD95^+$ cells was statistically significant in SLE patients compared with controls ($P<0.001$) the percentage of $CD4^+CCR5^+CD95^-$ cells was statistically significant in SLE patients compared with controls ($P<0.002$); the percentage of $CD4^+CX3CR1^+CD95^-$ cells was statistically significant in SLE patients compared with controls ($P<0.018$). Altered apoptosis is a common finding in autoimmune diseases including SLE. We found in our study that the expression of chemokine receptors on viable T-helper cells significantly different in the secretion of cytokines when compared to the cells resistant to apoptosis as evidenced by the increased expression of death-receptor CD95. These expanded $CD95^+$ primed T-helper cells most likely reflect the presence of activated lymphocytes as a consequence of abnormalities in the peripheral deletion of activated lymphocytes. These persistently activated lymphocytes might play a role in the induction of autoimmunity in SLE.

P5.12.20
Role of CCRL2 in the pathogenesis of mouse experimental arthritis

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Rheumatoid Arthritis (RA) is an autoimmune-mediated disease characterized by chronic inflammation and leukocyte recruitment into the inflamed joints. Chemokines and chemokine receptor activation are known to represent a major component of the effector molecules involved in the onset of RA.
CCRL2 [Chemokine (CC motif) receptor-like 2], also known as L-CCR, is a seven transmembrane protein that shows a high homology degree with many members of the CC chemokine receptor family. CCRL2 possesses a non-canonical DRYLIVE motif in the second intracellular loop that makes it to resemble like a nonsignaling chemotactic receptor (e.g. D6, DARC, CCX-CKR). CCRL2 is expressed by LPS-stimulated murine macrophages, neutrophils, mast cells, dendritic cells, glial cells, astrocytes and microglia and is up-regulated in human RA synovial neutrophils.
In order to elucidate the role of CCRL2 in RA, CCRL2 KO mice were tested in the model of collagen-induced arthritis. Only few CCRL2 KO mice displayed the pathological signs characteristic of arthritis with reduced leukocyte infiltration, synovial hyperplasia and joint erosions and with a significant reduction in visual scoring and paw thickness. Moreover, CCRL2 KO mice showed a marked delay (about ten days) in the onset of the disease and histological features reminiscent of recent joint damage. Conversely, the level of specific anti-collagen II IgG in the serum was similar in CCRL2 KO and control mice. The mechanisms underling the protection of CCRL2 mice in RA models are currently under investigation and will help to define whether CCRL2 may represent a new therapeutic target for RA.
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P5.12.21
Collagen-specific TCR Repertoire usage in RA and cytokine secretion

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic joint inflammation, associated with HLA-DR4. Collagen II specific T cells repertoire in DR4+ patients is characterized by a limited number of TCR-beta chain during the acute phase, in part enriched in Synovial fluid. The two more used TCR-beta chains are BV11 and BV13. PBMC from 85 RA patients (47 acute presentations and 38 remissions), cultured with or without collagen peptide, were examined by immunoscope. We also studied IL-17 and IL-13 secretion of collagen-specific individual T cells of 3 DR4+ patients after in vitro stimulation with the peptide and with or without bacteria-derived products. At acute presentation 6/15 patients showed BV11+ T cells and 7/15 showed BV13+ T cell, while after remission the number falls to 1/11 and 2/10, respectively. Some DR7+ patients displayed T cells using similar BV11 and BV13 chains. In this latter case, frequency of positive BV13 samples did not decrease with remission. We observed that 1/17 T cell clonotypes secreted IL-17 or IL-13 after stimulation with peptide. However, after stimulation with peptide in the presence of bacterial products, 3 more individual T cells became able to secrete IL-17. Thus, BV11+ and BV13+ cells are bystander of RA at acute presentation in DR4+ patients. The relative high frequency of these TCRs also in DR1+ and DR7+ patients is possibly due to similarities in peptide selection. In RA patients, secretion of the pro-inflammatory cytokine IL- 17 is modulated by exogenous (or endogenous) factors, possibly interacting with PRRs.

P5.12.22
Clinical and immunological characteristics of Senegalese patients with rheumatoid arthritis (RA)diagnosed according to American College of Rhumatology criteria of 1987

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Rheumatoid arthritis (RA) is an autoimmune disorder, systemic and inflammatory. Understanding of the immuno-molecular pathology of this entity has been considerable progress in recent years that have to revisit the diagnosis and care of patients. In Africa, few studies are available. The aim of our study was to investigate some immunological markers for diagnostic purposes. We conducted a prospective study of 78 patients received outpatient RA between June 2012 and January 2013. RA was diagnosed according to the criteria of the ACR (American College of Rheumatology) 1987. A questionnaire on sociodemographic data of the patient, disease activity at the time of the study, the Rapid Assessment score 3 and 5 RADAI was administered to each patient after informed consent. A blood sample for determination of parameters immunoserological was performed. The patients were aged between 19 and 83 old, with a disease duration of 6.2 years with a range from 2 months to 32 years. Clinical features, scores and RAPID3 RADAI 5 and immunological profile of patients were analyzed. Preliminary results have improved knowledge of Senegalese patients with RA.

P5.12.23
T676G polymorphims of the TNF receptor type II is associated with ankylosing spondylitis

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Tumor necrosis factor-alpha plays a central role in the pathogenesis s ankylosing spondylitis (AS), participating in the genesis of the inflammatory process. Both TNF-a mRNA and its protein have been identified in sacroiliac joints of patients with active disease. TNF-a is a pro-inflammatory cytokine exerting its actions through a link with two cellular surface receptors: TNFR1 and TNFR2. The TNFR2 could play a role in the promotion of the inflammatory process, and in the new bone formation, a characteristic of the AS. The M196R polymorphism of the TNFR2 has been associated with an impaired NF-KB signaling, and then it is associated with the inflammatory process. AS is a chronic inflammatory disorder of the axial skeleton involving mainly sacroiliac joints and lumbar spine, in which genetic factors play an important role. In this study we analyze the T676G polymorphism of TNFR2, which resulted in a non-conservative amino acid substitution (methionine-arginine) at position 196. Thirty three consecutive patients with AS, diagnosed according to the current criteria, and 52 healthy controls were included. DNA was extracted from each subject, and the T676G genotype was determined by PCR-SSCP according to the methods previously described. Our results showed that the 676-G/G genotype could have a protective role (OR 0.2778; 95%CI= 0 0.1112-0.6941), but the 676-T allele represents a risk factor (OR =4; 95%CI = 1.9054-8.3972). However, this conclusion requires further investigation, because of the limitation of a small sample size.

P5.12.24
Cytokines in relation to autoantibodies before onset of symptoms in systemic lupus erythematosus (SLE)

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Background. This study was performed in order to analyse cytokines in blood, and their relationship to autoantibodies in individuals who later developed SLE
Methods. Thirty-five patients with SLE (ACR criteria) who had donated blood to the Medical Biobank, Umeå, Sweden, before onset of symptoms, and 140 age- and sex-matched controls from the same biobank.were analysed for interferon (IFN)-α, interleukin (IL)-4, IL-9, IL-10, interferon inducible protein 10 (CXCL10/IP-10) and monocyte chemotactic protein-1 (CCL2/MCP-1), using a multiplex method. The associations of these cytokines to autoantibodies (ANA, ENA, anti-dsDNA and anti-histones) from the same blood samples (analysed earlier) were evaluated.
Results. The IP-10 levels were higher in the presymptomatic individuals than in controls, p=0.02, and had the most obvious association to autoantibodies, significantly correlated with ANA, SSA, Jo-1 and to autoantibody positivity over all (p=0.03, 0.01, 0.03 and <0.001 respectively). MCP-1 correlated with SSA, SSB and antibody positivity over all (p=0.009, 0.047 and 0.015 respectively), IFN-α with anti-SSB and antibody positivity over all (p=0.027 and 0.025). The levels of IFN-α, IP-10 and MCP-1 correlated with the number of autoantibodies in presymptomatic individuals (p= 0.066, <0.01 and 0.015 respectively). IL-10, IP-10 and MCP-1 increased between the presymptomatic sample and after SLE diagnose, (p=0.026, <0.0001 and <0.0001 respectively).
Conclusions. IP-10, MCP-1 and IFN-α had the clearest correlation with autoantibody formation before disease onset of SLE. Since secretion of IP-10 and MCP-1 are mediated by IFN- α, these findings support previous theories that the IFN-system is important in the early SLE pathogenesis and autoantibody formation.

P5.12.25
Identification of myeloid dendritic cells and plasmacytoid dendritic cells in the peripheral blood of rheumatoid arthritis

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Background: The study of peripheral blood dendritic cells (PBDCs) is increasingly reaching clinical relevance in a wide range of pathologies, including infectious, neoplastic and autoimmune diseases, because DCs are key antigen presenting cells that prime, polarize and regulate adaptive immune responses.
Objectives: To estimate quantitative and qualitative characteristics of dendritic cells subtypes in peripheral blood in patients with rheumatoid arthritis (RA).
Methods: Phenotypic characterization of immature mDC and pDC in the whole blood was estimated by the negative selection and the positive expression of HLA-DR, CD11c, CD123. To determine the ability of dendritic cells to mature stimulants R848 and lipopolysaccharide were used.
Results It was showed a significant quantitative imbalance of DC in peripheral blood in patients with RA, a significant decrease the pDC. In patients with RA mDC and pDC were characterized by a low expression of CD80, CD83 with high amount of CCR7-positive pDC in compare to the healthy donors. After stimulation percentage of CD80, CD83 and CCR7 positive cells was significantly increased in compare to the samples without stimulation in both groups.
Conclusions: The change in the ratio of subtypes of DC and low expression of CD83, CD80 on them, as well as high expression of CCR7 on peripheral blood pDCs in patients with RA may be explained by the mechanisms of general inflammation. Results of the assessment of maturation of dendritic cells in RA patients in response to TLR agonists indicate the ability of initially less mature mDCs and pDC respond adequately to the maturation.

P5.12.26
Extended characterization of human chondrocytes for establishment of co-culture systems with synovial fibroblasts under different culture conditions in the context of Rheumatoid Arthritis

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Rheumatoid Arthritis is the most common form of polyarthritis leading to massive joint destruction and increased morbidity of affected patients. Besides conventional treatment, biologic reagents are successfully used to efficiently slow down RA progression- although still some patients fail to respond and are highly dependent on alternative treatment options. Several different cell types are known to be involved in the development, progression and maintenance of RA. Different immune cells contribute to inflammatory processes, which activate synovial fibroblasts resulting in the development of a highly aggressive pannus tissue and finally cartilage/bone destruction. FLS and chondrocytes are important downstream effector cells and co-culture systems between both cell populations might represent a suitable way of modeling RA *in vitro*. In this study, different *in vitro* co-culture formats between human chondrocytes and RA-derived FLS were assessed on the basis of immortalized and primary-derived chondrocytes. In order to characterize these cells in terms of their chondrogenic phenotype, de-/re-differentiation potential and inflammatory state, extended gene and protein expression analyses have been performed by using qRT-PCR, FACS and immunofluorescence analysis. In addition, several co-culture formats (indirect/direct co-culture, e.g. RA-FLS conditioned medium, alginate beads or transwell system) were compared and also the impact of pro-inflammatory cytokines and anti-rheumatic drugs was assessed. All co-culture models resulted in the induction of an inflammatory chondrocyte phenotype represented by up-regulated gene expression of pro-inflammatory cytokines and MMPs reflecting the RA-diseased background. These *in vitro* co-cultures might serve as promising systems for testing potential anti-rheumatic drugs and studying intercellular mechanisms in RA.

P5.12.27
Interplay of myeloid cells and synovial fibroblast boosts the inflammatory response

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The interaction between synovial fibroblasts (FLS) and different myeloid subpopulations (monocytes and polarized macrophages) might be a contributor of the induction/aggravation of inflammatory processes in rheumatoid arthritis (RA) and other autoimmune diseases. In this study the interplay between monocytes layered over confluent fibroblasts was tested in a co-culture system analyzing several parameters: there was a massive increase in pro-inflammatory cytokine production (IL-6 or-8) and also matrix-metalloproteases (MMP1 and MMP3) in co-cultures compared to single cultured cells. Furthermore, expression of the adhesion marker ICAM-1 was also tremendously elevated on co-cultured fibroblasts in addition to the overall proliferation. Interestingly, the production of other cytokines/MMPs was only slightly elevated or even reduced in co-cultures. And there were no differences detectable between FLS from healthy donors or RA patients. To study whether the phenotype of the myeloid cells was essential for this boosted immune response, monocytes were compared with M1 and M2c polarized macrophages from the same donor: the response of M1 macrophages was in the range of freshly isolated monocytes or even higher, demonstrating a massively increased cytokine production in co-cultures. However, although there was a clear amplification in M2c co-cultures, the cytokine levels reached only about half of those from monocyte co-cultures and this was also true for the ICAM-1 upregulation. Similar effects were observed regarding the MMP production. Application of neutralizing antibodies to both adhesion molecules (such as e.g. ICAM-1) and different cytokines might help to disclose the interplay between these cell types and the signaling cascades involved in inflammatory processes.

P5.12.28
The candidate rheumatoid arthritis autoantigen aggrecan enhance IL-9 response in peripheral blood of Rheumatoid Arthritis' patients

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Rheumatoid arthritis (RA) is a chronic autoimmune disorder, characterized by sinovitis, leading to both cartilage and bone erosion. Recently, a citrullinated arthritogenic aggrecan peptide was identified in peripheral blood of RA patients and it has been demonstrated to activate Th17-mediated responses highlights the potential value of citrullinated aggrecan peptide specific responses as biomarkers of RA. In this study we evaluated CD4 T cell subsets percentage in RA patients and controls in response to citrullinated and non citrullinated aggrecan peptide and we investigated a possible role of IL-9, that wasn't studied in RA immune response. Peripheral blood mononuclear cells (PBMCs) were isolated from patients and controls and were stimulated with citrullinated e non citrullinated aggrecan peptide. After incubation cells were collected and stained with the following monoclonal antibodies: anti-CD4, anti-IFN-γ, anti-IL-17, anti-IL-9, anti-PU.1, anti-CD25, anti-FoxP3; flow cytometry analysis then was performed. The specific stimulation with the citrullinated peptide is characterized by production of proinflammatory cytokines and particularly IFN-γ and IL-17 that are actively involved in the articular damage in RA patients. Our results demonstrate an increase of CD4 T effector subsets after *in vitro* stimulation with citrullinated aggrecan, and provide the first evidence of a Th9 response in RA patients.

P5.12.29
Certolizumab Pegol in Rheumatoid Arthritis. Follow-up in latinoamerican experience

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Certolizumab pegol (CZP) is a TNF inhibitor, with efficacy in RA, (Rheumatoid Arthritis) clinical trials. Many clinical trials recruited patients from USA and Europe and therefore clinical experience in countries like Latin America is limited. This is the experience with CZP in daily clinical practice inColombia . We describe the characteristics of the first Latin-American patients receiving CZP in clinical practice. 150 clinical records of patients with RA were reviewed; . We used 400 mg in weeks 0,2,4 followed by 400mg every 4 weeks. DAS28 score was recorded before treatment initiation with follow-ups at weeks 12 and 24. Mean change in DAS28 score, EULAR response and change in disease activity according to DAS28 score were calculated. 150 patients were included. 125 were women, aged 21 to 80 years old (mean ±SD 54.8 ±13.2). Patients had used 2 Synthetic DMARDs Drugs, 35 patients had received one biological agents previously (23.3.%). Mean baseline DAS28 score was 5.50±0.64; after 12 and 24 weeks the DAS28 was 2.9±0.99 and 2.64±1.24, with a mean change of -2.5±0.96 and -2.6±1.25 respectively. In 110 patients (86.32%) the decrease (>/=1.2 points) was clinically significant at week 24. At the end of the follow up period 108 participants (72.63%) showed a good EULAR treatment response, 25 patients (17.79%) showed a moderate response and 17 were non-responders (11.33%).. Fifteen adverse events were reported which included a serious one. This experience suggest an adequate effectiveness and safety profile in latinamerican patients treated with CZP.

P5.12.30
Chondrocytes as mediators of citrullination and humoral immunity to citrullinated type II collagen in Rheumatoid Arthritis

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Collagen type II (CII) is the major component of articular cartilage targeted by the autoimmune response in RA. CII is arthritogenic in animal models, and anti-CII antibodies alone can induce arthritis in mice. The aim of this study was to investigate CII as a joint-specific target of the anti-citrulline response in RA. We identified potential citrullinated neoepitopes by high-resolution tandem mass spectrometry (MS) of CII treated in vitro with peptidyl arginine deiminase (PAD) 2, and showed that CII could be citrullinated in its native triple helical conformation. Based on the MS analyses, synthetic peptides were designed and analyzed for serum IgG reactivity in 356 RA patients. We identified two new citrulline-specific B-cell epitopes on CII that are recognized by autoantibodies in 40% of RA patients. We further affinity-purified citrulline-specific anti-CII antibodies from RA sera and proved the presence of post translationally-modified CII in arthritic cartilage. Finally, we provide evidence for PAD expression in chondrocytes as well as its localization in the pericellular matrix in RA cartilage, suggesting chondrocytes as local mediators of CII citrullination. We therefore propose the involvement of chondrocytes in provoking cartilage-directed anti-citrulline immunity contributing to the initiation and perpetuation of joint inflammation in RA.

P5.12.31
TNF-alpha and IL1-beta contribute to the increased severity of serum-induced arthritis in NOX2 deficient mice

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Oxidant stress has been implicated in the immune-mediated tissue damage in arthritis. To elucidate the role of reactive oxygen species (ROS) in modulating tissue inflammation, we established an arthritis model by intraperitoneally injecting the K/BxN serum into wild type and *ncf1*$^{-/-}$ CGD mice, which have deficiency in p47phox of NOX2. By comparing the joint inflammation index, we found that the serum-induced arthritis were more severe in CGD mice than in wild type mice. Neutrophil-dominant joint inflammation was marked in both strains of mice. By depleting neutrophils with anti-Ly6G antibodies, we found that serum-induced arthritis was abrogated in both *ncf1*$^{-/-}$ and wild type mice, which confirms previous reports that neutrophils play an important role in the induction and progression of serum-induced arthritis. We further analyzed the pro-inflammatory cytokines and chemokines to elucidate the possible mechanisms involved in ROS-mediated innate immunity in regulating the joint inflammation. We found that IL1β, IL6 and TNFα levels are higher in *ncf1*$^{-/-}$ than in WT control. Furthermore, we found that the blockade of either IL1β or TNFα may suppress the arthritic inflammation in both strains of mice while IL17-depletion suppressed the arthritic severity in WT, but not in *ncf1*$^{-/-}$ mice. In conclusion, IL17-type inflammation mediates the joint inflammation in mice with wild type NOX2 while IL1β and TNFα contribute to the increased severity of serum-induced arthritis in ROS deficient mice. These results indicate that ROS play an important role in modulating immune-mediated tissue damage in arthritis.

P5.12.32
TNFα-induced adipose-related protein (TIARP) functions as a negative regulator of inflammatory process in arthritis

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Objective
TIARP is a six-transmembrane protein induced by TNFα and IL-6. We found that TIARP is dominantly expressed in macrophages and synoviocytes of two arthritic mouse models (collagen induced arthritis; CIA and GPI-induced arthritis; GIA), although the pathogenic mechanisms of arthritis remain unclear. To elucidate the role of

TIARP in the development of arthritis, we have generated TIARP-deficient (TIARP-/-) mice.
Methods
We investigated several organs in TIARP-/- mice. To assess the role of TIARP in macrophages, peritoneal macrophages were cultured with TNFα. The levels of IL-6 production, apoptotic cell numbers, and NF-κB signaling were measured. We also examined the susceptibility of TIARP-/- to CIA and GIA. In addition, to examine the effects of IL-6 on the development of arthritis, mice were injected with anti-IL-6R mAb (MR16-1) after CII immunization. Moreover, the expression of STAT3, phosphorylated-STAT3 (P-STAT3) and SOCS3 in TIARP-/- macrophages after IL-6 stimulation were detected by Western blotting.
Results
We found 80% of TIARP-/- spontaneously developed weak synovitis with high concentration of IL-6 in serum. TIARP-/- macrophages with TNFα stimulation produced high amount of IL-6, and sustained degradation of IκBα. TNFα-induced apoptotic cells were significantly increased in WT but unchanged in TIARP-/-. TIARP-/- showed exacerbated inflammatory response in both CIA and GIA. Administration of MR16-1 significantly suppressed the progression of arthritis in TIARP-/- CIA. P-STAT3 was enhanced in TIARP-/-, whereas SOCS3 was comparable.
Conclusion
TIARP might be a negative regulator in autoimmune arthritis through the suppression of IL-6 production, NF-κB, STAT3 signaling, and the induction of apoptosis.

P5.12.33
Increase of PADI4 in the joints and anti-CCP antibody in the sera of a murine model for rheumatoid arthritis before the clinical onset of arthritis

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A knock-in mouse gp130F759 has a point mutation Y759F in gp130, a common receptor subunit for IL-6 family cytokines, and spontaneously develops arthritis like rheumatoid arthritis (RA) around 8 months old (MO). Due to interruption of the negative regulatory pathway mediated through Y759, tyrosine-phosphorylation of STAT3 is prolonged by the stimulation with physiological ligands. To find the earliest pathophysiological changes, we quantitatively analyzed the expression of genes for inflammatory cytokines and proteases in the joint before the onset of clinical arthritis. At 5 MO, when no obvious symptom of arthritis was detectable, the expression of MMP8 and IL-6, but not TNFα or IL-1β increased. Flowcytometric analyses of the cells from the synovium revealed presence of neutrophils, macrophages, eosinophils, erythroid and B cells, but not T cells, indicating that the increase of inflammatory cells at the synovium starts as early as 5 MO.
Furthermore, increased expression of peptidyl arginine deiminase type IV(padi4), whose SNPs have been reported as risk factor for RA in certain ethnic groups, was also found in the joints of gp130F759. Western blot analysis revealed the increases of PADI4 protein as well as phosphorylated-STAT3 in extracts from the joints of gp130F759 compared with control C57BL/6. Interestingly, the serum levels of anti-CCP antibody also increased in gp130F759 at 5 MO.
These data have suggested that the molecular mechanisms similar to RA are operating at the earliest phase of arthritis in gp130F759. Thus, the analysis of the relationships between these events will facilitate understanding of the pathophysiology of early RA.

P5.12.34
Promoter polymorphisms of the TNFα (-308 G/A) and IL-6 (-174G/C) genes predict therapeutic response to etanercept

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The aim of the study was to explore putative influence of - 308 G/A TNFα and -174 G/C IL-6 promoter polymorphisms (releated to TNFα and IL-6 level) on the therapeutic response to etanercept, a TNF-α blocker, in patients with rheumathoid arthritis (RA).
73 patients suffering from active RA were examined at the begining of etanercept treatment and 6- and 12-months following the begining of the treatment. The therapeutic response was estimated according to the European League Against Rheumatism response criteria. Patients were genotyped for -308 G/A TNFα and -174 G/C IL-6 gene polymorphisms by the PCR-RFLP method, and influence of the genotype to clinical response to etanercept was assessed.
Association between -308 G/A TNFα polymorphism and a response to etanercept after 6 and 12 months of therapy was not found. After 12 months the percentage of responders (patients who had DAS28 improvement > 1.2) was significantly increased in patients with the -174 GG IL-6 genotype (95.7 %) compared with those with the -174 GC IL-6 (75.6 %) and -174 CC IL-6 (44.4 %) genotype ($p = 0.006$). The best responders after 12 months were patients with -308 GG TNFα/-174 GG IL-6 genotype combination compared to the other genotype combinations ($p = 0.022$).
The patients with RA exhibiting -308 GG TNFα/-174 GG IL-6 genotype respond better to etanercept than patients with other genotypes examined. This finding suggests that this polymorphism combination should be considered as a genetic marker of responsiveness to TNF-α blockers in RA.

P5.12.35
Accumulation of CD4+CD28-PD-1lowOX40+ T cells and its clinical significance in rheumatoid and collagen-induced arthritis

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$CD4^+CD28^-$ T cells play a key role in the immunopathogenesis of Rheumatoid arthritis (RA). Co-inhibitory molecule of PD-1 has a crucial role in suppression of T cell responses. However, presence of PD-1 and absence of CD28 do not induce $CD4^+$ T cells into apoptosis in RA. In this study, we characterized a novel $CD4^+CD28^-PD\text{-}1^{low}OX40^+$ T cells subset and investigated its clinical significance in rheumatoid and collagen-induced arthritis (CIA). Peripheral blood samples were collected from RA, osteoarthritis and healthy subjects. CIA was induced in DBA/1 mice. After separation of mononuclear cells from peripheral blood of the patients or spleen of CIA mice, $CD4^+CD28^-PD\text{-}1^{low}OX40^+$ T cells were examined by flow cytometry. In RA patients and CIA mice, there were increased expressions of PD-1 and OX40 on $CD4^+$ T cells, with PD-L1 and OX40L expressions augmented on monocytes and B cells. Analyses showed that $CD4^+CD28^-PD\text{-}1^{low}OX40^+$ T cells were accumulated and closely correlated with clinicopathological features of RA patients and CIA mice. Moreover, immunosuppressive therapy significantly reduced the percentage of $CD4^+CD28^-PD\text{-}1^{low}OX40^+$ T cells. Additionally, $CD4^+CD28^-PD\text{-}1^{low}$ T cells displayed a phenotype of $CD45RA^{low}CD45RO^{high}CD25^{low}$. In summary, low PD-1 and enhanced OX40 expression implied longevity and persistent activation of these T cells both in RA patients and CIA mice. A phenotype of memory T cells and fine correlations with clinicopathological features suggested that these T cells may be involved in the immunopathogenesis of RA and CIA. Interventions with pathological functions of this T cells subset may provide a new therapeutic interesting in RA.

P5.12.36
Dendritic Cell ImmunoReceptor (DCIR) is a negative regulator in balancing bone metabolism

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C-type lectin receptors (CLRs) are important pathogen recognition receptors, which are capable of inducing a wide array of cytokine expression and are responsible for host defense against virus and bacteria. Dendritic cell immunoreceptor (DCIR) is a CLR that possesses immunoreceptor tyrosine-based inhibitory motifs (ITIM) docking site for tyrosine phosphatases, suggesting a functional involvement of DCIR in the regulation of the immune system. In line with the structural nature, Dcir−/− mice spontaneously developed autoimmune diseases with age, showing that DCIR maintains immune homeostasis in a negative manner. Here, we demonstrate that DCIR is a key negative regulator in osteoclastogenesis and bone metabolism. Osteoclasts (OC) expressed DCIR at the mRNA and protein levels and DCIR deficiency remarkably elicited OC formation in in vitro culture. As retroviral reconstitution of DCIR into Dcir−/− preOCs reduced OC formation at the comparable level with wild type, ITIM-mediated signal is required for proper osteoclastogenesis. We observed higher responsiveness to M-CSF in Dcir−/− preOCs, suggesting that DCIR may modulate signaling pathways downstream of c-Fms during OC formation. Interestingly, histomorphometric analysis revealed that Dcir−/− mice had the increased femoral bone volume accompanied with higher number of osteoclasts and osteoblasts. Moreover, calcein double-labeling showed a marked increase in bone formation rate in Dcir−/− mice, despise no expression of DCIR in osteoblasts. We will discuss DCIR-mediated regulation of bone metabolism that is regulated by a dynamic balance between osteoclasts and osteoblasts.

P5.12.37
Immunomodulatory role of Fasiola gigantica derived somatic antigens

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Helminth infections of live stock and humans in tropical and subtropical countries are known to polarize host immune responses toward anti-inflammatory Th2 type, which characteristically suppresses inflammatory mediators (like IFN gamma and TNF alpha) while promoting secretion of anti-inflammatory cytokines (like IL-4, IL-5, IL-10, IL-13). This shift in immune responses could be beneficial in autoimmune disorders where host immune environment is pro-Th1 type. In this context nothing is known about the effect of tropical liver fluke, Fasciola gigantica (Fg), derived products (in human or animals) on the rheumatoid arthritis (RA) which also affects a large population in Indian subcontinent. In this study we evaluated immunosuppressive and therapeutic effects of Fg somatic antigen on the collagen induced arthritis (CIA) in Wistar rats. The animals were primed with somatic antigen, one day prior to induction of CIA. Subsequently rats were separately immunized with collagen type II and different doses of parasite antigen. Controls were simultaneously run to avoid animal stock variations. Successful induction of CIA was evident in scoring of paw diameter and histopathological examinations. The results on the level of antioxidant enzyme SOD, level of NO (Nitric oxide), expression of MMP8 and the transcription factor NF kappa B, levels of TNF alpha and IL10 revealed pronounced immunomodulatory effect of Fg antigen. Taken together, it is concluded that Fg-derived products could be immunoprotective in this autoimmune model.

P5.12.38
Fibrinogen induced inhibition of osteoclastogenesis is reversed by citrullination of fibrinogen by peptidylarginine deiminase

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Background: There are increasing evidences that autoantibodies and immune complexes containing citrullinated fibrinogen are present in RA patients' sera and contribute to synovitis. We have in vitro data that fibrinogen can inhibit osteoclastogenesis from precursor cells. In this experiment, we investigated the effect of citrullinated fibrinogen, which are abundant in RA, on the in vitro osteoclastogenesis.
Method: Fibrinogen was citrullinated in vitro by reacting with rmPAD2. Fibrinogen, citrullinated or non-citrullinated, were applied with increasing doses to CD14+ cells, and osteoclastogenesis was induced in the presence of M-CSF and RANKL. To confirm a migration shift due to citrullination, western blotting was performed. The citrullinated sites of fibrinogen were analyzed using mass spectrometry.
Result: Fibrinogen inhibited osteoclastogenesis in a dose-dependent manner. In contrast, citrullinated fibrinogen via rmPAD2 did not show inhibition of osteoclastogenesis, which were evident with non-citrullinated fibrinogen. Several osteoclastogenesis related genes, especially DC-STAMP, were suppressed by fibrinogen during osteoclastogenesis, but restored by citrullinated fibrinogen. Citrullination of fibrinogen was confirmed by western blot analysis and mass spectrometry, showing peak changes between citrullinated and non-citrullinated fibrinogen at five sites in the α-chain, two in the β-chain, and one in the γ-chain. Western blot with anti-citrullinated antibody showed that proteins from RA synovial fluid were more citrullinated than those from OA synovial fluid.
Conclusion: Fibrinogen was citrullinated by PAD and confirmed by western blot and mass spectrometry. In contrast to fibrinogen, citrullinated fibrinogen did not show inhibition of osteoclastogenesis. These results may suggest that citrullinated fibrinogen can contribute to osteoclastogenesis in RA patients.

P5.12.39
Caspase-5 inhibition suppresses inflammatory responses in fibroblast-like synoviocytes with rheumatoid arthritis

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Background: Caspases form an conserved family of cytosolic, aspartate-specific, cysteine proteases. Beyond its role in apoptosis, certain caspases exert non-apoptotic functions, including cytokine maturation, cell proliferation, and NF-κB activation. This study investigated the effect of a selective caspase-5 inhibitor (Z-WEHD-fmk) on inflammatory response in fibroblast-like synoviocytes (FLS) in patients with rheumatoid arthritis (RA).
Methods: Caspase-5 expression in rheumatoid FLS were measured after stimulation either without or with pro-inflammatory agents, IL-1β (2 ng/mL), TNF-α (20 ng/mL) and lipopolysaccharide (1000 ng/mL) by real-time quantitative RT-PCR. The involvement of caspase-5 in inflammation and cellular proliferation was examined. The mRNA expressions of proinflammatory mediators after caspase-5 inhibitor treatment were determined by real-time quantitative RT-PCR, and FLS proliferation in response to caspase-5 inhibitor was measured by MTT assay. The levels of phosphorylated JNK, c-jun, ATF-2 and p-38 proteins were evaluated using western blot analysis and the DNA binding activity of NF-κB was measured using EMSA.
Results: Caspase-5 mRNA expression in rheumatoid FLS was increased by IL-1β, TNF-α and lipopolysaccharide stimulation. Caspase-5 inhibitor treatment induced significant reductions of proinflammatory mediators (IL-1β, IL-6, IL-18, COX-2, MMP-9 and CCL-2) from RA FLS. But, cellular proliferation of rheumatoid FLS was not suppressed by caspase-5 inhibitor. The phosphorylated JNK, c-jun, ATF-2 and p-38 were down-regulated and DNA binding activity of NF-κB was decreased following caspase-5 inhibitor treatment.

Conclusions: Caspase-5 inhibition suppresses various proinflammatory responses via modulation of NF-κB in RA FLS. These results suggest that inhibition of caspase-5 may represent a novel therapeutic approach in RA.

P5.12.40
Significant reduction of the Cia25/Pia42 interval, a new arthritis severity and joint damage locus that regulates the invasive properties of synovial fibroblasts. Cia25/Pia42

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Rheumatoid arthritis (RA) is a chronic and commonly disabling disease with a prevalence of 1% world-wide. Disease remission is rarely achieved with current treatments and little is known about the genes that control disease severity and joint damage. We have identified the arthritis severity and joint damage regulatory quantitative trait locus, *Cia25/Pia42* on rat chromosome 12, in an intercross between MHC-identical arthritis-susceptible DA and arthritis-resistant ACI rats. *Cia25/Pia42* regulates collagen-induced arthritis (CIA) and pristane-induced arthritis (PIA), two established models of RA. *Cia25/Pia42* regulates the synovial expression of IL-6, IL-1β, MMP-3 and other key mediators of arthritis pathogenesis. Here we report the genotype-guided breeding of twelve different DA.ACI(Cia25/Pia42) subcongenic strains. These new strains were studied in the homozygous state in PIA, and based on the subcongenic effect on arthritis severity we were able to reduce the gene-containing interval from 23Mb to 1.7Mb. The new and reduced interval contains 34 genes. We also studied the invasive properties of synovial fibroblasts from DA.ACI(*Cia25/Pia42*) congenics in an *in vitro* model of through Matrigel, a phenotype known to correlate with radiographic and histologic damage in arthritis. Synovial fibroblasts from congenics had an 80% reduction in invasion, compared with DA (p=0.02). Therefore, we have significantly reduced the *Cia25/Pia42* interval towards positional cloning, and identified evidence suggesting that at least part of its effect is mediated via synovial cell function and invasion. The discovery of the *Cia25-Pia42* gene has the potential to generate a new target for therapy in arthritis, and a new prognostic biomarker for RA.

P5.12.41
The arthritis severity locus Cia4 is an early regulator of IL-6, IL-1b and genes implicated in NFkB activation in synovial tissues of rats with pristane-induced arthritis

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Cia4 is a quantitative trait locus that regulates disease severity and joint damage in rats with pristane-induced arthritis (PIA). To identify cellular and molecular processes regulated by *Cia4,* synovial tissues from DA (severe erosive disease) and DA.F344(Cia4) congenic (mild non-erosive disease) rats were collected before and after onset of PIA and analyzed for gene expression. Before onset of arthritis, *Il6* levels were 135-fold higher in DA compared with congenics, and remained significantly increased. The *Il6* increase preceded a modest increase in *Il1b* (4.2-fold) suggesting that *Il6* could be driving cytokine expression and early histologic inflammatory infiltration. 187 genes had significantly different expression and included inflammatory mediators expressed in increased levels in DA such *Slpi* (10.94-fold) and its receptor *Plscr1* (2.31-fold), *Cd163* (5.85-fold), *Ccl7* (5.17-fold), and *Litaf* (2.09-fold). *Syk* or NFκB pathway activating and interacting genes were increased in DA synovial tissues. 59 genes implicated in cancer-related phenotypes were increased in DA, while genes involved in cell metabolism, transport across membranes and tissue protection such as *Acat1, Dgat1, Dhcr7, Slc25a29*, and *Slc1a1* were increased in *Cia4* congenics. 21 genes differentially expressed, or expressed in only one of the two strains were located within the *Cia4* interval, and could be the gene accounting for the arthritis effect. In conclusion, the *Cia4* interval contains a new arthritis gene that regulates the expression *of Il6*, *Il1b*, other inflammatory molecules known to mediate arthritis, and genes involved in cancer that may be implicated in synovial hyperplasia, invasion, and cartilage and bone destruction.

P5.12.42
The nuclear receptor NR1D1 suppresses the invasive properties of synovial fibroblasts from rheumatoid arthritis

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Rheumatoid arthritis (RA) is a common and chronic autoimmune disease. Arthritis severity and joint damage predict clinical outcome and the risk for disability in RA. Yet, little is known about disease severity and joint damage regulatory genes. We have identified a new nuclear receptor (NR) expression signature in synovial tissues that correlates with arthritis protection and development of mild and non-erosive disease. NR1D1 was the NR with the most significant difference in expression in synovial tissues, with an 8.75-fold increase in the protected rats, suggesting that increased levels and activity of NRs have a suppressive effect on disease severity and joint damage. We hypothesized that NR1D1 mediates arthritis protection at least in part via inhibition of the invasiveness of fibroblast-like synoviocytes (FLS) derived from RA patients and arthritic rats, an *in vitro* phenotype known to correlate with histologic and radiographic joint damage. FLS obtained from arthritic DA rats and RA patients treated with the NR1D1 agonist GSK4112 had a significant 70% and 60% reduction on their invasiveness through Matrigel ($p \leq 0.002$), respectively, compared with vehicle. GSK4112 treatment reduced numbers of thick actin filaments, numbers of elongated cells and the polarized formation of lamellipodia, all actin cytoskeleton and FLS morphologic changes required for invasion. GSK4112 did not significantly affect the IL-1β-induced expression of MMP-1, MMP-2 and MMP-3 in FLS. In conclusion, we have identified an association between increased synovial expression of NR1D1 and arthritis protection, and a new role for this gene in the regulation of FLS properties.

P5.12.43
TRPV2 is new suppressor of arthritis severity and joint damage

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Rheumatoid arthritis (RA) is a common and chronic disease associated with increased risk of developing disability. Disease remission is still rarely achieved and better treatments are needed. The fibroblast-like synoviocyte (FLS) has a central role in the formation of synovial hyperplasia and in articular damage. The FLS *in vitro* invasive properties correlate with disease severity and joint damage in rodents and patients with RA, yet, little is known about the genes regulating RA severity, FLS invasion and joint damage. We have identified significantly increased levels of the non-selective cation channel Trpv2 (transient receptor potential vanilloid subfamily, type 2 channel) in highly invasive FLS obtained from arthritis-susceptible DA rats. Knock-down of Trpv2 with siRNA unexpectedly further increased FLS invasion by nearly 4-fold suggesting that this gene is a suppressor of invasion. We next used the Trpv2-specific agonist O-1821 to treat FLS from DA rats and RA patients. O-1821 significantly reduced invasion by as much as 90% ($p<0.01$). O-1821 was then used to treat KRN serum-induced arthritis in C57BL/6 mice and it significantly reduced arthritis severity scores and preserved a nearly normal joint histology. In conclusion, we have identified a new regulator of arthritis severity and joint damage *in vivo*, and show that at least part of that effect involves suppression of the invasive properties of FLS both in rodents and patients with RA. These new discoveries should provide the basis for the development of new drugs targeting Trpv2 to better preserve joint architecture and improve outcome in RA.

P5.12.44
Decreased heart rate variability in patients with Behcet's disease

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The aim of this study was to investigate autonomous functions by means of heart rate variability (HRV), influence of conventional cardiovascular risk factors, and relationship with the disease activity in patients with Behcet's disease (BD).
30 patients with BD and 30 age- and sex-matched healthy controls were included in this study. All the participants were screened for basic cardiovascular risk factors. BD activity was studied with Behcet's disease current activity form (BDCAF) and acute phase indices. HRV was analyzed by 5-min-at rest ECG. Time and frequency domain parameters of HRV were calculated.
Patients with BD had decreased HRV in comparison to healthy controls as reflected by decrease of the standard deviation of normal R-R intervals (34.0±17.5 vs. 56.2±32.3 ms, respectively, p< 0.011) and of the HF power (120.0±79.9 vs. 343.5± 134.2 ms2, respectively, p< 0.006). A significant negative correlation of HRV parameters with BDCAF and CRP in BD was found.
Patients with BD had impaired autonomous cardiac regulation which is related to the presence of systemic inflammation and which could contribute to the increased cardiovascular risk in these patients.

P5.12.45
Upregulation of circulating soluble programmed death-1 is associated with genetic variants in patients with rheumatoid arthritis

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The Programmed death 1 (PD-1) gene is a critical regulator of T-cell activation and is also an important therapeutic target for autoimmune diseases. It had been showed that the human PD-1 gene has 5 splice variants, one of which is a soluble form lacking transmembrane domains. However, little is known about the regulation and the functional properties of soluble PD-1(sPD-1) variants. To better understand how sPD-1 could be involved in autoimmunity, we set out to characterize biochemical and functional properties of sPD-1. We first developed a specific, quantitative ELISA and then used it to detect the presence sPD-1 in serum and joint fluids from patients with rheumatoid arthritis (RA) and osteoarthritis (OA). The levels of sPD-1 occurred at high concentrations in sera and synovial fluid of RA patients and correlated with titers of rheumatoid factor, swollen joint counts and DAS 28 score in RA patients. Further characterization of soluble PD-1 revealed that it could functionally block the regulatory effect of membrane-bound PD-1 on T cell activation. Next, in correlation with their inflammatory status, we identified IFN-γ, TNF-α and IL-17 as inducers of sPD-1. Finally, the PDCD1+ 6370 single polymorphisms was found as a regulator of sPD-1 levels, serum sPD-1 levels in RA patients with GG genotype were significantly higher than those in AA genotype. Takentogether, the mechanism of antagonism of the function of PD-1 by soluble PD-1 may have important clinical relevance in the understanding of RA pathology.

P5.12.46
Antibodies against TIF1gamma in cancer associated myositis precede cancer symptoms and persist after cancer removal

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Objectives: To analyse levels of TIF1gamma antibodies in longitudinally collected sera taken before cancer diagnosis and after treatment of the malignancy in patients with Cancer Associated Myositis (CAM).
Methods: Serum samples and clinical data were available from 54 patients with CAM. Serum levels of anti-TIF1gamma antibody were tested by ELISA using a commercially available purified recombinant protein (OriGene, Rockville, MD).
Results: Sera from 16 (29.6%) patients (13 females and 3 males) were positive for anti-TIF1gamma antibodies in at least one serum sample. Serum samples taken before cancer diagnosis were available from 15 patients and 4 were positive for anti-TIF1gamma. One of these patients had detectable anti-TIF1gamma antibodies up to 5 years before cancer diagnosis. Of the 16 patients positive for anti-TIF1 gamma, 12 patients had died at time of our study, 7 within 1 year from cancer diagnosis. The 7 patients who died within one year had a mean antibody level of 1976 +/- 304 au, the 5 patients who died after more than 1 year had a mean antibody level of 1036 +/- 555 au (p=0.003). Four patients were still alive at time of the investigation, between 2-13 years after cancer treatment. Two of these became negative for anti-TIF1gamma antibodies.
Conclusions: Anti-TIF1gamma antibodies can be detected before clinical symptoms of cancer and may thus become a helpful marker to alert for cancer in patients with myositis. Levels of anti-TIF1gamma antibodies may be a prognostic marker for survival in CAM.

P5.12.47
Anti-Saccharomyces cerevisiae antibodies in rheumatoid arthritis patients

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Aim. To evaluated, retrospectively, the frequency of anti-Saccharomyces cerevisiae antibodies (ASCA) in patients with Rheumatoid Arthritis (RA). Patients and Methods. ASCA, IgG and IgA were determined by ELISA in sera of 131 patients with RA and 160 healthy subjects. Results. The frequency of ASCA (IgG and/or IgA) was significantly higher in RA patients than in the control group (12.8% vs. 3.7%, p = 0.003). ASCA IgG were significantly more frequent in RA patients than in healthy subjects (8.4% vs. 3.1%, p = 0.049). The same observation was found with ASCA IgA (6.1 % vs. 0.6%, p = 0.008). In RA patients, no significant difference between the frequency of ASCA-IgG and ASCA-IgA were found (8.4% vs. 6.1%). The mean level of ASCA-IgG was significantly higher in RA patients than in the control group (4.5±9.4 vs.2.2±2.8, p= 0.005). There was no significant difference of ASCA-IgA levels between RA patients and control group (4.7±10.3 vs. 3.5±1.7). In RA patients, mean ASCA-IgA level was similar to that of ASCA-IgG (4.7±10.3 vs. 4.5±9.4).
Conclusion. Patients with RA had a high frequency of ASCA compared to the control group.

P5.12.48
Anti-Saccharomyces cerevisiae antibodies in patients with systemic lupus erythematosus.

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Anti-Saccharomyces cerevisiae antibodies (ASCA) had been known to be specific for Crohn's disease but it has been found in many other autoimmune diseases like systemic lupus erythematosus (SLE). Furthermore, cross-reactive epitopes on β2-glycoprotein I (β2GPI) and Saccharomyces cerevisiae were found in SLE patients. The aims of this study were to evaluate the frequency of ASCA in patients with SLE and to compare it with that of anti-β2GPI antibodies (aβ2GPI). Sera of 116 patients with SLE were analyzed in this retrospective study. Sera of 160 blood donors were included as normal controls. ASCA IgA and IgG and aβ2GPI antibodies were determined by

ELISA. The frequency of ASCA (IgG and/or IgA) was significantly higher in SLE patients than in control group (31.9 vs. 3.7 %, $p < 10^{-6}$). ASCA IgG and ASCA IgA were more frequent in SLE patients than in control group (29.3 vs. 3.1 %, $p < 10^{-6}$ and 12.1 vs. 0.6 %, $p = 10^{-4}$, respectively). The mean level of ASCA IgG was higher than that of ASCA IgA (9.5 vs. 6.4 U/ml) but the difference was not statistically significant. The frequencies of aβ2GPI (IgG and/or IgA) and aβ2GPI IgA were significantly higher than those of ASCA (IgG and/or IgA) and ASCA IgA (54.3 vs. 31.9 %, $p = 5 \times 10^{-4}$ and 50.9 vs. 12.1 %, $p < 10^{-6}$, respectively). Increased ASCA IgG was observed in patients with SLE, suggesting a role of environmental stimuli in its pathogenesis.

P5.12.49
MiRNA expression profile of peripheral blood mononuclear cells from RA patients and their relationship with anti-TNF therapy

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Inhibition of inflammatory cytokines, including TNFalpha, can ameliorate Rheumatoid Arthritis (RA). Nevertheless, a significant proportion of patients are refractory to anti-TNF treatment and no reliable biomarkers are available to predict therapeutic response. The immune system is under complex genetic and epigenetic mechanisms, i.e. miRNA. Aim of this study was to investigate miRNA expressed in RA PBMCs and the effect of anti-TNFa treatment, with the final goal to identify novel disease biomarkers and potential therapeutic targets.
The miRNA profile was evaluated by TaqMan® Human miRNA Array in PBMCs from 4 active RA patients, before (T0) and 6 months after anti-TNFalpha therapy (T6) and from age-matched healthy controls (ND). All patients were responsive to therapy (mean DAS28 T0=5.63, T6=2.40). We identified 9 miRNA associated with the disease activity as differentially expressed between T0 and ND ($p \leq 0.05$), including miR-92a-1-5p (FC: -5.4), miR-639 (FC: 5.2), miR-801 (FC: 7.6), miR-124-3p (FC: 9.8), miR-572 (FC: 5.6), miR-630 (FC: 3.9), miR-922 (FC: 4.4), miR-7-1-3p (FC: -2.8), and miR-30d-5p (FC: -2.5). Interestingly, among these disease-associated miRNA, miR-124 has been previously associated with RA pathogenesis and it is involved in regulation of macrophage activation. The expression analysis identified 62 miRNA associated with response to treatment as differentially expressed between T0 and T6 ($p \leq 0.05$). These also include the disease-associated miR-7-1-3p and miR-30d-5p, which are significantly reduced in T0 as compared to both ND and T6.
MiRNA may be helpful biomarkers RA management and may offer further insights on the mechanism of action of TNFalpha-inhibitors.

P5.12.50
Podoplanin is upregulated at the Th17-mediated inflammation in SKG arthritic mice

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Recent advances in biomodifiers with early intervention strategy have dramatically improved the disease courses of rheumatoid arthritis (RA). In contrast, the diagnostics of RA is still lacking for objective and quantitative parameters. Recently, interleukin 17-producing helper T cell lineage (Th17) has been proven to be critical in the pathogenesis of chronic inflammation and autoimmune diseases. Therefore, we searched for Th17-specific biomarkers, useful for monitoring the pathophysiology of RA.
To identify the Th17-specific markers, (1) we improved culturing conditions of four murine helper T cell subsets, including Th17 cells, (2) identified more than one hundred Th17-specific candidate genes by comparative microarray analysis, and (3) validated the candidate markers in three autoimmune disease models (SKG arthritis, SCID colitis and experimental autoimmune encephalomyelitis (EAE)). Among them, twenty-five Th17-specific marker candidates, including IL17A and CCL20, were up-regulated in accordance with disease activities of all the three models. Gene ontology analysis revealed candidate markers, including twenty-nine cell-surface molecules. We focused podplanin, one of the Th17-specific cell-surface molecules, was highly expressed on SKG arthritic joint tissues by qRT-PCR. Moreover, we confirmed that podoplanin-positive Th17 cells significantly upregulated in the inflamed joint tissues of SKG arthritic mice by flow cytometrical (FCM) and immunohistochemical analyses.These results indicate that podoplanin would be a useful Th17 cell marker for diagnosing pathological conditions of Th17-related diseases, including RA.

P5.12.51
Allelic expression of 89G/A, 90T/C and 92G/C PADI4 polymorphisms in rheumatoid arthritis

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Introduction: Rheumatoid Arthritis (RA) is an inflammatory joint disease of unknown etiology where genetic factors have been associated with the onset and progression of the disease. Polymorphisms *89G/A, 90T/C* and *92G/C*, including the haplotype (GTG) of *PADI4* gene, have been associated with RA susceptibility in several populations. However, the allelic expression of these polymorphisms has not been reported. **Aim:** To assess the allelic expression of *89G/A*, *90T/C* and *92G/C PADI4* polymorphisms in RA. **Methods:** RA patients classified according to the 1987 ACR criteria and control subjects (CS), adjusted by age and sex, were included. The allelic expression was determined by ASTQ method in heterozygotes of *89G>A, 90T>C* and *92G>C PADI4* polymorphisms and mRNA expression of the *PADI4* gene was determined with qPCR in samples classified as homozygotes for the ACC and GTG haplotypes. The data was analyzed with STATA v 9.2 software and *p<0.05* was reported as statistically significant. **Results:** We found a high expression of 89A, 90C and 92C alleles in CS compared with RA patients (62.3% *vs* 46.7%, 55.6% *vs* 27.7% and 84.7% *vs* 43.9%, respectively) and a high expression of 89G, 90T and 92G alleles in RA patients compared with CS (53.3% *vs* 37.7%, 72.3% *vs* 44.4% and 56.1% *vs* 15.3%, respectively). Also, we observed in RA patients that GTG haplotype carriers has 3.29-fold more expression that ACC haplotype carriers (*p<0.001*). **Conclusion:** This study revealed that the 89G, 90T and 92G alleles and the GTG susceptibility haplotype was associated with increased gene expression in RA.

P5.12.52
Evaluation of anti-PADI4 antibodies in rheumatoid arthritis: Association with clinical parameters

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Introduction: Rheumatoid Arthritis (RA) is an inflammatory joint disease of unknown etiology where the antibody production has been associated with the onset and progression of the disease. PADI4 autoantibodies (anti-PADI4) been described, suggesting relevance in the clinical course of RA. Aim: To assess the association of the anti-PADI4 levels with clinical parameters in RA. Methods: RA patients classified according to the 1987 ACR criteria and control subjects (CS), adjusted by age and sex, were included. Serum levels of anti-PADI4 and anti-CCP antibodies were determined by ELISA method and rheumatoid factor (RF) was determined by turbidimetry. The data was analyzed with STATA v 9.2 and GraphPad v 5.04 software and p<0.05 was reported as statistically significant. Results: Levels of anti-PADI4 in the RA group were higher than CS (2660.7 U/mL vs

210.3 U/mL; p<0.001) and 41.7% of RA patients were positive to anti-PADI4 compared to 4.2% of CS (*p<0.001*). Moreover, we found a positive correlation between anti-PADI4 antibodies and disease evolution, ESR, hsCRP, RF and anti-CCP antibodies (*p=0.032*, *p<0.001, p<0.001, p<0.001* y *p<0.001*, respectively). Finally, we found that 30% of RA patients anti-CCP negative were anti-PADI4 positive and 50% of RA patients RF negative were anti-PADI4 positive. Conclusion: This study revealed that RA patients have high levels of anti-PADI4 and more positive for anti-PADI4 regarding CS and a high percent of RA patients anti-CPP or FR negative were anti-PADI4 positive. These suggest that anti-PADI4 antibodies could be used as a new serological marker of diagnostic and progression in RA.

P5.12.53
Study of IGA1 O-Glycosylation in a multi-ethnic population of IGA Nephropathy patients in Kwazulu Natal, South Africa

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Background - IgAN is a leading cause of chronic disease and end-stage renal disease worldwide. The pathogenesis is poorly understood. Immune complexes mask the ligand for the hepatic asialoglycoprotein receptor, preventing clearance, promoting selective mesangial deposition which show higher levels of degalactosylation.
Objectives - To study the O-glycosylation of serum IgA1 molecules in a multi-ethnic population of IgAN patients in KwaZulu Natal, South Africa.
Materials and Methods - An ELISA based lectin binding assay was used to measure and compare the level of IgA1 degalactoslyation between IgAN patients and controls. Participants included individuals of African, Caucasian, Coloured, Indian (predominantly) and mixed-race descent. 19 IgAN patients were recruited between 2005 and 2011; 53% were terminal. The mean A value corresponding to the degree of degalactosylation, for the IgAN group was compared to that of the normal control group for each test. Wilcoxom matched pairs test was used. The two-tailed p value was used to assess for statistical significance between the groups.
Results - When all the means of the tests were compared, the average means of the tests of the IgAN patients was 0.3678+- 0.0790 SEM which is statistically significantly greater than the normal controls which was 0.2969=-0.0586 SEM (p = 0.0076). IgAN patients exhibited abnormal IgA1 O-glycosylation with a greater level of terminal degalactosylation of IgA1 in comparison to normal controls.
Discussion - This finding is consistent with that of other populations globally; supporting a universal strategy for therapeutic or curative agents that target this aberrancy

P5.12.54
The mesenchymal stem cell supernatant therapy improves symptoms of collagen-induced arthritis in rats

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The Mesenchymal stem cells (MSCs)-mediated immunosuppression mainly acts through the secretion of soluble molecules. The immunosuppressive activity of MSC supernatants in prevention of rat collagen-induced arthritis (CIA) were examined in this study.
The model of Wistar rats with CIA was created using chicken type II collagen and Complete Freund's Adjuvant. Following this immunization, Rats treated with rat bone marrow derived MSC supernatants via tail vein.
Results this study showed that MSC supernatants suppresse paw swelling significantly (P<0.05) compared with the model group. This treatment also improved pathological changes, MSC supernatants - treated rats showed a significant improvement in synovial hyperplasia, inflammatory infiltration, cartilage and bone destruction and other symptoms. The levels of Anti-type II collagen antibodies and TNF-α significantly decreased in MSC supernatant treated rats compared to control group. The results of MTT assay showed that proliferative ability of splenocytes from MSC supernatant treated rats following exposure with chicken type II collagen significantly decreased compared with splenocytes from control group (P<0.01).
Taken together, we found that a single i.v. injection of MSC supernatants was able to improve symptoms of CIA.

P5.12.55
A monoclonal CCP-antibody derived from RA patients binds to several citrullinated antigens in the human cellular components

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Appearance of antibodies that bind to cyclic citrullinated peptides (CCP) (CCP-Abs) has been reported in the serum of rheumatoid arthritis (RA) patients. However, the physiological role of CCP-antibodies during the progression of RA are unclear. In this study, we tried to obtain monoclonal RA-derived CCP-antibody (RA-CCPAb) to identify the antigens in the cellular components to which the RA-CCPAb binds. To obtain the monoclonal RA-CCPAbs, we used an immunospot-array assay on a chip (ISAAC) technology in which we can detect antigen-specific antibody-secreting cells (ASCs) in human peripheral blood lymphocytes and can produce antigen-specific human monoclonal antibodies. We obtained two monoclonal RA-CCPAbs (IgG4) with the same amino acid sequence from RA patients. Western-blot analysis revealed many proteins to which the RA-CCPAb bound in the peptidyl-arginine- deiminase (PAD)-treated HEK293 cell lysate, but not in the PAD-non-treated lysate. Subsequently, we identified these proteins using immunoprecipitation with the RA-CCPAb followed by the mass technology. Several candidate proteins were identified using the nanoLC-MS/MS. To confirm the binding of the RA-CCPAb to the candidate proteins, we immunoprecipitated PAD-treated HEL293 lysate using RA-CCPAb and then examined whether the antibodies to candidate proteins can bind to the proteins. As a result, some antibodies bound to the candidate proteins under discussion. These data suggest that the RA-CCPAb recognizes several citrullinated proteins in the human cellular components. The analysis of antigens using the monoclonal RA-CCPAb should greatly contribute to the elucidation of the physiological roles of CCP-antibodies during the onset and progression of RA.

P5.12.56
Complement Regulatory Protein MCP; CD46 in Rheumatoid Arthritis

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Introduction: Rheumatoid Arthritis (RA) is a chronic inflammatory disease characterized by pain, swelling and progressive destruction of multiple joints, affecting approximately 1% of the adult human population. The growing importance of Membrane cofactor protein (MCP; CD46) not only as central membrane bound complement inhibitor but also as critical regulator of T-cell immunity, suggests that regulation of its expression may have significant effects on health and disease.
Objectives: Studies on animal models suggest disease modulating activity of Leukocyte CD 46 (L-CD46) in autoimmune disorders. We conducted a case-control study to explore the role of L-CD 46 in human RA.
Methodology: The L-CD46 expression in 40 healthy controls and 40 RA patients on different leukocyte subpopulations was observed by Flow cytometry and expression at mRNA level was monitored using Real-Time PCR. The clinical parameters Circulating Immune Complexes (CIC), Disease activity scores (DAS28), swollen and tender joint counts (SJC and TJC) were determined and correlated with L-CD46 expression in patients.
Results: In patients, the L-CD46 expression reduced significantly on lymphocytes followed by monocytes. Further L-CD46 transcripts declined significantly in patients. Significant inverse correlations of L-CD 46 expression on lymphocyte and monocyte surface and transcript level with DAS28, SJC and CIC were observed in patients.

Conclusions: Decline in the L-CD46 levels suggests failure in protective role of L-CD46 against complement mediated damage in RA patients. The negative correlations of L-CD46 with disease activity indices suggest its significant association with the disease state. Follow-up study confirming L-CD46 role as pivotal biomarker is warranted.

P5.12.57
Halofuginone ameliorates autoimmune arthritis by regulation the balance between Th17 and Tregs and the inhibition of osteoclastogenesis

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Objective: The small molecule halofuginone (HF) has been shown to inhibit fibrosis, angiogenesis, and tumor progression. The objective of this study was to evaluate the effects of HF in preventing autoimmune arthritis in mice.
Methods: The effects of HF on joint diseases were assessed by clinical scoring and histological analysis. Protein expressions were confirmed by immunohistochemistry, ELISA, flow cytometry, and/or western blotting. The mRNA expression levels of various molecules were determined by realtime-PCR. Proliferation of osteoclast precursors was assessed by bromodeoxyuridine (BrdU) uptake. Osteoclast differentiation and activity were determined by quantifying tartrate-resistant acid phosphatase (TRAP)+ multinucleated cells and area of resorbed bone.
Results: Treatment with HF suppressed the development of autoimmune arthritis and reciprocally regulated Th17 cell and Foxp3+ regulatory T (Treg) cells. These effects of HF on Th17 differentiation involved increased signaling of extracellular signal-regulated kinase (ERK), and reduction of signal transducer and activator of transcription 3 (Stat3) and nuclear factor of activated T cells cytoplasmic 1 (NFATc1) expression. Furthermore, HF induced expression of indoleamine 2, 3-dioxygenase (IDO) in dendritic cells (DCs), leading to reduced the production of Th17 cells. In addition, HF prevented the formation and activity of osteoclasts through suppression of transcription factors, such as activator protein-1 (AP-1) and NFATc1, and inhibition of cell cycle arrest of the committed osteoclast precursors via expression of ccnd1 encoding cyclin D1.
Conclusion: Taken together, our results suggested that HF is a promising therapeutic agent for the treatment of Th17 cells-mediated inflammatory diseases and bone diseases.

P5.12.58
Number of activated cytotoxic T-lymphocytes and natural killers in children with juvenile arthritis.

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Now participation of cytotoxic T-lymphocytes and natural killers (NK) in pathogenesis of various autoimmune diseases is actively discussed. One of markers of cytotoxic cells activation is CD38, participating in regulation of endocellular calcium concentration. The estimation of expression CD38 on T-lymphocytes and NK in children with juvenile arthritis was the purpose of our work.
Children of 7-17 years old are surveyed: 12 children with oligo- and 16 with polyarticulare juvenile arthritis, 6 children with system onset of juvenile arthritis, 12 children with juvenile spondiloarthopathy, 10 healthy children. Quantity of CD3+CD8+, CD3-CD16+/56+, CD3+CD8+CD38+, CD3-CD16+/56+CD38+, CD3-CD16+/56+CD8+CD38+ was estimated by flow cytometry with staining by monoclonal antibodies (Beckman Coulter, USA).
The number of T-cells (CD3+) and NK-cells (CD3-CD16+/56+) in all groups were the similar. Amount of T-cytotoxic cells (CD3+CD8+) was above in children with polyarticulary juvenile arthritis in comparison with the control group (p<0,05). The number of T-lymphocytes and NK with activation marker CD38 in children with arthritis in all cases was above control values (p<0,05). Amount of natural killers, simultaneously expressed CD8+ and CD38+, has been raised at juvenile spondiloarthopathy (p<0,05). It is considered that these cells possess the greatest cytotoxic activity and are capable to multiple killing of target cells. Thus, increase of the number of activated T-lymphocytes and natural killers in children with an autoimmune pathology testifies involving of these cells in pathogenesis of this diseases.

P5.12.59
Antibodies to citrullinated peptide in children with juvenile arthritis.

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Detection of antibodies to citrullinated peptides is widely used for diagnostics of rheumatoid arthritis in adults and demonstrates high sensitivity and specificity. Using of these tests is much less common in the diagnostics of juvenile arthritis (JA). The aim of our work was to investigate the frequency of anti-mutated citrullinated vimentin (anti-MCV) and anti-cyclic citrullinated peptide (anti-CCP) antibodies in children with juvenile arthritis.
The levels anti-MCV (Orgentec, Germany) and anti-CCP (Euroimmun, Germany) antibodies were determined by ELISA in 219 children with juvenile arthritis and in 25 healthy children.
Anti-MCV antibodies were found in 12% of children with oligoarticular JA (n = 114), 19% with polyarticular JA (n = 79), and 23% with systemic onset of disease (n = 26). The total frequency of anti-MCV antibodies was 18%, thus, the test sensitivity is low for the diagnostics of juvenile arthritis. High levels of anti-MCV antibodies were detected in 2 of 25 healthy children.
A simultaneous determination anti-MCV and anti-CCP antibody was held in 124 children with juvenile arthritis. Using the manufacturer's cut-off threshold 20U/ml for anti-MCV antibodies 19 children had concentrations of anti-MCV antibodies above normal, high levels of anti-CCP antibodies were observed in 11 ones. Increasing cut-off for anti-MCV antibodies up to 30U/ml the positive results were only in 11 patients, which shows the specificity of laboratory tests became equal. The concentration of anti-MCV antibodies was below 30U/ml in all healthy children. Thus, the cut-off for anti-MCV antibodies should be specified for the diagnostics of juvenile arthritis.

P5.12.60
The number of B-cells subpopulations and level of autoantibodies in children with juvenile arthritis

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It is known that increasing of the autoantibody level could have interconnection with the expansion of B1-cells (CD19+CD5+). The amount of B-memory cells (CD19+CD27+) could be also increased in autoimmune diseases. The aim of our work was to investigate the number of B-cells and their subpopulations and autoantibody level in children with juvenile arthritis (JA).
Children of 7-17 years old are surveyed: 33 children with oligo- and 32 with polyarticular JA, 23 children with juvenile spondiloarthopathy, 23 healthy children. Quantity of CD19+, CD19+CD5+, CD19+CD27+ was estimated by flow cytometry with staining by monoclonal antibodies (Beckman Coulter, USA). The level of antinuclear factor was investigated by indirect immunofluorescence (Euroimmun, Germany).
The levels of antinuclear factor were higher in all children with arthritis than in healthy children (p<0,05). But the number of B-cells (CD19+) in patients with oligoarticular onset and with juvenile spondiloarthopathy were less in comparison with the control group (p<0.05). The amounts of CD19+CD5+ and CD19+CD27+ in children with oligoarticular JA were more than in healthy children (p<0,05). Thus, the most considerable changing of B-cells subpopulations was observed in children with oligoarticular JA. It could be connected with level of antinuclear factor which was the biggest in this group. And we found correlation (p<0.05) between antibody level and CD19+ and CD19+CD27+ in these children but not with CD19+CD5+. In patients

with polyarticular JA we also observed correlation of the level of antinuclear factor (p<0.05) with the amount of CD19+CD27+, and in patients with juvenile spondiloarthopathy with the amount of CD19+.

P5.12.61
IL12B gene haplotype influences the risk of ankylosing spondylitis in Romanians

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Ankylosing spondylitis (AS) is a chronic inflammatory disease with predominant involvement of the spine. Recent findings showed that variations of the genes coding for members of the Th17 axis influence the risk of AS. Among these genes, IL12B that encodes the common subunit p40 of the heterodimeric cytokines IL12 and IL23 was less studied. Our aim was to investigate the possible influence of IL12B gene single nucleotide polymorphisms (SNPs) on the risk of AS in Romanian population.
Four SNPs mapping to IL12B gene were genotyped in 168 consecutive AS patients and 161 healthy controls of Romanian ethnicity using the Sequenom MassARRAY platform (Sequenom, San Diego, CA). Statistical analyses were performed with PLINK software. The intronic SNP rs1363670 was associated with a lower risk of AS (OR 0.59, 95%CI 0.358-0.998, p=0.04). The other polymorphisms were not associated with AS at individual level. The SNPs were not in linkage disequilibrium in the studied population (D'<0.22). Four haplotypic combinations (AAAC, CAAC, ACAC, ACGG) of the investigated SNPs (rs32132227/rs2853694/rs1433048/rs1363670) had frequencies over 5% in either controls or patients. Of these, the haplotype AAAC was significantly overrepresented in patients compared with controls (35.5% versus 26.8%, p=0.01). In conclusion, our results suggest that a particular haplotype of IL12B gene may increase the risk of AS in Romanian population.
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P5.12.62
Decrypting the genomic signatures associated with Rheumatoid Arthritis: A systems immunological approach

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Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory disease characterized by synovitis, articular damage, and other associated problems in brain, lungs and vasculature. The inflammatory synovitis in RA patients is characterized by a rapid influx and proliferation of immune cells resulting in the excessive cytokine production, tissue damage and other co-morbidities. In the present study, we have identified various genomic signatures and biological pathways associated with RA using systems immunological approaches. Raw microarray expression (Affymetrix CEL) files in the public domain derived from studies on RA patients were downloaded from the Gene Expression Omnibus (GEO) and analysed by Genespring GX 12.5 software (Agilent, USA). The significantly expressed genes were selected by a standard cut-off at 2-fold increased expression compared with the normal controls. These differentially expressed genes were then classified based on Gene Ontology (GO) such as the expression of immune cell receptors, regulation of inflammatory response, cytokines and chemokines etc., Additionally, the differentially expressed genes were analysed by Ingenuity Pathway Analysis (IPA) software for the identification of canonical and novel pathways implicated in RA. The genomic signatures identified clearly showed the disparity of proinflammatory and anti-inflammatory cytokines and chemokines in RA synovia that augment the destruction of adjacent cartilages and sub-chondral bone erosions, ligaments and the laxity of tendons in RA. Hence, systems immunological dissection of high throughput data is necessary to understand various disease promoting gene signatures in RA patients and help to efficiently devise therapeutic strategies to control the disease and successfully prevent relapse and attain remission.

P5.12.63
Angiogenic T cell depletion in Rheumatoid Arthritis patients

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Since Angiogenic T cells (Tang) could have a role in endothelial repair through cooperating with Endothelial Progenitor Cells (EPC), we analyzed both populations in Rheumatoid Arthritis (RA) patients and their associations with disease specific parameters and traditional cardiovascular (CV) risk factors. Thus, Tang ($CD3^+CD31^+CD184^+$) and EPC ($CD34^+VEGFR2^+CD133^+$) populations were quantified by flow cytometry in peripheral blood samples from 81 patients with long-standing RA and 18 matched-healthy controls (HC). Clinical features and traditional CV risk factors were obtained from case records and IFNα serum levels were assessed by immunoassays.
Results showed a strong depletion of Tang (p<0.001) and EPC (p<0.0001) in RA patients. In HC, but not in patients, both populations were positively correlated (r=0.886, p<0.0001) and inversely related to LDL- and total-cholesterol levels. Moreover, gender, presence of diabetes, dyslipidemia, hypertension or obesity in patients did not influence significantly Tang, although a higher depletion was detected in smokers (p=0.013). However, Tang depletion was strongly correlated with disease activity (DAS28: r=-0.562, p=0.001) and IFNα serum levels (r=-0.329, p=0.003). Multiple regression analysis adjusted for traditional CV risk factors confirmed that only disease activity (β[95% CI]: -0.413[-1.008, -0.339], p=0.0001), age at diagnosis (-0.310[-0.088,-0.018], p=0.003), ANA positivity (-0.247[-1.959,-0.184], p=0.019) and smoking habit (-2.826[-2.189,-0.374], p=0.006) could predict Tang frequency. Finally, patients who had suffered CV events from their RA diagnosis (n=16) presented stronger Tang depletion (p=0.007) and increased IFNα levels (p=0.019) than those CV events-free. All these data indicate that disease-specific parameters, rather than traditional CV risk factors, are implicated in Tang depletion in RA patients.

P5.12.64
The +1858C>T/-1123G>C PTPN22 gen haplotypes and linkage disequilibrium in rheumatoid arthritis patients from Western Mexico

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INTRODUCTION: Rheumatoid arthritis (RA) is an autoimmune disease, characterized by inflammation of the synovial membrane and destruction of cartilage and bone. T lymphocytes trigger an abnormal response against self-antigens, which leads to the activation of autoreactive B cells and autoantibody production. A large number of genes have been proposed as candidates for the development of RA, like PTPN22, which encodes a lymphoid protein phosphatase (LYP), a potent inhibitor of T and B cell activation. The -1123G>C and the +1858C>T PTPN22 gene polymorphisms have been reported in association with RA susceptibility. Both polymorphisms have been found in strong linkage disequilibrium and CT haplotype, consisting of both risk alleles, was reported as susceptibility haplotype to autoimmune diseases. OBJECTIVE: To

study the +1858C>T/-1123G>C PTPN22 haplotypes and linkage disequilibrium in rheumatoid arthritis patients from the Western Mexico. METHODS: Genotypes for -1123G>C and +1858C>T PTPN22 polymorphisms were identified by PCR-RFLP. Statistical analysis was performed using the Arlequin program v3.0, following the Expectation-Maximization algorithm. RESULTS: The inference of haplotypes for polymorphisms -1123G>C and +1858C>T PTPN22 gene, showed a strong linkage disequilibrium of 98% in CS (X2 = 6.87, p = 0.008). The distribution of frequencies of haplotypes inferred and their association with RA, showed that CT haplotype was significantly associated with the disease (p = 0.040, OR 3.4, 95% CI 1.09-10.69). CONCLUSIONS: Polymorphisms -1123G>C and +1858C>T are in linkage disequilibrium, with a magnitude of D' = 0.98 in CS. The CT haplotype is associated with RA susceptibility in patients from Western Mexico.

P5.12.65
Anti-TNF treatment limits the prothrombotic features of platelets and neutrophils in rheumatoid arthritis

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Systemic inflammation contributes to the increased cardiovascular risk in rheumatoid arthritis (RA), in which ischemic heart disease is the leading cause of mortality and is not fully explained by traditional risk factors. Immuno-suppressive treatments, in particular anti-TNF agents, have been suggested to reduce the disease-related cardiovascular risk. Platelet activation and pro-inflammatory platelet-leukocyte heterotypic aggregates have been observed in both acute coronary syndromes and RA. The expression of Tissue Factor (TF), the initiator signal of the extrinsic pathway of the coagulation cascade, is regulated by TNF-alpha in neutrophils. Platelets also express functional TF upon activation.
The aim of our study was to analyze the *in vivo* effect of anti-TNF treatment in RA patients on platelet/leukocyte activation and TF expression. Platelets and neutrophils from RA patients (n = 42) were activated compared to those from control groups (osteoarthritis, n = 12; chronic stable angina, n = 37; age- and sex-matched healthy controls, n = 70), as demonstrated by expression of activation markers (platelet P-selectin expression and vWF content, neutrophil CD40L expression and MPO content) and by circulating heterotypic aggregates. Neutrophil and platelet TF expression was also significantly increased. Platelet P-selectin and TF expression were particularly high in untreated patients. Anti-TNF treatments resulted in a significantly lower expression of activation markers and TF by platelet and neutrophils and quenched heterotypic aggregates formation.
In conclusion, circulating platelets and leukocytes from RA patients are activated and express TF. Anti-TNF treatment partially rescues these phenomena, suggesting a role of endogenous TNF-alpha in the patients' prothrombotic phenotype.

P5.12.66
Comparative significance of YKL-40 in different types of arthritis

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Background: The high concentrations of YKL-40 is a potential novel biomarker of disease activity and poor prognosis in patients with disorders characterized by inflammation and tissue remodeling. Little information has been obtained about serum and synovial YKL-40 levels in rheumatoid, psoriatic and gout arthritis. The aim of the study is to evaluate YKL-40 levels in these diseases and its association with proinflammatory cytokines TNF-α and IL-6.
Methods: The investigation involved 101 patients: 39 with RA (aged 53.18 ± 2.29), 14 with psoriatic arthritis (aged 49.36 ± 4.67), 8 patients with gout (aged 54.50 ± 5.32) and a control group of 40 age-matched healthy people. The concentrations of YKL-40, TNF-α and IL-6 were determined by ELISA.
Results: In all patients with arthritis, the concentration of serum YKL-40 was remarkably elevated compared to the serum level in the control group (P≤0.01). A strong association between serum and synovial levels of YKL-40 and serum TNF-α in patients with RA (P≤0.01) was detected. In this group of patients, a significant relationship between serum and synovial levels of TNF-α and IL-6 was also determined (P=0.001). In patients with psoriatic and gout arthritis, no evidence of correlation between levels of YKL-40 and proinflammatory cytokines was observed.
Conclusion**:** In conclusion, the results suggest that YKL-40 could be involved in disease activity and pathogenesis of rheumatoid arthritis but not in the other types of arthritis studied. The levels of the glycoprotein might reflect different pathogenetic routes in inflammatory joint diseases.
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P5.12.67
A positive feedback mechanism of IL-6 through phosphorylated and unphosphorylated STAT3 in inflammatory arthritis

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In rheumatoid arthritis (RA), autoimmune joint inflammation is driven by pro-inflammatory cytokines, including TNF and IL-6. However, the molecular mechanisms linking these cytokines with disease activity are unknown. STAT3 is phosphorylated (P-STAT3) upon IL-6 signalling, leading to gene transcription of STAT3 target genes including STAT3 itself. Unphosphorylated (U)-STAT3 complexes with NF-κB to activate κB-dependent genes, including IL-6. We showed previously that CD4+ T-cells of early RA were distinguished by an expression signature of STAT3-inducible genes, which correlated with serum IL-6 levels. In the current work, we used flow cytometry to determine basal and IL-6-stimulated induction of P-STAT3 in whole blood of 11 healthy controls (HC), 40 recent-onset RA, 22 non-RA inflammatory arthritis and 35 non-inflammatory arthritis patients. Serum cytokine levels were measured by chemiluminescence. Constitutive P-STAT3 and total (P-STAT3 and U-STAT3) levels were significantly higher in T cells of RA patients compared to HC, and P-STAT3 and total-STAT3 levels were positively associated. Constitutive T cell P-STAT3 was positively associated with measures of disease activity and with serum IL-6 and TNF. Consistent with the high unstimulated P-STAT3, IL-6-stimulated P-STAT3 was negatively associated with serum IL-6 and TNF. Hence P-STAT3 appears to reflect inflammatory burden across a range of inflammatory arthropathies. In longitudinal analyses of RA, constitutive P-STAT3 correlated with response to treatment. The associations between systemic IL-6, constitutive P-STAT3 and U-STAT3 are consistent with a positive feedback mechanism for IL-6 through both forms of STAT3 in inflammatory arthritis, which may underlie the potent therapeutic effect of IL-6 inhibitors.

P5.12.68
Anti-mutated citrullinated vimentin antibodies of IgA isotype during rheumatoid arthritis

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Objectives. We seeked the presence of anti-mutated citrullinated vimentin antibodies of IgA isotype (IgA anti-MCV) during rheumatoid arthritis (RA) and compared their diagnostic value with those of anti-mutated citrullinated vimentin antibodies of IgG isotype (IgG anti-MCV), anti-cyclic citrullinated peptide antibodies of IgA isotype (IgA anti-CCP), anti-cyclic citrullinated peptide antibodies of IgG isotype (IgG anti-CCP), IgA, IgM, and IgG rheumatoid factors for RA.
Methods. 82 RA patients with early and established RA and 191 controls were tested. Twenty five patients with recent onset disease

were evaluated 1 year after diagnosis to assess the predictive value of these antibodies for radiological damage.
Results. IgA anti-MCV were present in 42 (52.4%) patients and IgA anti-CCP in 45 (54.9%) patients. Specificity of IgA anti-MCV and IgA anti-CCP were 94.8 % and 86.4% respectively.
Out of 12 RA patients who had neither IgG anti-MCV nor IgG anti-CCP, 6 patients have IgA anti-MCV and 3 patients have IgA anti-CCP. There was no association between IgA anti-MCV or IgA anti-CCP and disease duration or radiological damage. Anti-MCV IgA gave the highest combined sensitivity when they were combined with IgG anti-CCP (91.5%).
Their sensitivity reached 89% when they were combined with IgG anti-MCV.
Conclusion. IgA anti-MCV are very specific of RA. Given the relatively low cost of anti-MCV kits, the high specificity of IgA anti-MCV and the high combined sensitivity of IgA anti-MCV/ IgG anti-MCV, it could be interesting to combine anti-MCV IgA and anti-MCV IgG tests.

P5.12.69
Genetic susceptibility to rheumatoid arthritis in the Tunisian population: case of PADI4 and REL genes

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To study genetic susceptibility in rheumatoid arthritis we assessed two single nucleotide polymorphisms (SNPs) located in the REL locus: rs13031237 rs6727504 and two SNPs located in PADI4 locus, padi4 94 (rs2240340) and padi4 96 (rs1748032).
Two hundred and nineteen RA patients were genotyped by PCR-RFLP and MS-PCR.
For REL locus, SNP rs6727504 was not polymorphic. Regarding SNP rs13031237, G allele was the most frequent and was detected in 66.4% of RA patients and 73% of controls and among all genotypes GT was the most frequent in RA patients (46.1%) and GG (55.7%) was the most common in controls. SNPrs13031237 was significantly associated with RA. The minor allele T was not associated with the disease. However, GT genotype was significantly associated with the disease with an OR = 1.71 (IC95%: 1.26-2.32), P = 0.018. Besides, no evident association with anti-CCP or erosion was found. For PADI4, the most frequent haplotypic combinations found in our series were (CT/TC) and (CT/CT). No single-point association between genotype and allele with susceptibility for RA was detected. Haplotype analysis revealed no evident association with RA even after stratification of patients by sex, presence of erosion, and of anti-CCP.
Our study is the first to examine the potential role of REL and PADI4 polymorphism in the Tunisian and the North African population. Taken together our results indicate that PADI4 polymorphisms and SNP rs13031237 lacks association with RA in the Tunisian population and genotype seems to be in linkage disequilibrium with another polymorphism of susceptibility to be determined.

P5.12.70
The development of antibodies specific for carbamylated protein precedes disease onset in mice with collagen induced arthritis

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Introduction: Antibodies against citrullinated proteins (ACPA) are a characteristic of rheumatoid arthritis. Recently, we described antibodies recognizing another type of post-translationally modified proteins, i.e. homocitrulline containing proteins. Because homocitrulline is post-translationally formed by a process called carbamylation, we named these novel auto-antibodies Anti-Carbamylated Protein Antibodies (anti-CarP antibodies). As no information is present on the mechanisms underlying the break of tolerance and hence the induction of anti-CarP-responses, we now analyzed their appearance in collagen induced arthritis (CIA).
Methods: CIA was induced in DBA/1 and C57Bl/6 mice by immunization with type II collagen in CFA. Arthritis severity was monitored and Anti-CarP levels were determined by ELISA. The specificity of the ELISA was validated using inhibition assays.
Results: Anti-CarP antibodies were not detected in non-immunized mice. However, unlike ACPA, they were readily detectable in mice suffering from arthritis. The onset of clinical symptoms was preceded by an increase in serum anti-CarP IgG2a levels. Although all immunized C56Bl/6 mice had anti-CII antibodies, only 60% developed arthritis. Anti-CarP IgG2c could only be detected in arthritic mice, but not in the non-arthritic immunized mice. Inhibition assays confirmed the ELISA results regarding the specificity of the antibodies for carbamylated proteins.
Conclusion: DBA/1 mice with CIA develop anti-CarP antibodies before disease onset and in C57Bl/6 mice the presence of these antibodies is associated with disease development. These results indicate a break of B-cell tolerance to carbamylated proteins which appears to be associated with the induction of chronic inflammation.

P5.12.71
Aryl hydrocarbon receptor genetic polymorphisms are associated with Rheumatoid Arthritis

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Rheumatoid Arthritis (RA) is a chronic inflammatory arthropaty with unknown etiology. RA development is associated with the pathogenic CD4+ lymphocyte T helper 17 (Th17). Then, the knowledge of the mechanisms related to Th17 generation and function is essential to disease control. For instance, it was described that Th17 express the aryl hydrocarbon receptor (AhR) which acts as Th17 function regulator. AhR is a ligand-dependent transcription factor that is activated by organic compounds as halogenated aromatic hydrocarbons present in smoking. The aim of this study was to evaluate the role of AhR in RA development. We evaluated the distribution of two AhR single nucleotide polymorphisms (SNPs) in sex/smoking pared samples of healthy individuals (104) and RA patients (104). Experimental arthritis was accessed by mBSA-induced arthritis (AIA) in C57BL/6 or AhrKO mice, and by collagen-induced arthritis in DBA1/J mice (CIA). Mice were treated i.p. with Vehicle, FICZ (AhR agonist) or CH223191 (AhR antagonist). To access experimental arthritis we evaluated: articular hiperalgesia, articular histopathology and Th17 frequencies. This study was approved by Human and Animal Ethics Committees from HCFMRP/USP. We found an AhR haplotype overrepresented in RA patients. This haplotype contains a SNP that enhanced AHR transactivation function and was related to higher Th17 frequencies in RA patients. In experimental arthritis, FICZ exacerbated AIA and CIA, whereas CH223191-treated or AhrKO mice developed a less severe form of AIA and Th17 frequencies. Our data suggest that AhR could be associated with RA development by enhancement of Th17/IL-17 signalling. Financial Support: FAPESP (2011/02505-7); TIMER (HEALTH-F4-2011-281608)

P5.12.72
Premolis semirufa caterpillar-bristles toxic components induce intense cellular and humoral immune responses

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The caterpillar of the moth *Premolis semirufa*, commonly named *Pararama*, is endemic of the Amazon basin. Accidental contact with these caterpillar bristles causes local symptoms such as heat, pain, edema and itching which last for three to seven days; however, after multiples contacts, it may induce to a chronic inflammatory reaction with immobilization of the affected joints. Specific treatment for this disease does not exist. Despite public health hazard of *pararama* caterpillar poisoning, little is known about the immune response

induced by this envenomation and its potential contribution to the development of the chronic disease.
Here we have evaluated the immune response against caterpillar-bristles toxic components, after intraplantar injection of the toxins into the mice hind footpads, for seven times, every two weeks. Data obtained showed a significant edematogenic response and an intense infiltration of inflammatory cells to the envenomation site. Immunohistochemical and immunofluorescence analyses showed the presence of a large number of neutrophils and macrophages in the paw tissues of the envenomated animals. The bristle extract was able to induce high specific antibody titers, but not autoantibodies (anti-DNA or anti-collagen type II), and proliferation/migration and activation of T and B lymphocytes in the lymph nodes. Evaluation of the cytokines production demonstrated elevated levels of IL-6, IL-10, IL-12, IL-17 and IL-23 after the 7th bristle extract inoculation. These data show that the intense cellular and humoral immune responses, induced by bristles toxic components, may play an important role in the establishment of the disease associated to the pararama envenomation.

P5.12.73
Association of the 22 genotype of enhancer HS1,2A of the Ig heavy 3′ regulatory region (IgH3′RR-1) with non response to DMARDS and response to Rituximab in rheumatoid arthritis patients.

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The aim of the study was to evaluate a possible association between the polymorphism in the enhancer HS1,2A of the IgH3′RR-1 (Giambra V., Genes 2005) and the response to therapy in 535 RA patients at different phases of RA (326 with an early RA (ERA), treated according to a tight control strategy; 89 TNF-treated patients with a long-standing RA (TNF-treated-LSRA) and 120 seropositive LSRA patients treated with Rituximab (RTX-treated-LSRA), not responsive to previous DMARDs and/or TNF-blockers).
The frequency of the 2/2 genotype was similar in ERA patients (28%) and in RTX-treated LSRA (22%, *p=0.17*), but lower than in TNF-treated LSRA (40%, *p=0.03* vs ERA). In ERA patients carrying the 2/2 genotype of HS1,2A enhancer, the percentage of sustained-EULAR-remission at 6-month FU (13%) was lower compared with subjects without the 2/2 genotype (26%, OR(95%CIs):0.40(0.18-0.91). ERA patients carrying the 22 genotype showed a more severe disease at baseline and a higher percentage of subjects treated with TNF-blockers at 6 months FU compared to patients without the 22 genotype. There was no association between the HS1,2A enhancer polymorphism and the response to therapy over time, in TNF-treated-LSRA patients. On the contrary, the percentage of good-EULAR response was more prevalent in RTX-treated-LSRA patients carrying the 2/2 genotype compared to subjects without the 22 genotype (42% vs 20%, OR:2.90(1.15-7.32)). The demonstration of the presence of a binding site for NF-kB in the allele*2 of HS12A enhancer (Frezza D., Ann Rheum Dis 2012), could explain the more aggressive phenotype in ERA patients poorly responsive to DMARDs.

P5.12.74
TRAF-6 dependent signaling pathway is essential for TNF-related apoptosis-inducing ligand (TRAIL) induces osteoclast differentiation

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Human osteoclast formation from mononuclear phagocyte precursors involves interactions between tumor necrosis factor (TNF) ligand superfamily members and their receptors. Recent evidence indicates that in addition to triggering apoptosis, the TNF-related apoptosis-inducing ligand (TRAIL) induces osteoclast differentiation. To understand TRAIL-mediated signal transduction mechanism in osteoclastogenesis, we demonstrated that TRAIL induces osteoclast differentiation via a Tumor necrosis factor receptor-associated factor 6 (TRAF-6)-dependent signaling pathway. TRAIL-induced osteoclast differentiation was significantly inhibited by treatment with TRAF-6 siRNA and TRAF6 decoy peptides in both human monocytes and murine RAW264.7 macrophage cell lines, as evaluated in terms of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells and bone resorption activity. Moreover, TRAIL-induced osteoclast differentiation was also abolished in TRAF6 knockout bone marrow macrophages. In addition to induction of NFATc1, treatment of TRAIL also induced ubiquitination of TRAF6 in osteoclast differentiation. Thus, our data demonstrate that TRAIL induces osteoclastic differentiation via a TRAF-6 dependent signaling pathway. This study suggests TRAF6-dependent signaling may be a central pathway in osteoclast differentiation, and that TNF superfamily molecules other than RANKL may modify RANK signaling by interaction with TRAF6-associated signaling.

P5.12.75
Prevalence of hyperprolactinaemia in antiphospholipid syndrome

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Aim: The aim of our study was to measure PRL level in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) with or without secondary antiphospholipid syndrome (APS) and correlation between PRL levels and antiphospholipid antibodies presenting.
Materials and methods: The study included 31 patients with autoimmune diseases like SLE (16) and rheumatoid arthritis (15) age: 41-68 years old and 20 age matched subjects as control group. All patients were females, Serum PRL concentration was determined by electrochemeluminescent method, anti nuclear antibodies (ANA) were determined by IIF, anti-dsDNA by ELISA and IIF kits and antiphospholipid antinodes were determined by ELISA. Patients with end-stage renal disease and patients who take medications that might cause HPRL, such as antidepressants were excluding from this study.
Results: Hyperprolactinemia -HPRL (> 20 ng/ml) was found in 8 patients (25,8%). The mean PRL level was higher in SLE than in patients with RA and control group (24,2±12 ng/ml, 14.5 ng/ml and 10,5±7 ng/ml). Only 5 patients (3 with SEL and 2 with RA) fulfilled the Sapporo APS classification criteria. HPRL was more common among patients who had b2GPI type G ($p<0.05$), but was not related to the presence of anticardiolipin antibodies both types G and M.
Conclusions: Hyperprolactinemia may be a marker of some APS. More research is required to elucidate the mechanisms involved in PRL immune effects in patients with APS.

P5.12.76
Deficiency of prostaglandin D2 receptor CRTH2 deteriorates adjuvant-induced arthritis

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Cyclooxgenase-metabolites, prostaglandins are known to be involved in the development of arthritis. However, the role of prostaglandin D2 (PGD2) in arthritis remains unknown. In this study, we investigated the contribution of one PGD receptor, chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) to the progression of adjuvant induced-arthritis (AIA). CRTH2 naïve (WT) or deficient mice (CRTH2-/-) were injected into the ankle joint with complete Freund's adjuvant (CFA, 150 microg). In both WT and

CRTH2-/-, CFA injection induced paw swelling. CRTH2-/- represented much more severe features than WT throughout the test period (~ day 11). RT-PCR analysis revealed that the mRNA expression levels of pro-inflammatory cytokines as well as Th1/Th17-producing cytokines in the paw of CRTH2-/- were higher than that of WT. Morphological studies showed that gene deficiency of CRTH2-/- accelerated infiltration of CD68-positive macrophage into the paw in the early phase of AIA (day 3), and infiltration of CD4- or CD8-positive T cell in the late phase (day 11). Treatment with a macrophage inactivator, gadolinium chloride, improved all the exacerbated symptoms of AIA observed in CRTH2-/-. These results indicated the protective roles for CRTH2-mediated signaling in arthritis by attenuating macrophage activation and Th1/Th17 responses.

P5.12.77
Association of PD1.1 and PD1.6 polymorphisms with ankylosing spondylitis

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Ankylosing spondylitis is a chronic inflammatory disease included in the spondyloarthritis group. It is considered as autoimmune disease, in which environmental and genetic factors are involved. Among the genes associated with autoimmune diseases, PD1 has been considered as a candidate because of their role in regulation for the immune homeostasis and in the maintenance of the peripheral tolerance through secondary co-stimulatory signaling in T cells. PD1.1 and PD1.6 are two single nucleotide polymorphisms, at -600 G/A (rs36084323), and +8669 G/A (rs10204225) respectively, that have been associated with others autoimmune diseases such as rheumatoid arthritis, type I diabetes mellitus, and systemic lupus erythematosus. In this work, we have determined and compared the frequencies of PD1.1 and PD1.6 polymorphisms in a group of 31 patients with AS diagnosed at HGZ No. 1 from IMSS according to the current criteria, and 90 healthy subjects as a control group. DNA was extracted form each subject and genotypification of PD1.1 and PD1.6 was carried out by using specific primers by bidirectional PCR amplification of specific alleles. Frequencies were determined, and odds ratio and CI95% were calculated. Our results showed no differences in the frequencies of neither PD1.1 (genotype AG or GG) nor PD1.6 between groups were found. However, the genotype PD1.1-AA showed statistically differences between groups (OR = 9.4286, 95%CI= 1.3648-65.1380), suggesting that it could be a risk factor, but it is necessary to increase the sample size in order to validate these association.

P5.12.78
IL-6R blockade enhances CD39+ efficient regulatory T cells in Rheumatoid Arthritis Patients

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Introduction: Tocilizumab is a humanized anti-IL-6 receptor monoclonal antibody, which binds to circulating soluble and membrane-expressed IL-6 receptor, inhibiting IL-6 binding. Tocilizumab is an efficient therapy for adults with Rheumatoid Arthritis (RA) in whom the most common treatments like DMARDs or TNF inhibitor have failed.
However, the consequences of anti-IL-6R treatment on cells and particularly on Treg and Th17 phenotype and activation remain unclear.
Patients and methods: 15 patients with severe and active RA were treated with Tocilizumab monthly. Peripheral blood was recovered for each patient at day 0, as well as 1 and 3 month after treatment. Peripheral blood mononuclear cells (PBMC) were purified for intracellular staining of FoxP3 and IL-17.
Results: Frequencies of IL-17 secreting cells were not significantly modified by Tocilizumab therapy, and no difference was observed between responders and non-responders (0.53%±0.22% and 0.23%±0.09%, respectively). Concerning the Tregs cells, CD39 is constitutively expressed on 50% of human CD25+FoxP3+Tregs cells in healthy controls. The percentage of CD39+cells among CD4+CD25+FoxP3+Tregs was significantly higher in the responders than in the non-responders after 3 months of therapy (72%±4% versus 44%± 8%, $p<0.05$). Comparison of the suppression capacity of both CD39- versus CD39+ Treg showed that both populations presented an equivalent capacity to supress the activation of effector cells in vitro.
In conclusion, the present study establishes that IL-6 pathway inhibition by anti-IL-6R antibody therapy in arthritis involves Treg cells. The induction of a CD39+ FoxP3+ Treg population was found to be associated with an attenuation of disease severity in RA.

P5.12.79
Prediction rheumatoid arthritis outcome in patients with unclassified arthritis considering enzymatic serum activity

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The aim of our preliminary study was to develop new method for predicting the outcome of unclassified arthritis (UA) in rheumatoid arthritis RA evaluating the influence of clinical, laboratory data, autoantibodies presence, enzymatic properties of serum.
36 patients with UA were examined prospectively at 6 and 12 months after first visit. DNAse and hyaluronidase serum activity was determined by rivanol clot prevention test.
In 30.56% patients with UA was developed RA, in 11.11% - not RA, in 30.56% - UA, 27.78% patients were recovered. Median of serum hualuronidase activity in RA outcome patients was 4.50 (95%CI 3.00-5.00), in other outcomes patients - 2.00 (95%CI 2.00-3.00), p=0.0007. Median of serum DNAse activity in patients with RA outcome was 4.75 (95%CI 2.89-5.00), in other outcomes - 2.75 (95%CI 2.00-3.61), p=0.0059. 63.64% RA outcome patients and 8.33% other outcomes patients were anti-CCP positive, $p<0.01$. Predictive model that included all three variables had the highest rates of sensitivity (90.00%), specificity (92.31%) and the area under the ROC-curve (0.96), and meets the criteria of excellence quality for expert scale. The model was tested in a control group (retrospective analysis), the agreement between the predicted results and held outcomes were noted in 89% of patients.
We evaluated influence of different factors on UA outcomes. According our study DNAse and hyaluronidase serum activity play a certain role in development of RA. The use of detection of serum DNase and hyaluronidase activity may serve an additional way for the prediction outcome of UA in RA.

P5.12.80
Nuclease IgG activity toward different DNA substrates in patients with rheumatoid arthritis

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Autoimmune mechanisms of rheumatoid arthritis (RA) are regarded as a certain immune regulation disbalance with high production of autoantibodies. The main goal of our study was to assess putative nuclease activity of RA IgG toward different DNA substrates.
IgG samples of 3 patients with RA (1 male and 2 female with disease duration 8, 6 and 16 weeks respectively) and two healthy person were examined. All patients were fulfilled 2010 RA Classification Criteria.
IgG were purified from the sera by combined method of protein A column affinity chromatography. The methods for activity assessment were developed and tested. For the assessment of nuclease IgG activity it has been used thymus bovine DNA, plasmid dsDNA and Cl. trachomatis DNA as reaction substrates. DNAse activity was registered with agarose gel electrophoresis.
It has been shown that RA IgG samples gydrolysed all DNA substrates and demonstrated different patterns of DNA degradation.

Healthy control IgG had not DNAse properties. All ERA samples gydrolysed low and high polymeric fraction thymus bovine DNA and changed down the mobility of Cl. trachomatis DNA due to nick cuts. One sample rendered full dsDNA hydrolysis, whereas two others displayed multiple nick cuts of super-coiled plasmid dsDNA fraction.
For the first time we confirmed the presence abzyme IgG activity against different DNA substrate in patients with RA. The different patterns of DNA degradation upon abzyme action were confirmed. Futher it seems worthy to study other DNA substrates to assign various kinds of DNAse IgG activity to certain subsets of RA.

P5.12.81
Reactive arthritis of Yersinia etiology: diagnostics and immunological analysis

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Autoimmune manifestations of yersiniosis can be associated with the defeat of the functions of almost all organs and systems. There are limited data on the diagnostics of autoimmune diseases (secondary immunodeficiencies) of *Yersinia* etiology in literature. This study is devoted to the improvement of verification methods of rheumatoid arthritis, caused by Yersinia. First for this purpose ELISA test-system with pore-forming protein F, species-specific protein of the outer membrane of *Y. pseudotuberculosis* and *Y. enterocolitica,* as antigen was used. The presence of specific anti-porin antibodies (Abs) was determined by ELISA in sera of almost half patients (44 women and 12 men) with rheumatoid arthritis. Immunological analysis showed the high level of IgG and low level of IgM anti-porin Abs. This observation is consistent with the capacity of *Yersinia* to give a prolonged antibody response that is a central issue in the pathogenesis of *Yersinia*-induced reactive arthritis. It is also shown that level of Abs against some auto-Ags, components of host's own cells, in the sera of the patients differs from the normal physiological level. It may be due to significant pathological changes in the host. The reason of higher level of auto-Abs in 57.1% of patients is the active pathological process increasing its intensity and/or duration, i.e. the polyclonal activation of the immune system. A decrease in overall immunological reactivity (immune suppression) in 42.9% of patients is accompanied by infringement clearance process leading to chronic self-poisonings with exchange products.

P5.12.82
The protective role of intracellular reactive oxygen species in autoimmune arthritis

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Rheumatoid arthritis (RA) is a polygenic systemic autoimmune disease characterized by chronic inflammation in the joints. Recently, a SNP in the human *NCF4* gene was found to be associated with RA, yet functional studies are missing to determine conclusively whether *NCF4* is a RA susceptibility gene. This gene encodes the $p40^{phox}$ regulatory subunit of the NOX2 complex, which is responsible for the production of reactive oxygen species (ROS) generated by phagocytes as a defense mechanism against invading microbial pathogens. Whereas the role of the NOX2 complex during the innate immune defense is well established, its function in the adaptive immune system remains less well understood. The identification of the *NCF1* gene, encoding the $p47^{phox}$ organizer subunit of NOX2, as a RA susceptibility gene in rats has shown that ROS can regulate autoimmune responses. Recent data suggest now that $p40^{phox}$ takes stage in regulating specifically the intracellular levels of ROS by retaining the NOX2 complex at the phagosomal membrane via binding of its PX domain to phosphatidylinositol-3-phosphate ($PtdInsP_3$). We hypothesize that polymorphisms in the *NCF4* gene that reduce the intracellular production of ROS could modify antigen processing in antigen presenting cells and directly or indirectly regulate the activation status of arthritogenic T cells. Functional studies in mice carrying a mutation in $p40^{phox}$ that prevents its binding to $PtdInsP_3$ point towards the concept that intra- rather than extracellularly produced ROS regulate the development of chronic inflammation in autoimmune arthritis.

P5.12.83
Anti-inflammatory effect of palm tocotrienol fractions in arthritis

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Arthritis is an inflammatory joint disease that results in destruction of the articular cartilage. In the present study, palm tocotrienol fractions from palm oil have shown to possess anti-inflammatory effect and provided a powerful new nutrient for reducing arthritis. Well-characterized models systems derived from bovine joint tissue were used in order to examine the proposed link between palm tocotrienol fractions and anti-inflammatory effects in arthritis. Interleukin-1 (IL-1α) used not only to stimulate chondrocytes and to synthesize cartilage matrix-degrading proteinases, but they also regulate matrix protein synthesis, inflammatory markers and cell proliferation. Chondrocytes isolated from the knee articular cartilages of bovine were treated with or without tocotrienol fractions. The actions of palm tocotrienol fractions were monitored by examining mRNA and protein levels of cytokines (COX-2, IL-B, IL-6), metalloproteinase enzymes (MMPs) and tissue inhibitor metalloproteinase enzyme (TIMPs) by qPCR, ELISA, GAG and Western blot analysis. Results demonstrated that palm-tocotrienols strongly inhibited the inflammatory response with gamma-tocotrienol being the most effective to treat osteoarthritis. The results of this study revealed that the use of palm tocotrienols significantly down regulated the production of COX-2, IL-β, IL-6 and MMP-3 in arthritis. On the other hands, palm tocotrienol fractions induced TIMPs that produces an anti-inflammatory effect to block inflammation directly in arthritis. These findings show that palm tocotrienol fractions may be of potential therapeutic value in regulating the joint destruction in arthritis.

P5.12.84
Association of the +49-A/G polymorphism of CTLA-4 in mexican patients with ankylosing spondylitis

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Ankylosing spondylitis (AS) is the prototype of an interrelated group of rheumatic diseases now known as spondyloarthritides, in which environmental and genetic factors are involved. CTLA-4 is a costimulatory molecule involved in the regulation of T cell activation. Theoretically, polymorphism within CTLA4 that lead to reduction of CTLA-4 expression may cause autoimmune T cell clonal proliferation and therefore contribute to the pathogenesis of autoimmune diseases. The +49-A/G polymorphism (rs231775) has been associated with autoimmune diseases such as Grave's disease, type II diabetes mellitus, and multiple sclerosis. Taking into account that genetic factors have been involved in the pathogenesis of AS, and that T cells could play an important role because of their implication in peripheral tolerance, we analyze the possible association of the +49-A/G polymorphism with AS. Thirty one consecutive patients with AS, diagnosed according to the New York criteria, and 67 healthy controls were included. DNA was extracted from a peripheral blood sample, and the +46-A/G genotype was determined by allele-specific PCR, by using previously reported primers. Frequencies for each genotype were obtained, and add ratio, 95% CI were determined. No statistically differences in the frequencies of GG, and AG genotypes were found between groups. However, the frequencies of the AA genotype could be considered as a risk factor (OR = 3.3102, 95%CI = 1.3068-8.3849). Although these data should be considered as preliminary, because of the small sample size, it is a strong candidate gene to be associated with AS.

P5.12.85
Implication for the detection of the joint lesion associated factors and EBV infection in patients with rheumatoid arthritis

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To analyze the implication for the detection of joint lesion associated factors and EBV infection in RA so as to explore the relationship between the pathogenesis of RA, joint lesions associated MMP-3 and TIMP-1 were detected by ELISA, while clonal expansion of RA self-reactive T cells induced by synthesized EBV peptides was analyzed by [^{3}H]-TdR incorporation anlysis. The results showed that the anti-collagen II Ab were found both in serum and SF of RA, while in SF, the level of IgG Abs. in RA was significantly higher than that in the OA. The levels of joint lesion associated MMP-3 and TIMP-1 in the SF of RA were both higher than those in serum of RA. Moreover, the anti-EBV gp110 Abs. were detected in most of RA SF and peripheral blood of RA, The self reactive T cells from RA were found to have marked auto-reactivity to synthesized EBV gp110 peptide. From these results, it is concluded that the EBV infection has some relationship with abnormal expansion of T/B cells in RA, especially the abonormal high levels of MMP-3,TIMP-1 and anti-CII Abs. Possibly, these abnormality may be caused by the mechanism of “molecular mimicry”, the self-reactive cells in RA may cross-react with EBV and peptides during the course of viral infection, and lead to the self-tissue destruction and finally cause activation and development of RA. The results suggest that the anti-virus therapy and immune-regulation may be one of the new idea for the prevention and treatment of RA.

P5.12.86
A CD19/CD3 bispecific TandAb, AFM11, facilitates safe and potent killing of CD19$^+$ cells mediated by T-cells

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CD19, due to broader expression on B-cell subtypes, is an attractive alternative to CD20 as a target for treatment of B-cell malignancies. T-cells are tumor-killing effectors that cannot be recruited by full-length antibodies. The CD3 RECRUIT TandAb AFM11, a bispecific tetravalent antibody with two binding sites for both CD3 and CD19, enables T-cells to potently and specifically kill CD19+ tumors and possesses advantageous PK properties. *In vitro* assays demonstrate higher AFM11 target cytotoxicity relative to a bispecific tandem-scFv. CD8+ T-cells dominate early cytotoxicity (4 hrs) while after 24 hrs both CD4$^+$ and CD8$^+$ T-cells equally contribute to tumor lysis with EC_{50} of 0.5-5 pM. AFM11 exhibits similar cytotoxicity at Effector:Target ratios from 5:1 to 1:5 and facilitates T-cell serial target killing. AFM11 activates T-cells only in the presence of CD19+ cells. In PBMC cultures AFM11 induces CD69 and CD25 expression, T-cell proliferation, production of IFN-γ, TNF-α, IL-2, IL-6, and IL-10. Depletion of CD19$^+$ cells from PBMC abrogates these effects indicating strict CD19$^+$ target-dependent T-cell activation. Thus, AFM11 should not elicit the devastating cytokine release observed when full-length antibodies bind CD3. One week co-incubation with AFM11 does not inhibit T-cell cytotoxicity and thus it does not induce anergy. In a NOD/scid xenograft model reconstituted with human PBMC AFM11 exhibits a dose-dependent growth inhibition of Raji tumors; a single dose of AFM11 exhibits similar efficacy as five daily injections. AFM11 is an efficacious drug candidate for the treatment of CD19+ malignancies with an advantageous safety profile and anticipated dosing regimen.

P5.14 Psoriasis and skin diseases

P5.14.01
Genome-wide linkage scan for psoriasis susceptibility loci in multiplex Tunisian families

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Psoriasis is a relapsing chronic inflammatory skin disease affecting all populations groups with a peak prevalence of 3% in northern European and Scandinavian Caucasians. Epidemiologic studies have implicated a genetic component to psoriasis. In the last 12 years multiple genomewide linkage analyses have identified putative susceptibility loci on several chromosomes, with a major locus in the MHC region. In order to investigate the genetic basis of familial psoriasis in Tunisian population we undertook a genome-wide linkage scan in seven multiplex psoriatic families from Tunisia. Following SNP genotyping on the Affymetrix 10K SNP array, we performed non-parametric linkage (NPL) multipoint analyses to identify genotypes and obtain evidence for linkage with psoriasis across the genome. No chromosomal region gave consistent evidence for linkage, providing evidence for genetic heterogeneity in Tunisian psoriasis families. Significant evidence for linkage of psoriasis to chromosome 2p12 was seen in one family. We also identified several regions of tentative psoriasis linkage on chromosomes 2q, 4q, 6p, 11q, 12q, 9q and 13q. One family exhibiting suggestive evidence for linkage to 17q25 (PSORS2) was identified and all affected members were shown to harbor a p.Gly117Ser mutation in *CARD14* (caspase recruitment domain family, member 14), recently described to lead to psoriasis in a large family from the U.S. Our results support the genetic heterogeneity of psoriasis in the Tunisian population, provide confirmatory evidence for a novel psoriasis locus at chromosome 2p12 and identify a psoriasis family with a mutation within PSORS2.

P5.14.02
Absence of functional or quantitative defects of peripheral regulatory T lymphocytes in patients with Alopecia areata

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Alopecia areata (AA) is characterized by a reversible form of patchy or complete hair loss associated with T-cell infiltration of hair follicles. It has been hypothesized that AA is a cell-mediated autoimmune disease but the mechanism underlying the loss of self-tolerance is unknown. A deregulation of regulatory T cell biology could be a possible hypothesis. We analyzed the percentage as well as the suppressive effects of peripheral regulatory T cells in 25 patients with AA and 20 sex- and age-matched healthy controls. The percentage of CD4+CD25bright T cells within the peripheral compartment was analyzed by flow cytometry. The suppressive effects of peripheral Tregs on proliferation and interferon (IFN)-γ production of conventional CD4+CD25- T cells were tested in our patients and controls after stimulation with an anti-CD3 antibody. We did not find any intrinsic functional or quantitative defect in peripheral regulatory T cells of patients with AA. Our data did not support the involvement of a peripheral regulatory T cell deregulation in the pathogenesis of Alopecia areata.

P5.14.03
Atypical pemphigus

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We diagnosed 300 cases of pemphigus vulgaris (PV) and foliaceus (PF), collecting clinical data, skin and blood serum. Methods used were: direct immunofluorescence (DIF); indirect IF (IIF) on normal human skin (NHS); ELISA for Desmoglein (DSG) 1-3 and bullous pemphigoid (BP) antigens 180-230; transfected cells for DSG1-3. Atypical clinical presentations were observed in case of herpetiform pemphigus (HP). HP demonstrated IgA intercellular (IC) deposits, anti DSG3 or IC staining in absence of DSG1-3 reactivity (Desmocollin+). Concurrent neoplasia were detected in 2 HP patients. Localized oral pemphigus vulgaris (OPV) were also observed, IgA and IgG mucosal deposits were demonstrated in 2 cases (1 case associated with thyroid neoplasia). In 2 exceptional cases OPV and PF firstly occurred during pregnancy. Patients affected by chronic lymphatic leukemia showed aggressive paraneoplastic pemphigus or a common OPV. In children PV, PF and a case of IgA pemphigus were observed. Typical cases of subcorneal pustolosis like IgA pemphigus were also observed in adults. Looking at serology anti DSG1-3 and anti BP180-230 were simultaneously detected by ELISA in some patients (aggressive cases?). Complete clinical remission was observed in patients with persistent high titre of antibodies. IIF on NHS stained lower part of epidermis (DSG3+) or upper part of epidermis (DSG1+). The specificity of cells transfected by DSG1-3 and BP230 was also confirmed in all cases analyzed. In conclusion the majority of atypical pemphigus cases should be identify and classify by old and new technologies, while antibody titre by ELISA not always was in correlation with clinical evolution.

P5.14.04
Neutrophil depletion by two different antibodies in a hapten-induced inflammation model in mice

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Neutrophils are thought to play a role in contact hypersensitivity (CHS) model which is a T-cell mediated immune reaction in response to epicutaneous application of a hapten. For depletion of neutrophils a rat anti-mouse antibody (clone RB6.8C5) has previously been used. However this antibody has recently been shown to deplete not only neutrophils but also a population of $CD11b^{+}LyC^{+}$ cells which are most likely monocytes. The impact of neutrophil-depletion may therefore have been overstated. The objective of this study was to evaluate the consequences of specific neutrophil-depletion by comparing administration of the RB6.8C5-antibody with a neutrophil-specific depletion using monoclonal antibodies in the dinitrofluorobenzene (DNFB)-induced CHS model.
For induction of CHS, mice were sensitized on day 0 by application of 0.5% DNFB on the abdominal skin and five days later mice were challenged by applying 0.3% DNFB on the ear. Ear thickness was measured on day 1-3 post-challenge. Antibodies and their isotype-controls were administered intraperitoneally a day prior to challenge and level of depletion was confirmed by flow cytometry.
Flow cytometry confirmed that RB6.8C5 depletes both neutrophils and a $CD11b^{+}LyC^{+}$ monocyte-population whereas neutrophil-specific depletion only targets neutrophils. Furthermore, administration of both antibodies results in significantly reduced inflammation however the RB6.8C5-antibody mediates the highest suppression. Thus, it can be concluded that treatment with the RB6.8C5-antibody in the CHS model results in a non-neutrophil specific depletion and therefore most likely overstates the relative importance of neutrophils in this model; specific depletion of neutrophils confirmed a role for these cells in contact hypersensitivity.

P5.14.05
Secretory leukocyte protease inhibitor (SLPI) controls the immunogenicity of DNA deposits in psoriasis

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Plasmacytoid dendritic cells (pDCs) emerged as a pivotal cell type in autoimmune and chronic autoinflammatory conditions such as SLE and psoriasis. The contribution of pDCs to these disorders is largely attributed to the production of IFNI by these cells following activation of TLR9 by endogenous DNA. The delivery of self-DNA to endosomal TLR9 was reported to require coupling of the DNA to several proteins/peptides primarily of neutrophil origin, including; LL37 and HMGB. A candidate source of these molecules are neutrophil extracellular traps (NETs), which release nucleic acids into the extracellular environment, generating a weblike structure composed of DNA as well as nuclear, granular and cytoplasmic proteins. In these studies we validated the concept that inhibitor of serine proteases-Secretory Leukocyte Protease Inhibitor (SLPI), produced by keratinocytes in injured skin or released along with DNA and serine proteases after neutrophil activation, contribute to psoriasis by inducing pDCs to secrete IFNI. Here we demonstrate that circulating neutrophils of psoriasis patients express more SLPI and its target enzymes (neutrophil elastase and cathepsin G) compared to healthy individuals. We also show that neutrophils infiltrating lesional skin of psoriasis patients were further enriched in elastase, released NETs and localized in the vicinity of pDCs. Whereas DNA deposits in psoriatic skin stained for neutrophil elastase, cathepsin G and SLPI, a mixture of SLPI with neutrophil DNA and elastase or cathepsin G, strongly stimulated production of IFNI in pDCs in TLR9-dependent manner. Thus, our data define a novel role for SLPI as pDC-regulating molecule in psoriasis.

P5.14.06
Skin inflammation is induced by weathered marine crude oil exposure

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The 2010 BP oil spill in the Gulf of Mexico is undoubtedly the worst marine oil spill in US history. Oil spill clean up workers tend to suffer a myriad of health effects, including contact dermatitis. Since nothing is known concerning the effects of exposure to weathered crude oil, a mouse model was used to investigate this pathology. Denuded skin of C57 and Balb/c mice was exposed to weathered oil or acetone (control) for 7 days. Histopathology showed inflammation, primarily characterized by epidermal thickening and neutrophil infiltration which was most pronounced in Balb/c mice. Analysis of skin inflammatory cytokine expression showed that oil exposure increased IL-6, CXCL1, CCL2, and CCL4 protein levels, the extent of which varied based on mouse strain. M1 macrophage marker (CD86) mRNA expression was increased in oil exposed skin, but not the M2 marker CD206. Since the source of irritancy of weathered oil is not known, but may be associated with bacterial content, microbial populations analyzed. Most were unclassified (42%), but identified species included environmental and hydrocarbon degrading bacteria, as well as several *Pseudomonas* species which are opportunistic human pathogens. Thus, it appears that weathered oil is indeed a skin irritant, and characteristic inflammation may depend on genetic background. Further, the contribution of bacterial load on irritancy is not known, but pathogenic bacteria appear to reside in weathered oil.

P5.14.07
The Ying & Yang of p40 in psoriatic plaque formation in mice

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Cytokines are mediators as well as regulators of inflammatory immune responses. The myeloid cell derived cytokine IL-23 is a critical player in psoriatic pathogenesis. Monoclonal antibodies (mAbs) directed against the common subunit p40, targeting both IL-12 and IL-23, is currently used therapeutically with impressive efficacy in treating psoriasis.
Recently, it was shown that AldaraTM cream, a drug used in the treatment of basal cell carcinoma, is a potent inducer of psoriatic plaque formation in mice and (inadvertently) human patients. Its mode of action in mice implicates TLR7 dependent induction of the proinflammatory cytokines IL-17A, -17F and -22.
Although in accordance to the successful therapeutic regime in human patients, anti-p40 mAbs treatment dramatically reduced psoriatic plaque formation in this mouse model, a mild degree of lesion formation persists. Since anti-p40 mAbs blocks pathogenic IL-23 as well as IL-12, we hypothesised a conflicting role of the two sister cytokines in psoriatic plaque formation.
While IL-23p19 mutant mice are resistant to induction of disease, we found that skin inflammation was highly increased in mice defective in IL-12 signalling (IL-12null or IL-12Rb2null). These more severe lesions exhibit an even more prominent IL-17 cytokine signature and a change in the composition of infiltrating innate lymphocytes. The effect was IL-12 specific as local injection of recombinant IL-12 during disease development reverted the alterations in IL-12null mice.
Taken together, our findings suggest a protective role of IL-12 in psoriatic inflammation and stress the need of specificity, when targeting the IL-23/IL-17 axis therapeutically.

P5.14.08
Essential role of ITAM-Syk-IL-1 axis in skin dendritic cells for the sensitization of allergic contact dermatitis

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Allergic contact dermatitis (ACD) is a skin inflammation caused by T cells reactive to environmental and industrial allergens. Optimal T cell priming requires co-stimulatory signals from DCs that are activated through innate immune sensors. However, the innate immune pathways through which various contact allergens activate skin DCs are obscure. We show that stimulation of DCs with chemical contact sensitizers (haptens) but not irritants induces activation of the tyrosine kinase Syk. This not only induces the synthesis of pro-IL-1α/β but also engages the NLRP3 inflammasome, resulting in DC release of bioactive IL-1α/β, which is essential for the sensitization of pathogenic T cells. We found both CARD9 and MyD88-deficient mice failed to induce contact hypersensitivity (CHS) to a hapten, TNCB. DAP12, Syk and CARD9 but not MyD88 were essential for TNCB-induced IL-1 secretion by DCs, whereas the IL-1R1-MyD88 signaling but not CARD9 was required for T cells to differentiate into effector T cells producing IL-17 and IFN-γ. The hapten-induced inflammasome activation in DCs was dependent on ROS generation, which was dependent on DAP12-mediated Syk activation but not on the CARD9/BCL10 complex. The CARD9/BCL10–mediated signaling selectively controlled pro-IL-1 synthesis after hapten stimulation by regulating NF-κB activation. Finally, DC-specific deletion of CARD9, DAP12, Syk or NLRP3 but not MyD88 in mice by our newly-developed MixC-Treck system resulted in impaired CHS sensitization. Thus, our results identified an essential molecular axis of innate immunity required for the sensitization of ACD.

P5.14.09
αE integrin augments Arthus reaction by modulating the function of CD8+ T cells

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Arthus reaction is induced by the deposition of immune complexes, which leads to acute inflammatory responses mimicking vasculitis. This inflammatory process is mediated by cell adhesion molecules that recruit various leukocytes to a site of inflammation. αE integrin is present in the form of αEβ7 integrin heterodimer. Whereas the expression of αE integrin is limited to subpopulation of lymphocytes and dendritic cells, β7 integrin is expressed on most leukocytes and is essential for their migration to gut-associated lymphoid tissues. Although αEβ7 integrin-positive dendritic cells are well known to play important roles in intestinal immunity, the role of αEβ7 integrin in Arthus reaction is not known. In this study, we investigated the role of αE and β7 integrin in peritoneal and cutaneous reverse passive Arthus reaction. The reaction was significantly attenuated in β7 integrin deficient mice and, surprisingly, also in αE integrin deficient mice. Infiltration of neutrophils was decreased in both mouse strains with reduced Il-6 levels, whereas the number of infiltrating mast cells was decreased only in β7 integrin deficient mice. The decrease of Arthus reaction in β7 integrin deficiency could be explained by defective recruitment of mast cells that express β7 integrin, however, mast cells did not express αE integrin. Instead, we found that the number of CD8+ T cells was decreased by αE integrin deficiency after peritoneal Arthus reaction, and the adoptive transfer of αE integrin-expressing CD8+ T cells rescue the reaction. Overall, these results indicate an important role of αE integrin in peritoneal and cutaneous Arthus reaction.

P5.14.10
IRF-2 haploinsufficiency and a TLR 7/8 stimulator synergize in the development of psoriasis-like skin inflammation

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Psoriasis is a T-cell-mediated immunological skin disease with a complex pathogenesis where both genetic and environmental factors are involved. Interferon (IFN) regulatory factor (IRF)-2 is one of the potential susceptibility genes for psoriasis. IRFs are a family of transcription factors regulating expression of pro- and anti-inflammatory genes. IRF-2 binds the same regulatory sequences as IRF-1, suppressing transcription of IFN-inducible genes. It was previously reported that IRF-$2^{-/-}$ mice spontaneously developed an inflammatory skin disease resembling human psoriasis. In this study, we applied TLR 7/8 ligand imiquimod (IMQ), which induces a psoriasis-like skin inflammation critically dependent on the IL-23/IL-17 axis, on the backs of IRF-$2^{+/-}$ and wild-type (WT) mice to know whether combination of IRF-2 gene status and environmental stimulus (IMQ) would cause severer skin lesions. IRF-$2^{+/-}$ mice showed a larger cumulative skin score (erythema, scaling, and skin thickness) than WT mice after daily application of IMQ (day 0-5). Increased mRNA levels of TNF-α, IL-12/23 p40, IL-23p19, and inducible nitric oxide synthase (iNOS) were detected on day 2, while TNF-α, IL-12 p35, IL-17A, and iNOS mRNA levels were increased on day 5 in IRF-$2^{+/-}$ mice compared to WT mice. Peritoneal macrophage stimulated with 5.0 μg/ml IMQ for 24 hours expressed significantly higher mRNA levels of TNF-α and IFN-α than non-stimulated macrophages. Interestingly, macrophages from IRF-$2^{+/-}$ mice expressed a higher levels of these cytokines. Our results suggest that IRF-2 haploinsufficiency and a TLR 7/8 stimulator synergize in the development of Th17-associated skin inflammation, which may serve as a good model of human psoriasis.

P5.14.11
High B-cell-activating factor (BAFF) levels in Tunisian pemphigus patients

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Background: High serum BAFF (B cell activating factor belonging to the TNF family) (sBAFF) level was observed in several autoimmune diseases such as lupus, Sjögren syndrome and rheumatoid arthritis. Its expression in autoimmune blistering diseases is controversial. The aim of our study was to determine sBAFF levels in pemphigus vulgaris (PV) and foliaceus (PF) patients.
Patients and methods: sBAFF levels were analysed in 50 PF patients, 36 PV patients and 38 healthy controls using an enzyme linked immunosorbent assay.
Results: The mean value of sBAFF in healthy subjects was 759 pg/ml. SBAFF levels were significantly increased in pemphigus patients (means 982 pg/ml in PV and 875 pg/ml in PF $p<0.0001$). High levels of sBAFF were observed in 14 (28%) PF patients and 11 (30,5%) PV patients ($p=0,004$ and $p=0,003$ respectively). Mean sBAFF levels were significantly higher in PF patients who did not receive yet corticosteroid treatments and in PF and PV patients with severe lesions. There was a significant correlation of sBAFF levels with anti-Dsg1 antibodies titres in PV ($p=0,007$) patients and a correlation, with a p value near the significance threshold, in PF patients ($p=0,079$).
Conclusion: High sBAFF levels were observed in pemphigus patients, suggesting a role in disease pathogenesis.

P5.14.12
IgE levels and immune response in bullous pemphigoid and mucous membrane pemphigoid

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Bullous pemphigoid (BP) and mucous membrane pemphigoid (MMP) are autoimmune blistering diseases characterized by subepidermal blisters, linear deposit of immunoglobulins and/or C3 and circulating autoantibody directed against the basal membrane zone (BP180 and BP230).
The growing interest regarding the potential pathogenic role of IgE class autoantibodies in the pemphigoid group is supported by presence of elevated blood IgE levels.
We conducted a retrospective study on patients affected by BP (n=90) and MMP (n=36). BP sera were examined for the presence of IgE, IgG and/or C3c by indirect immunofluorescence (IIF) and correlated to blood IgE levels. A perilesional skin biopsy was performed in order to test the deposition of IgE, IgG, IgA, IgM and/or C3c along the dermo-epidermal junction.
We therefore studied the specificity of serum anti-BP IgE testing BP (n=24) sera on commercial slides with biochips (BP180) or transfected cells (BP230).
In BP circulating IgE level resulted higher than 160 U/ml in 65 out of 90 patients (72%) (median value 672). In BP and MMP direct immunofluorescence (DIF) showed IgE deposits in 67%-75% and Indirect IF on salt skin split in 77%-16% of the patients respectively. Specificity of serum anti-BP IgE was confirmed on 18/24 sera (75%) by using biochip-slides and transfected cells.
Our findings indicate that specific IgE autoantibodies provide an additional criterion for diagnosis of pemphigoid, both BP and MMP, suggesting an important pathogenetic role of IgE-mediated immune response in these diseases.
Demonstration of IgE pathogenicity should be useful for new biological and immunomodulating strategies.

P5.14.13
Keratinocyte-restricted Rabgef1 gene deletion results in the development of lesions resembling atopic dermatitis, as well as systemic inflammation, in vivo

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RabGEF1, a guanine nucleotide-exchange factor for Rab5 GTPase, has been shown to negatively regulate mast cell activation *in vitro*. In addition, RabGEF1-deficient mice develop morbidity and severe skin inflammation associated with marked increases in skin mast cell numbers. These findings suggest that over-reactive skin mast cells may contribute to the observed skin pathology *in vivo*. In order to identify the cell type(s) which contribute to skin inflammation when RabGEF1 is absent, we attempted to delete *Rabgef1* gene specifically in three major skin cell types, namely mast cells, myeloid cells and keratinocytes; thus, *Rabgef1* floxed ($^{fl/fl}$) mice were crossed with mice expressing the Cre-recombinase under the influence of the promoter of Mcpt5, LysZ or K14, respectively, and the efficiency and specificity of Cre-mediated *Rabgef1* gene deletion were assessed by single-cell genomic PCR. Unexpectedly, Mcpt5-Cre;*Rabgef1*$^{fl/fl}$ and Lysz-Cre;*Rabgef1*$^{fl/fl}$ mice appeared normal without phenotypic abnormalities. However, K14-Cre;*Rabgef1*$^{fl/fl}$ mice were normal at birth but developed morbidity and skin inflammation after 2-3 days, and died between 1 and 8 weeks of age. Skin pathology was characterized by epidermal hyperplasia, keratinocyte differentiation defects, and death of keratinocytes, followed by dermal infiltration of eosinophilic granulocytes, increases in mast cell numbers, and increased pro-inflammatory cytokine expression. Mice surviving 6-8 weeks also displayed signs of systemic inflammation, such as lymphadenopathy and splenomegaly, blood eosinophilia and neutrophilia, and elevated levels of serum IgE. Further experiments will attempt to determine the mechanism by which keratinocyte-restricted RabGEF1 expression plays an essential role in maintaining skin immune homeostasis *in vivo*.

P5.14.14
Evidence for vitamin D deficiency and increased prevalence of fractures in autoimmune bullous skin diseases

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Vitamin D deficiency plays a role in autoimmune diseases and risk of fractures. No data are available on vitamin D levels and vertebral fractures in autoimmune bullous skin diseases.We studied 13 consecutive inpatients with untreated PV (age 53·5 ± 14·3 years), 15 with BP (age 76·9 ± 12·4 years) and 28 age-, body mass index- and sex-matched controls. The 25-hydroxyvitamin D (25-OHD) levels and presence of vertebral fractures on spinal X-ray were assessed in all subjects. In patients with PV, 25-OHD levels were lower (mean ± SD 12 ± 4·4 ng mL(-1)) and prevalence of severe hypovitaminosis D higher (62%) than in controls (mean ± SD 22·2 ± 11·7 ng mL(-1) , P = 0·012; 23%, P = 0·0047, respectively). The prevalence of fractures was 54% and 31% in patients with PV and controls, respectively. Patients with BP showed lower 25-OHD levels (mean ± SD 9·6 ± 7·2 ng mL(-1)) and higher prevalence of severe hypovitaminosis D (73%) than controls (mean ± SD 22·6 ± 18·7 ng mL(-1) , P = 0·022; 27%, P = 0·01, respectively). The prevalence of fractures was higher in patients with BP than in controls (67% vs. 33%, respectively, P = 0·068). The low 25-OHD levels found in PV and BP suggest a role for this agent in their pathogenesis. The increased prevalence of fractures should be taken into consideration in patients who must be given corticosteroids.

P5.14.15
Expression of pemphigus vulgaris antigen desmoglein 3 on pancreatic cells

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Pemphigus vulgaris (PV) is autoimmune disease mediated by the formation of IgG4 antibodies to desmoglein 3 (DSG3). Autoanibodies target desmosomes in skin epidermis and mucosa inducing intraepithelial splitting. Other organs and tissues are not affected in PV. Recently we have identified DSG3 expression in pancreatic adenocarcinoma cell lines BxPC-3, AsPC-1, M3T4, Colo357, Su86.86. Earlier it was thought that the major desmosomal protein in the epithelium of internal organs is DSG2. The aim of this work was to study DSG3 expression in pancreatic biopsy and in the epithelial cells of different origin. Cryosections of pancreas from a patient with chronic pancreatitis or various cell lines grown on cover glasses were stained with anti-DSG3 antibody and analyzed by confocal microscopy. Human pancreas expressed DSG3 as well as DSG2. Cell lines from kidney (HEK293) and liver (HepG2) did not express DSG3; some breast cancer cells (HBL-100) but not others (BT474) expressed DSG3; melanoma A375 and Mewo did not express DSG3. These data show for the first time expression of pemphigus antigen in internal organs and rise the question of IgG4 traffic to various tissues not affected in PV.
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P5.14.16
Activation-induced cytidine deaminase promotes oncogenesis of ultraviolet light-independent squamous cell carcinoma of the skin

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Most squamous cell carcinoma (SCC) of the skin develops after ultraviolet (UV) light-induced DNA damage and repair errors. However, significant portion of SCC appears to occur independently from UV light because SCC often occur in light-protected areas such as chronic ulcers and burn scars underneath clothing, and oropharyngeal mucosa. Such UV-independent SCC is speculated to have a causal link with chronic inflammation. However, how chronic inflammation leads to cancer is unclear. We propose here that activation-induced cytidine deaminase (AID) is a mechanistic link between chronic inflammation and SCC. AID is an enzyme essential for DNA cleavage involved in immunoglobulin class switch and somatic hypermutation. We found that tumor frequencies were elevated by transgenic expression of AID from keratin14 promoter, and decreased by genetic deletion of AID in a mouse skin cancer model using 7,12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol 13-acetate (TPA). Significant numbers of mutations were observed in the Trp53 gene of SCC developed spontaneously in AID transgenic mice. Human primary cultured keratinocytes and SCC cell line expresses AID after stimulation with LPS, TPA, poly(I:C), or inflammatory cytokines such as TGF-β and TNF-α. Overexpression of AID in human keratinocyte cell line caused point mutations in TP53 gene. These results suggest that AID is a DNA damaging factor involved in inflammation-associated SCC.

P5.14.17
Role of Interleukin-1 processing and signaling in imiquimod-induced psoriasis-like skin inflammation

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The pathogenesis of inflammatory skin diseases such as psoriasis involves the release of numerous proinflammatory cytokines, including IL-1. To highlight the potential role of IL-1 α and β in the induction and maintenance of psoriasis-like skin inflammation in mice, we assessed the expression of IL-1 family members after topical application of the TLR7/8 agonist imiquimod (IMQ) in C57BL/6 mice. Daily skin application of IMQ in wild type (wt) mice induced overexpression of IL-1α and IL-1β mRNA, followed by induction of IL-1 receptor antagonist expression. IMQ-treated IL-1α- or IL-1β-deficient mice display inflamed, red and scaly skin lesions, and epidermal hyperplasia, similar to IMQ-treated wt mice. Furthermore, IMQ induced expression of IL-23, IL-17 and IL-22, antimicrobial peptides b-defensin3 and S100A9, and chemokine CXCL3 is similar in mice deficient for IL-1α or IL-1β and wt mice. The absence of NALP3 or ASC in knock-out mice does not affect the inflammatory response to IMQ, demonstrating a modest role of NALP3/ASC inflammasome and downstream IL-1b processing in IMQ model. However, IMQ-induced dermatitis was partially reduced in mice deficient for IL-1RI. Finally, we showed that IMQ-induced skin inflammation was totally abolished in MyD88-deficient mice (the adaptator molecule shared by IL-1 and TLR signaling pathways) which indicate that, as expected, IMQ stimulation is largely TLR-dependent. Our data show that in IMQ model, IL-1α and IL-1β have a functional redundancy as demonstrated by the decreased development of skin inflammation in the absence of IL-1RI signaling.

P5.14.18
TSLP and CD40-ligand promote IL-23 production in human psoriasis

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Thymic stromal lymphopoietin (TSLP) is a keratinocyte-derived dendritic cell (DC)-activating cytokine expressed in atopic dermatitis (AD). TSLP plays a central role in the pathophysiology of allergy by initiating and sustaining pathogenic T helper (Th) 2 responses. Here, we report the unexpected finding that TSLP is involved in the physiopathology of human psoriasis, an auto-immune skin disease. In situ, TSLP was strongly expressed by keratinocytes of untreated psoriasis lesions, but not in normal skin. TSLP synergized with CD40-ligand (CD40L) to promote DC activation, and pathogenic IL-23 production by blood and skin DCs. Although CD40L was equally expressed in psoriasis and AD, we could demonstrate that IL-4, an important component of the Th2 inflammation in AD, inhibited IL-23 production induced by TSLP and CD40L. Our results identify TSLP as a novel player within the complex psoriasis cytokine network, and a potential early inducer of IL-23 production. We propose that inhibiting TSLP in psoriasis patients may contribute to decreasing DC activation and shutting down the production of pathogenic IL-23.

P5.14.19
Role and mechanisms of action of fibroblast growth factors in epidermal barrier function: Implications for inflammatory skin disease

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A large percentage of the population suffers from inflammatory skin diseases such as psoriasis and atopic dermatitis, but the molecular and cellular mechanisms underlying the pathogenesis of these diseases are not fully understood yet. Recently, our laboratory identified a novel and unexpected function of fibroblast growth factors (FGFs) in the control of epidermal barrier function and cutaneous homeostasis. Mice lacking FGF receptors (FGFR) 1 and 2 in keratinocytes have a severe phenotype with features resembling atopic dermatitis that results from impairments in tight junction formation. To identify the cellular mechanisms underlying these abnormalities, we mated the double knockout mice with mice lacking either T cells or mast cells (Cre-Master mice), since the number of these immune cells is strongly increased in the absence of FGFR1 and FGFR2 in keratinocytes. The phenotypes of the triple mutant mice suggest that both types of immune cells functionally contribute to the abnormalities caused by FGFR deficiency. To characterize the skin of these mice by flow cytometry, we have established a procedure to efficiently isolate immune cells from the dermis and epidermis. This allows us to obtain a fairly complete picture of the cutaneous immune cell composition of the triple mutant mice and to determine if and how the loss of T cells or mast cells affects other immune cells in the skin, the epidermal and dermal structure, as well as proliferation and differentiation of epidermal keratinocytes.

P5.14.20
Di methyl fumarate (DMF), is there a possibility for broader use in psoriasis treatment?

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Background: Fumaric acid esters were introduced for treatment of Psoriasis, a chronic inflammatory disease of the skin, more than fifty years ago. The oral preparation that is now used mainly contains DMF. This compound involves in suppressing the expression of proinflammatory cytokines, and also deviation of Th1-driven immune responses toward Th2 subset.
Objective: As DMF involvement in suppression of Th1 cells has been figured out, we decided to investigate its probable same effect on Th17 subset activity. Methods: We isolated lymphocytes from peripheral blood of 35 psoriatic patients with no immunosuppressive medication history, and also 35 normal controls. Lymphocytes were cultured in the presence or absence of DMF. mRNA expression of IFN-γ as a marker for Th1 activity, IL-4 for Th2 activity, and IL-17, IL-22 and GM-CSF as markers for Th17 development and function were determined by Real time PCR.
Results: As showed by previous studies, after DMF treatment, we detected a decrease in IFN-γ expression and also an increase in IL-4 expression. A decrease in IL-17, IL-22 and GM-CSF after DMF treatment was also detected.
Conclusion: An increase in IL-4 mRNA expression and a decrease in mRNA expression of IL-17, IL-22 and GM-CSF besides IFN-γ show the DMF ability in suppressing Th17 in addition to Th1 activity . Therefore, DMF may have promising effects for the treatment of autoimmune diseases in which Th1 and Th17 subsets have a dominant role.

P5.14.21
An orally bioavailable CCR6 antagonist confers protection against IL17-mediated inflammation

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The contribution of dysregulated IL17 expression to the pathology of various immune-related diseases is well documented. The clinical benefit derived from blocking IL17 signaling, provides support for targeting IL17 as a therapeutic approach in alleviating certain immunological disorders. Several reports indicate that the majority of IL17-secreting cells in human and mice co-express the chemokine receptor CCR6, thereby making it an attractive drug target. We have generated a potent orally bioavailable CCR6 antagonist, CCX9664, that inhibits CCL20-mediated chemotaxis of both human and mouse CCR6+ cells. The utility of CCR6 inhibition was tested in preclinical models of psoriasis, whereby CCX9664-treated cohorts were completely protected against imiquimod (IMQ) induced skin thickening (1.26 ± 0.05 mm for IMQ + Veh vs.1.03 ± 0.02 mm for IMQ + CCX9664 group; non-IMQ treated mice 1.06 ± 0.02 mm). Histological analysis of the skin confirmed the protective effect of CCX9664 compared to vehicle. CCX9664 also significantly reduced ear-thickening induced by intradermal injections of IL-23 (0.26 ± 0.0008 mm in IL-23 injection + vehicle vs. 0.20 ± 0.0004 mm in IL-23 + CCX9664; p=0.0001 vs.0.2 mm in PBS injection group). The mechanism of action for CCX9664 is unique from other therapeutics targeting IL17 such that inhibition of CCR6 disrupted the positioning of infiltrating leukocytes into the epidermis upon skin-damage, thereby protecting against epidermal hyperplasia. Thus pharmacological inhibition of CCR6 with an orally bioavailable small molecule antagonist inhibits IL17-driven inflammation in psoriasis models and may have therapeutic utility in other IL17-dependent indications.

P5.14.22
The inhibitory effects of yuzu seed oil to atopic dermatitis model mice by oral administration

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In this study, we examined the effects of yuzu seed oil to anti-atopic dermatitis (AD) using NC/Nga mice, a model animal of human AD. Yuzu (*Citrus junos Sieb.* ex Tanaka) is a typical Japanese citrus fruit with a desirable smell. Yuzu was brought via Korea from China to Japan more than 1,000 years ago. We have established an AD model in NC/Nga mice by repeated local exposure of mite-antigen for analyzing anti-AD effect. Two kinds of yuzu seed oil (crude oil and distilled oil) were used. We administered yuzu seed oil (1 ml/kg) for 4 weeks into tick-antigen treated NC/Nga mice. Surprisingly, these mice inhibited the development of AD-like skin lesions in these mice as exemplified by the significant decrease in the total skin severity scores and the decrease in hypertrophy and hyperkeratosis. Furthermore, the oral administration of yuzu seed oil to NC/Nga mice depressed the elevation of serum IgE levels. The serum IgE levels in the yuzu seed oil oral administrated NC/Nga mice with control (water), the crude oil and the distilled oil, were 1.89±0.475 (mean±SE; n=5) μg/ml, 0.57±0.092 and 0.78±0.204, respectively. Significant differences were found in the crude oil group (p=0.0004) and the distilled oil group (p=0.0007) between control group.
In conclusion, yuzu seed oil is a good candidate to be used as an alternative medicine against AD. Now, we are doing histological analysis of NC/Nga mice after yuzu seed oil oral administration and are confirming the depletion of mast cells and/or eosinophils.

P5.14.23
IL-17A-producing CD8$^+$ T cells in psoriasis: correlation with psoriasis severity and relationship with MAIT cell lineage

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Our observation that IL-17A-producing CD8$^+$ T cells are overrepresented in psoriatic lesions is in line with the general consensus that T cells and IL-17A are critically involved in psoriasis. Here we provide further proof that CD8$^+$ T cells are involved in the pathogenesis and analyzed T cells derived from peripheral blood and lesional dermis and epidermis by flow cytometry. The frequency of IL-17A-producing CD8$^+$ T cells in blood correlated with psoriasis severity. Such correlation was not found for IL-22-producing CD8$^+$ and CD4$^+$ T cells. Of note, a remarkable higher frequency of skin-homing molecule CLA was found in IL-17A and/or IL-22 producing CD8$^+$ blood T cells of psoriasis patients. However, this feature was also observed in healthy individuals. IL-17A-producing CD8$^+$ T cells in blood have been described to belong to the mucosa-associated invariant T (MAIT) cell lineage, expressing the invariant T cell receptor Vα7.2-Jα33 chain, CD161 (high) and IL-18Rα. We unequivocally show the presence of MAIT cells in psoriatic as well as healthy skin. IL-17A-producing CD8$^+$ MAIT cells were only present in psoriatic lesional skin, suggesting contribution to psoriasis development. IL-17A was also expressed by a considerable proportion of CD8$^+$ T cells without MAIT cell characteristics, likely containing MHC class I-restricted psoriasis-associated autoreactive T cells. In conclusion, we provide supporting evidence that implicates conventional CD8$^+$ T cells in the pathogenesis of psoriasis and describe the presence of innate MAIT cells in psoriatic lesions as an additional source of IL-17, a feature that was not appreciated before.

P5.14.24
Skin involvement in cutaneous and systemic vasculitis

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Cutaneous vasculitides are a heterogeneous group of inflammatory disorders affecting skin blood vessels. They may be triggered by several factors, such as infection or drug, or may be related to underlying disease, notably connective tissue or malignancies. However, vasculitis occurs without any demonstrable triggering agents in a relevant number of patients. On the other hand, vasculitic skin lesions may manifest as a component of vasculitis affecting also internal organs; in someone of these patients, skin involvement occurs initially as the sole sign of disease, leading to consider cutaneous vasculitis a diagnosis of exclusion. In this review, we have focused on the most common variants of cutaneous vasculitis, including cutaneous small vessel vasculitis and urticarial vasculitis as well as Henoch-Schönlein purpura, a systemic form in which however skin involvement often predominates. We have also argued on livedoid vasculopathy, a cutaneous entity which, although nonfrankly vasculitic in origin, is frequently associated with connective tissue disease. Finally, we have analyzed the variety of cutaneous manifestations that may develop during the course of the main systemic vasculitides, such as Wegener's granulomatosis, Churg-Strauss syndrome and polyarteritis nodosa.

P5.14.25
Topical application of the yuzu seed oil inhibits mite-antigen induced atopic dermatitis in NC/Nga mice

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The yuzu (*Citrus junos Sieb.* ex Tanaka) is a citrus fruit and this fruit was brought to Japan from China about a thousand years ago. The yuzu have been traditionally used as not only the food but also a folk remedy for a cold, gout, skin care and rheumatism in Japan. It is known that the yuzu contains a lot of material, such as limonene, hesperidine, auraptene and pinene, which contributes to health maintenance.
In this study, we show the effects of yuzu seed oil for topical application on the progress of atopic dermatitis (AD)-like skin lesions in NC/Nga mice induced by mite-antigen. We used two kinds of yuzu seed oil (crude oil and distilled oil). We evaluated skin dermatitis severity, ear thickness, serum IgE levels, histopathological examination and histamine level in AD-mice treated with the yuzu seed oil. The yuzu seed oil inhibited atopic dermatitis symptoms and signs in the antigen-treated Nc/Nga mice. Topical application of yuzu seed oil significantly inhibited the increase of the clinical skin severity score and the ear thickness in comparison with the negative control group (olive oil). Especially, the crude oil (1.24±0.11)μg/g (mean±SD.; n=5) and the distilled oil (1.00±0.07) strongly decreased the histamine levels in the skin lesions of the control group (1.84±0.13) at 4 weeks after antigen challenge compared to the negative control group, respectively.
In conclusion, yuzu seed oil is a good candidate to be used as an alternative medicine against atopic dermatitis.

P5.16 Liver immunology

P5.16.01
Contribution of quanta-lite® M2 EP (MIT3) test in primary biliary cirrhosis immunological diagnosis

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Introduction: PBC is a chronic auto-immune hepatopathy, characterized by an inflammatory destruction of the small intra-hepatic common bile ducts. In 90-95% of cases, PBC is accompanied by production of M2 AMA. These AMA, usually, are sought by IFA on LKS. However, they can be specifically detected by ELISA (MIT3) using as antigen the Pyruvate Dehydrogenase Complex (PDC).
Objective: The aim of this study is to compare results obtained using ELISA Quanta-Lite® M2 EP (MIT3) for detection and semi-quantitative measurement of AMA (M2) with those obtained using IFA in PBC immunological diagnosis.
Methods: Our study related to 217 sera of patients with suspected PBC. Among these sera : 174 (80%) are AMA (-) using IFA and 43 sea (20%) are AMA (+). All the sera were also tested using ELISA Quanta-Lite® M2 EP (MIT3) as well as sear of 100 healthy controls.
Results: Concordance rate between IFA and ELISA Quanta-Lite® M2 EP (MIT3) for AMA detection were 81%. Among 174 sera IFA (-) (LKS): 140 (81%) were MIT3 (-), on the other hand 34 sera (19%) showed a positivity of MIT3 test; while on 43 sera IFA (+): 36 (84%) were MIT3 (+) and 7 (16%) MIT3 (-). Quanta-Lite® M2 EP (MIT3) has a specificity of 98%: on the 100 healthy controls tested by ELISA: only 2 showed positivity, whereas 98 were MIT3 (-).
Conclusion: A good concordance rate exists between IFA and ELISA (MIT3) for AMA (M2) detection, with, however, a better sensitivity of ELISA compared to IFA.

P5.16.02
Interleukin-6 and C-reactive protein are overexpressed in the liver of perinatal deaths diagnosed with fetal inflammatory response syndrome

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Anatomopathologic studies have failed to define the Fetal Inflammatory Response Syndrome (FIRS) as cause of fetal death. Here, liver fragments of perinatal autopsies were collected at a University Hospital from 1990 to 2009 and classified according to cause of death, perinatal stress and gestational age (GA) of the fetus. The IL-6, TNF-alpha and C-Reactive Protein (CRP) expression were immunostained respectively with primary antibody. Cases with Congenital Malformation, Ascending Infection and Perinatal Anoxia showed increase respectively of IL-6, CRP and TNF-alpha. Prematures presented higher expression of IL-6 whereas term births showed higher expression of CRP. Cases classified as acute stress presented higher expression of IL-6 and TNF-α and cases with chronic stress presented higher expression of CRP. GA correlated negatively with IL-6 and positively with CRP and TNF-alpha. Body weight correlated negatively with IL-6 and positively with CRP and TNF-alpha. Despite the diagnosis of FIRS being clinical and based on serum parameters, the findings in the current study allow the inference of FIRS diagnosis in the autopsied infants, based on an in situ liver analysis of these markers

P5.16.03
Critical role of invariant natural killer T cells in xenobiotic induced murine model of primary biliary cirrhosis

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Primary biliary cirrhosis (PBC) is an organ-specific autoimmune liver disease characterized by the presence of anti-mitochondrial antibodies (AMAs) and the destruction of small intrahepatic bile ducts with portal inflammation. The major autoantigen is the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2). We have developed a murine model of PBC based upon immunization with 2-OA-BSA. To define the role of iNKT cells in PBC, we examined PBC disease progress of α-GalCer, an iNKT cell activator, and 2-OA-BSA co-immunized wild type mice and 2-OA-BSA immunized iNKT cell deficient $CD1d^{-/-}$ mice. We found that simultaneously activation of iNKT cells by α-GalCer leads to a profound exacerbation of portal disease in 2-OA-BSA immunized mice, including increased production of AMAs, a significant increase in both $CD4^+$ and $CD8^+$ T cells, portal inflammation, bile duct damage, and fibrosis after 12 weeks. In addition, significant increases of IFN-γ and IL-17 production of recombinant mouse PDC-E2 (rmPDC-E2) stimulated T cells as well as supernatant anti-PDC-E2 antibodies of rmPDC-E2 stimulated B cells in 2-OA-BSA/α-GalCer immunized mice were noted. In 2-OA-BSA immunized $CD1d^{-/-}$ mice, decreased $CD4^+$ T cells and B cells in the liver were observed. In addition, IFN-γ production of liver mononuclear cells in 2-OA-BSA immunized $CD1d^{-/-}$ mice were also decreased. These results suggest that iNKT cells are critical in the early pathogenesis of PBC. With activation, iNKT cells secrete amounts of cytokines to recruit immune cells to the liver and then accelerate bile duct damage.
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P5.16.04
Deranged neutrophil phagocytic capacity on cirrhosis and hyperammonemia is recovered by the blue agave inulin effect on cirrhotic patient gut microbiota

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Intestinal bacterial overgrowth and endotoxemia play an important role on the cirrhosis complications like spontaneous bacterial peritonitis and hyperammonemia. The increased susceptibility to infection of cirrhotic patients is by peripheral blood neutrophil deranged phagocytosis and increased proinflammatory cytokine production. The short chain fatty acids (lactic, acetic and butyric acid) are bacterial metabolic products which can unfold complex carbohydrates, blue agave inulin. Several papers have been considered that butyrate is the preferred energy source for colonocytes and it get stronger tight junctions between them. Finally, Butyrate inhibits inflammatory responses through NFκB inhibition. The aim of this research was to stablish the blue agave inulin effect on cirrhotic and hyperammonemia patients through reducing serum levels of ammonium and TNF-α, restoration of the phagocytic capacity of peripheral blood neutrophils and increasing colonization resistance against invading pathogens by gut microbiota modifications. The study included sixteen cirrhotic patients of whatever etiology but with hyperammonemia/Minimal hepatic encephalophaty. Two groups were formed by 8 patients randomised. One group was treated with inulin, and the second with lactulose. Ammonium levels was measured by spectrophotometric method, phagocytic index and phagocytic rate were for modified Cunningham assay, microbiota was assessed by RT-PCR. Finally, TNF-α was mesured by ELISA kit. Inulin or lactulose don´t decrease ammonium arterial levels (P=0.125 y 0.344 respectively) same as TNF.alpha (P=0.268 and P=0.422). However, just inulin increased phagocytic index and phagocytic rate (P=0.016 and 0.031). The Inulin effect of 5 days of treatment doesn't decrease arterial ammonium but restore neutrophil phagocytic capacity.

P5.16.05
Disruption of chemokine-glycosaminoglycan interactions protects the liver in the murine Concanavalin A-induced acute hepatits model

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Autoimmune-mediated liver injury is responsible for the pathogenesis of immune-, viral- and drug-associated liver diseases, conditions that constitute a significant health problem worldwide. The purpose of this research was to investigate the therapeutic potential, in liver disease, of inhibitors that target chemokine interactions with glycosaminoglycans (GAGs). Because of the key role played by GAG-attached chemokines in the migration of leukocytes into inflamed tissues, we predicted that interference with the binding of chemokines to GAGs, would lead to reduced leukocyte infiltration and decreased liver damage in a Concanavalin-A (ConA)-induced model of acute hepatitis in mice. Administration of a synthetic peptide that interferes with the binding of chemokines to GAGs into ConA-treated mice resulted in significant protection against liver damage. Peptide-treated mice showed a substantial reduction in areas of hepatocyte necrosis upon histological examination, and had significantly lower serum levels of liver enzymes (ALT and AST) as well as hepatocyte apoptosis compared to mice that received only ConA. Immunofluorescent staining of liver slices showed that this protection was correlated with reduced hepatic levels of IFNγ, IL-17 and TNFα as well as with significant reductions in the numbers of infiltrating T leukocytes and myeloid cells (both granulocytes and monocytes). Moreover, among the infiltrating T cells, peptide-treated animals showed reduced percentages of $IFN\gamma^+$-$CD4^+$ and $IFN\gamma^+$-$CD8^+$ cells. These results suggest that targeting chemokine/GAG interactions in vivo decreases the recruitment and activation of leukocytes and has potential as anti-inflammatory therapy in liver diseases.

P5.16.06

Development of a new murine model for autoimmune hepatitis useful for pathogenic studies and preclinical trials

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Background and aims: Autoimmune hepatitis (AIH) consists of an immune-mediated destruction of hepatocytes due to the loss of the immunologic tolerance against self-antigens. Understanding the molecular mechanism underlying AIH is critical to improve current therapies. Animal models resembling human AIH are essential. In the present work a murine model of AIH was developed.
Methods: Adenoassociated viruses expressing constitutively and hepatospecifically murine IL-12 or luciferase (AAVIL-12/AAVLuc) were intravenously injected in C57BL/6 mice at a very low dose. At different time points after treatment, transaminases, cytokines and presence of auto-antibodies were analyzed. Animals were sacrificed 30 and 60 days post-treatment for histopathological analysis of the liver and study of the intrahepatic lymphocytic populations. To determine the role of CD4+ and CD8+T cells they were depleted using specific antibodies.
Results: Mice treated with AAVIL-12, but no AAVLuc, developed anti-nuclear (ANA) and anti-smooth muscle actin (ASMA) antibodies, but no anti-mitochondrial or anti-LKM. Livers showed an initial portal hepatitis that, 60 days post-treatment, progressed into an erosive necrosis with a concomitant rise in serum transaminases. We observed an increase in $CD8^+$ and $CD4^+$ cells and depletion experiments showed that both were essential for the development of AIH.
Conclusions: AAV-mediated expression of low levels of IL-12 in the liver leads to the break of immunological tolerance to self-antigens giving rise to ANA and ASMA and to an erosive necrosis in the liver with significant lymphocytic infiltration. This scenario mimics human type I AIH and constitutes the first reported model without the use of transgenic animals.

P5.16.07

High frequency of antiphospholipid antibodies in primary biliary cirrhosis

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Background: The aim of our study was to evaluate, retrospectively, the frequency of serum autoantibodies of antiphospholipid syndrome (APLS) in Tunisian patients with primary biliary cirrhosis (PBC).
Materials and methods. We analyzed 80 PBC sera (all positive in anti-mitochondrial antibodies) and 80 sera from blood donors. ELISA was used to determine antibodies against cardiolipin and beta2 glycoprotein I.
Results: The frequency of antiphospholipid antibodies was significantly higher in PBC patients than in controls (70% vs 5%, $p<10^{-6}$). The frequency of aCL (IgG or IgA or IgM) was significantly higher in PBC patients than in the control group (23.7% vs 3.7%, p=0.0005). The frequencies of aCL IgA and aCL IgM in PBC patients sera were significantly higher in patients than in the control group (10% vs 0%; p=0.003 and 20% vs 2.5%; p=0.001 respectively). Two patients out of 80 (2.5%) had aCL IgG, aCL IgA and aCL IgM and none of the control group had this three isotypes. The frequency of aβ2GPI (IgG or IgA or IgM) was significantly higher in PBC patients than in the control group (70% vs 1.25%; $p < 10^{-6}$). The frequencies of aβ2GPI IgG, aβ2GPI IgA and aβ2GPI IgM in PBC patients sera were significantly higher in patients than in the control group (12.5% vs 0%, p=0.003; 62.5% vs 1.2%, $p<10^{-6}$ and 21.2% vs 0%, $p<10^{-4}$ respectively).
Conclusion: Autoantibodies related to APLS were present in the majority of patients with PBC, reflecting the ability of these antibodies to engage mediators of damage.

P5.16.08

CD8 T cell responses to T cell apoptosis-derived antigens as a marker of chronic immune activation and liver inflammation in chronic HCV infection

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The mechanisms responsible for chronic liver inflammation and fibrosis observed in several patients with chronic hepatitis C are not completely elucidated. Current opinion is that liver damage is caused by HCV-specific T cells attacking HCV-infected hepatocytes. However, they represent only a minority of the about 10-20 billions lymphocytes that generally infiltrate an infected liver. We have previously shown that T cell apoptosis creates a wide assortment of caspase-cleaved peptides, which can be cross-presented by dendritic cells to a broad repertoire of autoreactive CD8+ T cells specific for apoptotic antigens. This event takes part to the chronic immune activation (CIA) observed in chronic infections. We show here increased T cell apoptosis and high frequencies of CD8 T cells specific for self-apoptotic and HCV antigens (as detected by dextramers of class I molecules complexed with the relevant paptides or by IFN-γ-ELISPOT assay) in the periphery of chronically-HCV infected patients. This phenomenon is even more represented in inflamed livers. Importantly, a significant reduction of HCV-CD8 T cell frequencies since the first month of therapy (Peg-IFNα/ribavirin) and a decrease of self-reactive CD8 T cells since the third month are seen. Interestingly, we observe a linear correlation between the self-apoptotic (but not the virus-specific) CD8 T cell frequencies and the Ishak score (liver inflammation/fibrosis scoring system) on the liver biopsies. Altogether, these data suggest a possible involvement of self-apoptotic CD8 T cells in liver inflammation/fibrosis in chronic hepatitis C.

P5.16.09

Activation of $CD11b^+$ Kupffer cells/macrophages as a common cause for exacerbation of TNF/ Fas-ligand-dependent hepatitis in hypercholesterolemic mice

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We have reported that the mouse hepatic injury induced by either α-galactosylceramide (α-GalCer) or bacterial DNA motifs (CpG-ODN) is mediated by the TNF/NKT cell/ Fas-ligand (FasL) pathway. $F4/80^+$ Kupffer cells can be subclassified into $CD68^+$ subset with a phagocytosing capacity and $CD11b^+$ subset with a TNF-producing capacity. $CD11b^+$ subset increase if mice are fed high-fat and cholesterol diet (HFCD). The present study examined how a HFCD affects the function of NKT cells and $F4/80^+$ $CD11b^+$ subset and these hepatitis models. After the C57BL/6 mice received a HFCD, high-cholesterol diet (HCD), high-fat diet (HFD) and control diet (CD) for four weeks, the HFCD mice increased surface CD1d and intracellular TLR-9 expression by the $CD11b^+$ population compared to CD mice. Hepatic injury induced either by α-GalCer or CpG-ODN was more severe in HCD and HFCD mice compared to CD mice, which was in proportion to the serum TNF levels. In addition, liver cholesterol levels but not serum cholesterol levels nor liver triglyceride levels were involved in the aggravation of hepatitis. The FasL expression of NKT cells induced by both reagents was upregulated in HFCD mice. Furthermore, the liver
mononuclear cells and purified $F4/80^+$ $CD11b^+$ subset from HFCD mice stimulated with either reagent *in vitro* produced a larger amount of
TNF than did those from CD mice. Intracellular TNF production in $F4/80^+$ $CD11b^+$ cells was confirmed**.** The increased number of $F4/80^+$

CD11b$^+$ Kupffer cells/macrophages by HFCD and their enhanced TNF production thus play a pivotal role in TNF/NKT cell/FasL dependent hepatic injury.

P5.16.10
In vitro infection with Hepatitis C Virus increases the phenotype and the suppressive activity of the human regulatory T cell

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Hepatitis C virus (HCV) infection is characterized by a high risk of chronicity, which may progress to cirrhosis and hepato-cellularcarcinoma. We have shown that natural regulatory T cell (Treg) play an important role in this disease progression and are associated with the severity of the HCV recurrence after liver transplantation. In this context, one essential question remains to know whether HCV infection could alter Tregs function thus promoting liver pathogenesis.
Tregs were isolated from blood of healthy donors and infected with HCVcc/JFH-1. Internalization of HCV protein was confirmed by immunofluorescence and western-blotting. Expression of several cell markers associated to Treg phenotypic and function was analyzed using flow cytometry and Q-PCR. The Treg activity is evaluated by suppression tests (MLR) and correlated with cytokines secretion profile by ELISA.
We showed that infected Treg significantly increases the expression of virus receptors and that HCV proteins (Core, NS5A) are well internalized. We also showed that HCV infection promotes the Treg proliferation and probably the recruitment of infected Treg by infected liver cells. In addition, HCV infection potentiates the "suppressive phenotype" of Treg showing a significant increase of markers associated with their phenotype and immunosuppressive function. These results were correlated with increased suppression activity and increased secretion of immunosuppressive cytokines (IL-10, TGF-β).
This work showed for the first time that HCV is able to infect human Treg and promote their recruitment and suppressive activity, contributing to one mechanism by which HCV could escape the immune system thus leading to a worsening of the disease.

P5.16.11
Disproportionate decline of liver infiltrating regulatory T cells under therapy for autoimmune hepatitis

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Background & Aims: Autoimmune hepatitis (AIH) is a chronic autoimmune inflammation of the liver usually requiring life-long immunosuppression. The mechanisms responsible for the disease initiation are largely unknown. A contribution of deficient regulatory T cells in the peripheral blood was controversially discussed recently. So far investigations in the target organ of the autoimmune attack have been limited to single parameter analysis in untreated AIH.
Methods: We analyzed the pattern of liver infiltrating T, B and regulatory T cells (Treg) semi-quantitatively with a simultaneous multicolour immunofluorescence before and under therapy in patients with AIH. Data was correlated with disease activity and clinical outcome.
Results: Intrahepatic CD4+ cells dominate over CD8+ at diagnosis, but with increasing disease activity the CD4+/CD8+ ratio approaches 1. Treg enrich in liver infiltrates in untreated AIH independent from disease activity but may not be sufficient to balance the overwhelming infiltration of effector T cells (Teff). But more important immunosupressive therapy with steroids and azathioprin caused a disproportional loss of intrahepatic Tregs and better treatment response seems be associated with higher intrahepatic Treg/Teff ratios. Intraportal B cells correlated with serum IgG suggesting an autochtonous intahepatic production. This might be regulated by Tregs as serum IgG negatively correlated with Treg/B cell ratio.
Conclusion: Intrahepatic Tregs are rather enriched than numerically deficient in untreated AIH. The disproportional decrease of Tregs during immunosuppressive therapy might explain the high relapse rate after discontinuation of therapy. Therapies increasing intrahepatic regulation might be better suited for long-term control of AIH.

P5.16.12
IFNγ/STAT1-mediated tissue factor expression contributes to T cell-mediated hepatitis via induction of hypercoagulation in mice

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Concanavalin A (Con A) treatment induces severe hepatitis in mice in a manner dependent on T cells, IFN-γ and TNF. Treatment with the anticoagulant heparin protects against hepatitis despite normal production of IFNγ and TNF. Here, we investigated molecular and cellular mechanisms for hypercoagulation-mediated hepatitis. After Con A challenge liver of wild-type mice showed prompt induction of Ifnγ and Tnf, followed by mRNA expression of tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1), which initiate blood coagulation and inhibit clot lysis, respectively. The mice developed dense intrahepatic fibrin deposition and massive liver necrosis. In contrast, Ifnγ-/- mice and Ifnγ-/-Tnf-/- mice neither induced Pai1 or Tf, nor developed hepatitis. In wild-type mice TF blockade with an anti-TF mAb protected against Con A-induced hepatitis, whereas Pai1-/- mice were not protected. Both hepatic macrophages and sinusoidal endothelial cells expressed Tf after Con A challenge. Macrophage-depleted wild-type mice reconstituted with hematopoietic cells including macrophages deficient in STAT1 essential for IFNγ signaling, exhibited substantial reduction of hepatic Tf and of liver injuries. This was also true for macrophage-depleted Stat1-/- mice reconstituted with wild-type macrophages. Exogenous IFNγ and TNF rendered T cell-null, Con A-resistant mice deficient in recombination-activating gene 2, highly susceptible to Con A-induced liver injury involving TF. Collectively, these results strongly suggested that pro-inflammatory signals elicited by IFNγ, TNF and Con A in both hepatic macrophages and sinusoidal endothelial cells are necessary and sufficient for the development of hypercoagulation-mediated hepatitis.

P5.17 Autoinflammatory diseases

P5.17.01
The proliferative response to PHA and SEB stimulation of peripheral blood mononuclear cells from oral lichen planus patients

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Oral lichen planus (OLP) is a chronic inflammatory disorder of oral mucosa, which represents T-cell-mediated autoimmune disease. It has been suggested that clones bearing specific T cell receptor repertoires may be involved in the pathogenesis of OLP. This study assessed the *in vitro* proliferative response of lymphocytes from OLP patients to phytohemagglutinin (PHA) and to *Staphylococcus aureus* enterotoxin B (SEB) stimulation. Peripheral blood mononuclear cells (PBMC) were obtained from 8 women clinical and histopathological diagnose of OLP (45 ± 14 yrs old), and 8 healthy female blood donors (41±16 yrs old) (CTRL). At the time of blood collection patients had at least one of four clinical manifestations: atrophic, bullous, erosive or reticular OLP, and were not in corticoid therapy for at least 3 months. PBMC were stained with 5,6-carboxyfluorescein diacetate

succinimidyl ester (CFSE, 10mM) and then stimulated *in vitro* with PHA (5µg/mL) or SEB (100ng/mL) for 5 days (37°C, 5% CO_2). Cell proliferation (PI) was evaluated using flow cytometry. The proliferative response to PHA stimulation was increased (P = 0.04, Student's t test) in OLP (PI = 1.87 ± 0.95) compared to CTRL (PI = 1.15 ± 0.48). Also, in response to SEB stimulation we observed a tendency (P = 0.06 Student's t test) to increased proliferation in OLP (PI = 1.18 ± 0.58 OLP versus 0.72±0.29 CTRL). Our results show increased proliferative response of PBMC from OLP patients to PHA, a polyclonal mitogen, and SEB, a repertoire restricted mitogen, thus suggesting a general, repertoire unrestricted, lymphocyte activation in OLP.

P5.17.02
Posttranscriptional Gene Regulation of IL-17 by the RNA-binding Protein HuR

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Interleukin 17 (IL-17A) is a proinflammatory cytokine produced by activated CD4+ Th17. Th17 cells are major contributors to autoimmune diseases, including multiple sclerosis. Although the transcriptional control of IL-17 and Th17 differentiation have been well studied, posttranscriptional gene regulation is unclear. The RNA-binding protein (RBP) HuR regulates the stability of many target mRNAs via binding the AU-rich elements (ARE) present in the 3' untranslated region (UTR), of many pro-inflammatory cytokines such as IL-4, IL-13 and TNF. Since IL-17 mRNA also possesses HuR binding motifs, we investigated whether HuR can regulate Il-17 expression. We demonstrated that HuR directly binds IL-17 mRNA 3' UTR by using RNA immunoprecipitation methods as well as biotin pull down assays. Th17 cells from $HuR^{fl/fl}$ KO mice have decreased IL-17 steady-state mRNA and protein expression compared to wild type Th17 cells. IL-17 mRNA stability was decreased by 3-fold in HuR KO mice. We used the experimental autoimmune encephalitis (EAE) model of neuroinflammation to investigate whether HuR plays a role in CD4+ Th17 differentiation and disease initiation. Mice with adoptive transfer of HuR KO $CD4^+$ T cells had delayed onset, reduced severity of EAE symptoms, as well as reduced CNS inflammation and decreased IL-17 production by Th17-producing cells recovered from the CNS. Our results reveal HuR-controlled posttranscriptional regulatory mechanisms of Th17 differentiation and direct effects upon EAE initiation. These findings could provide novel therapeutic targets for treatment of IL-17- mediated autoimmune neuroinflammation and also potentially other diseases in which IL-17 plays a major role.

P5.17.03
Endogenous IFN-γ prevents escalation of normal immunity into a Th17-driven inflammation reminiscent of systemic juvenile idiopathic arthritis

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Whereas the mechanisms that initiate and maintain strong inflammatory reactions against endogenous and exogenous stimuli are well documented, regulatory circuits that prevent such reactions from escalating to harmful proportions are less well understood. Here we have studied endogenous IFN-γ as a proposed key player in such circuits. Mice were given heat-killed mycobacteria in Freund's adjuvant, an almost innocuous immunological challenge in wildtype mice. In the absence of IFN-γ, normal immunity escalated into a persistent systemic inflammatory syndrome. Elevated IL-6 production and cascading Th17 activation resulted in the development of arthritis, weight loss, anemia, neutrophilia, thrombocytosis, lymphadenopathy, splenomegaly, and abundant immature blood cells and hemophagocytes, all features reminiscent of systemic juvenile idiopathic arthritis (sJIA) in humans. The lack of a suitable animal model is a major drawback in the investigation of sJIA. The underlying pathogenic mechanism is not understood but prolonged stimulation of immune cells and excessive production of cytokines are considered to be important in the pathogenesis of the disease. Here we descibe an animal model of sJIA. The data point IFN-γ as an important counter-regulator of IL17, and suggest that a failure to produce sufficient IFN-γ during normal immunological events may be a primary driver of systemic juvenile idiopathic arthritis development.

P5.17.04
Risk factors of asthma in preschool children

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Asthma is the leading cause of chronic inflammatory illness in children. Common symptoms include wheezing, coughing, chest tightness, and shortness of breath. Asthma is caused by a combination of environmental and genetic factors. The prevalence of asthma in children, for unknown reasons, has increased in many countries over recent years steadily. The International Study of Asthma and Allergies in Childhood (ISAAC), was founded to maximize the value of epidemiological research into asthma and allergic disease, by establishing a standardized methodology and facilitating international collaboration. To plan effective interventions to reverse this trend we need a better understanding of the risk factors for asthma in early life. Multiple factors were associated with asthma symptoms in preschool children. Factors which increased the risk of asthma were: atopy or presence of allergies, having a parent with a history of asthma or allergies, low birth weight, having had a serious respiratory infection in the first 2 years of life, male gender, lower air conditioning, using feather pillow and blanket bedding and exposure to tobacco smoke before or after birth. Breast feeding and consumption of polyunsaturated fats decreased the risk of asthma. Also, change in environmental factors and sedentary life style from first year of life may prevent the development of childhood asthma.

P5.17.05
Pertussis toxin- driven IL-1β production is required for highly encephalitogenic GM-CSF$^+$ T cell generation in EAE pathogenesis

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IL-1β is a pleiotropic cytokine and plays an important role in several inflammatory disorders in humans and in animal models. In our study we showed that mice deficient for IL-1β or for a component of the inflammasome (ASC), after immunization with myelin antigen (MOG) emulsified in complete Freund's adjuvant (CFA) and treated with a double injection of pertussis toxin (PTX), did not develop experimental autoimmune encephalomyelitis (EAE). Autoreactive T cells were primed in wild-type (WT), $IL\text{-}1\beta^{-/-}$ and $ASC^{-/-}$ mice. While in WT mice T cells proliferated extensively and acquired a multifunctional phenotype ($IL\text{-}17^+IL\text{-}22^+IFN\gamma^+GM\text{-}CSF^+$), in knockdown mice the cells expanded poorly and showed reduced capacity to produce inflammatory cytokines. The same defects were observed both in $Myd88^{-/-}$ and $CCR2^{-/-}$ animals, as well as by depleting Gr^+ myeloid cells. We found that the induction of multifunctional T cells in WT mice was dependent on the presence of PTX. After PTX injections, IL-1β secretion was induced by $CD11c^+$ and $Gr1^+$ myeloid cells which were highly recruited in secondary lymphoid organs. These data supported the notion that the disease-inducing effect of PTX was due to its ability to induce recruitment of $Gr1^+$ myeloid cells, production of IL-1β and differentiation of pathogenic multifunctional T cells.

P5.17.06
Expression of Suppressor of Cytokine Signalling (SOCS) 1 and 3 in Behçet's Disease

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Behçet's disease (BD) is an autoinflammatory disease of unknown aetiology where a pro-inflammatory cytokine profile is a key feature. Suppressor of Cytokine Signalling molecules (SOCS) 1 and 3 belong to a family of eight intracellular proteins which have been shown to negatively regulate cytokine signals through the JAK-STAT pathway and have profound effects on many immunological processes. SOCS 1 deficient mice develop severe skin and eye lesions, reminiscent of BD, through recruitment of TH1 and TH17 cells into these non-lymphoid tissues. We have investigated the expression of SOCS1, 2 and 3 in Neutrophils and PBMCs in BD and healthy controls (HC) along with TH1 and TH17 cytokines and the Stats and Transcription factors associated with these pathways. Proteins were examined using western blot and mRNA expression was quantified by RT-PCR. Cytokine profiles of serum were investigated using multiplex bead analysis and cytokine secreting cells were enumerated by ELISPOT. Stats 1, 3 and 5 and the transcription factors t-bet and ROR γt were assayed using PhosFlow FACS analysis.
Results: SOCS3 was down regulated in PBMCs but up regulated in Neutrophils in BD patients compared to healthy controls. SOCS 1 and 3 show inconsistent bands in western blot analysis in both Neutrophils and PBMC's. IL-6, IL-23 and IL-17 ELISPOTS were detected after stimulation with LPS and human heat shock protein. Transcription factors and Stats showed altered levels in BD compared HC and also between active and inactive disease episodes, consistent with a failure of SOCS function in BD.

P5.17.07
Analysis of neutrophil function in severe and moderately affected Chronic Fatigue Syndrome subjects

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Research has commonly shown that people with Chronic Fatigue Syndrome (CFS) have immune system abnormalities. Decreases have been found in neutrophil respiratory burst in a CFS cohort. Reduced ability of neutrophils to eliminate pathogens can contribute to the impaired immune function in CFS subjects. It is unknown whether neutrophil function is more impaired in patients severely affected by CFS. The purpose of this study was to examine neutrophil activity including phagocytosis and respiratory burst in patients with severe CFS in comparison to those with moderate symptoms and non-fatigued controls. CFS subjects were characterised using the CDC Case Definition and the International Consensus Criteria for CFS. CFS subjects were then grouped as either moderate or severely affected based on health and quality of life questionnaires. Blood samples were taken from age and sex matched non-fatigued controls, moderately affected and severely affected CFS subjects. Response to *E.coli* bacteria was measured in neutrophils by assessing phagocytic activity and respiratory burst, these were then analysed on the flow cytometer. Preliminary data from CFS subjects showed differences in neutrophil function based on respiratory burst and phagocytic activity when compared to the control group. These results suggest that differences in neutrophil function in CFS patients may contribute to CFS related immune dysfunction.

P5.17.08
To investigate the roles of IL-12 family cytokines on islet graft rejection and tolerance

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IL-12 family cytokines are mainly produced by antigen presenting cells and have crucial roles in the control of T helper (Th) cell differentiation and function. It has been reported that Th1 and Th17 cells are linked to the graft rejection whereas Th2 and Treg cells are correlated to graft tolerance. In this study, we aim to investigate whether IL-12 family cytokines modulate the differentiation of Th cells and subsequently influence the survival of islet grafts in diabetic recipients. Bone marrow-derived dendritic cells (BMDCs) from IL-12 knockout, IL-23 or IL-27 knockdown mice displayed similar capacity to induce T cell proliferation. However, BMDCs from IL-12 knockout or IL-23 knockdown mice showed slightly reduced ability to promote Th1 and Th17 differentiation. In contrast, BMDCs from IL-27 knockdown mice enhanced Th1 and Th17 development. In transplantation experiments, the survival of islet grafts was prolonged in IL-12 knockout or IL-23 knockdown recipients and was reduced in IL-27 knockdown mice after diabetogenic T cell transfer, as compared with the control recipients. Histological examination of grafts from IL-12 knockout or IL-23 knockdown recipients displayed less severe infiltration than grafts from control group. Our findings suggest that IL-12 and IL-23 mediate the islet grafts rejection through promoting Th1 and Th17 differentiation, respectively. IL-27 acts as a regulatory cytokine to dampen immune responses and reduction of IL-27 leads to increased T cell activation and early graft rejection.

P5.17.09
Characterization of tonsil infiltration and gene expression profile of innate sensors in PFAPA Syndrome

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The syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) is the most common periodic fever disease in young children. The etiology of this disorder is still unknown. Palatine tonsils are sites where innate immunity leads to onset of the adaptive immunity, mediated by B and T lymphocytes.
We aimed to investigate differences in leucocytes subpopulations and innate receptors gene expression of palatine tonsil cells from patients with PFAPA in order to understand the pathogenesis of this inflammatory condition. We have collected tonsil tissue from 2 groups of pediatric patients undergoing tonsillectomy: PFAPA patients (n=20), and patients who had indication of bacterial tonsillitis (control group, CG) (n=16) . We have performed staining of subpopulations on tonsil cells and tissues using flow cytometry and immunohistochemistry. We have analyzed NLRs, and RLRs gene expression by quantitative real-time RT-PCR.
FACS analysis demonstrated a higher number of naïve and a significantly lower percentage of effector memory $CD4^+$ and $CD8^+$ T cells in PFAPA patients compared to CG. Remarkably, we observed a considerably recruitment of NK cells in tonsils of PFAPA patients with respect to CG. In particular, we discovered a significant expansion of $CD56^+CD16^-$ and $CD56^+CD16^+$ NK cell subsets when compared to CG. Moreover, in PFAPA patients we demonstrated a significant increase in the gene expression of NALP1 and NALP3 when compared to CG. These results indicate a possible involvement of NK cells and of innate receptors in pathogenesis of PFAPA supporting the crucial role of the innate immunity.

P5.17.10
Autoantibodies characteristics of patients with autoimmune liver disease in Uygur people

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Objective To study the clinical significance of immunological characteristics in patients with autoimmune liver disease in Uygur people. Methods A total of 51 patients with autoimmune liver disease in Uygur people were examined for anti-nuclear antibodies (ANA), anti-mitochondria antibodies (AMA), and anti-smooth muscle antibodies (SMA) using immunofluorescent assays. Soluble liver antigen (SLA)/liver-pancreas antigen (LP), anti-liver kidney microsomal antibody-1 (LKM-1), liver cytosolic-1 (LC-1), subtype of AMA (M2), as well as ANA profile were detected by an immune blotting assay and an enzyme-linked immune absorbent assay, respectively. Cytokines were tested by flow cytometry. Results Out of the 51 patients with autoimmune liver disease in Uygur people, 20 cases were diagnosed with autoimmune hepatitis (AIH). The AIH group could be divided into 3 subtypes according to the difference of the autoantibodies as I (65%), II (20 %), III (10 %). 31 cases were diagnosed with primary biliary cirrhosis (PBC), who were 97% AMA positive and 94% M2 positive. Conclusion Autoimmune liver disease is a progressive liver inflammation accompanied by production of characteristic antibodies. Detection of autoantibodies is useful clinically for identifying and classifying liver diseases.

P5.17.11
BAFF, κ and λ free light chains, C4d and IgG in serum and relations with peripheral blood lymphocyte populations in patients with primary Sjögren's syndrome

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Serum markers and their relations with peripheral blood lymphocyte populations` (absolute counts) of patients with primary Sjögren's syndrome (pSS) with (1pSS) or without (2pSS) extra-glandular manifestations were investigated.
Blood samples for flow cytometry and immunoassay were collected from 30 1pSS, 23 2pSS patients and 35 healthy controls.
Significantly increased levels of B-cell activating factor (BAFF) and IgG in patients with pSS were observed, results between pSS groups were comparable. Complement fragment C4d levels in all groups were similar. κ free light chains (FLC) levels were significantly increased in pSS patients likewise in 2pSS group in comparison to 1pSS group. λ FLC levels were significantly higher only in 2pSS group compared to control group. κ/λ FLC ratio in all groups were comparable. Correlations in 1pSS group were observed among κ, λ FLC and BAFF levels. Correlations were detected in 2pSS group between κ and λ FLC levels, κ/λ FLC ratio negative correlated with λ FLC and C4d levels. In 1pSS BAFF levels negative correlated with CD3+, CD4+, CD8+, CD8brightCD57-CD27+ and CD19+ cells. κ/λ FLC ratio correlated with CD3-CD16+56 and CD19+ cells. C4d levels correlated with CD8+, CD8brightCD57-CD27+ and CD3+CD16+56 cells. In 2pSS group only negative correlation between λ FLC levels and CD4+ cells was observed.
Despite the fact that between pSS groups only κ FLC levels differed, distinct linkage of investigated markers with peripheral blood lymphocyte populations` were observed. The distributions of markers investigated in 1pSS and 2pSS patients groups may indicate several ongoing mechanisms during the progression of the disease.

P5.17.12
Antinuclear antibodies without identified antigenic target: what is their diagnostic value?

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The objective of this study was to determine the clinical relevance and the diagnostic significance of positive ANA without identified antigenic target by the usual characterization technique.
It was a retrospective study conducted in our laboratory during 18 months. The inclusion criteria were the presence of an ANA titer ≥ 1/320 with negative characterization result. ANA screening was performed by indirect immunofluorescence (IIF) on Hep2 cells. Each positive serum was tested by IIF on Crithidia luciliae (anti-native DNA) and by immunodot (Euroimmun®, profile 3). Sera of systemic lupus erythematosus (SLE), myositis, and scleroderma patients were tested for antisynthetase antibodies.
Sera of 90 patients were studied: 18 men and 72 women. Drug-induced ANA was found in eight patients. The most frequent clinical symptoms were joint (56.7%), cutaneous (54.4%) and constitutional symptoms (45.6%). The diagnosis of an autoimmune disease was suspected in 49 patients (54.5%) and confirmed in 30 (33.3%) including 20 cases of connective tissue disease: myositis (n = 6), scleroderma (n = 5), Sjögren's syndrome (n = 3), SLE (n = 4), rheumatoid arthritis (n = 6) and antiphospholipid syndrome (n = 4). Other autoimmune diseases were less frequent. The anti-Ku antibody was detected in the majority of patients with connective tissue disease.
Our study demonstrated the diagnostic value of the presence of ANA even in the absence of known antigenic target, confirmed the role of the IIF as "gold standard" test for ANA screening, and suggested the usefulness of the addition of Ku antigen in the immunodot classic profile.

P5.17.13
Cyclophosfamide plus Rituximab treatment in a case of plurirefractory cutaneous sarcoidosis with large vessels vasculitis

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Sarcoidosis is a multi-systemic disease; its clinical course ranges from an incidental finding to a life-threatening disease, sometimes associated with other immunological diseases. Cutaneous involvement is present in about 20% of patients. We report a case of atypical, aggressive and therapy-resistant cutaneous sarcoidosis. A 57-y old Caucasian male was referred to our attention with a diagnosis of cutaneous sarcoidosis; the patient reported joint pain in the hands, feet, ankles and shoulders and a transient Raynaud's phenomenon. Physical examination revealed papules and plaques on the limbs. Seriated skin biopsies showed granulomatous and giant-cellular inflammation consistent with sarcoidosis (quantiferon test and PCR for atypical mycobacteria were negative). Lymphocyte subpopulations in the BAL were normal. HRCT showed enlarged bilateral mediastinal lymph nodes. PET-CT scan was negative. Laboratory findings showed an increase of inflammatory indexes, sACE, 24-h calciuria and phosphaturia. Patient was unsuccessfully treated with several regimens of treatment: steroids, methotrexate plus hydroxychloroquine, anti-TNFα, pulse i.v. cyclophosphamide and PUVA therapy, but a disease progression was observed with recrudescence of the skin lesions, the onset of severe large vessels vasculitis (an angio-CT showed an extensive involvement of the major abdominal and lower limbs arteries) and ocular involvement confirmed by episcleral biopsy. A cyclophosphamide plus rituximab regimen was initiated followed by surgical revascularization, observing until today (6 months later) a complete remission on skin and vascular disease. Despite classically sarcoidosis well respond to first-line steroid therapy, a wide spectrum of classic

immunosuppressant and biologic drugs, including rituximab, should be used in resistant patients.

P5.17.14
CD8+ T cells in the pathogenesis of Systemic Sclerosis

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T lymphocytes play an important role in systemic sclerosis (SSc), a connective tissue disease characterized by inflammation, fibrosis and vascular damage. Its most characteristic feature is cutaneous fibrosis that is attributable to excessive deposition of collagen and other connective tissue components by activated dermal fibroblasts. Although the pathogenesis is still unclear, this fibroblast activation is believed to result from their interaction with immune mediators, such as T cell-derived cytokines, and other growth factors. We recently found that dysregulated production of the profibrotic cytokine IL-13 by peripheral blood effector CD8+ T cells correlates with more severe forms of cutaneous SSc and is associated with defects in the molecular control of IL-13 production, such as the aberrant expression of the transcription factor GATA-3. The following are our most recent results. Firstly, we found high numbers of IL-13+ and CD8+ T cells in the skin lesions of early SSc patients. Secondly, we established that increased numbers of CD8+ T cells expressing skin homing receptors and producing IL-13 are found in the peripheral blood of SSc patients compared to normal controls. Finally, we demonstrated that CD8+ T-cell supernatants from SSc patients induce collagen production by normal skin fibroblasts and that this is inhibited by the addition of an anti-IL-13 antibody. We conclude that IL-13-producing CD8+ T cells are directly involved in the initiation and maintenance of dermal fibrosis in SSc. These new insights into disease pathogenesis suggest novel therapeutic targets that may be exploited for the treatment of SSc.

P5.17.15
Anti-BPI (bactericidal permeability increasing) in autoimmune vasculitis

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Anti-neutrophil cytoplasmic antibodies are autoantibodies directed against neutrophil lysosomal enzymes, accompanying vasculitis. Indirect immunofluorescence distinguishes two aspects: cytoplasmic (C-ANCA) and perinuclear aspect (P-ANCA). These antibodies are respectively directed against the proteinase 3 (PR3) and the myeloperoxydase (MPO). Recent studies showed the existence of other types of antibodies in vascular involvement, among these, those directed against the BPI (bacterial permeability increasing), cytotoxic protein specific of Gram (-) bacteria.
This work aims to evaluate the frequency of anti-BPI in 40 subjects (32F/8M) aged between 12 and 40 years with vasculitis, as well as the sensitivity of the technique used for their detection. The determination of antigenic targets in case of positive ANCA is performed by ELISA. 30 sera from healthy subjects, with no autoimmune disease served as controls. All the studied sera are PR3 (-) and MPO (-). 52,5% of them are BPI (+), 35% have a c-ANCA fluorescence and 17,5% have a p-ANCA fluorescence. 17, 5% of the sera have no anti-BPI antibodies. Finally, 30% of these sera are ANCA (-).
We conclude that BPI is a major target antigen for ANCA with a clear predominance of the cytoplasmic aspect. The presence of anti-BPI in the control population reflects the high sensitivity of ELISA test used, which requires confirmatory tests such as Western Blot.

P5.17.16
Association of enhanced Tim-3 expression on monocytes with disease activity in systemic lupus erythematosus

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Background: SLE is a typical chronic autoimmune disease. Considerable evidences support the notion that impaired clearance of apoptotic cells is respossible for the development of SLE. It is found that Tim-3 is also expressed on innate immune cells such as macrophage and dendritic cells, and it can mediate of the uptake, engulfment of apoptotic cells and plays a role in immune tolerance.
Aims: To investigate expression of Tim-3 in PBMC from SLE patients and healthy controls and analyze correlation between Tim-3 expression level and disease activity of SLE.
Methods: FITC-conjugated anti-human CD14 and PE-conjugated anti-human Tim-3 were used to detect Tim-3 expression by flow cytometric analysis. Annexin-V/PI cells were analyzed by flow cytometry.
Results: We found that expression of AnnexinV+ cells in peripheral blood from SLE patients was significant higher than that of healthy controls, and the expression of AnnexinV+ cells in peripheral blood positively correlated with SLEDAI scores and negatively correlated with C3. Expression of Tim-3 on CD14+ cells from active SLE patients with SLEDAI>5 increased compared with healthy controls, and the expression of Tim-3 on CD14+ cells in peripheral blood positively correlated with anti-dsDNA. Positive correlation between CD14+-Tim3+ cells and AnnexinV+ cells was found in peripheral blood from SLE patients.
Conclusions: Our results suggest that Tim-3 might participate in the pathogenesis of SLE by inducing CD14+ monocytes defects in eliminating apoptotic cells.
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P5.17.17
Effects of Vitamin D on regulatory T cells in lupus patients

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Introduction: Systemic lupus erythematosus (SLE) is a chronic, systemic, autoimmune disease with unknown aetiology, leading to an array of different clinical presentations. Regulatory cells are involved in the pathogenesis of SLE, and depletion of regulatory T cells has been shown to cause autoimmune diseases in animal models. Vitamin D as an environmental factor has anti-inflammatory and modulatory effects on the immune system, and Vitamin D deficiency was also suspected in the pathogenesis of SLE. In this research we studied the immunomedulatory effects of vitamin D on regulatory T cells (Tregs) by flow cytometry method, and on the expression level of its predominant cytokine genes in SLE patients by Real-Time PCR.
Materials and Methods: We enrolled 30 SLE patients (14 patients were new cases with active SLE and had received no therapeutic intervention). PBMCs isolated using Ficoll-Hypaque, and cultured in the presence of vitamin D. Then Regulatory T cells were analysed by flow cytomtry using a commercial Treg staining kit (eBioscience). RNA extracted using Tripure (Roche) , cDNA synthesized and the expression levels of FOXP3, and IL-6 analysed by Real Time-PCR.
Results: In the presence of Vitamin D the percentage of regulatory T cells and the expression level of FOXP3 increased significantly in PBMCs from SLE patients.
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P5.17.18
Oral changes in Wegener granulomatosis(WG)

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Wegener granulomatosis (WG) is a rare multisystem autoimmune disease of unknown etiology. Its hallmark features include necrotizing granulomatous inflammation and pauci-immune vasculitis in small- and medium-sized blood vessels. The pathologic hallmarks of Wegener granulomatosis are vasculitis of the small- to medium-sized vessels, "geographic" necrosis, and granulomatous inflammation, particularly in the airways. The initial pathologic lesion is that of the granuloma believed to be caused by cellular immune processes. These criteria from the American College of Rheumatology are used for enrolling patients in studies and should not be considered as diagnostic criteria. These criteria were developed before antineutrophil cytoplasmic antibody (ANCA) testing was in widespread use as a diagnostic test for WG.They are: nasal or oral inflammation, abnormal chest radiography findings, urinary sediment, granulomatous inflammation on biopsy.
Case report:
The patient comes for gingival hyperplasia, and looseness of the teeth. From history of the patient is suffering from schizophrenia, high blood pressure, diabetes and asthma has long away. The patient was subjected to biochemical, hematological tests, immunologic, viral serology, HIV and hepatitis, and a biopsy was taken.
Results showed decreased hemoglobin and hematocrites, high elevated leukocytes, eosinophils, basophils, neurophils, lymphocytes and monocytes, very high CRP and elevated levels of protein and leukocytes in the urine, decreased albumin, alpha and beta globulins were increased, positive EBV, CMV IgG, IgM and c-ANCA. A gingival biopsy was made, conferming the diagnosis of WG. The patient was referred for further treatment.

P5.17.19
Epidemiology study of patients with suspected and diagnosed Celiac Disease

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Background: Celiac disease (CD) affects 0.5% of the population and is more prevalent in women than men (3:1). Regarding age, there are two peaks, 1-3 and 30-50 years, which is higher the number of cases diagnosed.
Mehods: 2590 patients (985 children and 1605 adults) with suspicions or diagnostic of CD were included in the study between January-May 2013. Prevalence of CD markers (anti-tissue transglutaminase (tTG) IgA and IgG and anti-deamidated gliadin peptide (PDG) IgG antibodies), clinical features and biopsy were evaluated.
Results: Prevalence of positive markers of CD in children was 15% (148/985): 148 PDG IgG, 39 tTG IgA and 15 tTG IgG; 73 were female and 75 male. Positive biopsies (Marsh classification) were 23/52 (12/23: 1-3 years-old and 11/23: 3-17 years-old children). Main Symptoms: anaemia, pondostatural delay, malabsorption syndrome, chronic diarrhea, abdominal pain and constipation. Prevalence of positive markers of CD in adults was 6,9% (111/1605): 111 PDG IgG and 12 tTG IgA, 74 were female and 37 male. Positive biopsies were 18/35 (7/18: 18-29 years-old, 9/18 in 30-50 years-old and 2/18: 51-87 years-old adults). In addition to the classic symptoms these patients had polyneuropathies, liver diseases, polyarthralgia, pruritus, irritability and recurrent miscarriage.
Conclusions: CD in children showed the same prevalent in both gender, affecting more between ages 1-3 years and predominantly gastrointestinal symptoms. However, in adults is more common in female, the diagnosis is made mainly before 50 years and the symptoms are more variable. Screening of CD should be performed only with antiDGP IgG antibody determination.

P5.17.20
Dense fine speckled patterned antinuclear antibody in patients with fibromyalgia

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Fibromyalgia (FM) is characterized by chronic widespread pain and various manifestations. There has been reported high prevalence of psychologic distress, comorbidity, inflammatory autoimmune characteristics in patients with FM. Despite of its high prevalence, there is still no standardized laboratory test to suggest FMG or its severity. Anti-dense fine speckled antibody (anti-DFS Ab) Ab is found in 10% of healthy individuals, but only in a tiny population of with autoimmune rheumatic disease. It was initially identified as an atypical anti-nuclear antibody from a patient with interstitial cystitis. It has been studied in many specific rheumatic diseases. But there is no report in FM patients. FM patients (n=39) were recruited and 17 systemic lupus erythematosus patients and 19 healthy individuals were recruited as control groups. Especially for physical measurement, we evaluated weight, BMI, disease duration, tender point, Fibromyalgia Impact Questionnaire (FIQ), VAS (Visual Analogue Scale) and somatic symptoms. Anti-DFS Abs were measured with ELISA kit.
There is no statistically significant differences and relativity between anti-DFS Abs and age, BMI, disease duration, tender point, and FIQ. The level of anti-DFS Ab in FM patients did not differ from those in SLE patients and controls. However there was a significant correlation between the anti-DFS Ab level and VAS, fatigue. The prevalence of anti-DFS Ab in Korean FM patients was quite undetermined. In this study, the anti-DFS Ab level in FM patients was correlated with VAS and fatigue. In future, we should evaluate the autoimmune feature in FM.

P5.17.21
Expression and function of the NLRP3 inflammasome in Behçet's disease

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Behcet's disease (BD) is a systemic inflammatory disorder with diverse clinical manifestations. A new concept to BD is 'autoinflammation'. We explored the expression of the different components of the NLRP3 inflammasome in tissue and serum samples from patients with BD.
The expression of NLRP3, apoptosis-associated speck-like protein containing CARD domain (ASC) and caspase-1 in erythema nodosum lesions and serum of 25 BD and 25 non-BD patients were examined by immunohistochemistry and Western blot. The release of IL-1β, IL-18 from PBMCs and monocytes after stimulation with LPS, ATP and caspase-1 was evaluated in 20 BD patients and 10 healthy control (HC) by ELISA. The mRNA expressions of NALP3, ASC and caspase-1 were examined by Real time-PCR.The immunohistochemistry showed NLRP3 and ASC expression was higher in BD patients. When PBMCs from BD patients were stimulated with LPS, IL-1β secretion increased compared to control. With ATP, IL-1β release was more increased compared to LPS stimulation alone. NLRP3, ASC, caspase-1 mRNA was up-regulated in active BD compared to inactive BD and HC. When caspase-1 inhibitor, zYVAD-fmk, was treated on PBMCs, IL-1β secretion was decreased compared to LPS stimulation. When ATP was added to LPS primed PBMCs treated with zYVAD-fmk, IL-1β secretion was only slightly decreased. In conclusion NLRP3 inflammasome was upregulated both at the mRNA and protein levels in BD patients. Also in active BD, ATP stimulation in LPS primed PBMCs induced mRNA components of the NLRP3 inflammasome with increased IL-1 β production. However, caspase-1 inhibition was unsuccessful in decreasing IL-1β secretion.

P5.17.22
The cytokine production of peripheral blood mononuclear cells reflects the autoantibody profile of patients suffering from type 1 diabetes

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Type 1 diabetes (T1D) is an autoimmune disease characterised by the destruction of pancreatic beta-cells. Proinflammatory Th1 response is considered as the most pathological. Specific autoantibodies can be detected a long time before the disease clinical onset. This study describes the influence of synthetic diabetogenic autoantigens GAD65 and IA2 on the cytokine response of peripheral blood mononuclear cells (PBMC) obtained from T1D patients with regards to their antibody profile.
The study has been carried out on T1D patients (n = 40) positive for anti-GAD65 and/or anti-IA2 autoantibodies. Using flow cytometry we measured the cell type ratio in PBMC samples. The cells have been stimulated by three different concentrations of mentioned antigens. Their IFN-gamma and IL-17 production has been detected by ELISPOT assay.
In the case of both cytokines we have found a significant interaction between the way of stimulation (type and antigen concentration) and the patient's antibody profile ($p = 0.003$ IFN-gamma; $p < 0.001$ IL-17). The IA2 autoantibodies level correlates positively with the proportion of Tc cells and negatively with the proportion of Th cells ($p = 0.004$; $p = 0.001$). The IFN-gamma production correlates positively with the proportion of T cells and negatively with the proportion of NK cells in PBMC ($p = 0.001$; $p = 0.001$).
Our results show that patient´s autoantibody profile reflects the type of his/her cellular responses too. This approach can be helpful in designing immunointervention strategies aimed at preventing T1D onset. Such an immunointevention should apparently be individualised.
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P5.17.23
Glucocorticoid cooperates with complement prevent the development of systemic lupus erythematosus via maintenance of homeostasis of plasmacytoid dendritic cell

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Controlling the accumulation of type I interferon-producing activated and mature pDC (ampDC) is important for impeding the progression of systemic lupus erythematosus (SLE). Complement and glucocorticoid have been considered protective and therapeutic for SLE, here we investigated the effect of complement, glucocorticoid, and their interplay on regulating ampDC accumulation. PDC exhibit decreased expression of complement regulatory proteins (CRP) and increased sensitivity to complement-mediated cytotoxicity (CMC) along with maturation and activation. Nuclear acid-containing immune complexes trigger and enhance CMC to ampDC by downregulating CRP, but reduced complement activity in SLE impairs the CMC to ampDC, leading to the accumulation of ampDC. Glucocorticoid-treated pDC alive are immature but highly resistant to CMC because of expressing high levels of CRP despite glucocorticoid induces a large number of pDC deaths. Downregulation of P53, as a target of hsa-let-7b/7i which expression is dependent on P38 pathway by TLR9 or CD40, is responsible for the decreased CD59 expression on ampDC, while glucocorticoid blocks the activation of P38 pathway. So, CMC contributes to immature pDC homeostasis via deleting ampDC, which is facilitated by glucocorticoid. Together, our study outlines novel mechanistic explanations for protective role by complement and the therapeutic role of gucocorticoid in SLE.

P5.17.24
Accelerated spontaneous apoptosis of neutrophils in patients with familial Mediterranean fever

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The hallmark of acute inflammatory attacks in Familial Mediterranean fever (FMF) is inappropriate neutrophil activation and their influx to the serosal membranes. The mechanisms of neutrophil apoptosis in FMF are poorly understood. To investigate apoptotic behaviour of resting neutrophils and neutrophils cultured for 3 and 24 hours with TNF-α, LPS, MDP, CSK4 and colchicine, we measured apoptotic rate of circulating neutrophils in whole blood of 7 FMF patients in remission period and 7 healthy subjects using flow cytometry. FMF neutrophils displayed a higher rate of constitutive ($p<0.05$) and spontaneous ($p<0.01$) apoptosis of the cells cultured in media for 24 hours compared to healthy cells. Cultured healthy neutrophils were significantly more sensitive to TNF-α, LPS, MDP, and CSK4, while diseased neutrophils showed no differences in apoptotic rate after inducers exposure. Short cultivation (3h) of healthy cells resulted LPS- and TNF-α-induced inhibition of apoptosis ($p<0.05$). Thus, neutrophils of FMF patients undergo spontaneous apoptosis at an accelerated rate *ex vivo*. Our findings suggest that enhanced inducible apoptosis in FMF neutrophils is likely to be mediated by the intrinsic signaling pathways.

P5.17.25
Comparison between enzyme-linked immuno-sorbent assay and multiplex assay for serological markers of Celiac Disease detection

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Objective: To compare ELISA with multiplex assay for anti-deamidated gliadin peptide (anti-DGP) IgG and anti-tissue transglutaminase (anti-tTG) IgA antibodies detection in order to identify the best according to sensivity and specificity.
Methods: 101 patients with suspicion or diagnostic of celiac disease (CD) were selected. Each serum was tested by ELISA (Orgentec Diagnostika) and multiplex assay (Bioplex 2200, Bio-Rad) for the anti-DGP IgG and anti-tTG IgA autoantibodies quantification. The association with clinical features was examinated by systematical review of the clinical histories. Also previous CD markers determinations, biopsy and HLA features were studied.
Results: Compared to the clinical, biopsy and HLA features, the ELISA showed a sensitivity of 96.15% and a specificity of 38.09% (Positive predictive value (PPV): 79.36%; Negative predictive value (NPV): 80%), whereas multiplex assay had a sensitivity of 79.59% and a specificity of 100% (PPV: 100%; NPV: 68.75%) for anti-DGP IgG antibodies detection. Furthermore, the ELISA showed a sensitivity of 68.42% and a specificity of 100% (PPV: 100%; NPV: 93.18%), and multiplex assay had a sensitivity of 100% and a specificity of 96.34% (PPV: 86.36%; NPV: 100%) for anti-tTG IgA antibodies testing.
Conclusions: Taking into consideration the previous CD markers determination and clinical, biopsy and HLA features, ELISA showed a higher sensitivity for anti-DGP IgG antibodies, but specificity was better for multiplex assay. In addition, ELISA showed a higher specificity and multiplex had a better sensitivity for anti-tTG IgA antibodies detection.

P5.17.26
The prevalence of wheezing and its relationship with some environmental factors in children aged 6-7 in Yazd

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Backgrounds: Asthma is an inflammatory disease of conducting airways which affects quality of life of children, also posing a high level of burden on health care system. Avoiding exposure to environmental allergens is a well-known method for control of symptoms of asthma.
Objectives: To measure the prevalence of wheezing among children who were exposed to different environmental factors.
Methods: In this cross sectional study, parents of 2740 children aged 6-7 attending selected schools in Yazd, received the International Study of Asthma and Allergies in Childhood (ISAAC) written questionnaire. The children were classified according to exposure to cigarette smoking (mother vs. father vs. babysitter) and keeping cats and dogs (in first year of life vs. past 12 months) at home.
Results: Prevalence of wheezing was 10.9 (12.9% among boys and 8.4% among girls) ,and only 3.8% claimed physician assessed asthma diagnosis.
Prevalence of wheezing was significantly increased in those with the history of keeping cats at home for past 12 months.(12.2% vs. 8.5%. p value : 0.03) ,in addition having a smoker mother was associated with increased wheezing.(2.4% vs. 1%. P value: 0.04).
Other associations were not statistically significant.
Conclusion: A smoker mother and keeping cats at home in past 12 months were associated with increase of wheezing among children of our study

P5.17.27
Psychoneuroimmunologycal study in Cuban in patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic disabling disease with a complex clinical presentation and course. Patients and clinicians widely believe in the relationship between stress and this disease, but the evidence supporting this association is limited. This study aimed to assess the behavior of psychological stress and immunological marker in these patients. Sixty people participated in this study, thirty were patients with SLE from the Nephrology Institute in Cuba and equal number of control subjects with the following diagnoses: (rheumatoid arthritis, primary Sjogren's syndrome, fibromyalgia). To evaluate of stress, we used the stress vulnerability inventory (Miller and Smith), personality inventory (Eysenck), self-esteem (Coopersmith) and personal strength test (Bagés and Kobasa). The new Systemic Lupus International Collaborating Clinics (SLICC) and the 1997 American College of Rheumatology (ACR) systemic lupus erythematosus classification criteria were also evaluated. Eight patients were classified as LES and tree were excluded when the new SLICC classification was applied instead of 1997ACR criteria. The inclusion was due to the presence of biopsy proven lupus nephritis in the existence of antinuclear antibodies. The exclusion was because they had only clinical criterion. On the other hand, the mainly characteristic of personality in SLE patients were introverted (92.8%) obsessive (85.7%) and melancholic(64.2%). Both groups presented the same behavior according to personal strength and stress vulnerability. Nevertheless the SLE patients showed statistically significant reduction in self-esteem ($p<0.05$). The right characterization of SLE patients should be including both the new SLICC classification criteria as psychological study.

P5.17.28
CAPS monocytes are highly susceptible to oxidative stress: effects on cytokine secretion, reversal by antioxidants

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Cryopyrin associated periodic syndromes (CAPS) are autoinflammatory diseases where NLRP3 mutations result in increased IL-1β secretion upon TLR stimulation. We recently showed that monocytes from CAPS patients exhibit a dramatic suppression of IL-6 and of IL-1receptor antagonist (IL-1Ra) production. The deficient secretion of these cytokines coupled to increased IL-1β release may explain the severity of the IL-1-related clinical manifestations and the predominant implication of innate immunity in CAPS.
CAPS monocytes also display a mild level of stress at baseline. We then investigated the functional link between stress and altered cytokine production. Our results show that: i secretion of IL-1Ra and IL-6 is restored in CAPS monocytes by antioxidants that prevent oxidative stress but it is not induced in normal monocytes; ii. the mild stress level at baseline makes CAPS monocytes prone to be activated by low doses of TLR agonists, unable to stimulate healthy monocytes. iii. CAPS monocytes display autophagy defects due to the stress state. In agreement with the inverse correlation between autophagy and IL-1β secretion, these defects result in increased IL-1β secretion. In conclusion, CAPS monocytes display a higher susceptibility to TLR stimulation, due to the presence of stress at baseline. This condition: i. affects the response to TLR agonists, lowering the threshold required to activate CAPS monocytes; ii: impairs the normal autophagic process resulting in increased IL-1β secretion; iii. causes the rapid induction of oxidative stress upon exposure to full doses of TLR agonists, leading to defective production of cytokines downstream of IL-1β.

P5.17.29
Loci associated with N-glycosylation of human IgG show pleiotropy with autoimmune diseases and haematological cancers

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After analysing glycans attached to human immunoglobulin G in 4095 individuals we performed the first genome-wide association study (GWAS) meta-analysis of the glycome of an individual protein. Nine genetic loci were found to associate with glycans with genome-wide significance. Of these, four were glycosyltransferases which participate in IgG glycosylation, thus the observed associations were biologically founded. The remaining five genetic loci were not previously implicated in protein glycosylation, but the most of them have been reported to be relevant for autoimmune and inflammatory conditions and/or haematological cancers. A particularly interesting gene, IKZF1 was found to be associated with multiple IgG N-glycan traits. This gene has been implicated in numerous diseases, including systemic lupus erythematosus (SLE). We explored biomarker potential of N-glycans in 101 cases with SLE and 183 matched controls and demonstrated substantial discriminative power. Our study shows that it is possible to identify new loci that control glycosylation of a single plasma protein using GWAS. The results may also provide an explanation for the reported pleiotropy and antagonistic effects of loci involved in autoimmune diseases and haematological cancer.

P5.17.30
B-cell depletion in neuropsychiatric disease in MRL/lpr mice

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Neuropsychiatric disease in one of the earliest clinical manifestations in systemic lupus erythematosus (SLE). However, the mechanisms leading to neuropsychiatric SLE (NPSLE) are not fully understood, and the optimal treatment yet to be defined. Since antibodies play an important role in lupus pathogenesis, B-cell depletion has been proposed as a directed treatment approach. In lupus, a compromised blood brain barrier may allow for the passage of circulating autoantibodies into the brain, where they then can induce neuropsychiatric abnormalities. To conclusively determine if indeed B-cells and/or autoantibodies are instrumental in the pathogenesis of murine NPSLE, we evaluated B-cell depleted (JhD/MRL/lpr) and wild type MRL/lpr lupus mice, the latter of which are known to develop several cardinal features of human neuropsychiatric disease. Comprehensive neurobehavioral assessment including forced swim, anhedonia, open field, object recognition, object placement, and social preference were employed to evaluate the neuropsychiatric manifestations in JhD/MRL/lpr as compared to MRL/lpr mice. We confirmed that peripheral B-cells were substantially depleted in JhD/MRL/lpr mice, and that autoantibody levels were very low in serum and cerebrospinal fluid. Nevertheless, B-cell depleted MRL/lpr mice surprisingly had no attenuation of key neuropsychiatric features, including depression-like behavior and cognitive dysfunction. Nevertheless, an increased number of rears were observed in JhD/MRL/lpr mice, indicating higher motor activity. Thus, constitutive depletion of B-cells was not sufficient to ameliorate lupus-associated neuropsychiatric disease, at least in the MRL/lpr strain. Whether conditional B-cell ablation later on in life will have the same effect remains under investigation.

P5.17.31
Induction of immunoglobulin G Fc-sialylation is associated with disease remission in CIDP

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Humoral immune responses mediate peripheral nerve damage and represent important pharmacological targets in chronic inflammatory demyelinating polyneuropathy (CIDP), the most common treatable acquired chronic polyneuropathy. IgG Fc-linked glycan structures determine antibody-mediated effector functions and were identified as a molecular switch shifting IgG activity from a pro-inflammatory to an anti-inflammatory pathway. Here, we demonstrate that patients with CIDP show higher levels of IgG-Fc N-glycans lacking terminal galactose and sialic acid residues as compared to demographically matched healthy individuals and that induction of Fc-sialylation is associated with clinical disease remission. Furthermore, CIDP patients show altered expression levels of both activating and inhibitory Fc-receptors. Treatment with intravenous immunoglobulins (IVIG) can partially restore normal expression levels and may therefore reset the threshold for immune-complex mediated cell activation. Our findings underline the importance of IgG Fc-linked glycan structures and Fc receptor expression in human autoimmune diseases and support the concept that the clinical response to antibody-mediated immunotherapies in CIDP can be improved by Fc-glycan modification.

P5.17.32
Expression QTL analysis from primary immune cells of a multi-ethnic cohort identifies novel disease-causing regulatory effects

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The extent to which human genetic variation drives inter-individual variation in immune function is critical to the systematic dissection of altered immune function in disease. We have performed an expression quantitative trait locus (eQTL) study of highly purified CD4+T cells and CD14+CD16- monocytes, representing adaptive and innate immunity, respectively, from a multiethnic cohort of 650 healthy individuals representing Caucasian, African American, and East Asian ancestry. Here, we report small differences in cis-eQTLs among populations, primarily due to population divergence in allele frequencies. We demonstrate that meta-analysis across populations facilitates fine mapping of putative causal regulatory variants. Using a joint-analysis for both cell types, we estimate that 63% of cis-eQTLs are shared between the two cell types, though effect sizes often vary. When exploring the functional impact of disease-associated genetic variation, we observe that susceptibility alleles for inflammatory diseases display a preponderance of regulatory effects in T cells, suggesting that altered T cell function may play a larger role in these diseases. In contrast, for Alzheimer's and Parkinson's disease (PD) susceptibility alleles, we observe a striking enrichment of regulatory effects in monocytes: suggesting the innate immune system appears to play an important role in these neurodegenerative diseases. Finally, we report of and replicate trans-eQTLs and highlight trans-regulatory effects in PD. Overall, this multiethnic exploration of both arms of the immune system identifies many novel eQTLs, highlights a remarkable level of cis-eQTL sharing and provides important new insights into the role of immune cell populations in neurodegenerative, inflammatory, and other diseases.

P5.17.33
Antibody profile and their relationship with clinical manifestations in patients with Systemic Lupus Erythematosus

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The autoantibodies which are produced in systemic lupus erythematosus (SLE) patients have different clinical and pathological importance, and are used for diagnosis, activity evaluation, prediction of tissue involvement, and in the prediction of prognosis. Some of the autoantibodies are produced years before organ involvement and can be used as a tool for prediction of an organ involvement.
Methods & Materials: In this project the sera of 98 SLE patients were studied (76 female, 22 male). Autoantibody profile was performed on sera of all participants using a commercial kit (EUROIMMUN; Germany). By using this kit autoantibodies against nRNP/Sm, Sm, SSA, SSB, Ro-52, CDENP, Jo-1, Scl-70, Nucleosome, Histone, Rib.p-Protein were assessed.
Results: anti-SSA and anti-Ro-52 with prevalence of 44.6% and 39.3% were the most prevalent autoantibodies in all patients, but anti-Scl70 and anti-Jo-1 were not observed in any of the patients in our study. In patients with kidney involvement anti-nucleosome with prevalence of 43.4% was the most prevalent antibody. Anti-dsDNA, and anti histone autoantibodies were related to active phase of SLE disease. In female SLE patients the number of autoantibodies increased in comparison to male patients, and SSA, SSB, Ro-52 were more prevalent in females.

P5.17.34
1,25 Dihydroxy vitamin D modulatory effects in patients with systemic lupus erythematosus

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Objectives: Systemic lupus erythematosus is an autoimmune disease that is complicated by autoantibody mediated organ involvements. In SLE immune tolerance breakdowns and some environmental and genetic factors are involved in the pathogenesis of SLE. In recent year's disorders in regulatory suppressive lymphocytes has been suspected. Vitamin D has been implicated as an environmental immunomodulatory factor which targets various immune cells and may play a role in immune regulation via increasing and activating regulatory cells and its related molecules.
Immunosuppressive effects of vitamin D have been investigated in various studies, however the mechanism by which it induces its modulatory effects is not well understood.
In this investigation we studied the mechanisms by which vitamin D may regulate the immune responses in SLE patients.
Materials & Methods: Study group comprised of 25 SLE patients, and 20 age and sex matched controls. lymphocytes cultured with 1,25 dihydroxyvitamin D3, and CD4+CD25+FOXP3+ cells were analyzed by flowcytometry methods before and after incubation. RNA was extracted and after cDNA synthesis the expression levels of FOXP3, IL-17, IL-6, IL-12, IFN-γ, TGF-β genes was studied by Real-Time PCR method.
Results: 1,25 dihydroxyvitamin D_3 increased CD4+CD25+FOXP3+ cells, and the expression levels of FOXP3, TGF-β, and IFN-γ genes increased, while IL-6, and IL-17 decreased significantly in SLE patients.
Conclusions: 1,25 dihydroxyvitamin D_3 modulates inflammatory immune resposes in lupus patients by increasing the regulatory T cells subsets.

P5.17.35
Effects of Vit D on Th17 cells and related cytokines in lupus erythematosus patients

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Introduction: Systemic lupus erythematosus is a multisystem inflammatory autoimmune disease characterized by autoreactive T cell activation against self-antigens and subsequent production of autoantibodies causing inflammation and tissue damage. Emerging data suggests that Th17 cells are the main pathogenic cells involved in SLE that secrete proinflamatory cytokines and increased frequency of these cells in peripheral blood is correlated with disease activity. Environmental and genetic factors are involved in the pathogenesis of SLE disease. Vitamin D deficiency has been proposed as a potential environmental factor triggering SLE. In the present study we investigated the effects of vitamin D on modulation of Th17 cells and related cytokines.
Materials and Methods: Blood samples were obtained from 30 patients with SLE. Lymphocytes were isolated using Ficoll-Hypaque. Isolated lymphocytes were cultured in the presence and in the absence of vitamin D. After incubation the IL-17 secreting cells were analyzed by flowcytometry. RNA was extracted using Trizol, cDNA synthesized, and the expression levels of IL-17, IL-6, IL-23 genes was analyzed by Real-time PCR.
Results: 1,25 dihydroxyvitamin D decreased IL-17 secreting cells, and the expression levels of IL-17, IL-23 genes in SLE patients.

P5.17.36
Disease modeling and drug discovery using induced pluripotent stem cells from CINCA syndrome patients

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Chronic infantile neurological cutaneous and articular (CINCA) syndrome is an IL-1-driven autoinflammatory disorder caused mainly by NLRP3 mutations. The pathogenesis of CINCA syndrome patients who carry NLRP3 mutations as somatic mosaicism has not been precisely described because of the difficulty in separating individual cells based on the presence or absence of the mutation. Here, we report the generation of NLRP3-mutant and non-mutant induced pluripotent stem cell (iPSC) lines from two CINCA syndrome patients with somatic mosaicism, and describe their differentiation into macrophages (iPS-MPs). We found that mutant cells are predominantly responsible for the pathogenesis in these mosaic patients because only mutant iPS-MPs showed the disease relevant phenotype of abnormal IL-1beta secretion. Next, after confirming that the existing anti-inflammatory compounds inhibited the abnormal IL-1beta secretion from iPS-MPs, we started candidate compound screening for treating CINCA syndrome and other NLRP3-related inflammatory conditions using a recently-established feeder-free differentiation protocol. Our results illustrate that patient-derived iPSCs are useful for dissecting somatic mosaicism, and that NLRP3-mutant iPSCs can provide a valuable platform for drug discovery for multiple NLRP3-related disorders.

P5.17.37
Distribution of peripheral blood lymphocyte populations in patients with primary Sjögren's syndrome

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Distribution of lymphocyte populations' in peripheral blood mononuclear cells (PBMCs) of patients with primary Sjögren's syndrome (pSS) without (1pSS) or with (2pSS) extraglandular manifestations were investigated.
30 1pSS, 23 2pSS and 35 healthy controls were enrolled in the study. PBMCs were stimulated in whole blood with PMA and ionomycin, PBMCs labelled for CD3, CD4, CD8, CD19, CD16+56, CD25, CD27, CD57, Foxp3 and IL-17A and analyzed by flow cytometry.
Decline in CD3+ T cells in PBMCs of patients with pSS was detected. The reason for this was significantly decreased absolute counts of CD4+, CD8+ and CD3+CD16+56 cells in patients with pSS. Significant decrease in absolute counts of CD4+IL17A+ and CD8brightCD57-CD27+ cells in patients with pSS were observed as compared to control group. Absolute counts of CD4+25brightFoxp3 and CD19+ cells were similar in all three groups and CD3-CD16+56 cells was significantly decreased only in 1pSS group. All results between 1pSS and 2pSS groups were comparable. However, different correlations between lymphocyte populations in 1pSS and 2pSS groups were found. Only in 1pSS the correlation between CD3-CD16+56 and CD3+CD16+56, CD3-CD16+56 and CD4+25brightFoxp3, CD4+ and CD19+, CD4+ and CD8brightCD57-CD27+ cells was registered. Whereas, in 2pSS the correlation between CD4+ and CD4+IL-17A+, CD8+ and CD3+CD16+56, CD4+IL-17A+ and CD8brightCD57-CD27+ cells was revealed.
Declining and variations of T-lymphocyte subsets in pSS patients` could be the result of increased apoptosis and/or migration to inflamed tissues of CD4+IL-17A+, CD8brightCD57-CD27+, CD3+CD16+56 and
CD3-CD16+56 (only in 1pSS group) cells. We suppose that these cell populations play an important role in pSS pathogenesis.

P5.17.38
Protease activation receptors - A new link between IgE mediated and non-IgE mediated inflammatory reactions

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Background: Food derived proteases are known to have a central role in inflammatory reactions via activate protease activation receptors (PAR's) on various cells types. The interplay with IgE-mediated reactions however is not understood. We investigate in this study the effect of a novel crustacean protease on the up-regulation of PAR's in the presence of IgE-receptor activation.
Methods: Trypsin (PT) from black Tiger prawn (Penaeus monodon) was purified by HPLC/C18 chromatography and activity studied using zymography. The impact of PAR's (PAR-1, -2, -3, -4) on IgE mediated degranulation were analysed using a humanized rat basophilic leukemia (RBL-2H3) cells, while PT stimulation on the four PAR-receptors was evaluated using a human lung epithelial cell line (A549). The expression level of PAR-receptors and cytokines was quantified by qPCR and cell signaling pathways explored by western-blotting.
Results: A novel PT is highly resistant to autoproteolysis. Stimulated RBL cells demonstrated after IgE-Antigen stimulation or exposure to PT very strong up-regulation of IL4/IL13 mRNA level. In addition IgE stimulation enhanced the expression of PAR-2 but none of the other three receptors. In contrast PT stimulation of A549cells boosted both expressions of PAR2 and PAR4 and 9 different cytokines. Both cell types utilized the MAPK pathway.
Conclusions: This is first study to demonstrate the up-regulation of PAR-2 receptor via activation of IgE-receptors on RBL cells besides enhanced up-regulation of IL-4/IL-13 cytokines. These cytokines favor the production of more IgE antibody in a Th2 driven milieu, demonstrating the crucial link between IgE and non-IgE mediated pro-inflammatory reactions.

P5.17.39
Identification of a novel cold-associated autoantigen, heterogeneous nuclear RNP-K, in patients with secondary Raynaud's phenomenon by proteomic approach.

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Objective: To identify cold-associated autoantibodies in patients with Raynaud Phenomenon (RP) secondary to connective tissue diseases (CTDs).
Method:Indirect immunfluorescence staining was performed on non-permeabilized cold-stimulated normal human dermal microvascular endothelial cells (dHMVECs), using patients' sera. Cold-induced alterations in cell surface proteome were analyzed by iTRAQ analysis. Serological proteome analysis was applied to screen cold-associated autoantigens. Prevalence of the candidate autoantibody was determined by ELISA in 231 patients with RP secondary to CTDs (systemic sclerosis, systemic lupus erythematosus or mixed connective tissue disease), 10 patients with primary RP and 27 healthy controls.
Results: Enhanced cell surface immunoreactivity was detected in cold-stimulated dHMVEC cells, when incubated with sera from patients with secondary RP. By iTRAQ analysis, many proteins including heterogeneous nuclear RNP-K (hnRNP-K) were found increased on the cell surface of dHMVECs after cold stimulation. By SERPA approach, hnRNP-K was identified as a candidate autoantigen in patients with secondary RP. Cold-induced translocation of hnRNP-K to cell surface was confirmed by immunoblotting and flow cytometry. By ELISA analysis, patients with secondary RP represented a significantly higher prevalence of anti-hnRNP-K autoantibody (28.4%, 52 of 183) than patients without RP (8.3%, 4 of 48, P = 0.0019), patients with primary RP (0%, 0 of 10, p = 0.0396) and healthy controls (0%, 0 of 27, P = 0.0003).
Conclusions: By comprehensive proteomics, we identified hnRNP-K as a novel cold-associated autoantigen in patients with secondary RP. Anti-hnRNP-K autoantibody may potentially serve as a biomarker for RP secondary to various CTDs.

P5.17.40
Increased levels of IL-17F but not IL-17A in patients with immunosuppressive therapy for autoimmune hepatitis (AIH).

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AIH is an inflammatory condition of the liver in which the mechanisms that operate in tissue destruction and/or tissue repair are still unknown. Corticosteroids are the mainstay of therapy and, either alone or in combination with azathioprine. This treatment has been shown to improve survival rates and quality of life. Patients on corticosteroid therapy evolve to remission but some of them experience an exacerbation after withdrawal of drug(s). Relapse is common and occurs in a significant percentage of the patients within 6 months. In this study, we analyzed the serum levels of Th17-associated cytokines in 55 Mexican patients with confirmed AIH diagnosis under different treatment regimens (prednisone, prednisone-azathioprine or azathioprine), comparing against healthy donors (HD). The results were analyzed by Mann-Whitney U or Kruskal-Wallis tests, using Sigma Plot statistics software. A value of $P<0.05$ was considered significant. Elevated levels of IL-17F (98.5 pg/ml), IL-21 (161.83 pg/ml) and IL-23 (39.49 pg/ml) were detected in patients under treatment. In contrast, very low or undetectable levels for these cytokines were measured in the HD group (IL-17F: undetected, IL-21: 12.46 pg/ml, IL-23: 8.52 pg/ml). There were not differences between the values for IL-17A in the AIH and HD groups (35 pg/ml vs. 23 pg/ml) and IL-22 (29 pg/ml vs. 18.46 pg/ml). Collectively, these results suggest a differential effect of the Immunosuppressive therapy on Th17 cytokine production. IL-17F could play an important role in the pathogenesis of AIH in patients under conventional immunosuppressive therapy.

P5.17.41
Amplification of T cell autoreactivity by OX40/OX40L functional complex on peripheral CD4+ T cells in Graves' disease and clinical significance

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Persistent activation of autoreactive T cell is crucial in the development of Graves' disease (GD) since the prodution of autoantibody (TRAb) is T-cell dependent. It was reported that some costimulatory receptors and their ligands such as CD28 and CD80 coexpressed on activated T cells as well as T cells from some autoimmune diseases, could enhance T cell autocrine stimulation. Because OX40/OX40L pathway plays a very important role in the antigen priming T cells and effector T cells, in this study we address whether OX40/OX40L was expressed on T cells from 124 patients with GD. It was very interesting to find that OX40 and OX40L were abnormally coexpressed on $CD4^+$ T cells of peripheral blood in GD patients. Then we also evidenced these OX40, OX40L expression and coexpression levels on $CD4^+$ T cells were all notably higher in $TRAb^h$ group than that in $TRAb^n$ group and changed with TRAb alteration after therapy. This means that the expression levels were closely correlated with TRAb levels. In addition, blocking OX40/OX40L signal in vitro inhibited T cell proliferation, which suggested OX40 and OX40L coexpressed on $CD4^+$ T cells could enhance $CD4^+$ T cell proliferation in GD by self-enhancing loop of T cell activation independent of APCs. The data of confocal microscopy and coimmunoprecipitation further showed that OX40 and OX40L formed functional complex which may facilitate signal transduction

from OX40L to OX40 and contribute to the pathogenesis of GD. Our results indicated targeting OX40/OX40L signal could be a new therapeutic approach in patients with GD.

P5.17.42
Increased frequency of Th17 cells in patients with dermatomyositis

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DM (dermatomyositis) is a subtype of inflammatory myopathies,which is a rare autoimmune disease of skeletal muscle. Although the pathogenic mechanism of DM is still unclear, it was considered as a CD4+T cells driven disease. It was reported that IL-17, a key cytokine of Th17 cells, has been detected in the inflammatory patients. But there is no study focusing on the alteration of Th17 cells in the peripheral blood of patients with DM. In this study, we investigate the roles of Th17 cells in the pathogenesis of DM.
The percentage of Th17 cells was significant increased in PBMCs from patients with DM compared with healthy controls. qRT-PCR analysis displays an enhanced expression of IL-17mRNA in the PBMCs from DM patients, with lower expression in healthy controls. The expression of RORC mRNA in PBMCs from DM patients is significant higher than that from healthy controls. We found that there was a positive correlation between the percentages of Th17 cells and serum level of CK, but not with level of LDH. In addition, we found that DM patients have significant increased serum concentration of IL-6 and TGF-βin comparison with healthy controls. In addition, serum concentrations of IL-1βis higher in DM patients compared with healthy controls, but this difference did not reach statistical significant.
In summary, our data collectively suggest that there is an enhanced frequency of Th17 lymphocytes in DM patients, and these cells might participate in the pathogenesis of the inflammatory phenomenon observed in this condition.

P5.17.43
Plasma microparticles and LL-37 levels regulate Behcet's Disease severity

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Microparticles (MPs) are small vesicles secreted from immune and non-immune cells. The number of MPs was found to be elevated in rheumatic diseases such as rheumatoid arthritis, systemic sclerosis, and vasculitis. However, the relationship between Behçet's Disease (BD) and level of MPs has not been examined.
This study aimed to characterize the cell source, cell activation state and quantity of MPs in Behcet's patients with different clinical features. We also attempted to identify MP mediated immune activation of BD patients PBMCs. MPs isolated from peripheral blood plasma of 72 patients with BD and 22 healthy controls were stained with Annexin-V together with specific cell surface markers of CD3, CD14, CD31, CD42a, CD69, and CD105. Isolated MPs incubated with healthy PBMCs to assess IL-6 production. The concentration of LL-37 in subjects` plasma and MPs were determined by ELISA.
Data suggested that platelets are the major source of MPs. Furthermore, the number of MPs/ml plasma in active BD patients increased by 2 fold and by 5 fold with respect to inactive BD and healthy individuals, respectively. PBMCs efficiently took up syngeneic patients` MPs and MPs stimulation led to IL-6 secretion proportional to disease progression/severity. The plasma and MP-associated LL-37 levels were the highest for active BD patients and correlated with cytokine production.
This study suggested that MP levels and source along with persistence and cargo plays a pivotal role in regulating the disease activity and may contribute to the pathogenesis of BD.

P5.18 Inflammatory bowel diseases

P5.18.01
Modulation of macrophage differentiation by crotoxin from *Crotalus durissus terrificus* in acute intestinal inflammation in mice

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Macrophages are important cells to maintain homeostasis between host and commensal microbiota and also play essential role mediating inflammatory reaction against pathogenic agents. Macrophages, including 'classical' (M1) and 'alternatively' activated (M2) have been studied as a target for the treatment of inflammatory bowel diseases such as Crohn's disease. Crotoxin (CTX) is the main component of the *Crotalus durissus terrificus* rattlesnake venom and has immunosuppressive effect. Here, we evaluated the induction of M1 and M2 macrophage populations by CTX on 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice. After 18 hs of the TNBS-colitis induction, the mice were treated with CTX or PBS. After 24 or 48h of the treatment the peritoneal lavage and mesenteric lymph node cells were collected for flow cytometry analysis. Clinical and histological scores showed that the CTX-treatment decreased the disease progression in TNBS-colitis induced mice. Higher percentage of $CD11b^{+}CD68^{+}$ (M1) cells was observed in peritoneal lavage at 24 and 48h as well as in the lymph nodes after 48h of PBS administration in TNBS-colitis mice compared with the CTX administered in TNBS-group. The highest percentage of $CD11b^{+}CD206^{+}$ (M2) cells was found in peritoneal lavage and mesenteric lymph nodes collected at 24 and 48h after the CTX treatment of TNBS-induced mice. As previously observed this macrophage population (M2) is able to produce IL-10 which can modulate the intestinal inflammatory response. Our preliminary results suggest a modulatory role of CTX in the macrophage differentiation in acute intestinal inflammation induced by TNBS in mice. Financial Support: FAPESP 2010/05701-9 and CNPq

P5.18.02
The role of IFITM genes in the pathogenesis of the gastro-intestinal tract

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The Interferon inducible transmembrane protein family (IFITM) includes several highly homologous family members. These genes are involved in multiple molecular and cellular processes, such as, early development, immune response to pathogens, viral restriction, inflammation, malignant transformation, cellular adhesion and cell cycle control. Several findings associate between IFITM genes and pathogenesis in the gastro-intestinal (GI) tract. IFITM2 and IFITM3 genes were shown to be over-expressed in colitis-associated cancers, and in severely inflamed mucosa. Moreover, polymorphisms of the IFITM3 gene were found to be associated with susceptibility to ulcerative colitis.
Our research aims at investigating the molecular pathways governing IFITM genes expression and their role in colon related diseases.
To study the role of IFITM genes in colitis, we induced acute colitis by administration of Dextran Sodium Sulfate (DSS). Colitis severity was monitored and assessed in IFITM3KO (knockout) mice. The severity of the disease was evaluated by daily weighting, endoscopic scoring, and histological analysis. We have shown that the absence of a single IFITM3 allele, results in a significantly severe acute colitis. Gene expression analysis in colons of healthy IFITM3KO mice revealed that these mice predominantly express pro-inflammatory genes, such as IL-beta and IL-6.
Further investigation of the mechanisms, by which IFITM genes are regulated, as wells as their role in GI pathogenicity, are of great importance in attributing to our understanding of the role that immunity plays in complex processes of inflammation and malignancy.

P5.18.03

Profile of ANCA (Anti-Neutrophil Cytoplasmic Antibodies) and ASCA (Anti-Saccharomyces Cerevisiae mannan Antibodies) in patients with IBD (Inflammatory Bowel Diseases): Crohn's disease and Ulcerative Colitis

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Introduction: ANCA (Anti-Neutrophil Cytoplasmic Antibodies), directed against the cytoplasmic granules of neutrophils. They are associated with various antigenic targets and found during a set of inflammatory pathologies. ASCA (Anti-Saccharomyces cerevisiae mannan Antibodies), react with yeast "Saccharomyces cerevisiae". They are directed against mannose residues of the cell wall of S. cerevisiae and found in patients with Crohn's disease (CD) and ulcerative colitis (UC). Objectif: To study the involvement of ANCA and ASCA in CD and UC Moroccan patients and establish ANCA/ASCA profile in these complementary pathologies. Materials and methods: We tested sera from 89 patients of Gastroenterology Department, Ibn Rochd University Hospital of Casablanca. The presence of ANCA was investigated by IIF on slides of neutrophils cytocentrifuged and fixed in ethanol or formalin-acetone and ELISA: ANCA-combi Kits. The presence of ASCA was investigated by ELISA: ASCA (IgG+IgA) Kits. Results: ASCA showed a significant association (p= 0.0000003) with CD and p-ANCA with UC (p= 0.00071). The combination of both tests ASCA/p-ANCA leads to higher association than tests alone. c-and a-ANCA pattern were also found and showed an association with these digestive diseases complicated with underlying infectious diseases. Beside PR3 and MPO, we identify other antigenic targets (Lactoferrin, Bactericidal Permeability Increasing Protein, Cathepsin G, Elastase) associated with different patterns in IIF. Conclusion: The application of both ANCA and ASCA tests may be helpful in our country for the differential diagnosis between CD and UC. It should however find a possible association with an infectious disease suggesting an infectious etiology underlying these inflammatory diseases.

P5.18.04

Glucocorticoid-induced leucine zipper (GILZ) regulates intestinal immune homeostasis and anti-inflammatory effects of glucocorticoids

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Inflammatory bowel disease (IBD) comprises a group of diseases of the gastrointestinal tract. Glucocorticoids (GCs) are one of the most widely used drugs in IBD treatment. Glucocorticoid-induced leucine zipper gene (GILZ) is rapidly induced by GCs. We have recently generated a conditional knock out mice of gilz gene (gilz cKO). To determine whether deletion of gilz influences the susceptibility to chemichally-induced colitis, we compared the development of dinitrobenzene sulfonic acid (DNBS)-induced colitis in wild-type (wt) and gilz cKO mice. The severity of colitis was markedly more severe in gilz cKO mice compared to wt controls, as measured by increased mortality, body weight loss, diarrhea score and colon shorteining and increased post-injection recovery time. Levels of TNFα were significantly higher in mesentheric lymph nodes of DNBS-treated gilz cKO compared to wt mice. These results suggest that GILZ absence in T cells dampens a normal intestinal immune response.
The DNBS-induced colitis was not cured by dexamethasone (DEX) treatment in gilz cKO mice as occurs in wt mice, evidenced by lack of DEX effect on body weight loss and overall clinicall score in gilz cKO mice. Thus, lack of GILZ precludes efficient anti-inflammatory effects of GC.
Finally, the exacerbated inflammation of DNBS-induced colitis in gilz cKO mice was fully prevented by recombinant TAT-GILZ protein administration, suggesting that pharmacologic modulation of GILZ expression can represent a new strategy for the treatment of IBD.

P5.18.05

Polymorphisms of NOD2/CARD15 and PTPN22 in Algerian adult inflammatory bowel disease (IBD)

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Background:
Susceptibility to IBD, a complex genetic disorder, is influenced by common variants at many loci. The aim of this preliminary work is to highlight the presence of allelic variants in NOD2, the first susceptibility gene described as being linked to IBD, and the allele of a minor gene, PTPN22 which has been unequivocally confirmed by GWAS as conferring susceptibility to IBD and many autoimmune diseases.
Methods:
The SNP-s for NOD2 (C2104T, G2722C, 3020insC) and PTPN22 (C1858T) were investigated by PCR-RFLP, in a case-control study. 37 Algerian IBD patients were genotyped and compared to 39 healthy controls and 19 DT1 Algerian patients. All participants provided written informed consent before participation.
Results:
The three risk alleles for NOD2 (C2104T, G2722C, 3020insC) are present in IBD patients (4, 06%, 5,40%, 1,35%) and are more frequent than in controls (3,84%, 1,92% , 0%). The frequencies of the three alleles were higher in CD compared to UC patients (16,22% vs 1,70%), and thus support the literature data. For PTPN22 gene, (C1858T) allelic variant frequencies are (1,25%, 5,27% and 0%) in IBD, T1D controls and healthy controls, respectively and appear to be much less frequent compared to the neighbor Tunisian population.
Conclusion:
In this preliminary study on Algerian population, we present for the first time, allelic variants frequencies of two susceptibility genes, NOD2 and PTPN22, which seem to be associated with IBD. It would be interesting to extend our sampling in order to better evaluate the impact of those two loci in IBD disorder.

P5.18.06

Autoimmune diseases association study with the KIAA1109-IL2-IL21region in a Tunisian population

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Autoimmune diseases share many genetic factors resulting in similarity of disease mechanisms. For instance polymorphisms from the KIAA1109-IL2-IL21 block in the 4q27 chromosome, has been associated with a number of autoimmune phenotypes. Here we performed a haplotype-based analysis of this autoimmune related region in Tunisian patients. Ten SNPs (rs6534347, rs11575812, rs2069778, rs2069763, rs2069762, rs6852535, rs12642902, rs6822844, rs2221903, rs17005931) of the block were investigated in a sample set consisting of 93 systemic lupus erythematosus (SLE), 67 ulcerative colitis (UC), 39 Crohn's disease (CD) patients and 162 healthy control subjects of Tunisian origin.
In SLE population, haplotypes AGCAGGGTC, AGAAGAGTC, AGAAGGGTC and AGCCGAGTC provided significant evidence to be associated with SLE risk (P = 0.013, 0.028, 0.008 and 0.021, respectively).
In the UC population, haplotypes AGCCGGGTC and AGCAGAGTT have a susceptibility effect for UC (p = 0.007and p= 0.028, respectively).
In the CD population, haplotype CAGGCC showed a protective effect against the development of CD (p = 0.038). Haplotypes GAGA, AAGGTT and CGGGCC provided significant evidence to be associated with CD risk (P = 0.048, 0.0004, 0.041, respectively).
Our results replicate and extend the association found in the *KIAA1109 /IL2/IL21* gene region with autoimmune diseases, implying

that this locus is a general risk factor for multiple autoimmune diseases.

P5.18.07
Frequency of NOD2/CARD15 and MDR1 gene polymorphisms in a cohort of Algerian pediatric Crohn's disease patients

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Objective: The aim of this study is to describe for the first time, the allele frequency of most relevant SNPs in two candidate genes for inflammatory bowel disease, NOD2 which encodes an innate immune receptor and MDR1 which encodes a membrane transport protein, in Algerian Pediatric Crohn's disease patients.
Material and methods
15 unrelated pediatric CD patients and 39 healthy controls were studied. Clinical data and personal information were collected after obtaining informed consent from each participant. NOD2 (C2104T, G2722C, and 3020insC) and MDR1 (C3435T, C1236T, G2677T/A) polymorphisms were analyzed using PCR-RFLP method.
Results: The mean age of diagnosis is 10±3,5 years old with a sex ratio of 1,5 (M/F). 33,33% of parents are consanguineous and 36,36% of patients have family history of CD. The frequency of ileitis, Ileocolitis and colitis is 50%, 41,66% and 8,33% respectively. 45,45% had surgery and 33,33% extraintestinal manifestations. The 2104T allele of NOD2 gene is more frequent in CD patients than controls but neither G2722C nor 3020insC mutations are found in patients. Interestingly, only the 3435T and 1236T alleles of MDR1 gene are significantly more frequent in patients compared with controls (45,45% vs 31,25% and 46,66% vs 38,23%, respectively).
Conclusion: Our study shows that allelic variants of NOD2 and MDR1 are present in the Algerian population and their frequency varies between CD patients and controls. An increase of sampling is required to understand the impact of these genes in CD. Exploring MDR1 polymorphisms may contribute to explain inter-individual differences in pharmacokinetics of drugs.

P5.18.08
miR-132/212 cluster knockout ameliorated DSS-induced colitis

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MicroRNAs (miRNAs), a 20- to 25-base-pair single-stranded non-cording RNA fragments, are important post-transcriptional regulators. By modulating the expression of numerous target mRNAs mainly at the post-transcriptional level, these miRNAs have been involved in most, if not all, biological processes as well as in the pathogenesis of a number of diseases including human ulcerative colitis. Our previous work demonstrated that Aryl hydrocarbon receptor (Ahr) has important roles in differentiation or activation of immune cells and autoimmune disease. Subsequently, our group found mir-132/212 cluster was driven and participated in differentiation of immune cells such as Th17 cells and macrophages in condition of the Ahr activation. However, the role of mir-132/212 in autoimmune diseases remains unknown. To investigate the function of mir-132/212 in immune system, we produced DSS-induced colitis in Wild type (WT) mice and examined mir-132/212 expression by real time-PCR. Interestingly, expression of mir-132/212 was upregulated in DSS administrated mice colon. Furthermore, mir-132/212 KO mice showed mild symptoms of DSS-induced colitis compared to WT mice.These results suggest that mir-132/212 has anti- inflammatory function in DSS-induced colitis. We further examine the effect of mir-132/212 deficiency to pathological and biological alterations and its target genes.

P5.18.09
Overexpression of IL-9 and its receptor in Crohn's disease

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Th9 cells have been recently defined as the main producers of IL-9. However, the role of this cytokine and its receptor in Crohn's disease, one of the two major forms of inflammatory bowel disease, has not been addressed so far. The capacity of cultured peripheral blood lymphocytes to produce IL-9 upon stimulation has been investigated by ELISA whereas the expression of the IL-9/IL-9R was monitored by flow cytometry and immunofluorescence. Spontaneous apoptosis in cultured granulocytes has been addressed by flow cytometry using annexin V and 7-AAD, and various concentrations of IL-9 were used to modulate this process. Stimulated Crohn's disease lymphocytes produced significantly more IL-9 compared to cells from healthy controls. Intracellular anti-cytokine staining demonstrated that IL-9-producing cells represent a subset of lymphocytes that is distinct from IL-17+ cells in Crohn's disease. Patients expressed significantly more IL-9R on their peripheral blood cells compared to controls. Functionally, IL-9 addition decreased the rate of spontaneous granulocyte apoptosis in a dose-dependent manner. Nevertheless, granulocytes from Crohn's disease biopsies stained positive for the IL-9R suggesting a possible pathogenic implication of these cells in situ. Our present results identify IL-9-producing cells as a distinct subpopulation of lymphocytes in Chron's disease patients. Moreover, the high levels of IL-9 production and/or IL-9R expression by peripheral blood cells in Crohn's disease might define therapy-resistant cases and thus IL9/IL-9R could become novel disease markers and/or future therapeutic targets.

P5.18.10
Integrity of the splenic nerve is required to mediate the vagal anti-inflammatory effect on colonic inflammation in a murine DSS-induced model of colitis

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Background: Subdiaphragmatic vagotomy enhances colonic inflammation in a Dextran Sodium Sulfate (DSS)-induced mouse model of colitis. In sepsis, vagal control of the splenic nerve was proposed to mediate the vagal anti-inflammatory effect. To which extent this neuronal circuitry is involved in modulating DSS-induced colonic inflammation remains unknown.
Aim: We therefore investigated the role of the spleen and splenic innervation in modulating the immune system during colonic inflammation.
Methods: A time-course preliminary study was performed to collect evidence supporting immune activation in the spleen during colonic inflammation. C57BL/6 mice were exposed to DSS (2%) for 5 days and sacrificed at day 1, 3, 12 and 21. Denervation of the spleen or Sham operation was performed prior to DSS treatment to evaluate the role of splenic innervation in modulating colonic inflammation.
Results: At day 12, enhanced expression of CD80 on splenic macrophages and increased percentages of splenic neutrophils and regulatory T cells (T regs) were observed in the spleen of DSS-treated mice compared to water-exposed animals. Spleen denervation did not affect the splenic response. However, an increased Disease Activity Index (i.e. inflammatory score, diarrhea score, visible fecal blood), suggesting a higher colonic inflammation, and enhanced expression of colonic CD45 mRNA, showing a higher cell infiltration, were observed in spleen-denervated DSS-treated mice. The colonic expression of FoxP3 mRNA, a Treg marker, was also increased in these mice.
Conclusion: Our study suggests that splenic denervation increases colonic inflammation. Further experiments are currently conducted to confirm the role of the spleen in DSS-induced colonic inflammation.

P5.18.11
Neutrophil gelatinase B-associated lipocalin - matrix metalloproteinase-9 (NGAL-MMP-9) complex as a surrogate serum marker for mucosal healing in ulcerative colitis

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Objective. The current standard for assessing intestinal inflammation and mucosal healing after therapy in inflammatory bowel diseases (IBD) is endoscopy. However, a need exists for non-invasive, accurate serum markers. Several studies have shown that MMPs are predominant proteases in IBD. Serum NGAL-MMP-9 complex levels were studied as a surrogate marker for mucosal healing in ulcerative colitis (UC) patients.
Design. Serum NGAL-MMP-9 complex levels were determined from 66 infliximab (IFX)-naïve patients with active UC before and 4-6 weeks after first IFX infusion and from 40 healthy controls (HC), using zymography analysis and sandwich ELISA. The response to IFX was defined as complete mucosal healing at control endoscopy. Data were analyzed with the use of non-parametric tests in SPSS Statistics 20.0 software and p-values <0.05 were considered significant.
Results. At baseline, serum NGAL-MMP-9 complex levels were significantly increased in UC patients versus HC (103.8 vs 42.4 ng/ml; p<0.0001), whereas in more than 50% of the patients CRP levels were normal. NGAL-MMP-9 levels decreased after therapy in UC responders (median range: 116.3 to 32.0 ng/ml; p<0.0001) and non-responders (median range: 94.7 to 54.1 ng/ml; p= 0.0469). NGAL-MMP-9 complex levels correlated more (τ=0.317) with endoscopic activity as measured by the Mayo endoscopic subscore than CRP titers (τ=0.299). ROC analysis defined a NGAL-MMP-9 complex cut-off value of 97.7 ng/ml predicting complete mucosal healing with high specificity (93%).
Conclusion. NGAL-MMP-9 complex outperforms CRP in assessing mucosal healing after treatment with IFX in UC patients and is suggested as a new serum marker for UC.

P5.18.12
Rip-2 mediated control of pathogenic and protective T cell function during chronic colitis

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Inflammatory bowel diseases (IBD) are poorly understood conditions which are characterized by chronic inflammation of the gastrointestinal tract and deregulation of T cell homeostasis. Since mutations to Nod2 have been linked to an increased susceptibility to IBD in humans and mice, my hypothesis is that innate Nod1&2 signals, conducted through the signalling adaptor molecule Rip2, are important for regulating pathogenic and protective adaptive T cell function during chronic colitis.
The T cell transfer model of colitis and Rip2 deficient mice were used to investigate the role of innate Nod1&2 signals in T cell function during colitis. Pathogenic CD45RB^{hi} T cell were injected alone or with protective CD45RB^{low} T cells into Rag1$^{-/-}$ or Rip2$^{-/-}$Rag1$^{-/-}$ mice. The severity of colitis was quantified by measurement of weight loss; analysis of mesenteric lymph node, lamina propria and spleen lymphocyte numbers, intracellular cytokines and surface markers by flow cytometry; pathological scoring of colons; screening of colon explant supernatants via Luminex and ELISA; and analysis of inflammatory mediators via RT- qPCR.
Rip2$^{-/-}$Rag1$^{-/-}$ mice had an increased rate of disease onset as indicated by rapid weight loss, high pathological scores at week 4 and increased frequency of IFN-γ^{+} colon infiltrating lymphocytes. Furthermore, co-injection of protective CD45RB ^{low} T cells did not protect the Rip2$^{-/-}$Rag1$^{-/-}$ mice even though normal frequencies of FoxP3^{+} regulatory T cells were found in the colon. Further investigation of this novel finding will lead to a better understanding of the mechanisms controlling both pathogenic and regulatory T cell populations in the gut.

P5.18.13
Significance of IL-9 levels in inflammatory bowel disease

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The primary aim of this study was to evaluate serum IL-9 levels in patients with inflammatory bowel disease (IBD); the secondary aim was to analyse the levels of interferon-γ (INF-γ), Th2 cytokines (IL-4, IL-5 and IL-13), and IL-6, and their relationships with the clinical features of the disease.
Methods: Venous blood samples of 43 IBD patients (20 with Crohn's disease [CD] and 23 with ulcerative colitis [UC]) were analysed by means of quantitative enzyme-linked immunosorbent assays using purified anti-human IL-4, IL-5, IL-13, INFγ, IL-9 and IL-6 antibodies, and the laboratory findings were statistically correlated with clinical expression.
Results: All of the patients were negative for IL-4, IL-5 and IL-13. Forty (93%) were positive for INFγ, thus confirming the presence of Th1 in both UC and CD; INF-γ also correlated with disease activity (p=0.045). Eighteen patients (41%) were clearly positive for IL-9, which was associated with a severe prognosis (p<0.001), and 72.2% of the IL-9-positive patients were also IL-6 positive, which suggests that pro-inflammatory cytokines play a role in the disease. There was a significant correlation between disease severity and IL-9 in the CD patients (p<0.001), but not in the UC patients (p = 0.1).Conclusion: Our findings confirm the presence of the common Th1 cytokines underlying both UC and CD. Regardless of the diagnosis, clinical severity seems to be associated with the detection of serum IL-9, which suggests the presence an alternative T cell population with inflammatory function that conditions the prognosis of patients with IBD

P5.18.14
The role of thymic stromal lymphopoietin (TSLP) in intestinal pathologies

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Thymic stromal lymphopoietin (TSLP) is an IL-7 like cytokine produced by epithelial, stromal cells and some hematopoietic cells. TSLP has been shown to play a major role in several homeostatic immune responses but also in the pathogenesis of allergic and autoimmune disorders. In the gut, it is expressed by intestinal epithelial cells and educates non-inflammatory DCs the have reduced ability to produce IL-12p70 and drive the differentiation of inducible Tregs. Conversely, overexpression of TSLP and TSLP-induced Th2 responses are often associated with strong allergic manifestations, like asthma and atopic dermatitis, and with the progression of breast, pancreatic and lung cancer.
In humans, two homologous isoforms of TSLP are expressed, the "long" isoform (159 AA) and the "short" isoform (63 AA). Even though the most well characterized form of TSLP so far is the long one, we found the putative promoter for the long isoform to be almost completely inactive in most of the cell lines present in the UCSC database. On the contrary, the promoter region for the short isoform seems to have a high capacity to bind a number of different transcription factors and the protein is readily expressed in various tissues. We found that the short isoform is the only one expressed by intestinal epithelial cells under steady-state conditions and it is downregulated in inflammatory bowel disease. The long isoform instead shows an opposite trend, in fact it is expressed by epithelial cells only in inflamed ulcerous tissue.

P5.18.15
Vanin-1 (VNN1), an epithelial pantetheinase that regulates intestinal inflammation

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Vanin-1 molecules are epithelial pantetheinases and regulators of intestinal inflammation in mouse. Vanin-1 modulates PPARγ anti-inflammatory potential in gut mucosa and exerts pro inflammatory or cytoprotective properties depending of the tissue context. We showed that Vanin-1 is tightly regulated in the mouse gut in normal and colitis conditions and that PPARγ regulates its expression. We investigated the contribution of human VNN1 to IBD and showed the constant upregulation of VNN1 in the colon of IBD patients, establishing VNN1 as a new marker of IBD. The sequencing of VNN1 regulatory regions in a cohort of 480 IBD patients and healthy controls enabled the identification of 3 VNN1 SNPs statistically associated to the disease. These polymorphic positions in the VNN1 gene locus are direct targets for nuclear factors and include a PPARγ binding site. In addition, we described a rare VNN1 variant associated with very high VNN1 levels and severe colitis. We developed transgenic mice, lacking or overexpressing Vanin-1 and analyzed their response to DSS -induced colitis. Preliminary results show that Vanin-1 expression levels could be correlated to the severity of colitis.

P5.18.16
Assessment of loss of response to Infliximab therapy in inflammatory bowel diseases using antibodies to Infliximab and trough Levels

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Currently the management of inflammatory bowel disease (IBD) patients on infliximab therapy who lose response is mostly empirical: an increase of dose up to double or a decrease of treatment intervals is the common clinical practice, with a consequent increase of drug use and high costs. Measuring trough levels (TL) and antibodies to infliximab (ATI) may be of great benefit in optimizing therapy. We aimed to evaluate the relationship of TL and ATI assay with loss of response and infusion reactions in an Italian IBD unit.
Methods: Sera were obtained from 82 IBD patients on infliximab maintenance therapy from at least 6 months. Patients were classified as being or not in clinical response. The occurrence of acute reactions to infliximab infusions was recorded. ATI and TL were measured by a commercial ELISA (Immundiagnostik AG, Bernsheim, Germany).
Results: ATI were detected in 18 patients (22%), 50% of which were in clinical response as compared to 80% of patients without detectable serum ATI levels (p=0.0271 by χ2 test). 5/18 (28%) of ATI positive patients had infusion reactions as compared to 2/64 (3%) ATI negative (p=0.0047 by χ2 test). 60/82 patients had infliximab TL >0,1 µg/ml and 80% of them were in clinical response, as compared with 54% of patients with TL lower than 0,1 ug/ml (p=0.0043 by χ2 test). Median TL were not significantly different among clinical responders and non-responders.
Conclusion: The combination of ATI and infliximab TL assay could help in managing loss of clinical response in IBD.

P5.18.17
Mannose receptor contributes to intestinal inflammation

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Macrophage mannose receptor (MR) plays a role in innate immune responses. It is a multifunctional pattern recognition receptor that recognises a large number of micro-organisms including various bacteria, fungi, and viruses. We describe a novel role for the MR in LPS mediated cytokine responses and show that this receptor contributes to intestinal inflammation.
We studied MR mediated cytokine production in human and mouse macrophages. LPS stimulation of MR deficient macrophages showed at least a 50% decrease in IL-6, IL-12 and TNF-α production. Furthermore, incubation with MR blocking antibody gave a similar reduction in IL-6 in human monocyte derived macrophages. Following induction of dextran sodium sulphate (DSS) mediated colonic injury in MR deficient mice and WT mice we found that MR deficient animals had significantly reduced inflammation. Colon weights, pathology scores and levels of pro-inflammatory cytokines in the colons showed that MR deficient mice hardly had any inflammation. Furthermore, colons of MR deficient animals had significantly reduced influx of macrophages, DCs and neutrophils during DSS induced colitis. Using fluorescent immunohistochemistry we show that both in human and mouse the number of MR expressing macrophages increases during colonic inflammation.
Our data is the first to show that MR plays a significant role in LPS mediated macrophage responses and suggests that this is the underlying cause of reduced intestinal inflammation after DSS induced colitis in MR deficient mice. Since we found an increase in MR positive cells in IBD patients it is likely that MR plays a role in the pathogenesis of IBD.

P5.18.18
Microscopic colitis patients demonstrate a mixed Th17/Tc17 and Th1/Tc1 mucosal cytokine profile

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Data on the local cytokine profile in collagenous colitis (CC) and lymphocytic colitis (LC) is limited. This study investigated the T helper (Th) cell and cytotoxic T lymphocyte (CTL) mucosal cytokine profile at messenger and protein levels in CC and LC patients. Mucosal biopsies from CC (n=10), LC (n=5), and CC or LC patients in histopathological remission, (CC-HR, n=4), (LC-HR, n=6), ulcerative colitis (UC, n=3) and controls (n=10) were analyzed by real time PCR and Luminex. Mucosal mRNA but not protein levels of IFN-γ and IL-12 were significantly up regulated in CC, LC and UC patients compared to controls. Transcription of the Th1 transcription factor T-bet was significantly enhanced in CC but not LC patients. mRNA levels for IL-17A, IL-21, IL-22 and IL-6 were significantly up regulated in CC and LC patients compared to controls, albeit less than in UC patients. Significantly enhanced IL-21 protein levels were noted in both CC and LC patients. IL-6 protein and IL-1β mRNA levels was increased in CC and UC but not LC patients.
Although at lower magnitude, IL-23A mRNA was upregulated in CC and LC, whereas TNF-α protein was increased in CC, LC and UC patients. Neither mRNA nor protein levels of IL-4, IL-5 or IL-10 were significantly changed in any of the colitis groups. LC-HR and especially CC-HR patients had normalized mRNA and protein levels of the above cytokines compared to LC and CC patients. In conclusion, LC and CC patients demonstrate a mixed Th17/Tc17 and Th1/Tc1 mucosal cytokine profile.

P5.18.19
Involvement of mast cells in the development of colitis-associated colon cancer

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The chronic inflammatory process contributes multi-step tumorigenesis of colorectal cancer in the inflammed colon. The aim of this study was to examine whether mast cells regulate colitis-associated colon cancer (CAC) in the mast-cell-deficient mice (W/W^{v})

and C57BL/6 wide-type (WT) mice. *W/W^v* and WT mice (each n=10) intraperitoneally given azoxymethane (AOM) were provided 2.5% dextran sodium sulfate (DSS) in drinking water, repeated for 3 cycles. Colon tissues were used to examine the development of colon cancer, various gene expression, and activation of MAPK signals. As a result, macroscopic and microscopic analysis showed that W/W^v mice were significantly attenuated in colon tissue injuries and the occurrence of colon cancer induced by AOM+DSS compared to WT mice. In particular, the expression of cancer proliferating proteins, including Ki-67 and β-catenin and the inflammatory makers, including COX-2 and iNOS, were dramatically decreased in in colon tissues of W/W$^{v(+)}$ mice. Moreover, W/W^v mice revealed the attenuation in MAPKs phosphorylation including JNK, ERK, and p38 in the colon tissues. Thus, these results suggest that mast cells could affect the generation of colitis-associated colorectal cancer through pathological multiple mechanisms

P5.18.20
Whey and Soy protein supplements improves body composition in patients with Crohn`s disease under azathioprine and anti-TNF- α therapy

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Introduction: Cohn's disease (CD) is a chronic inflammation of the gastrointestinal tract. Obesity is becoming common among CD patients and it is well known that adipose tissue produces pro-inflammatory cytokines creating a favorable environment for disease progression.
Objective: Evaluate the effects of two nutritional supplements on nutritional status and disease's activity of CD patients.
Methods: A double-blind, randomized clinical trial study was conducted with two groups of patients: one was supplemented with whey protein enriched with TGF-β (WP-TGF-β) and the other was supplemented with soy protein (SP). Bioimpedance analysis, anthropometric measurements, Crohn's Disease Activity Index (CDAI), and dosages albumin, prealbumin and C-reactive protein (CRP) were performed at the beginning of the intervention and after 8 and 16 weeks. Spearman's correlation, Student's t-test, Friedman test and Analysis of Variance were used to analyze data.
Results: Forty-one patients received WP-TGF-β or SP; 29% were overweight or obese. There were no significant differences between the groups before and during the study, but both supplements improved body composition. Triceps skin fold ($p< 0.001$) and body fat percentage ($p=0.001$) decreased, whereas mid-arm muscle circumference ($p=0.004$), corrected arm muscle area ($p=0.005$) and lean mass percentage ($p=0.001$) increased. CDAI was positively correlated to CRP levels, and both negatively correlated to albumin and pre-albumin.
Conclusion: These different sources of protein reduced body fat. Although the underlying mechanisms are still unknown, we suggest that these supplements contribute to a reduction the inflammatory cytokine secretion of the adipose tissue, preventing CD relapse.
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P5.18.21
The role of FIBCD1 in intestinal homeostasis and inflammation

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We have recently identified and characterized FIBCD1 as a homotetrameric transmembrane protein expressed by epithelial cells in the gastrointestinal tract. Moreover, we have shown that FIBCD1 is an endocytic receptor that binds selectively and calcium-dependently to acetylated structures but not their non-acetylated counterparts.
We have produced fibcd1-/- mice and investigated the role of FIBCD1 in gut homeostasis and in a mouse model for inflammatory bowel disease (IBD). The unchallenged fibcd1-/- mice have an increased intestinal permeability and display a reduced intestinal mucosal layer compared to wild type littermates. When challenged in a chemically induced colitis model, fibcd1-/- mice exhibit significantly elevated weight loss, reduced colon length and increased cytokine levels compared to that of wild type littermates.
The observed phenotypes of fibcd1-/- combined with the binding affinity toward acetylated structures led us to investigate a potential interaction between FIBCD1 and mucosal components. Using in vitro assays we demonstrate that FIBCD1 binds solubilized mucus with high affinity in a calcium-dependent manner. Moreover, we identify the glycosaminoglycan (GAG) chondroitin sulphate A as a potential mucosal component through which FIBCD1 interact.
Taken together our results suggest that FIBCD1 play a role in the maintenance of intestinal homeostasis during steady state and inflammatory conditions, through interactions with mucosal components containing chondroitin sulphate A.

P5.18.22
Protective role of HLADRB1*04 allele against ulcerative colitis in Iran

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Crohn's disease (CD) and ulcerative colitis (UC) are two major clinical manifestation of inflammatory bowel disease (IBD) with uncertain etiology thought to be triggered by interactions between various environmental, genetic, and immunologic factors. Previous studies on the association of UC and human leukocyte antigen (HLA) class II genes suggested a role for HLA DRB1 gene polymorphisms with this disease. Several studies reported that HLADRB1*1502 and HLADRB1*0101 are in association with UC in the different populations such as Japanese. The aim of this study was investigation of association between UC and HLA-DRB1 gene polymorphisms using molecular technique. 95 healthy controls and 85 patients with ulcerative colitis disease were enrolled in our study. Molecular technique based on PCR-SSP was recruited to detect HLA-DRB1 gene polymorphisms. Allelic analysis showed that HLA-DRB1*01 and HLA- DR B1*15 are not in association with UC in our patients whereas the common HLA-DRB1*04 allele is significantly associated with the disease ($p<0.05$). According to the results of this study, HLADRB1*04 allele seemingly has protective role against the ulcerative colitis disease in our patients, in Iran. Genetic factors also vary from region to region due to differences in race, ethnicity and geographical conditions. Future research on genetic susceptibility to ulcerative colitis in different regions in Iran might be helpful to people who are prone to this disease.
Key word: HLA DR B1, ulcerative colitis, HLA DRB1*04 , Iran, PCR-SSP

P5.18.23
SHIP represses auto-inflammation and is required for intestinal immune homeostasis

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Crohn's Disease (CD) is an inflammatory bowel disease characterized by inflammation in the gastrointestinal tract. It has been suggested that CD may result from a primary immune dysfunction in macrophages. Macrophages contain multi-protein complexes, inflammasomes that mediate the production of IL-1β, a key driver of auto-inflammation. The SH2 domain-containing inositol 5´-phosphatase, SHIP, is a hematopoietic-restricted negative regulator of class I PI3-kinases (PI3K). We have reported that SHIP-/- mice develop spontaneous intestinal inflammation that shares key

pathological features with CD. Herein, we demonstrate that ileal homogenates from SHIP knockout mice contain high levels of IL-1β. Ex vivo SHIP-/- macrophages produced significantly more IL-1β than SHIP+/+ upon inflammasome activation and increased IL-1β production was dependent on TLR activation of the p110α subunit of class I PI3K. Intestinal pathology was dramatically reduced in SHIP-/- mice by depleting macrophages with clodronate-containing liposomes or by treating mice with the IL-1 receptor antagonist, anakinra. Furthermore, we show that SHIP protein levels are low in PBMCs and ileal biopsies in a subset of subjects with ileal CD. A single nucleotide polymorphism (SNP) in the autophagy-related gene, ATG16L1, is a susceptibility locus for CD and we have found that reduced SHIP expression correlated with expression of the ATG16L1 SNP. Importantly, PBMCs from subjects with low SHIP levels produced more IL-1β than PBMCs from subjects with high SHIP levels. Taken together, our data suggest that CD may be caused by auto-inflammation in some people and that these people may be amenable to treatment with anakinra or other IL-1/IL-1R antagonists.

P5.18.24
Multispecific anti-neutrophil cytoplasmic antibodies in chronic inflammatory bowel diseases: what does that mean?

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Background: Neutrophilic proteases and cationic proteins have antimicrobial and immunomodulatory effects. Recent data suggest an association between gut inflammation and immune response to commensal or pathogenic bacteria in inflammatory bowel diseases (IBD). We analyzed specificity of anti-neutrophil cytoplasmic antibodies (ANCA) in IBD patients and its clinical significance.
Methods: Data from 52 ulcerative colitis (UC) patients with 32 Crohn's disease (CD) patients were compared. Primary sclerosing cholangitis (PSC) was present in 12/84 patients. ANCA, ANA and anti-smooth muscle antibodies (ASMA) were detected by IIF. ANCA were tested by ELISA for proteinase 3 (PR3), myeloperoxidase, bactericidal/permeability increasing protein (BPI), elastase, cathepsin G, lysozyme and lactoferrin.
Results: pANCA were more frequently present in UC than in CD patients ($p<0.001$). ANCA titer correlated with the disease activity only in UC patients ($p<0.05$). UC patients more frequently had two or more ANCA specificities compared to CD patients ($p<0.01$). Patients with UC more frequently had ANCA specific to BPI ($p<0.01$) and PR3 ($p<0.05$) compared to CD patients. 14/22 UC patients with multispecific ANCA concomitantly had ANA and ASMA. Multispecific ANCA in medium and/or high concentrations were associated with long-lasting ($p<0.05$) and left-sided UC ($p<0.001$). Multispecific ANCA with ANA and ASMA had sensitivity 67% for PSC.
Conclusion: Higher concentrations of multispecific ANCA in long-lasting, left-sided UC suggest an influence of bacterial stimulation on the break of tolerance. Multispecific ANCA with ANA and ASMA could be markers for PSC. ANCA specific to several antigens may worsen inflammation by reducing antimicrobial capacity of neutrophil proteases and cationic proteins.

P5.18.25
Phenotypic and functional characterization of intraepithelial and lamina propria inflammatory cell infiltrate in autoimmune enteropathy

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Autoimmune enteropathy (AE) is a rare syndrome characterized by severe villous atrophy, lamina propria (LP) infiltration by T lymphocytes and increased intraepithelial lymphocytes (IEL).
We characterized phenotype and function of inflammatory cells obtained from duodenal biopsies at disease onset and after steroid therapy.
Biopsies' analysis revealed an extensive infiltration of IEL mainly expressing CD4-CD8+CD103+. These cells produced high amounts of IFN-γ and a relevant amount of them coexpressed IL-17. Steroid treatment reduced the number of IEL CD4-CD8+CD103+ as well as the amount of IFN-γ and IL-17. Within LP CD4+ T cells, we found the same proportion of CXCR3+ and CCR6+ and a relevant number of CXCR3+CCR6+ cells. LP CD4+ T cells expressed high levels of IFN-γ, IL-17 and IL-22, while CD8+ cells expressed mainly IFN-γ.
After therapy we observed an increased number of CXCR3+ and a decreased number of CCR6+ and CXCR3+CCR6+ cells. Nevertheless, the levels of IFN-γ were dramatically reduced while IL-17 and IL-22 were increased. Steroid treatment was not affecting CD8+ cytokine production. In LP we found two population of dendritic cells (DCs), one expressing cell myeloid marker CD11c and CD103, the other being CD11c- CX3CR1+. Steroid treatment induced an increase of CX3CR1+ DC and a decrease of CD11c+ CD103+ DC.
Our data demonstrated that active AE leads to a massive infiltration by CXCR3+, CCR6+ cells and activated CD8+ T cells in LP, which is reduced after steroid therapy.
These results could be relevant for the identification of novel therapeutic targets in patients with AE.

P5.18.26
Decrypting the role of S1P/SphK axis in the dextran sulphate sodium-mediated colitis in mice

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Ulcerative colitis (UC) is one of the common types of inflammatory bowel disease (IBD) in humans. The aetiology is still not well defined. Dextran sulphate sodium-mediated colitis in mice is a well-studied model for human UC. In the present study, we have specifically decrypted the role of sphingosine 1 phosphate (S1P) and sphingosine kinase (SphK) in the development of DSS-colitis in mice. For acute colitis induction, the wild-type (WT) C57BL/6j, SphK1-/- and SphK2-/- mice were given 3.5% (weight/volume) DSS (molecular weight 36-50 kDa; ICN Biomedicals, Aurora, Ohio, USA) in their drinking water from day 0 for consecutive 7 days. The control mice were given with normal drinking water. The body weight, stool consistency, and rectal bleeding were monitored daily using the modified method of Cooper and colleagues. Body weight loss was calculated as the difference between the predicted body weight and the actual body weight on a particular day. All the mice were sacrificed on day 7. The blood samples were collected for the isolation of serum for S1P and multiplex cytokine assays. The colons were dissected and properly cleaned for morphological, histological and molecular analyses. The serum S1P levels were significantly elevated in the WT mice administered with DSS compared with SphK1-/- and SphK2-/- mice. Furthermore, both SphK1-/- and SphK2-/- mice were protected from body weight loss, colon shortening, and other pathological changes. Our results vouch for the importance of designing specific therapeutic strategies to target S1P/SphK axis in human UC in the near future.

P5.18.27
Systems immunological strategies to decipher the genomic expression patterns in dextran sulphate sodium-mediated acute colitis

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Dextran Sulphate Sodium (DSS)-induced colitis in mice is a thoroughly studied model for human UC. In the present study, we

have deduced the genomic signatures and biological pathways associated with DSS-induced colitis in mice using systems immunological approaches. Raw microarray expression (Affymetrix CEL) files (GSE22307) in the public domain derived from mouse colon tissue in response to DSS induction at day 0, 2, 4 and 6 were downloaded from the Gene Expression Omnibus (GEO) and analysed by Genespring GX 12.5 software (Agilent, USA). The significantly expressed genes were selected by a standard cut-off at 2-fold increased expression compared with the values on day 0. These differentially expressed genes were then classified based on Gene Ontology (GO) such as the expression of receptors, endopeptidases, regulation of inflammatory response, cytokines and chemokines as well as chemotaxis in the colonic epithelium of DSS-induced colitis in mice. Furthermore, the differentially expressed genes were analysed by Ingenuity Pathway Analysis (IPA) software. The Genespring GX12.5 analysis coupled with IPA to delineate the effect of DSS on the colonic epithelial cells has shown that it could potentially induce the expression of proinflammatory cytokines and chemokines, immune receptors, as well as the adhesion molecule expression in the colonic epithelium. Besides, it has also significantly induced the expression of transcription factors such as NFkB as well as the tissue destructive enzymes such as MMPs. Henceforth, designing and assessing biological therapies against these disease causing genomic signatures would help in the improvement of current therapeutic modalities against human UC.

P5.18.28
Dissecting the effect of resveratrol in dextran sulphate sodium-mediated colitis in Balb/c mice

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Resveratrol (3,5,4'-trihydroxy stilbene) is a phytoalexin. In the present study, we have dissected the therapeutic effect of resveratrol in dextran sulphate sodium (DSS)-mediated colitis in mice. For colitis induction, the male & female Balb/c mice were given 3.5% (weight/volume) DSS (molecular weight 36-50 kDa; ICN Biomedicals, Aurora, Ohio, USA) in their drinking water from day 0 for consecutive 15 days. The control mice were given only normal drinking water. The two treatment groups received 10 mg/Kg and 15 mg/Kg of resveratrol i.p in 200 µL respectively and 3.5% DSS in the drinking water. On the other hand, the positive control group received only 200 µL PBS i.p and 3.5% DSS in the drinking water. The body weight, stool consistency, and rectal bleeding were monitored daily using the modified method of Cooper and colleagues. Body weight loss was calculated as the difference between the predicted body weight and the actual body weight on a particular day. All the mice were sacrificed on day 15. The resveratrol treated mice given with DSS had significantly less body weight loss, colon shortening compared with the positive control. The amelioration of DSS-induced colitis in both male and female Balb/c mice could be ascribed to the decrease in local and systemic inflammation. Besides, it would be valuable to explore the therapeutic targets of resveratrol for the development of de novo therapeutics for ulcerative colitis in humans.

P5.18.29
TL1A/DR3 interaction is associated with pathogenesis of inflammatory bowel disease in adults and children

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TL1A is a proinflammatory cytokine and the ligand for death receptor 3 (DR3). These proteins are strongly up-regulated on activated cells of the immune system. TL1A/DR3 interaction costimulates T-cells and induces production of several proinflammatory cytokines (including IL-4, IL-13, IL-17A, IFN-γ) by these cells. TL1A and DR3 are linked to the development of Crohn's disease (CD) and ulcerative colitis (UC), although their exact pathological role remains unknown. In this study, we investigated the mRNA level of TL1A, IL-4, IL-13, IL-17A and IFN-γ in colon mucosal biopsies of paediatric and adult inflammatory bowel disease (IBD) patients. We found that expression of TL1A was significantly elevated in inflamed but not in non-inflamed colonic tissue of both CD and UC patients as compared to healthy individuals. Interestingly, we detected up-regulation of DR3 only in inflamed colonic tissue of paediatric but not adult patients. Up-regulation of TL1A mRNA was accompanied by elevated expression of proinflammatory cytokines and positively correlated with the expression level of IL-17A mRNA in inflamed and non-inflamed tissue of both paediatric and adult UC patients. Furthermore, TL1A and DR3 protein expression was localized by immunohistochemical technique not only to mucosa-infiltrating mononuclear cells but also to enterocytes. Our study also provides evidence for the first time that TL1A mRNA expression is significantly higher while DR3 mRNA expression is significantly lower in healthy adults compared to healthy children. These findings show that TL1A contributes to the development of IBD via induction of IL-17A expression and this observation may have therapeutic implications.

P5.18.30
Anti-TNF-alpha monoclonal antibody therapy of crohn's disease is associated with acute reduction of circulating classical and intermediate monocyte numbers

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Monocytes, recently classified as $CD14^{++}/CD16^{-}$ (classical), $CD14^{++}/CD16^{+}$ (intermediate) and $CD14^{+}/CD16^{++}$ (non-classical), play an as-yet poorly characterised role in inflammatory bowel disease (IBD) pathogenesis. In this study, proportions and absolute numbers of circulating monocyte subsets were determined by flow cytometry of fresh PBMC samples from 3 groups: **A**. Healthy controls (n=16); **B**. Crohn's Disease (CD) not receiving infliximab (n=6); **C**.CD receiving infliximab (n=19). For Group C, samples were analysed before and after anti-TNF-[[Unsupported Character - Symbol Font α]] (infliximab) infusions.
Total monocyte numbers were higher in Group A compared to Groups B and C, (15.0±3.4; 10.0±3.0 and 4.5±5.8 $x10^4$ cells/ml respectively; $p<0.0001$, one-way ANOVA). Group C total monocytes were further reduced to 1.7±1.4 $x10^4$ cells/ml following infliximab ($p=0.05$, paired t-test). Subset analysis indicated that classical monocytes were reduced to the greatest degree in CD patients (Group A 10.4±3.1; Group B 5.2±2.5; Group C, 2.0±2.1 pre-infliximab and 0.6±0.6 $x10^4$ cells/ml post-infliximab, $p<0.0001$). Similar trends were present for intermediate monocytes (Group A 3.4±1.8; Group B 2.7±1.3; Group C, pre-infliximab 1.5±2.2; Group C, post-infliximab 0.6±0.6 $x10^4$ cells/ml, $p<0.0001$), although the magnitude of the differences were not as great resulting in a proportionate increase in

intermediate monocytes in CD patient groups. Non-classical monocyte numbers demonstrated no significant differences between the groups.
Thus, Crohn's Disease is associated with reduction in total monocytes in the circulation that predominantly affects the classical subset. Infliximab infusion leads to further reduction in the classical and, to a lesser extent, intermediate subset suggesting that anti-TNF therapy directly targets specific monocyte populations.

P5.18.31
A crude extract of hydatid laminated layer prevents Dextran Sulfate Sodium induced-colitis in mice by Nitric Oxide Synthase2 down-regulation

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Inflammatory bowel diseases (IBD) are immunologically mediated diseases, they are more prevalent in developed countries but less frequent in countries where exposure to helminthes is common. Several studies suggest strongly a beneficial role of helminthes in IBD experimental models. Recent work of our team showed that Nitric Oxide (NO) production is strongly implicated in human IBD pathogenesis as well as in experimental colitis. *Echinococcus granulosus* is helminth which causes cystic hydatid disease; cysts of *E.granulosus* are able to survive for several years in chronic infection. Previously, our team showed that laminated layer extracted from the cyst wall induces parasitic protection by down-regulating NO production. The aim of the current study is to investigate the immuno-modulatory effect of the hydatid laminated layer in Dextran Sulfate Sodium (DSS) induced colitis model. An acute colitis was induced in Swiss mice using 2.5% DSS and the prophylactic effect of the laminated layer was investigated. A crude extract of laminated layer was daily administered orally starting five days before colitis induction. Colonic damage was evaluated histologically and NOsynthase2 expression was assessed by immunofluorescence staining. The production of NO was concomitantly measured in the supernatants of peritoneal macrophages (pMϕ) cultures. Interestingly, our results showed significant NO decrease levels in supernatant of pMϕ cultures in correlation with an important inhibition of colonic NOsynthase2 expression and decrease of mucosal alterations when compared with non treated mice.
Collectively, our findings suggest a potential preventive effect of the laminated layer by local NOsynthase2 down regulation in this model of colitis.

P5.18.32
Transfer of Myeloid Derived Suppressor Cells from neonatal tolerized IL-10 gene-deficient mice protects from development of intestinal inflammation.

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population characterized by the co-expression of CD11b and Gr-1 that exhibit potent immunosuppressive functions. We have previously shown that these cells greatly expand in IL-10 gene-deficient mice, tolerized as neonates to endogenous bacterial antigens, and are involved in the suppression of the bacterial antigen-stimulated T cell responses and, hence, reduced intestinal inflammation in these mice. To investigate whether MDSCs from tolerized mice can suppress colitis development in recipient IL-10 gene-deficient mice we isolated CD11b+ MDSCs by FACS from spleen cell cultures of 20 week old mice and transferred 106 cells per mouse by intra peritoneal injection into 8 week old IL-10 gene-deficient mice before the microscopic visible onset of colonic inflammation. Control littermates were injected with saline. Mice were analyzed 10 weeks later for macroscopic and histopathologic intestinal injury, pro-inflammatory cytokine and myeloperoxidase (MPO) release. Adopted transfer of CD11b+ MDSCs resulted in reduced incidence and severity of intestinal inflammation in IL-10 gene-deficient mice at age 18 weeks as judged by significantly reduced numbers in injury scores (0.8 ± 0.8 versus 4.4 ± 1.2 in controls) and colon weight/length ratio (49.3 ± 9.6 versus 76.7 ± 7.5 in control) as well as a decrease in intestinal MPO and IFNγ release compared to saline treated controls. Treatment with MDCS can protect IBD-prone IL-10 gene-deficient mice from developing disease. These results indicate that MDSCs play an immunoregulatory role in intestinal inflammation and that an enrichment of MDSCs may provide a therapeutic approach for IBD development.

P5.18.33
Infiximab Simultaneously Restore Foxp3$^+$Treg, Type 1-Like Treg and B cells in IBD

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Background: Infliximab (IFX) increases circulating Foxp3(+)T cells in patients(pts) with IBD. Co-expression of CD45RA & Foxp3 distinguishes resting & active Treg(rTreg&aTreg) from Foxp3(+)effector T cells(Teff). Subsets of B cells induce Tr1-like cells(Tr1L) and expand Foxp3+Treg.
We investigated these cells in IBD during IFX therapy.
Methods: Blood was taken from healthy controls(HC, N=37) and pts (N=99) and assessed by flow cytometry. Globe Assessment distinguished responders(RS, N=59) from non-responders (NRS, N=15).
Results: 1. Pts before therapy had low rTreg(0.43±0.080, p<0.001), aTreg(0.62±0.12, p<0.001), Foxp3Teff(2.38±0.27, p=0.002) , Tr1L(4.79±0.68,p<0.001) and B cells(0.17±0.02, p=0.002)(N=25), compared with HC(1.47±0.16),(2.40±0.17),(3.75±0.34),(16.82±1.7) and (0.27±0.02)(10e6/L blood mean±SEM for Tr1L, 10e9/L for others).
2. Compared with baseline, change was seen in rTreg(RS: 1.57±0.21, p<0.001; NRS: 1.14±0.24, p<0.001), aTreg(RS: 2.70±0.26 , p<0.001; NRS: 1.48±0.33,p=0.0057), Foxp3+Teff (RS: 3.19±0.24, p=0.09; NRS: 3.02±0.41,p= 0.25),Tr1L(RS: 26.09±2.21, p<0.001; NRS: 8.92±1.00, p=0.013) and B cells(RS: 0.25±0.03, p=0.035;NRS:0.14±0.01, p=0.31).
3. Significant differences between RS and NRS only for aTreg, Tr1L and B cells(p=0.0067, <0.001, <0.001).
4. CRP negatively correlated with rTreg, aTreg, Tr1L and B cells (p=0.0011, r=- 0.32),(p<0.001, r=- 0.40),(p<0.001, r=- 0.39) and(p=0.044, r=- 0.21).
5. B cells positively correlated with rTreg, aTreg and Tr1L(p=0.002, r=0.31), (p<0.001, r=0.49) and(p<0.001, r=0.37).
Conclusions: Foxp3T cells,Tr1L and B cells are decreased in active IBD and an increase in these cells (except for Foxp3Teff) correlates with biological response and/or with the clinical response. The positive correlation between B cells and others might suggest a cross talk between B cells and Tregs.

P5.18.34
BCG-specific lymphocyte proliferation and cytokine in patients with Crohn` s disease under anti-TNF- α and azathioprine therapy.

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Background: Anti-TNF-alpha, in combination with azathioprine, is used in patients with Crohn Disease (CD) and may result in an increased risk of infection and latent tuberculosis reactivation.
Objectives: To evaluate BCG-specific lymphocyte proliferation and cytokine production in cultures with peripheral blood mononuclear

cells (PBMC) from patients with Crohn's disease under anti-TNF- α and azathioprine therapy.
Methods: Thirty-two patients who were using anti-TNF-α and azathioprine (Group A) and 11 patients using only azathioprine (group B) were followed up at the Gastrocenter, UNICAMP, all of them with BCG scar. The comparison group consisted of 34 healthy individuals (group C) with BCG scar. BCG-specific lymphoproliferation and T-cell subsets CD4+, CD8+ and TCR γδ+ were analyzed by flow cytometry and cytokine concentration by ELISA. Data analysis was performed with Kruskal-Wallis test and nonparametric multiple comparison ($p < 0.05$).
Results: When compared to the healthy control group the patients from group A and group B showed significant reduction ($p < 0.001$) in the following parameters: BCG-specific lymphoproliferation, TCRγδ+ lymphoblasts and production of IFN-γ, TNF-α, IL-6 and TGF-β1. However, there was no statistical difference when the A and B patient groups were compared.
Conclusion: We conclude that BCG specific adaptive immune response in patients with CD treated with TNFα inhibitors and azathioprine is reduced and may contribute to an increase in the risk of infection and latent tuberculosis reactivation. Although BCG scar was present before starting therapy, the patients should be screened for possible latent infections.
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P5.18.35
Prevalence of IgA anti-zein antibodies in Mexican patients with celiac disease (CD) and irritable bowel syndrome (IBS)

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Introduction: A high prevalence of CD is found among patients with a clinical diagnosis of IBS. CD is a gluten-sensitivity autoimmune disease of the small intestine affecting genetically susceptible individuals worldwide. The gluten-free diet is not enough to treat Celiac Disease patients, because some of them present high inflammation, poor vitamin status, and leaky gut which persist on a gluten-free diet, suggesting there are cross reactivity or specific immune responses against related-prolamines in other cereals. In this study we evaluated the prevalence of IgA anti-zein antibodies (IgA-AZA) in a group of Mexican patients diagnosed with CD and IBS. Corn has been the basis of the Mexican diet for thousands of years, however the humoral response against zein has not been studied before. Methods: 23 CD and 366 IBS patients were included in this study. All of the IBS subjects met the Rome III criteria and were negative for the following tests: IgA h-tTG, IgA and IgG Gliadin II (INOVA diagnostics, Inc). IgA-AZA were measured by ELISA. Results: 4 patients with CD (17.39%) were positive to IgA-AZA. The prevalence among women was 15.78% (3/19) and for men 25% (1/4). 13 patients with IBS (3.55%) were positive to IgA-AZA. The prevalence among women was 4.16% (13/312). Conclusion: There is a high prevalence of IgA-AZA (17.39%) in Mexican patients with CD and even in patients with IBS. These results open the possibility to explore more about the contribution of the humoral response to zein in the immunopathogenesis to CD and IBS.

P5.18.36
NDST4 deficiency enhances acute colitis and colitis-associated tumorigenesis in mice

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NDST4 is one member of N-deacetylase/N-sulfotransferase (heparan glucosaminyl) (NDST) family, which are responsible for heparan sulfate (HS) biosynthesis on a core protein to form heparan sulfate proteoglycans (HSPGs). The HS chains of HSPGs bind to and regulate their functions of various growth factors, cytokines and chemokines. HSPGs ubiquitously reside on cell surface, inside the cell, and in the extracellular matrix. Importantly, the content and distribution of HSPGs are altered during tumorigenesis. Here we show that 30 (57.7%) out of 52 human colorectal carcinomas exhibited a dramatic reduction in NDST4 RNA transcripts, and that genetic loss of NDST4 was significantly associated with poor survival of patients with colorectal cancer. In addition, we generated an NDST4-knockout mouse strain, which developed and reproduced normally, while showed a marked increase in the thickness of the colonic crypts and the number of goblet cells of the colonic mucosa. Using the dextran sodium sulfate model of acute colitis, homozygous NDST4 mutants showed a significantly higher disease activity index and obviously hyperemic appearance in the cecum. In a colitis-associated cancer model, the size of colonic tumors induced by azoxymethane/dextran sodium sulfate was significantly increased in NDST4-deficient mice compared with wild-type mice. These results suggest that NDST4 might limit tumor-promoting inflammation and tumorigenesis by regulating the modification of HS chains of specific HSPGs in colon.

P5.19 Reproductive immunology

P5.19.01
HLA antigens and anti-sperm antibody

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Background: Anti-sperm antibodies (ASAs) leading to autoimmune male infertility. It is suggested that HLA antigen play key roles in the autoimmune diseases and antibody production. Therefore in this study we evaluated the serum ASA in men after vasectomy and investigated the correlation of ASA production with HLA class I and II in vasectomized men.
Methods: 110 vasectomized and 130 healthy men were examined for ASAs by Gelatin Agglutination Test (Kibrick) and indirect mixed antiglobulin reaction (MAR) test. Also their HLA-A, B, C, DR and -DQ frequency were investigated using microlymphocytotoxicity method in T cells and B cells.
Results: The ASA was detected in 95% vasectomized men, regarding that 53% have an ASA titer of 1/32 or 1/64. Whereas, the levels of ASA only in 13.8% normal men were positive with maximum titer 1/8. No statistically significant correlation was found between the high responder group and frequency of HLA antigens, but a statistically significant correlation was observed in the low responder group and frequency of HLA-A2 and DQw2 antigens. **Conclusion:**
Predisposition to produce ASA seems correlate with HLA class I and II antigens, so that men expressing HLA-A2 and DQw2 produced lower ASA than other populations, although understanding the impact of these findings requires further study.

P5.19.02
Multiple immune accentuations predict implantation failure and mark target group among IVF patients for intravenous immunoglobulin therapy

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Problem: Studies of immune markers that predict implantation failure and show indications for intravenous immunoglobulin therapy may be perspective for IVF results.
Method of study: Totally 244 women (≥3 IVF failures) in IVF program were investigated as two groups: 123 women not treated by IVIG and 121 women treated by IVIG. Flow-cytometry was used to study NK

cell cytotoxicity, lymphocyte subsets and expression of activating molecules on them.
Results: We selected some immune accentuations (IA) that predict implantation failure: elevated expression of CD56 and CD158 in T cells, decreased levels of T helper cells and increased levels of CD8 T cells, elevated expression of HLA-DR in CD8 T and NK cells, elevated NK cell count and NK cytotoxicity, decreased expression of CD158a and CD8 in NK cells. In women without or with isolated IA Pregnancy Rate (PR) and Birth Rate (BR) were 50.9% (27/53) and 33.9% (18/53). In contrast, patients with more than 2 IA had dramatically decreased PR and especially BR 21% (9/42) and 9.5% (4/42). Patients with more than 2 IA had not decrease of PR (43%, 20/46) and BR (30.4%, 14/46) if IVIG treatment was used. These indices were significantly higher in comparison to women not treated by IVIG. So IVIG treatment significantly increased PR and BR in women with multiple immune accentuations.
Conclusions: Multiple immune accentuations (more than 2) may serve as immune markers that predict implantation failure and at the same time mark target group for intravenous immunoglobulin therapy to improve results of IVF procedure.

P5.19.03
Features of the immune response and microbial colonization in children with extremely low birth weight

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101 newborns with extremely low birth weight (ELBW) from birth until 38-40 weeks of adjusted age were examined: 60 children with a gestational age of 23-27 weeks, and 41 infants - 28-31 weeks. The control group included 18 full-term infants. Cytokines and acute-phase proteins were determined using kits made by Vector-Best (Russia). The immune system of premature infants with ELBW at birth was characterized by leukopenia, neutropenia, decreased levels of lactoferrin, ferritin, haptoglobin, IFN-γ and increased expression of the apoptosis-related molecule CD95 and C-reactive protein (CRP) concentrations. The ratio of INF-γ-and IL-4-producing T cells is biased towards the Th-1 path. The children with ELBW at term had increased B-lymphocyte and NK-cell numbers and preserved Th1-dependent immune responses while maintaining leukopenia and neutropenia and reduced content of lactoferrin, ferritin and CRP. Children born at 23-27 weeks of gestation had decreased neopterin concentration, children born at term (28-31 weeks) had a reduced level of haptoglobin. All the children with ELBW at 5-7 days of life had a colonization of the intestine by gram-positive cocci. Coccal flora of the intestine pushed by the opportunistic pathogens for the gestational age full-term children (to a greater degree in children, born with a lower gestational age), while receiving antibiotic therapy and passive immunization which was associated with a violation of the local (reduced sIgA in coprofiltrates) and system (reduction of neopterin in the blood) immunity.

P5.19.04
Maternal-fetal immunotolerance from mice with pregnancy loss induced by adoptively transferring exosomes from T lymphocytes

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Objective: To explore the feasibility of exosomes from T lymphocyte as a non-cellular therapy that can replace the traditional cellular therapy on unexplained recurrent spontaneous abortion (URSA). Methods: Exosomes from splenocytes of BALB/c male mice were isolated by sucrose gradient ultracentrifugation and ultrafiltration. CBA/J (♀) without mating as non-pregnancy. CBA/J (♀) ×BALB/c (♂) as normal pregnancy, and CBA/J(♀) ×DBA/2 (♂) as URSA were randomly divided into groups of URSA, Cellular Therapy and Non-cellular Therapy. The rates of fetal absorption and pregnancy loss were calculated. A492 of induced proliferation were measured through MTT, the expressions of Th1-type (IFN-γ, IL-2) and Th2-type cytokines (IL-4, IL-10) were analyzed by flow cytometry. Through immunohistochemical staining, the expressions of immunosuppressors (TGF-β1, IL-10, COX-2) were analyze. Results: After both of cellular and non-cellular adoptive transferring, the rates of embryo adsorption and pregnancy loss, and the A492 of splenocytes decreased significantly (All $P<0.01$), in which the effects of non-cellular therapy exerted greatly more than cellular therapy ($P<0.05$). Conclusions: (1) Adoptive transfer of periphery lymphocytes or their non-cellular components can induce maternal-fetal immunotolerance, which should be helpful for normal pregnancy. (2) Adoptive transfer of non-cellular components from paternal lymphocytes can induce stronger maternal-fetal immunotolerance than T lymphocytes. (3) Peripheral lymphocytes or non-cellular components should initiate Th2 shift and local immunosuppressions in placenta, and induce efficient maternal-fetal immunotolerance. (4) Non-cellular components secreted by T lymphocytes from paternal individuals could become a biological product, which should replace traditional adoptive immunotherapy of lymphocytes.

P5.19.05
The predominance of Th17 lymphocytes and decreased number and function of Treg cells are present in preeclampsia

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Problem: The aim of the study was to estimate the prevalence of T lymphocytes producing IL-17, T regulatory cells and the immunosuppressive activity of Treg cells of patients with preeclampsia in comparison with healthy women in the third trimester of pregnancy. Material and methods: 34 patients with preeclampsia and 27 healthy women in third trimester of pregnancy were included to the study. The percentage of Treg cells and CD3+CD4+ T lymphocytes with intracellular expressions of cytokines were estimated using flow cytometry. The in vitro functional assays were performed with the use of Treg Cells Isolation Kit and 3H-thimidine. Results: The percentages of T lymphocytes producing IL-17A were significantly higher in preeclampsia when compared to healthy normotensive pregnant women in the third trimester of normal pregnancy. The population of Treg cells was significantly lower in the study when compared to the control group. There were no changes in the stimulation index of CD3+CD4+CD25- T lymphocytes of patients with preeclampsia during in vitro assay without Treg cells and after the addition of autologous Tregs. In normal pregnancy the stimulation index of CD3+CD4+CD25- T lymphocytes was significantly higher without Treg cells when compared to this response after addition of autologous Tregs. Conclusions: The results obtained suggest the up-regulation of Th17 immune response in preeclampsia. It seems that the decreased number and function of Treg cells may be responsible for the activation of inflammatory response in this disorder. In preeclampsia the predominance of Th17 immunity can act through the modulation of Th1/Th2 immune response.

P5.19.06
Effect of prenatal steroid treatment on the developing immune system

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Prenatal steroids have an undisputed positive effect decreasing neonatal morbidity and mortality by improving fetal lung maturation. Some concerns have been raised on long term consequences on the HPA axis and cognition, but there are no studies addressing effects on the immune system. The thymus is an essential organ for the development and selection of T cells, and thymocytes are extremely sensitive to steroids. Using a mouse model for prenatal steroid administration, we show here that betamethasone treatment to the mother has a profound effect on the thymus of the offspring. We find the thymus volume reduced, affecting mostly the developing CD4+ CD8+ double positive thymocytes, and a compensatory accelerated

transition of the earlier stages to replenish the depleted compartment. This effect lasts for at least three days, which correspond to a very relevant period for the selection of the T cell repertoire. Moreover, we show that low doses of betamethasone have similar effects on human thymocytes. Therefore, further studies are needed to analyze possible long-term consequences of this treatment on the immune system of the offspring.

P5.19.07
HLA-G6 and reproductive immunology

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Background:The definite cause of several pathologies of pregnancy including reproductive failure is still unknown. HLA-G6, a nonclassical class Ib HLA, is novel molecule in the tolerance between fetus and mother. The purpose of this study was evaluation of HLA-G6 soluble isoforms and also difference in total expression of these antigens in threatened miscarriage pregnant women termination to reproductive failure in comparison with control group.
Materials & Methods: The study sample enrolled 101 threatened miscarriage pregnant women in age of 20-32Y in first trimester without any physiological or genetic disorders related to reproductive failure as cases and 101 women with normal pregnancy by history of term pregnancy and no miscarriage as controls. We evaluated expression of HLA-G6 soluble isoforms on PBMCs by Real-time PCR.
Results: The results showed the significant decrease in expression level of HLA-G6 isofrms in threatened miscarriage pregnant women in comparison with controls ($p< 0.05$).
Conclusion: The different concentration of HLA-G6 molecule is necessary for saving of pregnancy. Therefore, it is concludedthat HLA-G6 is an important molecule as predictive marker in early stages of reproductive failure for setting a clinical test.

P5.19.08
Automated clustering of flow cytometry data identifies a Th0 population induced by influenza vaccination in pregnant women

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Pregnancy alters the immune system, causing increased susceptibility to pathogens such as Influenza virus, and/or increased disease severity. This may be related to the proposed feto-protective model of a shift in T effector cell responses away from Th1 (and possibly towards Th2) during the gestational period. We recently showed that Ki-67 is a sensitive marker of influenza vaccination responses in non-pregnant individuals. We investigated responses to influenza vaccination during pregnancy using Ki-67 expression to focus on vaccine-induced responses. Blood was collected from pregnant and non-pregnant subjects prior to vaccination, at 7 and 28 days post vaccination, and 12 weeks postpartum. We evaluated CD4 T cell cytokine secretion after in vitro stimulation with influenza vaccine and 16-color flow cytometry. Datasets were analyzed with SWIFT, our new, high resolution flow cytometry algorithm, that uses model-based clustering to objectively detect very small altered populations in large, high-dimensional datafiles. Assigning samples to cluster templates allows rigorous comparison between samples. Both pregnant and non-pregnant subjects responded to the vaccine, as we detected a transient influenza-specific Ki-67+ cytokine-secreting population at day 7 in both groups. We also discovered an unusual CD4 T cell population secreting high levels of IL-10, IL-4 and IFNgamma in response to influenza. These cells were present in pregnant day 7 samples, but not in pregnant day 0 samples, or day 0 or 7 samples of non-pregnant subjects. Thus pregnancy does not prevent the Ki-67 response to influenza vaccination, but does produce a distinctly altered cytokine pattern, consistent with a stronger Th0 phenotype.

P5.19.09
Inflammation as a cause of placental dysfunction in high-risk pregnancies

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Inflammation during pregnancy is associated with stillbirth and life-long diseases in surviving newborns. The mechanism underlying this association is mostly unknown. We hypothesize that cytokines induce placental dysfunction, resulting in fetal nutrient/oxygen deprivation. To test this hypothesis we determined the inflammatory profile in human placentas from high-risk pregnancy associated with reduced fetal movements (RFM) and investigated the effects of identified cytokines on placental function.
Methods: Term placentas were collected from women experiencing RFM and compared with uncomplicated pregnancies. ELISA and immunohistochemistry were used to determine cytokine levels in placental lysates and assess their localization respectively. Placental explants from normal pregnancies were treated with interleukin (IL)-1 and the effects on inflammation, hormone production and cell turnover were determined.
Results: Levels of IL-1β and IL-1 receptor antagonist (IL-1Ra) were increased in RFM pregnancies, while levels of IL-6 and TNF- remained unchanged. Placentas from pregnancies with RFM also showed a decreased expression of IL-10, suggesting a pro-inflammatory imbalance within the tissue. RFM placentas also had elevated numbers of CD45+ cells within placental parenchyma, which were highly positive for IL-1Ra. Treatment of placental explants with IL-1 led to decreased secretion of hormone (hCG) and immune activation.
Conclusion: This study identifies a pro-inflammatory profile in high-risk pregnancies associated with RFM, characterized by a predominant involvement of the IL-1 system and decreased anti-inflammatory IL-10. IL-1-treated placental explants showed altered function. These results emphasize the importance of inflammation-induced placental dysfunction in human high-risk pregnancies and subsequent fetal mortality and morbidity.

P5.19.10
Inhibition of TLR3 mediated direct natural killer cell activation by a macrolide antibiotic clarithromycin

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Anti-inflammatory benefits of macrolide antibiotics s have long been reported in patients with chronic pulmonary inflammatory disorders. They are also prescribed for pregnant women with premature labour and threatened miscarriage. However, their effects on female reproductive immune responses are so far unknown. Human uterine mucosa especially decidual tissues contain a large number of Natural killer (NK) cells. They provide resistance to viral infections and play important roles in immune -surveillance and possible regulatory roles for maintenance of pregnancy. However over activation of uterine NK cells is considered to be involved in pregnancy failures including recurrent spontaneous abortion and premature labour.
In the present study we investigated inhibitory effects of a macrolide antibiotic clarithromycin (CAM) on TLR3 mediated interferon-γ (IFN-γ) production by human mucosal CD56+ NK cell line KHYG-1 using ELISA and flow cytometry.
We observed dose and time dependent suppression of the Poly(I:C) and IL-2 +IL-12 induced IFN-γ production in KHYG-1 cells. Of interest, CAM showed no remarkable suppression of other cytokine production including IL-22. Taken together, we propose decidual natural killer cells as novel targets of clarithromycin other than its antibacterial effects by inhibition of bacterial protein synthesis by binding to the bacterial 50S ribosomal subunit . As the safety of clarithromycin for early pregnant subjects has been reported recently on large cohort clinical studies, its use for prevention of miscarriage can be justified.

P5.19.11
The export of T helper cells from thymus during pregnancy

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Recent thymic emigrants (RTEs) comprise an essential population of T cells, connecting thymic output to the recirculating T cell pool, which can be a useful measure of thymic function. Little is known about the role of thymus in human pregnancy and pregnancy-associated tolerance. In this study we explore the role of thymus in human pregnancy by analyzing the output of T helper cells from the thymus. DNA from isolated CD4+ blood cells from healthy 2nd trimester pregnant and non-pregnant women were analyzed by RT-PCR for the content of T cell receptor excision circles (TRECs), which are enriched in newly synthesized T cells. Further, whole blood was analyzed for the frequency of RTE T helper subsets in pregnant and non-pregnant women by flow cytometry, utilizing CD31 as a marker of recent thymic exposure. Pregnant women had significantly reduced levels of TREC in the CD4+ population as compared to non-pregnant women, indicating a reduced output of the CD4+ population from thymus. Next, we attempted to determine if the decreased output during pregnancy also included regulatory T cells (Tregs). Preliminary results suggest that pregnant women rather have an increased frequency of RTE Tregs. In conclusion, we show that the TREC levels in CD4+ cells are reduced in pregnant women, likely as a result of reduced output of the entire T helper cell population from thymus. However, the output of Tregs may be differently regulated in pregnant women, contributing to the establishment and maintenance of the fetal-tolerant environment in pregnancy.

P5.19.12
Expression of LAG-3, but not PD-1, is enriched on double negative T cells at the female genital tract

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Background: LAG-3 is an inhibitory protein expressed on activated T, NK and NKT cells that is associated with immune exhaustion, but its expression at mucosal surfaces is unknown. Engagement of its ligand, MHC class II, initiates APC maturation and activation, and LAG-3 expression inhibits allo-responses, making it a candidate for the modulation of immune activation at the genital mucosa, an issue of importance during HIV exposure.
Methods: The expression of LAG-3, PD-1, CCR5, CD69 and CD40L were quantified on CD4+, CD8+ and double negative (DN) T cell populations in 11 matched PBMC and CMC samples from Kenyan women. Cytokine and chemokine concentrations in plasma and CVL samples were quantified by bead array.
Results: Expression of LAG-3 was significantly higher among CMC T cell subsets compared to PBMC ($p<0.01$), and was highest among the DN T cell subset. PD-1 expression was only significantly increased on CD4+ T cells derived from CMC ($p<0.05$). LAG-3+ T cells expressed CCR5 and CD69, indicating that LAG-3 expression may be associated with activated and HIV-susceptible CD4 T cell subsets. Double negative T cell activation correlated with soluble markers of mucosal inflammation ($p<0.05$).
Significance: The expression of LAG-3 at the genital mucosa suggests that a better understanding of its function in mucosal immunity is required. The enrichment of LAG-3 on mucosal DN T cells could be related to the immunoregulatory function of these cells in the periphery. The co-expression of LAG-3 with CCR5 and CD69 suggests the association between LAG-3 and HIV susceptibility should be further examined.

P5.19.13
Reduced inhibitory NK cell receptors expression in women with recurrent spontaneous abortions (RSA)

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It has long been suggested that NK cells and their allorecognition system based on inhibitory and activating NK cell receptors and their ligands play an important role in pregnancy as well as in miscarriages.
Aim of this study was to investigate the expression of NK cell receptors in couples with unexplained RSA.
Material and methods: 32 individuals who experienced RSA (16 women and their partners) were studied. Other probable genetic factors have been excluded. Expression of inhibitory (KIR2DL1, KIR2DL2/3, KIR3DL1) and activating (KIR2DS4, NKp46, NKp30, NKp40) receptors in NK cells were determined by flow cytometry using four color direct whole blood immunofluorenscence.
Results: Percentages of inhibitory CD158a (KIR2DL1) and CD158b (KIR2DL2/3) receptors expression in NK cells was found significantly decreased in women with RSA compared to their partners (19.2% (5.3-44.1) vs 30.2% (14.0-61.3) of NK cells, $p=0.012$ and 19.6% (0.0-41.2) vs 28.8% (6.2-49.0) of NK cells, $p=0.014$, respectively). Full repertoire of the three inhibitory KIRs tested was found in 18.75% of women in comparison to 31.25% of men. In addition, in 12.5% of women no inhibitory receptor was detected, a finding that was not observed in the respective partners. It should be noticed that 56.25% of women were lacking at least one inhibitory receptor expressed by their partner.
Conclusions: Our findings show that NK cells in women with RSA express low levels and a narrow repertoire of inhibitory receptors in comparison to their partners. This could possibly contribute to elevated NK cytotoxicity and may interfering with RSA pathogenesis.

P5.19.14
Lipid bodies in preimplantation embryo health

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Lipid bodies (LBs) are dynamic cell organelles, present in virtually any cell type and are involved in a number of cellular procedures ranging from energy storage and generation to cell activation, antigen cross-presentation, oocyte maturation and early stage embryo development. Treatments, such as L-Carnitine administration, that affect LB function and survival where followed in order to investigate the role of lipid bodies in preimplantation embryo development and correlate embryo health to maternal physiology in BALB/c mice. LB number and distribution in the preimplantation embryos was evaluated using confocal microscopy and Third Harmonic Generation microscopy, a novel non-destructive imaging technique. Maternal physiology was examined through cytokine evaluation and MHC production/expression, parameters actively involved in embryo development, in blood serum and isolated spleen cells. Although a 7-day in vivo administration of L-Carnitine in mice has been shown to prevent pregnancy by inducing an inflammatory state associated with infertility, administration for 3 or 4 days was able to attain oocyte maturation and even preimplantation embryo development, reducing, however, the LB content of the cells. Correlating embryo health to maternal physiology, it was shown that L-Carnitine administration in addition to lipid body reduction also induced an increase in MHC II and anti-inflammatory cytokine production.

P5.19.15
Influence of THP-1 cells on endothelial cells tube formation in the presence of soluble placenta-derived factors

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Angiogenesis plays an essential role in formation of placental vascular network. It is regulated by placental macrophages and other cells of placenta which secrete different cytokines. The aim of the research was investigation of THP-1 cells influence on capillary-like tube formation of endothelial cells EA.Hy926 at presence of soluble placental products obtained from healthy pregnant women on 38-39 weeks of gestation (n=20) and from pregnant women with an preeclampsia on 38-39 weeks of gestation (n=20). Endothelial cells were seeded on Matrigel-coated 24-well plates (BD, USA) at density of 150000 cells/well, also were added placental tissue supernatants and 2,5% fetal bovine serum. To part of wells THP-1 cells were added (250000 cells/well), to part of wells - the cultural medium without THP-1 cells. Capillary tube formation was assessed 24 hours later using microscope AxioObserverZ1(100x). It is established that tube length formed by endothelial cells was higher ($p<0.001$) and number of cellular aggregate was lower ($p<0.05$) in the presence of placental tissue supernatants from women with preeclampsia compared with physiological pregnancy. There were no differences between compared groups when THP-1 cells were added to the system. Our data suggest that in comparison with physiological pregnancy preeclamptic placenta tissue secretes factors that lead to reduced activity of angiogenic process and domination of non-branching angiogenesis, which suggests the violation of angiogenesis at this pathology. Addition of THP-1 cells led to compensation of the observed effects. Work is sustained by grants of the President of the Russian Federation №НШ-131.2012.7, СП-3492.2013.4 and МД-150.2011.7.
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P5.19.17
Effect of high-dose dexamethasone in early pregnancy on pregnancy outcome

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In the present study, we aimed to evaluate the anti-inflammatory effects of high-dose dexamethasone in the window of implantation on pregnancy outcome.
Pregnant BALB/c mice were treated daily with dexamethasone as experimental group or an equivalent volume of saline as control group on days 0.5-4.5 of pregnancy. On day 13.5 of pregnancy, the pregnant mice were sacrificed and placental, decidual and blood were collected. Serum levels of progesterone, 17-β estadiole and ferritin determined with ELISA. The effect of decidual and placenta cell supernatants (DS and PS, respectively) on PHA or LPS-induced lymphocyte proliferation was investigated by MTT assay.
Results of this study showed a statistically significant higher abortion rate in the experimental compared to the control group. Dexametasone treated BALB/c mice showed the increasing amounts of progesterone and decreasing amounts of 17-β estadiol, but we didn't see a significant difference in ferritin concentration between the two groups. MTT assay results showed that dexamethasone significantly decreased ($P < 0.05$) LPS and PHA-stimulated splenocyte proliferation in the experimental group compared to the control group. PS from experimental group compared to control group showed no significant effect on LPS and PHA-stimulated splenocyte proliferation.
The present results demonstrate that maternal high-dose dexamethasone application in early pregnancy has pronounced effects on hormone levels and the immune state of the decidua, and finally on fetus resorption.

P5.19.18
Genetic polymorphisms of KIR2DL4 and LILRB1 in spontaneous abortion and control women in Polish population

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NK cells are the major leukocyte population in the decidua during the first few months of pregnancy. These cells express a variety of receptors, among them KIR2DL4 (killer cell immunoglobulin-like receptor) and LILRB1 (leukocyte immunoglobulin-like receptor subfamily B member 1), which are both capable of recognizing HLA-G expressed by placental trophoblast. Therefore, polymorphisms of KIR2DL4 (9A and 10A alleles) and LILRB1 (SNP G>A, rs 41308748) genes can affect susceptibility to spontaneous abortion.
We tested 152 women with recurrent spontaneous abortion (RSA, 3 or more abortions) and 77 with sporadic spontaneous abortion (SSA, 1 - 2 abortions) and 178 control women (with 2 or more healthy-born children). *KIR2DL4* 9A/10A alleles were distinguished in HRM (high resolution melting) method, and RFLP (restriction fragment length polymorphism) was used for *LILRB1* genotyping. Statistical odds ratios (OR), and significance (*p*) were generated from two-by-two tables using two-sided Fisher's exact test.
Frequencies of 9A and 10A alleles and genotypes of *KIR2DL4* in RSA women were similar to those in control women. Only in SSA women we observed increased frequency of 10A homozygotes (35%) of *KIR2DL4* in comparison to 25% in control women. However, this comparison did not reach significance. Also, distribution of alleles and genotypes of SNP G>A of *LILRB1* was similar in all tested groups. We found no association of *KIR2DL4* and *LILRB1* (SNP G>A, rs 41308748) with spontaneous abortion in Polish population.

P5.19.19
Potential role of circulating microRNAs as a biomarker for unexplained recurrent spontaneous abortion

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Unexplained recurrent spontaneous abortion (URSA), defined as three or more consecutive pregnancy losses before the 20th week of gestation, occurs in 1-5% of women of reproductive age. URSA is a complex disease, resulting from an array of diverse etiologies. Its diagnosis remains difficult because of lacking of specific indexes. MicroRNAs are small single-stranded RNA molecules which play an important role in the regulation of gene expression at the transcriptional level. Circulating microRNAs have been investigated in a wide variety of patient samples. Altered circulating microRNA expression in URSA patients was found using the miRCURY™ LNA Array (v.18.0), with 4 differentially up-regulated and 3 down-regulated microRNAs. These findings have been confirmed by real-time reverse transcription-polymerase chain reaction assays on select microRNAs, including miR-320b, miR-146b-5p, miR-204-3p and miR-22-5p.This is the first report that the expression of microRNAs is altered in peripheral circulating of patients with URSA, suggesting circulating microRNAs provide a new promising diagnostic tool for URSA.

P5.19.20
Pro-inflammatory and anti-inflammatory cytokine production patterns in intra-uterine growth restriction (IUGR)

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Intrauterine fetal growth restriction (IUGR) is a serious complication putting the fetus and neonate at high risk for mortality and morbidity and the child at permanent risk for numerous disorders. Definite causes remain unidentified in nearly 40-50% of IUGR; insufficient

blood flow to the placenta is the primary etiology, but restricted fetal growth is not explained by placental insufficiency alone. It is of interest to examine immunologic reactivity that may cause IUGR with and without placental insufficiency.
Cytokine production by mitogen-stimulated and trophoblast antigen-stimulated maternal peripheral blood lymphocytes from 36 women with IUGR and 22 women with normal fetal growth was assessed. Pro-inflammatory cytokines (IFNγ, TNFα, IL-8, IL-12, IL-18, IL-23) and anti-inflammatory cytokines (IL-4, IL-10, IL-13), were measured by ELISA.
Mitogen-stimulated lymphocytes from normal pregnancy produced higher IL-4 levels compared to IUGR lymphocytes suggesting a bias towards Th2-type immunity. Levels of IL-6, TNFα and IL-12 were higher in IUGR with placental insufficiency compared to IUGR without placental insufficiency indicating a pro-inflammatory bias in IUGR with placental insufficiency.
Trophoblast-stimulated IUGR lymphocytes produced higher IL-8 levels and lower levels of IL-13 than normal pregnancy lymphocytes. IL-8, IFNγ and TNFα were higher in IUGR with placental insufficiency than in normal pregnancy. IL-12 levels were higher and IL-10 levels were lower in IUGR with placental insufficiency than in IUGR without placental insufficiency. This pattern of maternal cytokine profile suggests a proinflammatory bias in IUGR compared to normal pregnancy and in IUGR with placental insufficiency compared to IUGR without insufficiency.

P5.19.21
Trophoblast cells induce and recruit iTregs by VIP production and through a TGF beta dependent pathway

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The inducible regulatory T cells (iTreg) population, essential for maternal tolerance of the conceptus, performs its suppressive actions in the critical peri-implantation phase of pregnancy. Vasoactive intestinal peptide (VIP) is synthesized and secreted by trophoblast cells and promotes anti-inflammatory and tolerogenic profiles through binding to specific receptors on immune cells. Here, we evaluated VIP contribution to the differentiation and recruitment of iTreg toward trophoblast cells. We used an in vitro model of maternal leukocyte-trophoblast cell interaction represented by cocultures of fertile women PBMC with human trophoblast cell line (Swan71). We observed that VIP increased the frequency of CD4+CD25+Foxp3+ cells after 48h of coculture (3.9±0.4 vs 8.3%±0.6, p<0.05) which was prevented by VIP antagonist. Moreover, iTreg differentiated upon interaction with trophoblast cells in the presence of VIP, suppressed the maternal alloresponse and increased CD4+IL10+ cell % but did not modulate IFNγ or IL-17 production. Getting insight into the mechanisms involved in iTreg differentiation, VIP induced the expression of the three isoforms of TGFβ in Swan cells with a peak at 12h and increased TGFβ1 secretion. The increase in iTreg frequency was prevented by an antiTGFβ Ab and VIP antagonist. Finally, Swan cells selectively recruited iTreg and the effect was higher in the presence of VIP through CCL5 (RANTES) production. These results suggest that VIP could have an active role in the immunoregulatory processes operating in the maternal-placental interface by contributing to the induction and recruitment of iTregs in a TGFβ dependent mechanism.
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P5.19.22
CD4(+) CD25(high) Foxp3(+) cells increased in the peritoneal fluid of patients with endometriosis

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CD4(+) CD25(high) Foxp3(+) cells and IL-6, IL-10, IL-17, and TGF-β in the peritoneal fluid of women with endometriosis. A total of ninety-eight patients were studied: endometriosis (n = 70) and control (n = 28). First, peritoneal fluid lymphocytes were isolated, and CD4(+) CD25(high) cells were identified using flow cytometry. Then, RT-PCR was performed to verify Foxp3 expression in these cells. Also, IL-6, IL-10, IL-17, and TGF-β concentration was determined. Of all the lymphocytes in the peritoneal fluid of women with endometriosis, 36.5% (median) were CD4(+) CD25(high) compared to only 1.15% (median) in the control group (P < 0.001). Foxp3 expression was similarly elevated in patients with the disease compared to those without (median, 50 versus 5; P < 0.001). IL-6 and TGF-β were higher in endometriosis group (IL-6: 327.5 pg/mL versus 195.5 pg/mL; TGF-β: 340 pg/mL versus 171.5 pg/mL; both P < 0.001). IL-10 and IL-17 showed no significant differences between the two groups. The peritoneal fluid of patients with endometriosis had a higher percentage of CD4(+) CD25(high) Foxp3(+) cells and also higher levels of IL-6 and TGF-β compared to women without the disease. These findings suggest that CD4(+) CD25(high) Foxp3(+) cells may play a role in the pathogenesis of endometriosis.

P5.19.23
Immune profile of Sudanese women with conception disorders: Possible role for T-helper cytokines

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Objectives: This prospective, case-control, cross-sectional and hospital-based study aims to determine the cytokines profile of Sudanese women with infertility and recurrent abortions.
Background: Unexplained female infertility, recurrent miscarriages, premature delivery and pregnancy-induced hypertension may all, in some cases be linked to immune and cytokines networks of early pregnancy. Understanding these cytokines and their actions could be the new area of conception disorders treatment.
Materials & Methods: Following informed consent, 25 women with a history of unexplained conception disorders (15 infertile women, 10 women with recurrent miscarriages) were compared with 75 pregnant women with history of success pregnancy as controls. Blood samples and peripheral blood mononuclear cells (PBMCs) were stimulated by phytohaemaglutinin (positive controls) and trophoblast antigen in an in vitro culture system and secreted cytokines were determined using the ELISA technique.
Results: Significantly high concentrations of INF-γ cytokine were seen in women with a history of unexplained conception disorders compared to those with history of normal pregnancies (*p=0.00*). Significantly higher concentrations of IL-4 were seen in women with history of normal pregnancy compared to those who had conception disorders (*p=0.04*).
Conclusions: Women with conception disorders showed predominantly Th1 type of immune response in PBMCs when stimulated with trophoblastic antigen compared to women who had a history of normal pregnancy. Immunomodulation could be introduced as a treatment modality to induce normal pregnancy.

P5.19.24
Intravenous immunoglobulin treatment on expanded CD56$^+$ cells in women with recurrent reproductive failure

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Natural Killer (NK) cells are key players in maternal tolerance, whereas expansion of circulating cytotoxic NK cells is associated with recurrent reproductive failure (RRF). Intravenous immunoglobulin (IVIg) represents an immunomodulatory therapy for this selected group of patients with immunological pathophysiology.
Objectives: To study the clinical effect of IVIg on clinical pregnancy and live birth rates in women with RM and/or RIF after IVF and circulating NK/NKT-like cells expansion.
Patients & Methods: Retrospective real-life study of 428 women with past medical history of RM (n=217) and RIF after IVF (n=211). Only women with NK or NKT-like expansion above 12% or 10% cut-off levels, respectively, were assigned for IVIg. 157 patients received IVIg every 3-4 weeks from the date of known pregnancy or embryo transfer to 35-week gestation.
Results: Women with RM under IVIg therapy showed a live birth rate of 96.3%. Women with RIF under IVIg therapy showed clinical pregnancy and live birth rates of 93.8% and 80.0%, respectively. Significantly lower pregnancy and live birth rates were observed for patients with RRF and NK/NKT-like cells expansion without IVIg.
Conclusions and discussion: In women with RRF of unexplained etiology and preconception NK or NKT-like cells expansion, clinical pregnancy and live birth rates are significantly improved by IVIg therapy. Immunomodulation with IVIg of a well-selected group of RRF patients with immunological alterations enhanced clinical pregnancy and live birth rates.

P5.19.25
Expression profiling of vitamin D receptor in reproductive tissues of pregnant mice

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The presence of vitamin D receptor (VDR) and the identification of localized vitamin D3 synthesis in placenta and decidua implicate the importance of vitamin D3 in reproductive function. There is, however, no data on the expression profile of VDR in the mouse placenta and endometrium throughout the pregnancy period. In the present work expression of VDR in reproductive tissues of pregnant mice at different gestational phases has been addressed. Expression of VDR was determined by semi-quantitative RT-PCR, Western blotting and immunohistochemistry. The results showed that VDR mRNA and protein were expressed in decidua, placenta and ovary throughout the pregnancy. VDR gene expression in placenta was significantly elevated in late pregnancy when compared to that of mid pregnancy. Additionally, VDR expression level in decidua rose significantly as pregnancy progressed from early to mid stages. VDR expression in decidua of pregnant mice was higher in comparison to endometrium of non-pregnant mice. Immunohistochemical analysis revealed that VDR protein is consistently expressed by luminal and glandular epithelial cells of decidua, giant cells, glycogen rich cells and labyrinth cells of placenta and by almost all follicular cell types of ovary. Surveying the expression of VDR at the protein level by Western blotting confirmed PCR results. It seems that expression of VDR in reproductive organs is finely tuned during pregnancy indicating its eminent role in reproductive biology

P5.19.26
Immune cells functional disorder in case of preeclampsia

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Preeclampsia is associated with functional disorder of immune cells that can affect their phenotype and influence their functions.
The aim was to assess expression of surface receptors on lymphocytes, NK- and NKT-cells, monocytes and its adhesion to endothelium.
Peripheral blood was obtained from 75 healthy non-pregnant women, 69 healthy pregnant women (38-39 weeks) and 79 pregnant women with preeclampsia (38-39 weeks). Peripheral blood mononuclear cells (PBMC) were stained with antibodies to CD3, CD4, CD8, CD16, CD56, CD14, CD11a, CD11b, CD11c, CD18, CD29, CD49d, CD31, CD44, CD47, CD54, CD58, CD62L, integrin β7, HLA-DR, CD119 and analyzed by FACSCantoII.
The adhesion of monocytes and lymphocytes to the intact and TNFα activated endothelial cells increased in preeclampsia as compared with physiological pregnancy. The amount of CD8+ T-lymphocytes and CD4+ T-lymphocytes expressing CD29, CD49d and CD54 decreased in case of preeclampsia comparing with healthy pregnant women. There was also a 6,5-fold increase of CD18 expression intensity by CD8+ T-lymphocytes. In case of uncomplicated pregnancy and in case of pre–eclampsia the amount of NK–cells expressing CD29 and CD49d was elevated. At preeclampsia the amount of NK–cells expressing CD11c, integrin β7 and CD47 and also expression of CD31 and CD11c by NKT-cells increased. The amount of monocytes expressing adhesion molecules didn`t differ between physiological pregnancy and preeclampsia. But the amount of CD119+ monocytes and their CD119-expression intensity were elevated in case of preeclampsia.
These results indicate that preeclampsia is associated with phenotypical changes of PBMC that facilitate their adhesion to uterine vessel endothelium.

P5.19.27
Influence of placental secretory factors to endothelial cell and monocyte phenotype and monocyte transendothelial migration activity

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Monocyte regulate placental development, but molecules that control differential monocyte migration to placental and decidual tissue during normal pregnancy and local placental inflammation during preeclamptic pregnancy are not defined yet.
Placental tissue samples were taken from healthy pregnant women on 9-11 weeks (abortion) and 38-39 weeks of gestation, pregnant women with preeclampsia on 38-39 weeks of gestation (both after Cesarean section) and were incubated in DMEM/F12, 10% FBS. The ECs line EA.hy926 was grown in DMEM/F12, 10% FBS. Monocyte cell line THP-1 was grown in RPMI-1640 medium, 10% FBS. Cells were incubated with placental supernatants to characterize its influence to ECs phenotype and transendothelial migration activity.
Third trimester placental supernatants caused decrease of CD54 ECs expression in comparison to first trimester placental supernatants, simultaneously monocyte transendothelial migration activity and CD11a monocyte expression were lower with third trimester placental supernatants in comparison with first trimester one. Placental supernatants of women with pregnancy, complicated preeclampsia, caused increased CD119 ECs expression in comparison to normal pregnancy supernatants. Simultaneously monocyte transendothelial migration activity was higher to placental supernatants of women with pregnancy complicated preeclampsia in comparison to normal pregnancy one and was accompanied with increased monocyte CD11b expression. Discovered ECs and monocyte cells phenotype alterations can contribute to monocyte migration regulation during normal pregnancy and to local placental inflammation initiation during

pregnancy, complicated preeclampsia. The work was sustained by the Ministry of education and science of Russian Federation GK №02.740.11.0711 and grant of President of Russian Federation №NSH-3594.1010.7 and MK-1580.2013.7.

P5.19.28
Parameters of cellular immunity in pregnant women with threatened abortion in early pregnancy, against the low level of progesterone

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Results. In pregnant women with threatened abortion in the first trimester due to low level of progesterone, compared with rates of women with physiological pregnancy, was showed an increase in production of natural killer cells CD16+ (21.43% (19.2%-23.7%) and 8.91 % (8.2% - 9.6%), respectively, p<0,01), as well as the increase in the total population of T-lymphocyte CD3+ (53.9% (50.8%-56.9%) and 44,65% (42.59%-46.4%), respectively, <p 0,01) with the disturbance of the balance in the subpopulation composition of T-lymphocytes in the direction CD4+, which manifested by increased immunoregulatory index (IRI, CD4 + /CD8 +; 2,66 (2.36 - 2.78) and 1.63 (1.49-1.89), respectively, p<0,01).
Conclusions. By pregnant women with the presence of threatened abortion against low level of progesterone, was found an activation of cellular immunity, shown with overproduction of CD16+ natural killer cells and the prevalence of the potential helper T lymphocytes (CD4+) of the suppressor (CD8 +), which is extremely unfavorable to the gestational process in the future.

P5.19.29
Polymorphisms -725 C>G>T, -716 T>G of the HLA-G gene in spontaneous abortion and control women in Polish population

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Human leukocyte antigen - G (HLA-G) has been found to contribute to modulation of maternal immune system during pregnancy and acceptance of the semi allogeneic fetus. Therefore, HLA-G is an attractive candidate for genetic studies in reproductive failure such as spontaneous abortion. We investigated whether -725C>G>T and -716T>G genetic polymorphisms in the promoter region of the HLA-G are risk factors for spontaneous abortion in Polish population. We tested 229 spontaneous abortion women: 152 with 3 or more spontaneous abortions (RSA), 77 with sporadic abortion (SSA, with 1 or 2 abortions), and 178 control women (with 2 or more healthy-born children). Temperature gradient gel electrophoresis (TGGE) was used for -725C>G>T, -716T>G typing. Statistical odds ratios (OR), and significance (p) were generated from two-by-two tables using two-sided Fisher's exact test or three-by-two tables.
We observed significant differences in genotype distribution for -716T>G polymorphism (p = 0.009). GG genotype was almost two-fold frequent in sporadic abortion women in comparison to control women. Carriers of -716G allele had over two-fold higher risk to sporadic abortion in comparison to non-carriers (p=0.006, OR=2.46, 95% CI = 1.35-4.51).
Our data suggest that genetic variation within HLA-G influence the risk of sporadic abortion.

P5.19.30
Spontaneous miscarriages are explained by the stress/glucocorticoid/lipoxin A4 axis

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Despite various suspected causes, ranging from endocrine and genetic to infectious and immunological aspects, the molecular mechanisms of miscarriage still remain enigmatic. This report provides evidence that downregulation of 11β-hydroxysteroid dehydrogenase type 2 (HSD2), the key enzyme inactivating glucocorticoid activity, insults the pregnant inflammatory milieu by inhibiting the biosynthesis of lipoxin A4 (LXA4), a metabolite of arachidonic acid, leading to an early loss of the pregnancy. Both LXA4 and its biosynthetic enzymes were found to be decreased in women with spontaneous miscarriages, along the murine miscarriage model. Replenishing LXA4 reversed LPS-induced miscarriages in mouse models whilst blocking LXA4 signaling resulted in miscarriages in the pregnant mice. The protective effect of LXA4 might be explained by LXA4's role in regulating uterine and placental inflammatory factors and mast cells. The underlying molecular mechanism involved miscarriage-inducing infections or stresses that downregulated the expression of HSD2 but not HSD1, resulting in increases in glucocorticoid activity and decreases in LXA4. In conclusion, these findings suggest that the stress/glucocoticoid/lipoxin A4 axis might be a common pathway through which miscarriages occur. <!--EndFragment-->

P5.19.31
The role of systemic inflammation in the development of pregnancy and obstetric complications

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Systemic inflammation (SI) is a typical process integrating some phenomena such as systemic inflammatory reaction, microthrombosis, tissue alteration, distress of neuroendocrine system.
Objective. To evaluate the role of SI in the development of critical pregnancy and obstetric complications.
Patients and methods. The study included follow groups: patients with pre-eclampsia, examined during the third trimester (n=22), patients with pre-eclampsia, examined during the first day after labor (n=15), patients with hemorrhage during labor above 10-15% of a blood volume without (n=13) and with hemorrhagic shock (n=26). Control group - 24 healthy women. A comparison group - patients with normal labor (n=12). Levels of interleukins-6,8,10, TNFα, C-reactivity protein, cortisol, myoglobin, troponin I, D-dimers were measured in plasma. On the basis of these markers we calculated, using our own procedure, integrated criterion - scale of SI, ranging 0 to 9.
Results. SI was diagnosed only in patients with hemorrhagic shock (76,9%). MODS (50%) and lethal outcomes (29,6%) were detected only in this group too. Meanwhile, SI developed in 100% dead persons and in 84,6% MODS-patients. Pre-eclampsia was attended an increased levels of proinflammatory cytokines and CRP (as systemic inflammatory reaction manifestation), but not other phenomena and SI in whole.
Conclusion. The hemorrhagic shock is a damage factor, caused SI development. SI determines state severity, probability of MODS and lethal outcomes.

P5.20 Tumor immunity and immunosurveillance

P5.20.001
Anti-tumor specific T-helper cell priming by CIITA-driven MHC-II-positive tumor cells in dendritic cell-depleted transgenic mice

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We previously showed that anti-tumor protective immunity can be obtained by injecting Balb/c mice with H-2^d tumor cells of different histological origin expressing MHC-II molecules after transfection with the MHC-II transactivator CIITA. CIITA-tumor vaccinated mice potently rejected tumor challenges with untransfected, MHC-II-negative parental cells, and specific anti-tumor protection could be transferred to naïve recipients by immune CD4+ T helper (TH) spleen cells. These data indicated that MHC-II expression in tumor cells is instrumental to trigger TH priming, subsequent anti-tumor effector response and T cell memory *in vivo*. However the above data did not formally demonstrate that MHC-II-positive tumor cells could serve as antigen presenting cells (APC) for TH priming *in vivo*, a function generally attributed to dendritic cells (DC). By using CD11c.DTR transgenic mice (kindly donated by Natalio Garbi, University of Bonn, Germany) that can be conditionally depleted of DC, we now demonstrate that CIITA-driven MHC-II-positive H-2^b LLC tumor cells are dramatically reduced in their growth *in vivo* in absence of DC and as result of TH triggering and subsequent generation of anti-tumor effector T cell response, both CD4 and CD8. These results strongly suggest that tumor cells expressing CIITA-driven MHC-II molecules play a crucial role as APC in TH cell priming *in vivo*. The implications of these findings for the common belief of MHC-II-restricted T cell priming and in relation to novel strategies of anti-tumor vaccination will be discussed.
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P5.20.002
Domination of Th17 subpopulation of lymphocytes during tumor progression.

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Background: Inflammatory responses play a decisive role in the early stages of tumor development, Progress, Invasion and metastasis. Inflammatory mechanism is also effective in immune surveillance and efficacy of the treatment. The tumor microenvironment consists of a complex network of various cell types including tumor-infiltrating lymphocytes. Tumor infiltrating lymphocyte, have a vast and dynamic relationship with the cancer cells. Similar to human, Th17 cells are found to infiltrate tumors in murine models of cancer. Th17 cells are known with potent pro-inflammatory activities, which are mainly mediated by its cytokines. The purpose of this study was to explore whether there is a linkage between the infiltration of Th17 cells and tumor progression in an experimental model of murine melanoma.
Methods: After induction of tumors in mice using B16F10 melanoma cell line subcutaneously, tumors and draining lymph nodes were extracts at 3 different time points during tumor progression. We quantified expression pattern of functional group of genes associated with Th17 Using Real-time PCR.
Results: Real-time PCR analysis of gene expression in tumor microenvironment and draining lymph node revealed increased expression of related genes, suggesting significant increase in Th17 infiltrating cell during tumor progression.
Conclusions: Along with the increase of Th17 cells, a pro-inflammatory microenvironment is performed, which contributed to the tumor progression. This tumor is characterized by a pro-inflammatory cytokine microenvironment. Evaluation of different subpopulation of T lymphocytes infiltrating the tumor site could be a valuable prognostic factor showing tumor progression and novel therapeutic strategies could be designed to change the microenvironment.

P5.20.003
Relation between Hepatitis B virus DNA load and quantitative HBsAg in HBV infected patient's sera

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Hepatitis B virus (HBV) infection is a serious global health problem. Aim of this study was to find out correlation between quantitative Hepatitis B surface antigen (HBsAg) and HBV-DNA load in HBV infected patient's sera. HBV DNA test was performed by real time PCR using Cobas TaqMan48 (Roche, USA) and HBsAg quantitation by chemiluminiscence EIA (Roche, Switzerland). 280 HBV infected Bangladeshi patient's blood samples were tested for this study having viral load from low to high. Samples were divided among 5 groups, i.e., HBV DNA not detected (30), HBV DNA levels < 6 (50), > 6 to < 50 (50), > 50 to < 2000 (75), and > 2000 IU/mL (75) accordingly. HBsAg levels were found to be in between < 6.25 and > 16.25 IU/mL in this study. < 6.25 IU/mL is the sensitivity of the method. For practical constraint, we preferred to take > 16.25 IU/mL as upper limit of detection,
High levels of HBsAg were detected mostly in patients with HBV DNA above 2000 IU/mL. A very few samples with undetectable HBV DNA had HBsAg levels above detection limit. Overall correlation between quantitative HBsAg and HBV DNA load was not significant. Weak correlations were found in HBV patients with DNA level low to moderate. However, significant correlation ($p < 0.01$) was observed between quantitative HBsAg and group 5 (HBV DNA above 2000 IU/mL). Thus, Quantitative HBsAg assay appears to have a limitation because it cannot fully reflect the replication status of HBV.

P5.20.004
BRCA1 gene deletions and duplications in Omani Breast cancer cases

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Breast cancer (BC) is the most common cancer reported in females in Oman and usually occurs at a relatively younger age, presents at an advanced stage and behaves aggressively. BC occurs in hereditary and sporadic forms. Although germ-line mutations in BRCA1 and BRCA2 genes are rare in sporadic cases compared with hereditary cases, somatic molecular alterations are common. In this study, we investigated the potential association of molecular alterations with sporadic BC in Omani patients.
Methods. Fourty three sporadic BC cases were selected based on age ≤ 40 years, or bilateral breast cancer, or estrogen and progesterone receptor negative status, and HER-2/neu negative (Triple Negative phenotype) status. DNA extracted from peripheral blood was screened for molecular alterations using multiplex ligation-dependent probe amplification (MLPA).
Results. Genomic deletions and duplication in the BRCA1 gene were identified in four female patients. Two patients carried exon 1 and 2 deletions and two showed exon 1 and 2 duplications.
Conclusions. The detected BRCA1 gene deletions and duplications were previously reported in Italian cases with breast/ovarian cancer, which supports that different populations share common breast cancer molecular mechanisms.

P5.20.005
Interplay of Th17/Treg cells in immunopathogenesis of Urothelial carcinoma of bladder

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BACKGROUND: Bladder cancer is 2nd most common urologic malignancy amongst males after prostate cancer. Urothelial carcinoma of bladder accounts for 90% of all bladder cancer.

Crosstalk is observed between Th17 and Treg cells in inflammatory and autoimmune diseases. Recent studies suggest potential impact of Th17 & Treg cells in cancer. Probable role of these cells are not well studied in bladder cancer. Therefore, we aim to assess circulatory levels and molecular expression of Th17 and Treg related cytokines and transcription factors respectively, to understand their role in immunopathogenesis of bladder cancer.
METHODOLOGY: 20 patients with muscle invasive urothelial carcinoma of bladder (confirmed by clinical and histopathogical diagnosis) and 15 healthy individuals as controls were included. Serum IL-17, IL-23, IL-6 and TGF-β levels were analyzed by ELISA and molecular expression of IL17, RORγt, TGF-β and Foxp3 was observed by RT-PCR in blood and tissue.
RESULTS: Significantly higher circulatory levels of IL- 6 have been found in patient as compared to controls ($p<0.001$). Levels of IL-23, IL-17 and TGF-β were elevated in patients in comparison to controls. Molecular expression of IL17, RORγt and TGF-β was significantly high ($p<0.05$) whereas change in Foxp3 expression was insignificant.
CONCLUSION: These findings indicate possible interplay between Th17 and Treg cells in urothelial carcinoma of bladder. Further, data can be validated in larger patient cohort for better understanding, role of these cells in this disease which might be an aide to formulate a more efficient therapeutic strategy with Th17 & Treg cells as a target in future.

P5.20.006
Association some of the major histocombatibility complex class II alleles with breast cancer among Jordanian females

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Major Histocombatibility Complex (MHC) Class-II represents tumor antigenic peptides on the cell surface that is recognized by T lymphocytes. MHC molecules play a key role in the immune response to breast cancer tumor antigens.
The aim of this study is to determine whether MHC class-II alleles could confer susceptibility or resistance to breast cancer among Jordanian females. This study is a case control study that is consisted of 52 breast cancer patients and 40 healthy controls. DNA was extracted, nucleic acid were amplified for HLA-DQB1 and HLA-DRB1 alleles by muliplex INNO-LiPA kit and alleles typing was carried out by reverse hybridization. Comparison of HLA-DQB1 and HLA-DRB1 alleles' distribution was evaluated by paired t-test and chi-square. Risk factors were expressed by odd ratio with 95% confidence interval. Significant negative correlations were observed between HLADQB1* 02 alleles and breast cancer ($p = 0.022$). While no significant association were observed among HLADQB1* 03, 04, 05 and 06 or among HLA-DRB1 * 01, 03. 04, 07, 08, 10, 11, 13, 14, and 15. Four breast samples alleles could not been identified for any allele by LiRAS® for LiPA HLA software; which may suggests new alleles.
In conclusion, screening HLA-DQB1 alleles among breast cancer females may predict high risk females.

P5.20.007
Expression of CD95 and caspases activity in peripheral blood lymphocytes during breast cancer development

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Rationale: Breast cancer is the most frequent cancer in women. It is known that breast tumorigenesis is accompanied by suppression of anticancer immune response. One way of this process is susceptibility of immune cells to apoptosis in which caspases play the main role. The aim of this study was to evaluate CD95 expression and caspase-8, -9, -6, -3 activities in peripheral blood lymphocytes (PBL) of women with the diagnosed breast cancer depending on a neoplasm stage.
Methods: Gene expression was assessed by qPCR. Caspases activity was measured using fluorogenic substrate. Cellular apoptosis was evaluated by means of cytofluorometric assay.
Results: No changes in caspases activity and CD95 expression were observed during the first stage of breast cancer. Caspase-6 was the only caspase to exhibit lower levels of activity. Beginning from the second stage of cancer, activity of casp-8 and casp-3 sharply increased (max St IIIA), activity of casp-9 and casp-6 changed insignificantly. In addition, the number of CD95+ cells was increased authentically (max St III B). Levels of membrane-associated phosphatidylserine in PBL significantly elevated during the tumor progression, apopotosis was activated.
Conclusions: 1. Apoptosis in PBL is possibly activated during breast tumorigenesis. The increase of CD95+ cells and intracellular activity of casp-8 and casp-3 is supportive of Fas-mediated apoptosis.
2. Differences in functional caspases activity in PBL might be used as a predictor marker of breast cancer development.
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P5.20.008
Serum Amyloid A impacts the growth of melanoma cells and may be a link between inflammation and cancer outcome

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Serum amyloid A (SAA), an inflammatory protein, has been called attention due its increased concentrations in plasma of cancer patients. Recently, it was suggested an immune modulation mediated by SAA in melanoma patients (Nat Immunol., 11:1039-46, 2010). Previous study from our group showed increased proliferation of human glioma lines in the presence of SAA. Furthermore SAA also affected migration and invasion (Mediators of Inflammation, 2013, in press). To verify if these findings could be extended to other tumor we evaluate the effects of SAA (5 or 10 µg/mL) on proliferation, migration, invasion and cytokine release on melanoma cells (SK-Mel 19, 28, 103 and 147). We used [3H]-thymidine incorporation and clonogenic assay to evaluate proliferation, flow cytometry for necrosis and apoptosis, scratch test and Boyden chamber assay to evaluate mobility and ELISA for cytokine release. We observed that SAA inhibited proliferation without inducing cells death, however, by optical microscopy, we observed that SAA could cause morphologic alterations similar to cell senescence. Some of the cell lines seemed to be more sensitive to SAA in relation to cellular motility. In these cases it was observed decreased in migration and/or invasion. Furthermore, SAA induced the release of IL-6 and IL-8 cytokines on melanoma cells. Our finding demonstrated that SAA might, in some cases, impact melanoma growth and emphasizes the possibility to be one of the factors that links inflammation with cancer outcome.

P5.20.009
A relationship between soluble MICA and anti-MICA-Abs in nasophayngeal carcinoma patients

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A soluble form of the major histocompatibility complex class I-related chain A (sMICA), released from tumor cell-surface, inhibits CD8+ T lymphocyte cytotoxicity through down-regulation of the NKG2D activatory receptor with consequent tumor escape. Conversely, MICA antibody (MICA Abs) not only promotes dendritic cell-mediated cross-presentation of tumor cells resulting in tumor cells lysis through complement fixation but also by neutralizing sMICA isoforms, favor NK- and T cell- related cytotoxicity. Here, we evaluated the presence of MICA antibody (Abs MICA) in NPC patients and controls and their potential relationship with sMICA serum level and the disease.
157 NPC patients compared with 43 healthy individuals sera. Serum levels of sMICA were determined using MICA ELISA kits. MICA Abs was performed using LABScreen® assay.
We found that among the cohort (157), 66 patients (42%) produced high serum levels of sMICA (>80pg/ml) while only 12 (8%) were found positive for MICA Abs (>3). Further Analysis accounting both high sMICA levels (> 80 pg/ml) and positive MICA Abs (>3), showed that

among the 12 patients positive for MICA Abs, 6 of them have also high levels of sMICA, (3% vs 42%, p< 4.4 10 -17). Interestingly, the patients with positive MICA Abs presented all a non-advanced stage of tumor.
The inverse relationship observed here may reflect a neutralizing effect of MICA Abs on sMICA isoforms with consequent NK-cell anti tumor defense. Altogether, these data, if confirmed in a larger patient cohort, may pave the way to novel immuno-therapeutic interventions involving MICA Abs in NPC treatement.

P5.20.010
Tertiary lymphoid tissue (TLT) in colorectal cancer: lymph node-like aggregates supporting the immune response

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Tertiary lymphoid tissue (TLT) is a lymph node-like environment, which develops at sites of chronic inflammation and is involved in sustaining adaptive immune responses. Its occurrence in cancer is sporadically documented and its functional role in tumors is unknown.
Despite the recognized role of inflammation in colorectal cancer (CRC) and the evidence that a CD3+ T cell infiltrate correlates with a better prognosis in this tumor, the presence and biological function of TLT in CRC have been poorly investigated.
In human CRC, we identified highly organized lymph node-like aggregates, with compartmentalized B and T cell areas, a network of follicular dendritic cells, activated fibroblasts, lymphoid chemokines and haematic and lymphatic vessels. Notably, in a preclinical model of inflammation-driven colon carcinogenesis, TLT expansion was associated to CD3+ T cell increase, thus indicating an association among TLT and T cell infiltration in CRC.
In CRC patients, high density of TLT correlated with better prognosis. Intratumor lymphoid neogenesis occurred by formation of new vessels, including high endothelial venules and lymphatic vessels and correlated with increased T cell infiltration, suggesting an active contribution to T cell traffic to the tumor. Our results suggest that TLT is associated with a more favorable clinical outcome for CRC patients, rising the hypothesis that it may actively participate to leukocyte recruitment to the tumor and take part to the anti tumor immune response.

P5.20.011
Role of ATP and myeloid derived suppressor cells in neuroblastoma microenvironment.

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The biochemical composition of tumor microenvironment is crucial for the modulation of cancer cell growth as well as for the functions of immune cells. Recent findings have shown that solid tumors have an increased concentration of adenosine and extracellular ATP that may exert a pivotal role in the regulation and homeostasis of immunosuppressive cell populations.
Aim of this study was to investigate whether:
i) extracellular ATP is a component of neuroblastoma (NB) microenvironment,
ii) myeloid-derived suppressor cells (MDSCs) are involved in NB cell growth
iii) ATP exerts modulatory effects on MDSC functions.
The NXS2 murine NB cell line was stably transfected with plasma membrane luciferase probe and inoculated in mice. Bioluminescence imaging was used to detect extracellular ATP in living animals. MDSCs were phenotypically characterized in NB-bearing and healthy mice by flow cytometry and evaluated for the expression of immunosuppressive molecules such as arginase-1 and reactive oxygen species. The functional expression of P2X7 receptor was evaluated in MDSCs by PCR, immunofluorescence and spectrofluorimetric analysis. Extracellular ATP was detected in NB-bearing mice in amounts that increased as tumor progresses. The percentage of $CD11b^{+}/Gr\text{-}1^{+}$ cells was higher in NB-bearing mice compared to healthy animals and they produced higher levels of ROS and arginase-1. Finally, ATP was able to exert some modulator effects i.e. cytokines release and induction of apoptosis on MDSCs through interaction with the functional P2X7 receptor. Our findings identified MDSCs and ATP as new players of NB microenvironment, thus providing new information for the design of novel therapeutics approaches.

P5.20.012
Antitumor activity of synthetic phosphoethanolamine in experimental melanoma

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The low responsiveness of melanoma to traditional treatments together with its increasing incidence makes the development of new therapeutic strategies against this type of cancer extremely important. In this study, we used a murine melanoma model to evaluate the effects of synthetic phosphoethanolamine (PEA) on the development of this tumor. *In vitro*, PEA had an inhibitory effect on the proliferation of B16F10 cells, inducing apoptosis after 24 to 72h stimulation. *In vivo*, oral treatment of melanoma-bearing animals with different doses of PEA (10, 20 and 40mg/Kg) during 20 consecutive days resulted in reduced tumor volumes (at least 70% compared to the control) and in expressive macroscopic differences. PEA also induced a dose-dependent increase of apoptosis and decrease in tumor cell proliferation. The treatment also resulted in hematological changes, such as increased numbers of platelets, erythrocytes and leukocytes. Among leukocytes, we observed a higher proportion of lymphocytes and monocytes. In addition, PEA induced higher levels of the pro-inflammatory cytokine IL-6 and of the anti-inflammatory cytokines IL-10 and TGF-β, and it also induced a lower production of the pro-inflammatory cytokine IFN-γ. No differences were observed in the levels of IL-1β, TNF-α, IL-12p70 and IL-17 upon treatment. Our results demonstrate an inhibitory role of PEA in the development of melanoma, contributing to a better understanding of its antitumor activity.
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P5.20.013
Proteomic identification of Annexin A4 as a novel renal cell carcinoma associated antigen

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Early diagnosis remains the main strategy for reducing morbidity and mortality in renal cell carcinoma (RCC) patients, while high immunogenicity of these tumors renders cancer-associated autoantigens as attractive diagnostical biomarkers and promising targets for immunotherapy. In the present study we explored renal cell carcinoma autoantigenic repertoire by means of Serological Proteome Analysis (SEPRA) using A.704 and RCC68 cell lines and RCC patients/healthy donors' sera as primary (auto)antibody sources for two-dimensional Western-blot analysis. Proteins demonstrating differential immunoreactivity between groups of RCC patients and healthy donors were identified using LC-MS/MS analysis. Annexin A4 (ANXA4) was one of the most promising candidates and was selected for further examination of in vitro effector CD8+ T-cell response against five HLA-B (B13, B15, B18 and B27) - restricted ANXA4 peptides previously identified as a part of RCC-associated HLA class I peptidome. In a first screening, we were able to detect spontaneous effector CD8+ T-cell response against 2 HLA-B15-restricted peptides in 1 out of 7 HLA-B15+ carriers suffering from RCC. Moreover, ANXA4 was overexpressed in malignant versus normal kidney tissue (Oncomine database). Taken together, we identified a number of candidate autoantibody biomarkers of RCC and propose ANXA4 as a novel tumor associated antigen.

P5.20.014
Proliferative potential of double positive CD4+/CD8+ T lymphocytes in coculture with tumor cells

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Immune response involved in tumor progression is not fully understood yet. It seems that patients with tumors develop mechanisms to escape the immune surveillance, leading to abnormal anti-tumoral immune response. The purpose of this study was to investigate in vitro behavior of peripheral blood mononuclear cells from healthy individuals when placed in contact with breast cancer cells (SKBR3).
SKBR3 tumor cells were cultured in standard conditions and specific media. PBMCs were isolated from healthy donors using Ficoll density gradient and further cocultured with tumor cells for 5 days. Freshly isolated PBMCs were analyzed for expression of CD4, CD8, CD25, CD38, CD69, and HLA-DR. Adherent fraction and culture supernatant were harvested every day and analysed for the same phenotypical markers. Adherent SKBR3 were immunostained and flowcytometrically investigated.
We found that CD45 positive initial population was approximately 70% and remained constant. CD4+ T cells decreased progressively from 70% to 46%, while CD8+ T cells maintained constant proportion of 18%. From day two of coculture we identified presence of double positive CD4+/CD8+ T cells population with a tendency to increase. CD25 and HLA-DR, late activation markers, presented an increased expression towards day 5 of experiment. SKBR3 cells proliferation was inhibited and aggressive markers expression was decreased. We may conclude that occurrence and proliferation of double positive CD4+/CD8+ T cells population when in contact with tumor antigens can be associated with anti-tumoral response of T lymphocytes.

P5.20.015
***Cis*-acting genetic elements at or near the *Pas1* locus control *Kras2* mutations and gene expression in lung tumors from mice selected for acute inflammatory response**

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AIRmax (high response) and AIRmin (low response) mice, genetically selected for the intensity of acute inflammatory response (AIR), show divergent susceptibility for chemically-induced tumors, suggesting that genes which control acute inflammatory reactivity may modulate predisposition to tumorigenesis. AIRmax and AIRmin carry the resistance and susceptibility haplotypes, respectively, at *Pas1* (*Pulmonary adenoma susceptibility 1*), the major locus regulating susceptibility to lung tumorigenesis in mice. The oncogene *Kras2* maps at *Pas1,* and mutations of this gene are associated to lung tumor development. Our objective was to identify, through linkage analysis, chromosomal regions which control the mutability of *Kras2* and the expression levels of *Kras4A* and *Kras4B* isoforms in lung tumors of intercrossed F2 (AIRmax x AIRmin) mice. Mice were treated at 7 days of age with 300 mg/kg urethane and after 9 months lung tumors were excised for DNA (n=500) and RNA (n=110) extraction. *Kras2* activating mutations at codons 12, 13 and 61 were identified by DNA pyrosequencing. Expression levels of *Kras* isoforms and *Lyrm5* (also mapping inside *Pas1*) were determined by qPCR. A panel of 1449 SNPs (single nucleotide polymorphisms) distributed in the whole genome, was tested for association with the mutability and expression phenotypes. One region at chromosome 6 (near to *Pas1*) was associated with *Kras* mutations (LOD 4.67). The expression levels of *Kras4A*, *Kras4B* isoforms and *Lyrm5* showed association with *Pas1* in chromosome 6 (LOD 3.16, 2.36 and 8.43 respectively). Results suggest the participation of *cis*-acting elements at or near *Pas1* in the regulation of *Kras2* mutability and expression.

P5.20.016
Inhibition of cell cycle progression and apoptotic activity of Ganoderma lucidum in oral cancer cell lines

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In recent years, although the apparent improvement in the treatment of oral cancer with conventional strategies the rates of morbidity and mortality remain high. Current treatment for oral cavity cancer uses chemotherapeutic agents as Cisplatin, but it's possible that efficacy of cisplatin could be greatly increased in combination with natural compounds such as Ganoderma lucidum (GL) an oriental medical mushroom used in traditional Chinese medicine as a dietary supplement by cancer patients. In this study we analyzed the effects of GL on cell cycle progression, and apoptosis of two tumour cell lines (BICR-18 cell line - human larynx squamous cell carcinoma and FaDu cell line - human pharynx cell carcinoma) treated with cisplatin, and/ or GL. The cell cycle phase distribution, apoptotic events and expression of several molecular markers (e.g. p21,p53,Bax) in human tumour cells were assessed by flow cytometry. The apoptosis assay was carried out by using Annexin V-FITC/PI double staining kit, while cell cycle phases were investigated by using CycleTEST PLUS DNA reagent kit, than data were acquired on BD FACS Canto II flow-cytometer.
Cell cycle analysis showed that the growth inhibition effect was associated with G2/M arrest and up-regulation of p21 expression. In addition, GL induced apoptosis of oral tumor cells and an increase of the p53 and Bax proteins expression. Furthermore, the treatment with GL significantly amplifies the apoptosis induced by cisplatin in tumour cells analysed. Altogether, our findings suggest that G. lucidum exerts anti-tumor effects on oral tumor cells and enhances their sensitivity to cisplatin.

P5.20.017
Urethane-induced lung cancer and associated events

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Urethane-induced lung cancer is a model for human lung adenocarcinoma. Our aim was to identify markers in this model which could be used as key points for lung cancer therapy. BALB/c mice were injected with Urethane and followed for 120 days. Blood, bone marrow, and lung were harvested for evaluation. Histology showed that mice injected with Urethane (UR) presented lung nodules (number=5.25; area=14,570mm^2) whereas no nodule was observed in control mice (C). UR group presented an increase of myeloid-derived suppressor cells (MDSC, CD11+Gr-1+) in blood (UR=4.4% x C=2.9%), lung (UR=7.6 x C=4.5%), and bone marrow (UR=65% x C=60%). Alpha-smooth muscle actin (alpha-SMA) expression was observed in stroma and vessels of the lung tumor. The expression of TGF-beta was found in lung bronchioles epithelium both in C and UR groups. This marker was also observed in lung nodules. UR presented a higher expression of TLR4 protein in lung than control (UR=3.0$\pm$0.6, C=1.4$\pm$0.7) whereas TLR2 protein expression was similar (UR=0.72$\pm$0.04, C=0.74$\pm$0.07), and IL-6 protein expression decreased (UR= 0.21$\pm$0.04, C=0.61$\pm$0.13). Alpha-SMA is a marker of tumor-infiltrating myofibroblasts which facilitate tumor growth, invasion and metastasis by secreting growth factors and cytokines, produce matrix and matrix associated molecules. One of these factors is TGF-beta also found in our lung samples both in normal tissue and tumor stroma. TLR4 has a negative role when expressed in tumor and we found that UR group presented higher levels of TLR4 at the tumor site. In conclusion, new lung cancer therapies should focus on MDSC, alpha-SMA, TGF-beta and TLR4.

P5.20.018
SIGIRR as a novel HER2-Regulated gene in breast tumors

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Introduction: Transcriptional changes associated with overexpression of the receptor tyrosine kinase HER2 in human breast tumors were investigated using an immortalized mammary epithelial cell (HB4a) and its HER2 overexpressing variant (HB4a-C5.2). Global analysis of gene expression identified SIGIRR, a negative modulator of pro-inflammatory signals triggered by IL-1R and TLRs, as an upregulated gene in the C5.2 variant. Given the dual role of inflammation in tumor initiation and progression, we hypothesize that HER2-dependent SIGIRR overexpression in breast tumors may fine-tune inflammation and attenuate the antitumoral adaptive response. **Methods and Results:** qPCR and Western Blot analysis in C5.2 cells treated with Lapatinib confirmed that SIGIRR upregulation was dependent on HER2 activity. In agreement, bioinformatic analysis of public microarray data confirmed both SIGIRR and HER2 strong correlation. EMSA assays showed that shRNA-mediated knockdown of SIGIRR in C5.2 cells resulted in a 2-fold increase of NFkB activity upon IL-1β stimulation, confirming SIGIRR as a negative modulator of IL1R-dependent NFkB activation. To address the role of SIGIRR upregulation in tumoral milieu we investigated the expression of the cytokines IL-1β, IL-8 and TNF-α after IL-1β stimulus. The expression of all the above mentioned cytokines were 2 to 3-fold upregulated in SIGIRR knockdown clones and higher levels of IL-6 and IL-8 were also detected in conditioned medium. Higher expression of chemokines (CCL2, CSF2, CSF3 and CXCL10) was also observed in SIGIRR knockdown cells. **Conclusion:** Our results indicate SIGIRR as a novel HER2-regulated gene that may fine-tune inflammation and adaptive immune response in tumor microenvironment.
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P5.20.019
ANGPTL4 is involved in the metastatic hepatoma cells' survival in the third microenvironment

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The third microenvironment, which is also known as the vascular system, is of great importance during cancer cells' metastasis. Metastatic cancer cells have to conquer a lot of stresses including immune system of the body to survive in the vasculature before they arrive at the second sites. We established a three-dimensional cell model by using Poly-HEMA pre-coated plates to mimic the cells in the third microenvironment and found that these metastatic hepatoma cells were aggregated, non-proliferative, apoptosis-resistant, cell cycle arrested and much more invasive. They could settle down and proliferate again in suitable conditions. We analyzed the expression profile of the cell model and their control using microarray, which revealed that a lot of molecules were significantly different between the two groups. There are some increased apoptosis-related genes. One of these genes is ANGPTL4, which maybe help the metastatic cells acquire the ability of survival in the third microenvironment. We knockdown ANGPTL4 of metastatic hepatoma cells and found that cell viability of the metastatic hepatoma cells was decreased significantly and the percentage of dead cells was much higher after ANGPTL4 knockdown as compared with their control groups. Thus, we conclude that ANGPTL4 may play an important role in survival of metastatic hepatoma cells in the vascular system through a certain pathway.
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P5.20.020
Identification of Early Pancreatic Cancer-Associated Autoantibodies in Mouse and Human

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Pancreatic ductal adenocarcinoma (PDAC) has a dismal prognosis and diagnostic markers have not yet been defined. In this study we performed a SERological Proteome Analysis (SERPA) to identify antigens that elicit a humoral response in PDAC patients. Nineteen tumor-associated antigens (TAAs), mainly metabolic enzymes and cytoskeletal proteins, induced with high frequency specific autoantibodies in PDAC patients. The protein most frequently recognized was α-enolase (ENOA), a glycolytic enzyme that can localize on the cell surface as a plasminogen receptor. Further studies revealed that in PDAC ENOA is subjected to specific post-translational modifications and PDAC patients specifically produce antibodies against two Ser-419- phosphorylated isoforms of ENOA. By exploiting a genetically engineered mouse model (GEM) spontaneously developing PDAC, we applied the SERPA approach with mouse sera to validate circulating antibodies to TAAs as early diagnostic tools in PDAC. Eight antigens able to induce the production of autoantibodies in pancreatic intraepithelial neoplasia were identified and five of them induced specific IgG also in PDAC patients. Ezrin, a plasma-membrane cytoskeleton linker molecule, which displayed the highest frequency of autoantibodies in both GEM with early disease and resectable PDAC patients, was also recognized by prediagnostic PDAC sera. ROC analysis demonstrated that the combination of anti-Ezrin autoantibodies in association with antibodies to Ser-419-phosphorylated-ENOA and CA19.9 serum levels achieved approximately 97% diagnostic accuracy. In conclusion, our results demonstrate that the simultaneous capture of autoantibodies against a panel of pancreatic cancer-associated antigens might have diagnostic value in this tumor.

P5.20.021
CD4+CD25highFoxP3+ regulatory T lymphocytes in peripheral blood of gastric cancer patients during chemoradiotherapy

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Gastric cancer is often diagnosed at advanced stages, patients have regional node involvement and/or extension of tumor through the stomach wall, or into the adjacent organs. Preoperative chemoradiotherapy may downstage the tumor and potentially increase the rate of resectability, but it causes inflammation and immune imbalance. Furthermore, the disorder of immune homeostasis may influence the effectiveness of radiotherapy and impede tissue repair.
Treg cells play an active and significant role in the progression of cancer, and have an important role in suppressing tumor-specific immunity. They down-regulated the activity of effector function against tumors, resulting in T cell dysfunction in cancer-bearing host.
The current study was designed to investigate the changes in peripheral Treg cells in cancer patients and the influence of chemoradiotherapy on immunity function. The treatment consisted of radiotherapy and chemotherapy encompassed the stomach and regional lymph nodes.
We showed increased populations of Treg (CD4+CD25highFoxP3+) in peripheral blood T cells in patients with gastric cancer in comparison with healthy donors. We investigated the change in Treg cells' number in peripheral blood lymphocytes in patients undergoing chemoradiotherapy and compared it with reduction in the absolute number of T cells and changes in other lymphocyte subsets. These results are correlated with clinical data pertaining to tumor progression and therapy-related radiatoxemia.
Because of the fundamental role of Treg cells in suppressing anticancer immunity and diminished survival, the monitoring of T-regulatory lymphocytes ratio during conventional anticancer therapies could become an important clinical index useful in prognosis of cancer patients.

P5.20.022
Dendritic cells from mesothelioma patients are numerically and functionally impaired

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Malignant mesothelioma is an aggressive tumour affecting the mesothelium surrounding the pleura. Diagnosis occurs at a late stage of disease progression with death within twelve months. Current treatment options include surgery, chemotherapy and/or radiotherapy, however, no patients are cured and improved treatment strategies are needed. One possibility is immunotherapy. Dendritic cells (DC) are involved in inducing and maintaining adaptive immunity. Many immunotherapies target DCs yet their functional status in mesothelioma patients is unknown. Therefore, blood DC subsets from mesothelioma patients (n=48) and healthy age-matched controls (n=40) were analysed by flow cytometry. Mesothelioma patients had significantly lower $CD303^+$plasmacytoid (p)DC, $CD1c^+$myeloid (m)DC1 and $CD141^+$mDC2 numbers relative to controls. GM-CSF/IL-4-exposed monocytes from mesothelioma patients differentiated into monocyte-derived DCs (MoDCs) that expressed significantly lower levels of CD40, with decreased trends in CD11c/CD80/CD83/CD86 and HLA-DR expression compared with healthy-derived MoDCs. Mesothelioma-derived MoDCs demonstrated a significantly decreased capacity to process antigen. Stimulation with LPS+/-IFNγ induced incomplete maturation indicated by slight loss of antigen processing ability with limited upregulation of the maturation marker, CD83, and costimulatory molecules CD40/CD80 and CD86. Attempts to rescue DC function through CD40L stimulation did not fully restore CD1a/CD40/CD83/CD86 and HLA-DR expression and antigen processing function remained intact. Nonetheless, MoDCs from mesothelioma patients maintained their ability to induce allogeneic T cell proliferation. Our data suggests that mesothelioma patients have significant numerical and functional defects in their DC subsets and rescue with CD40L offers limited benefit. Furthermore, reduced capacity to process antigen and reduced costimulatory molecules suggests that T cells will be anergized/tolerized.

P5.20.023
High proportion of PMN-MDSCs within primary tumors correlates with disease aggressiveness in melanoma

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Immune escape was recently recognized as one of the hallmarks of cancer. A heterogeneous population of myeloid cells characterized by CD11b+/ Gr1+ accumulates in tumor bearing hosts and promotes tumor escape by limiting T cell responses. To account for their potent immune-suppressive properties on T and NK lymphocytes, these cells were named myeloid derived suppressive cells (MDSCs). Two main populations were identified according to their phenotype and their morphology: monocytic MDSCs (CD11b+, Ly6Chigh, Ly6G-) and PMN-MDSCs (CD11b+, Ly6C+, Ly6G+). To study their role in tumor escape, we use the model of spontaneous melanoma driven by the RET oncogene (MT/Ret mice). In this model, primary uveal melanoma disseminates early, but remains dormant for several weeks. Then, MT/ret mice develop local and finally distant metastases. Moreover, vitiligo occurs in nearly 40% of MT/Ret mice and is associated to a better tumor control. PMN-MDSCs are significantly more frequent than monocytic MDSCs within primary tumors. Here, we show that MT/ret mice developing melanoma-associated vitiligo displayed less PMN-MDSCs within tumors and in periphery than mice without vitiligo. Moreover, depletion of PMN-MDSCs with an anti-Ly6G antibody leads to a critical increase in the occurrence of vitiligo associated to a decrease in metastases incidence. Altogether, our data suggest that a high density of PMN-MDSCs infiltrating primary melanoma contributes to disease progression and may be a poor prognosis factor in humans.

P5.20.024
IL-18-Binding Protein (BP) is expressed in human ovarian cancer and may limit IL-18 activity

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Epithelial ovarian carcinoma (EOC) is associated to several immune-escape mechanims. IL-18, an immune-enhancing cytokine, which induces IFN-γ production, is studied in phase I-II trials of cancer immunotherapy. We reported that IL-18 processing is defective in EOC cells, which secrete the inactive precursor (pro-IL-18) in vitro. In addition, IL-18 levels were higher in EOC patients than in healthy women, particularly in the ascites. Hovever, only the 21 kDa inactive pro-IL-18 was detected and, accordingly, IFN-γ was not increased.
IL-18 and IFN-γ induce the production of IL-18 binding protein (BP), which can block IL-18 biological activity in a negative feed-back loop. Analysis of gene expression profiling indicated that IL18BP expression is increased in EOC tumors compared to normal ovary cells and cultured cell lines. In addition, we detected elevated levels of IL-18BP in the serum of EOC patients and even higher levels in the ascites, which suggested production at the tumor site. Immunochemistry of ascites cells or EOC tissues indicated IL-18BP expression in tumor cells and in reactive leukocytes. Cultured EOC cell lines show no constitutive IL-18BP production, while the same cell lines xenotrasplanted into ID mice showed IL-18BP production suggesting that IL-18BP expression in EOC may represent the outcome of a cross-talk between EOC cells and the

microenvironment. Indeed, different cytokines up-regulate IL-18BP expression in EOC cell cultures. High levels of IL-18BP may block the effects of endogenous or therapeutic IL-18 in EOC and contribute to the altered functionality of the IL-18 system in EOC.

P5.20.025
Proteolytic machinery involved in MICA and MICB processing in cervical Cancer

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Introduction: MICA and MICB are upregulated in epithelial tumors. The activating NKG2D receptor, expressed on NK and cytotoxic T lymphocytes, recognizes MIC molecules leading to anti-tumor responses. However, it has been demonstrated that tumor cells use proteolytic cleavage to shed MICA/B as a mechanism to evade immunosurveillance. **Objective:** To identify the expression profiles of ADAM10, ADAM17, MMP9 and Erp5 in cervical cancer cell lines and to associate these with soluble MICA/B levels. **Methods:** Three different cervical cancer cell lines were used (HeLa, SiHa and C-33A) as well as non-tumorigenic keratinocytes (HaCaT). The transcriptional expression of ADAM10, ADAM17, MMP9 and Erp5 was evaluated using qRT-PCR. Identification at the protein level was assessed by Western blot. MMP9 activity was detected through zymography. Soluble MICA/B were quantified by ELISA. **Results:** All cell lines, including HaCaT, express the proteolytic machinery capable of shedding MICA/B. Interestingly, it was observed that the ADAM10 active form, and its zymogen, are mainly expressed in HPV-positive HeLa and SiHa cells, as compared to C33A, an HPV-negative tumorigenic cell line. The strongest MMP9 enzymatic activity was found in SiHa cells, which interestingly showed the highest soluble MICA/B levels. HaCaT did not show significant soluble MICA/B. **Conclusions:** While proteolytic machinery was present in all cell lines, soluble MICA/B was only found in tumor cell supernatants. This finding suggests that MICA/B processing is more complicated than expected and that additional mechanisms (such as different MICA/B variants, glycosylation or palmitoylation states) could facilitate the proteolytic cleavage from the tumor cell surface.

P5.20.026
Analysis of lymphocyte subpopulations in blood from gastric cancer patients during radiotherapy

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Gastric cancer is not only the serious issue from the epidemiological point of view, but also therapeutically and diagnostically. The only reasonable solution of this problem seems to rely on individualized and combined modality treatment. The treatment results may also be significantly improved by identification of new diagnostic and predictive markers.
The aim of the following project is the analysis of lymphocyte subpopulation changes in gastric cancer patients during radiotherapy and influence of different ionizing radiation doses on lymphocyte functioning in vitro.
Material: peripheral blood collected from 30 gastric cancer patients, and 10 healthy volunteers. The lymphocyte subpopulation analysis was performed during the whole 35 day cycle of radiotherapy. Different lymphocyte subpopulation changes were analyzed before radiotherapy and after each treatment week.
Lymphocyte phenotype was analyzed using fluorochrome labeled antibodies against human surface antigens CD45, CD3, CD4, CD25, CD16+CD56, CD19 and CD8. Analysis of phenotype and percentage of different lymphocyte subpopulation was performed using flow cytometer FACSCanto.
Comparison of lymphocyte subpopulation in peripheral blood of gastric cancer patients before treatment and healthy volunteers showed statistically significant differences in quantity of undifferentiated lymphocyte T - cytotoxic/suppressor as well as lymphocyte B. Level of lymphocyte T and lymphocyte B in blood of healthy volunteers was respectively ten times and twice higher than in cancer patients.
Monitoring of the percentage of different lymphocyte subpopulation during the treatment and individual assessment of ionizing radiation susceptibility may become significant predictive marker allowing on cancer treatment individualization.

P5.20.027
Involvement of the long pentraxin PTX3 in skin carcinogenesis

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The role of the innate immune system on inflammatory carcinogenesis is a central issue for tumor biology and immunology. The long pentraxin PTX3 is a pattern recognition receptor that plays a fundamental role in regulation of the inflammation and in tissue remodelling. Available information in mouse and human suggest a protective role of PTX3 in cancer. In this study we showed that *Ptx3*-deficient (*Ptx3*$^{-/-}$) mice were more susceptible to DMBA/TPA-induced skin carcinogenesis than WT controls. Incidence and number of skin papillomas were significantly higher in *Ptx3* $^{-/-}$ mice compared to WT controls. ELISA revealed a strong PTX3 production in skin sample of WT mice during the acute phase of DMBA/TPA-induced cutaneous carcinogenesis. Furthermore immunohistochemical investigation showed PTX3 colocalization with lymphatic vessels and its specific deposition inside the skin papillomas. The deficiency of Ptx3 was associated to increased infiltration of neutrophils in skin papillomas and decreased infiltration of lymphocytes. Higher complement C3 deposition was observed in papillary lesions of *Ptx3*$^{-/-}$ mice compared to WT animals. Epidermal production of pro-inflammatory cytokines (IL-17 and TNFα) and chemokines, such as CXCL2, were increased in *Ptx3*$^{-/-}$ mice after TPA treatments. These data are in agreement with the protumorigenic role of IL-17 and CXCL2 in skin carcinogenesis. These results provide evidence that PTX3 is locally produced and has a protective role during skin carcinogenesis. The mechanisms of PTX3-mediated protection in this model could range from modulation of neutrophil recruitment to complement activation and are currently under investigation.

P5.20.028
Intra-tumoral B cells are able to organize into tertiary lymphoid structures and to develop a protective humoral immune response in lung cancer patients

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The major role played by the immune system in tumor immunosurveillance has been extensively documented. Several

clinical studies have notably demonstrated that a high density of effector T cells within the tumor is associated with an increased median survival. More recently, our team has described in some lung cancer (LC) patients the presence of tertiary lymphoid structures (TLS), composed of mature dendritic cells which home selectively in T-cell rich areas surrounded by B-cell follicles. By immunohistochemistry, we observed that these B-cell follicles are characterized by Ki-67+ proliferating germinal center B cells, a network of CD21+ follicular dendritic cells, and that a high density of follicular B cells is associated with favorable clinical outcome in early-stage LC patients. Consistent with their organization in TLS, we observed by flow cytometry all stages of B cell differentiation, namely a small proportion of naïve and germinal center B cells, and a larger proportion of memory and plasma cells. Importantly, we demonstrated in half of the patients considered that intra-tumoral plasma cells secreted IgG and/or IgA specific for several tumor-associated antigens (TAAs). For some patients, a specific reactivity of the antibodies has been observed against one to nine distinct TAAs among the 36 tested. Finally, a pilot study demonstrated that the antibody response developed against one of these TAAs was polyclonal. All together, these results demonstrate that B cell density represents a new prognostic biomarker for LC patient survival, and suggest a major role for TLS in the initiation of a protective humoral immune response.

P5.20.029
Development and optimization of an HPLC-UV assay for measurement of Indoleamine2.3-dioxygenase (IDO) activity

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Background: Indoleamine2.3-dioxygenase (IDO) is an intracellular enzyme that catalyses the initial rate-limiting step in tryptophan (Trp) degradation along the kynurenine pathway (Kyn). This enzyme plays a crucial role in regulating the immune response by inhibiting T-cell proliferation. This immunomodulatory function of IDO plays an essential role in different physiological, paraphysiological, and pathological states. Measurement of IDO activity in plasma could constitute a relevant biomarker for all these different states.
Objective: IDO activity has conventionally been represented as a ratio of Kyn to Trp. We developed a specific HPLC method with UV detection using experimental design methodology for simultaneous measurement of Trp and Kyn in human plasma in a single run.
Method: The chromatographic experiments were carried out on a VARIAN (ProStar). A strategy for experimental design (factorial design and desirability function) were used to allow analyses parameters (acetonitrile percentage, buffer pH and the flow rate of the mobile phase) to be simultaneously optimized in order to identify Trp and Kyn with a high resolution between peaks, reproducibility and short analysis time.
Results: Optimized analyses were run using the mobile phase consisting of 15mmol/L phosphate buffer (pH3.5) and 10.6% acetonitrile at a flow rate of 1.2ml/min. The eluate was monitored by the programmed wavelength detection setting at 360 nm from 0 to 6.5 min for Kyn and 280 nm from 6.5 to 8.5 min for Trp. Satisfactory precisions and recoveries were obtained by this method.
Conclusions: Experimental design methodology allowed to rationally and economically developing a simple, fast and reliable method.

P5.20.030
The heterogeneity of lung metastasis immune microenvironment according to the primary tumor location

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Introduction: Recent evidence suggests that the tumor microenvironment of the primary and metastatic tumoral lesions can be heterogeneous. In the two scenarios the immune contexture might have different influence and prognostic impact. This study aim's was to give a broad description of the immune contexture of some common lung metastatic tumors.
Material/Methods: Up-to-date, 36 metastatic tumors (11=leimyosarcomas, 8=osteosarcomas, 5=breast, 4=liver, 4=prostate and 4=thyroid) have been analyzed by IHC for CD8 and CD20 with the quantitative automatic software Calopix, and through manual counting for DC-LAMP. The density (# of positive cells/mm2) of the analyzed populations was compared by non-parametric statistics.
Results: We did not find significant differences among CD8+ cell densities' among the analyzed tumors (354±593 leimyosarcomas, 48±71 osteosarcomas, 185±169 breast, 73±56 liver, 242±221 prostate and 209±301 thyroid). Nonetheless, the density of CD20+ cells in the osteosarcomas (2.2±2.2) was significantly lower than in thyroid (40.8±11.6, $P<0.005$) and prostate metastasis (422±372, $P<0.005$); similarly, the leimyosarcomas (33.1±53.4, $P<0.05$) displayed lower values of CD20+ cells than their prostate counterparts (422±372). No other differences were found for CD20+ densities among the analyzed groups (20.9±11.5 breast and 38.9±23.2 liver). Finally, the DC-LAMP densities in thyroid metastasis were significantly higher (3.5±3.8, $P<0.05$) than those from leimyosarcomas (0.3±0.3). No other differences on DC-LAMP expression were found (0.4±0.7 osteosarcomas, 1.2±2.4 breast, 0.9±0.7 liver, 3.5±3.8 prostate).
Conclusions: These preliminary results provide evidence than the tumor immune contexture of different types of lung metastasis is heterogeneous, and could be strongly influenced by the origin of the tumoral lesion.

P5.20.031
Azacytidine and Everolimus therapy reduces the high frequency of terminally differentiated CD38+HLADR- CD8 T cells in relapsed AML patients.

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T cells have a central role in anti-tumor immunity as demonstrated by the graft-versus-leukemia effect, however functionally impaired CD8 T cells are found at increased frequencies in AML patients. The effects of novel epigenetic treatments in AML patients, azacitidine and everolimus on CD8 T cell subset frequencies have not been previously investigated. Here, we demonstrate that relapsed AML patients have elevated overall levels of CD8 T cells when compared to healthy donors. We mapped this increase as due to a single cell subset, characterized by the expression of CD38, a marker associated with activation. However, these CD38+ CD8 T cells did not co-express other activation markers such as HLADR. The CD38+HLADR- T cell subset was found to express high levels of the senescence marker, CD57, with higher expressions found within AML patients. This subset also had low levels of CD28 in AML patients than healthy donors. These cells were not exhausted T cells, as they did not express high levels of PD-1. This suggests that CD38+HLADR- CD8 T cell subset represents a terminally differentiated CD8 T cell population. Upon treatment, there was a significant reduction of this sub-population within week 1, and these levels remained low for up to three months of treatment. This reduction was observed to be significantly lower in patients who had stable disease when compared to patients with disease progression.

P5.20.032
Panobinostat in combination with azacitidine reduces the frequency of TNFR2+ Tregs in newly diagnosed acute myeloid leukemia patients.

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Acute Myeloid Leukemia provides an environment that enables immune suppression, resulting in functionally defective effector T cells. Regulatory T cells (Tregs) are significant contributors to the impaired anti-tumor immunity. Expression of the TNF receptor TNFR2 on Tregs identifies a potent regulatory subset, and since TNF is present at high levels within AML patients, we hypothesized TNFR2+ Tregs may be a functionally relevant Treg subset in AML. Furthermore, the effect of the emerging epigenetic treatment drug combination, panobinostat and azacitidine on T cells and Tregs, particularly TNFR2+ Tregs has not been previously investigated in AML or other blood diseases. Here, we demonstrate that TNFR2+ Tregs are the dominant highly functionally relevant Treg population within AML patients. Treatment with azacitidine and panobinostat resulted in a decrease in TNFR2+ Tregs, but not TNFR2- Tregs in AML patients. Moreover, patients who had a reduction in their TNFR2+ Tregs in both the bone marrow and peripheral blood responded positively to treatment. These patients consequently also showed increased IFNγ and IL-2 production by effector T cells isolated from the bone marrow. In vitro mechanistic studies suggested that panobinostat and not azacytidine, primarily and dominantly drives the reduction in Tregs. AML patients who responded clinically to panobinostat and azacitidine therapy had an immune profile associated with decreased TNFR2+ Tregs and increased effector T cells.

P5.20.033
The effects of Cancer Associated Fibroblasts on mechanisms of immune evasion in a rat chemical mammary carcinoma model

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Objective: Fibroblast cells turn into cancer associated fibroblasts(CAFs) in tumor microenvironment. Tissue fibroblasts have previously been shown to affect T-cell functions. However, studies investigating the effects of CAFs on T-cells are limited in the literature.
Methods: N-Nitroso N-Methyl Urea(NMU) induced experimental mammary carcinogenesis model was utilized. Tumors were harvested surgically under sterile conditions for CAF isolation. CAFs and healthy tissue fibroblasts were immunostained to evaluate expression differences of markers like α-Smooth-Muscle-Actin(αSMA) and Vimentin. DNA damage of peripheral blood cells due to NMU were analyzed by Comet Assays. Cocultures of CAFs with lymphocytes employing Carboxyfluorescein-succinimidyl-ester(CFSE) proliferation assays were performed. Surface and intracellular expressions of immune activation markers (CD25, IFN-γ) of T-cells cocultured with CAFs were analyzed. Levels of IL-4 were analyzed in CAF-splenocyte cocultures. Gene expression levels of several immune activation markers like CD25 and costimulatory molecules like CD28 in splenocytes were investigated.
Results: The immunostainings showed that CAFs had significantly higher levels of αSMA expression. Comet Assays showed that levels of DNA damage in tumor-bearing animals were similar to control levels. CAFs decreased proliferations of splenocytes in cocultures. Flow cytometry experiments showed that CAFs decreased intracellular expressions of immune activation markers like IFN-γ in T-cells. Supernatants of CAF-splenocyte cocultures were shown to have increased levels of IL-4. Splenocytes cocultured with CAFs were found to have decreased gene expression levels of costimulatory molecules.
Conclusions: A rat chemical breast carcinogenesis model was successfully employed and CAFs were stably propagated. Coculture experiments suggested an immunomodulatory role for CAFs on immunity against breast cancer.

P5.20.034
Effects of Silymarin on frequency and function of Myeloid-derived suppressor cells in animal model of tumor

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Background: Myeloid-derived suppressor cells (MDSC) are identified as heterogeneous population of immature myeloid cells (IMCs) including precursors of macrophages, granulocytes, and dendritic cells (DC). These cells accumulate during tumor formation; facilitate immune escape, and therefore promote tumor progression. Silymarin, a plant flavonoid isolated from the seeds of milk thistle (Silybum marianum), has been indicated to possess antioxidant, anti-inflammatory and immunomodulatory properties. We sought to determine silymarin's effects on the frequency and function of MDSC population accumulated during tumor progression.
Methods: We isolated MDSCs from spleen of tumor bearing mice treated with silymarin by MACS method. Then, the number of MDSCs and also their ROS production were evaluated using flow cytometry and compared with untreated group. Moreover, the silymarin's effect on NO production was analyzed using ELISA.
Results: Our results showed that silymarin had some positive effects on number and function of MDSCs.
Conclusion: Silymarin, as an anti-inflammatory drug, may exert beneficial effects on T cell responses against tumors and should be considered as an agent that affects the number and function of MDSCs. Further studies are needed to shed light on adjuvant anti-cancer effects of this compound which potentially could be used as combination therapy of cancer.
Key words: MDSC, Silymarin, Anti-inflammatory, ROS, NO

P5.20.035
Formation of vascular mimicry in human gastric cancer and the corelation with LOX and MMP-9

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To explore the corelations of lysyl oxidase (LOX) and matrix metalloproteinases-9 (MMP-9) with formation of VM in gastric carcinoma tissue. Gastric cancer tissues were collected from 49 patients with diagnosis of gastric cancer. According to metastasis (include lymph node and distant metastasis), gastric cancer tissues were divided into the group of metastasis and the group of non-metastasis. According to the invasion, gastric cancer tissues were divided into the group of T1 + T2 and the group of T3 + T4. The formations of VM were measured through CD34 and PAS double staining on gastric cancer tissue slices. The distributions of LOX and MMP-9 were observed using immunohistochemistry. Relative levels of LOX and MMP-9 were surveyed by Western-blot and immunohistochemistry. The locations of LOX and MMP-9 were detected by Immunofluorescence. Results showed, in gastric carcinoma tissues, the VM formation in group with metastasis were more than that in group without metastasis ($P<0.05$), the level of LOX and MMP-9 protein were higher in group with metastasis than that in group without metastasis ($P<0.05$); the expression of LOX in group of T3 + T4 were more abundant than in T1 + T2 group($P <0.05$). The levels of LOX was positively correlated with VM formation in gastric carcinoma tissues, the levels of MMP-9 was positively correlated with VM formation. The immunofluorescence results suggested that LOX often located in the area that MMP-9 expressed. The results suggest that VM promotes the invasion and metastasis of gastric cancer, LOX and MMP-9 may be facilitative factors in VM formation.

P5.20.036
Aberrant expression of circulating PD-L1+CD14+HLA-DR-/low cells in esophageal squamous cell carcinoma patients: Immunosuppressive characteristics and tumor clinical relevance

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Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of immune-suppressive cells which play an important role in tumor immune-escape. Recently, a population of $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs was identified in blood and tumor tissue of multiple types of cancer patients. In this study, we assessed the levels of $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs in peripheral blood and tumor-draining lymphnode (TDLN) from patients with esophageal squamous cell carcinoma (ESCC). The level of circulating $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs increased significantly in patients with ESCC and was correlated with lymphnode metastasis, TNM stage. Furthermore, circulating $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs decreased with the surgical excision of tumor. Additionally, $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs increased notablely in the tumor-positive TDLN comparing with corresponding tumor-negative TDLN. In vivo experiment showed that $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs could effectively inhibit autologous T cell proliferation and downregulate the production of IL-2, IFN-γ comparing with corresponding $CD14^{+}HLA\text{-}DR^{high}$ cells. Subsequently, we examined surface expression of B7-family molecules on $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs and found remarkable increase of PD-L1 expression. Furthermore, intervention PD-L1 pathway by a blocking antibody effectively reversed the suppression of autologous T cells. Our data indicate that a new population of $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs with PD-L1 high expression accumulated in peripheral blood and tumor-positive lymphnode of patients with ESCC. This population is of immunosuppressive characteristics and correlated with tumor burden, lymphnode metastasis and TNM stage. And our data also confirmed that PD-L1 plays a key role in T cell suppression mediated by $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs in patients with ESCC. PD1/PD-L1 pathway may be alternative immune checkpoint of tumor immune-escape mediated by $CD14^{+}HLA\text{-}DR^{-/low}$MDSCs.

P5.20.037
Microparticles: a road to antitumor immunity

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Tumor cells are capable of changing their cytoskeletal structure and resulting in plasma membranes encapsulating cytosolic elements and expelling them or downloading them into the extracellular space. These specialized subcellular vesicles are called microparticles (MPs) and come in different sizes of 100 to 1000 nm in diameter. In line with the cellular components, MPs may contain numerous tumor messenger molecules, enzymes, RNAs and even DNA. This study explored the possibility of translating tumor information into antitumor immunity through the MP pathway.
Despite the critical role of dendritic cells in antitumor immune responses by cross-presenting tumor antigens to tumor-specific T cells, we found that DCs were vulnerable to take up apoptotic tumor cells but strongly captured tumor cell-derived MPs. As a result, DCs upregulated CD80, CD86 and MHC class II, concomitant with the activation of JNK, ERK and NF-κB, leading to activating tumor specific T cells. Vaccination of murine H22 hepatocarcinoma tumor cell-derived MPs prevented H22 tumor rather than MCA26 colon cancer or B16 melanoma formation in mice in a CD8 T cell dependent manner. Consistently, B16 cell-derived MPs selectively affected B16 melanoma, suggesting that tumor cell-derived MPs are tumor antigen specific. Undoubtedly, tumor cells are the roots of antitumor immune responses, however previous tumor cell-based vaccines only generated limited progress. The present study probably discloses tumor cell-derived MPs as a new generation of vaccine, leading to effective antitumor consequence.

P5.20.038
Mapping on chromosome 17 a novel major locus determining kidney cancer susceptibility in mice

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Mouse lines derived by bidirectional phenotypic selection for inflammatory responsiveness differ in susceptibility to urethane-induced kidney carcinogenesis. High inflammation producing mice (AIRmax) are resistant whereas the low responder AIRmin are susceptible, showing 70% incidence of papillary kidney tumors (racemase positive) after sc treatment with 300 mg/kg bw urethane at 7 days of age. The difference is also observed in untreated 14 month-old mice, since AIRmax mice are unaffected and tumor incidence in AIRmin reaches 50%. Using a single nucleotide polymorphism (SNP) array for genome-wide linkage analysis, we mapped one quantitative trait locus (QTL) regulating kidney tumor incidence in an F2 (AIRmax x AIRmin) intercross population (n=693). This QTL mapped to chromosome 17 at 30 Mb (LOD score = 15.8, 13 Mb confidence interval), identified by SNP rs3693494 ($P= 2.1^{-16}$, per allele odds ratio = 2.9, 95% CI: 2.2 - 3.7). Several candidate genes mapping in this locus have been implicated in inflammation-related kidney pathologies in humans, such as *Pkd1,* encoding polycystin-1, responsible for the polycystic kidney disease and *Tsc2*, a tumor suppressor gene encoding tuberin that participates in signal transduction pathways involved in cancer development. Genes relevant to innate and acquired immunity such as the major histocompatibility complex (H2) and C2 and C4 complement system components also map to this QTL. Functional effects of allelic variants could contribute to the differential susceptibility to inflammation and kidney cancer of AIRmax and AIRmin mice.
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P5.20.039
Anti-tumor effect of BCG-LM via activated eosinophils.

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Mycobacterium tuberculosis (tubercle bacilli) and the related acid-fast bacteria including Mycobacterium bovis Bacille Calmett-Guerin (BCG) contain a lipoarabinomannan (ManLAM or LAM) and lipomannan (LM). LAM and LM have been reported to activate type-I inflammatory responses via dendritic cells (DCs) through Toll-like receptor 2. However, the cellular and molecular mechanisms underlying the antitumor effect of LAM/LM are not well analyzed. In order to study the antitumor effect of LAM/LM, a B cell lymphoma cell line A20 and an OVA-expressing A20-OVA tumor cells were subcutaneously inoculated into the flanks of the mice. The mice were treated by intra-tumor injection of LAM, LM, or BCG cell wall (CW), or LAM-, LM-, or CW-pulsed dendritic cells. The most potent antitumor effect was observed by the treatment with LM with massive infiltration of eosinophils. Eosinophils can be activated by LM. Thus, these results indicate that LM from M. bovis BCG exerts an antitumor effect via the activation of eosinophils.

P5.20.040
Inducible nitric oxide synthase from prostate cancer stem/initiating cells inhibits T cell priming

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Consistent evidence supports the concept that, irrespective of the cell-of-origin, many cancers are hierarchically organized as a normal tissue, and originate from a population of stem-like initiating cells (CSC). According to this theory, CSC are essential to tumor evolution

and recurrence, and indeed, it is reasonable to hypothesize that evolution has supported these cells and/or their niches with mechanisms of immune evasion. Here we provide experimental evidence that prostate CSC (PCSC) from the prostate of transgenic adenocarcinoma of the mouse prostate (TRAMP) mice induce anergy in T lymphocytes. Indeed, PCSC-conditioned T cells showed reduced activation of TCR and IL-2 pathways, that resulted in inhibited proliferation and cytokine production. Inducible oxide synthase (iNOS), a molecule that has been already implicated in prostate cancer aggressiveness both in TRAMP and human prostate cancer, appeared to be one of the mechanisms adopted by PCSC to inhibit T cells, as treatment of PCSC with the iNOS inhibitor L-NAME reduced their immunosuppressive activity. This phenomenon was restricted to T cells in the phase of priming or ristimulation, being activated T cells resistant to PCSC immune suppression. Even if other candidate immunosuppressive molecules expressed by PCSC, such as Galectin-3, are being currently investigating, all together, these findings confirm iNOS as a strategic target molecule for therapeutic applications against prostate adenocarcinoma.

P5.20.041
Inflammatory stress upregulates chemokine expression in uveal melanoma cell lines and increases monocyte migration

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Purpose: Uveal melanoma (UM) is the most common primary intraocular tumor in adults and the presence of infiltrating leukocytes is associated with a poor prognosis. This study investigates the effect of activated T-cells on UM cell differential gene and protein expression, and the ability to attract monocytes.Methods: T-cells were purified from healthy human donors, activated with anti- CD3/CD28 beads and co-cultured over three UM cell lines for 64 h in a membrane insert. Supernatants were collected and RNA was purified from the UM cell lines. Gene expression analysis was performed with microarrays. Protein expression in the supernatants was quantified with a multiplex bead array. For the migration assay, CD14+ monocytes purified from healthy human donors were added in the upper chamber of a transwell plate. Supernatants were added to the lower chamber and plates were incubated for 2.5 h. Migrated cells were counted using flow cytometry.Results: Gene expression analysis of UM cell lines co-cultured with activated T-cells resulted in an upregulation of chemokines such as CXCL8, CXCL9, CXCL10, CXCL11, CCL2 and CCL5. The expression of these was confirmed on the protein level. This increase of chemokines coincided with increased monocyte attraction towards co-culture supernatants in a migration assay.Conclusions: Soluble factors derived from activated T-cells shift the UM cell-transcriptome towards a more inflammatory state, including the upregulation of several chemokines, which lead to an increased migration of monocytes. Therefore, UM cells might actively participate in generating an inflammatory environment around the tumor which corresponds to a worse prognosis.

P5.20.042
Interleukin-33/ST2 Axis Promotes Breast Cancer Progression and Angiogenesis by Intratumoural Accumulation of Immunosuppressive and Natural Helper Cells

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Interleukin-33 through binding to its receptor ST2 promotes Th2 responses, but whether IL-33 signaling affects antitumour immunity is not fully understood. Using 4T1 mammary tumour model that shares many characteristics with human breast cancer we demonstrate that IL-33 is expressed in endothelial and tumour cells. Administration of IL-33 accelerated tumour growth and development of lung metastases which was associated with increased intratumoural accumulation of CD11b+Gr-1+ myeloid derived suppressor cells, CD4+Foxp3+IL-10+ Tregs and IL-13 expressing Lin-Sca-1+ natural helper cells. A marked reduction of NKp46+FasL+ tumoricidal cells with pronounced increase of PD-1 expressing NK cells were observed in tumours after IL-33 treatment. A deficiency of the ST2 gene significantly reduced myeloid suppressor cells and highly enriched FasL expressing NK cells at tumour site. IL-33 promoted intratumoural proliferation and neovascularization which was attenuated in ST2-deficient mice. Higher number of immune suppressor cells and IL-10 expressing immature CD11c+ dendritic cells in spleens and increased serum levels of IL-10 and IL-13 were observed only in IL-33 treated tumour-bearing mice. A significantly reduced NK cell, but not CD8+ T-cell cytotoxicity in IL-33-treated mice was observed and the dispensable role of CD8+ T cells in mammary tumour progression was confirmed by their in vivo depletion. We show a previously unrecognized role for IL-33 in promoting breast cancer progression through increased intratumoural accumulation of immunosuppressive cells and neoangiogenesis and by diminishing innate antitumour immunity. IL-33 may be therefore considered as an important mediator in regulation of breast cancer progression.

P5.20.043
The role of memory T lymphocytes during growth of SL2 tumours in DBA/2 mice

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The purpose of this study was to evaluate the role of central memory (TCM) and effector memory (TEM) T lymphocytes in the murine DBA/2-SL2 concomitant immunity system.
Tumours were implanted by injecting 107 SL2 lymphoma cells subcutaneously on the left (primary tumour) and the right side (secondary tumour) of the chest with the interval of 2 days. Peripheral blood was collected from the tail vein of individual mice on day 0 (prior tumour implantation) and on day 9 after primary tumour implantation. Percentages of TCM (CD8+CD44highCD62L+) and TEM (CD8+CD44highCD62L-) lymphocytes in the CD8+ subset in peripheral blood of tumour-bearing mice were analysed by flow cytometry. The weights of the tumours were measured after sacrificing the mice on day 9.
The percentage of TEM lymphocytes in the CD8+ subset significantly increased in peripheral blood of tumour-bearing mice on day 9 of experiment compared to day 0. The percentage of TCM lymphocytes was lower on day 9 compared to day 0. No correlation between the level of TEM lymphocytes in peripheral blood and the mass of primary or secondary tumour was found. Significant negative correlation was found between levels of TCM in peripheral blood and the mass of the secondary tumour.
Thus, expanded TEM lymphocytes do not influence the growth of tumours in the murine DBA/2-SL2 concomitant immunity system. TCM seem to inhibit the growth of the secondary tumour, despite their failure to expand during tumour growth.

P5.20.044
Myeloma with biclonal IgG lambda and IgD kappa in serum : a case report

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IgD myeloma is a rare entity (1-3%). Kappa subtype contributes only 10-30% of IgD myelomas and biclonal gammopathies involving IgD are even more rare. In fact, biclonal bands constitute only 3-4% of all myelomas.
Case report : We report the case of a 57 years old women, who presented inflammatory low back pain and experienced a severe decline in general health. The symptoms were present 3 months before diagnosis. Laboratory test abnormalities included an elevated erythrocyte sedimentation rate (100mm /h), normochromic normocytic aplastic anemia, hypercalcemia (2,92 mmol/l), hyper-β2-

microglobuline (2,87 mg/l). Serum protein electrophoresis showed hypergammaglobulinemia with a monoclonal peak in gamma region. Immunofixation detected an IgD Kappa band and a faint IgG lambda one. Bone marrow aspirated from the sternum was found to contain 30% of malignant plasma cells. Radiograph magnetic resonance imaging (MRI) disclosed myelomateous infiltration of dorsolumber spine and spondylolisthesis, with no evidence of spinal cord compression.
IgD myeloma is a particular severe form of myeloma often associated with poor prognosis. The pathogenesis is unclear. This case presents interesting findings regarding prognosis: We discuss, on one side, pronostic significance of biclonal gammopathy with IgD-Kappa comparing to the monoclonal IgD myelomas. On the other side, we analyze particularities of the IgD Kappa compared to the typical IgD lambda.

P5.20.045
Effect of SAA1, SAA2 and SAA4 knockdown on proliferation and invasion of glioblastomas multiformes cells

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Serum amyloid A (SAA) is an immunomodulatory protein produced in acute and chronic inflammatory process, such as cancer. Our research group showed that glioblastomas multiformes - A172 and T98G cells - produce SAA and have proliferation, migration and invasion affected by SAA treatment (Knebel, et al. Mediators of Inflammation, 2013, in press). To understand the significance of SAA produced by the tumor itself or by an inflammatory process, SAA1, SAA2 and SAA4 genes knockdown were prepared by lentiviral system using five different plasmids for each SAA gene. ^{3}[H] thymidine incorporation and Boyden chamber assay were used for proliferation and invasion measurements, respectively. Among the five plasmids of each gene, we were able to selected at least one that present a gene inhibition greater than 88%. SAA1 and SAA2 knockdown inhibited proliferation and invasion of glioblastomas multiformes cells when compared to the wild type. However the SAA4 knockdown only inhibited the A172 proliferation. Although preliminary, our data may suggest that SAA1 and SAA2 contribute more than SAA4 to tumor invasiveness. Addition of recombinant SAA (Peprotech) revert the effects of silencing in most but not all cases. At this point it is crucial to identify the roles of each SAA genes and the genetic or cellular uniqueness that outline SAA responsiveness to identify the potentiality of SAA as a therapeutic target in cancer.

P5.20.046
Inactivation of tumor-specific CD8+ CTLs within tumor-infiltrating lymphocytes by tumor-infiltrating immunosuppressive tolerogenic dendritic cells

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Insufficient activation of tumor-specific class I MHC molecule-restricted CD8+ cytotoxic T lymphocytes (CTLs) will permit tumor growing in vivo. DEC-205+ dendritic cells (DCs), having the ability to cross-presenting capacity, can present captured tumor antigens in association with class I MHC molecules alongside costimulatory molecules, and induce the priming and activation of tumor-specific CD8+ CTLs. It has been suggested that reduced levels of costimulatory molecules on DCs may be a cause of impaired CTL induction and that some tumors may induce the downregulation of costimulatory molecules on tolerogenic DCs. To examine such possibilities, we established two distinct types of murine hepatoma cell lines, Hepa1-6-1 and Hepa1-6-2 from Hepa1-6 cells, and confirmed that they display similar antigenicities, as well as identical surface expression of class I MHC molecules. Hepa1-6-1 had the ability to grow continuously after subcutaneous implantation into syngeneic C57BL/6 mice and did not prime CD8+ CTLs. While, Hepa1-6-2 cells, which display reduced levels of adhesion molecules, failed to grow in vivo and efficiently primed CTLs. Moreover, Hepa1-6-1-derived factors converted CD11c+ MHC-IIhigh DEC-205+ DC subsets into tolerogenic cells, with downregulated costimulatory molecules and impaired cross-presenting capacities. These tolerogenic DCs appeared to inhibit the induction of tumor-specific CD8+ CTLs and suppress their cytotoxic functions within the tumor. Taken together, the findings presented here provide a new method of cancer immunotherapy using the selectivesuppression, depletion, or alteration of immunosuppressive tolerogenic DCs within tumors.

P5.20.047
Systemic changes of cellular immune parameters as observed in patients with preinvasive/invasive cervical cancer before and after treatment

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Experimental data accumulated to date suggest that individual's immune status serves as a background creating permissive conditions for HPV persistence and cervical cancer initiation. Immune disorders occurring both at local and systemic levels during cervical cancer development may comprise changes not only in subpopulation composition of immune cells but also changes of their functional state as, for example, increased susceptibility to apoptosis. Induction of apoptosis-related processes in circulating lymphocytes, their contribution to cervical cancer progression and applicability as predictive immunologic markers requires thorough investigation. In this work we were aimed to: a) analyze changes of subpopulation composition of peripheral blood lymphocytes (cytotoxic and regulatory T-cells, T-helpers, natural killer cells, B-cells) at successive steps of squamous cervical cancer progression (severe dysplasia, carcinoma in situ, microinvasive carcinoma and invasive cancer stages II-IV); b) to measure the changes of CD95-expression and activity of caspases; c) to trace the restoration of these parameters after surgical and immunomodulatory treatment by alloferon-alfa. We found that progression of cervical cancer, beginning from early precancerous lesions, is accompanied by significant reduction of circulating cytotoxic and helper T-cells and increase of regulatory T-cells (Tregs), natural killers and B-lymphocytes. Gradual up-regulation of proteolytic activity of extrinsically regulated initiator capase-8 and its downstream effector caspases -3 and -6 as well as surface CD95 expression was observed. Complex immune-based treatment resulted in more efficient normalization of Tregs, NK- and B-cells in patients with pre- and microinvasive lesions. The work was supported by the Government of the Russian Federation (contract numbers 11.G34.31.0052, 2012-1.2.1-12-000-1014-027)

P5.20.048
Repurposing Na^+/K^+ pump blockers for *in vitro* suppression of tumor development

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It has become clear that progression of carcinomas depend not only on alterations on epithelial cells, but also on changes of microenvironment. Solid tumors survival and development is often based on vascular network sustaining such an intense metabolic process. Main purpose of our study was investigation of effects of Na/K pump blockers on tumor cells and tumor associated fibroblasts (TAF).
TAFs and breast tumor cell line SK-BR3 were used. Ouabain, Na^+/K^+-ATPase inhibitor, was added in cell culture media for 24 and 48 hours in concentrations ranging from 10 µM to 1 nM, and amount of cellular utilization was determined by HPLC. MTT-based viability assay showed decreased proliferation rates for higher concentration of Ouabain. Flowcytometric analysis investigated expression of phenotypical markers, including CD106, CD90, CD44, CD29, CD117, CXCR4 for TAFs, and Her2, CD44, VEGF-R and CD29 for SK-BR3 cells. Gene expression of α1 and β1 subunits of Na^+/K^+ pump, as well as VEGF were determined using qRT-PCR method. The results

showed that VEGF secretion was significantly reduced in Ouabain-treated SK-BR3 and TAFs. Although level of expression for α1 catalytic subunit was increased in SK-BR3, we could not find presence of corresponding protein, reflected also in high levels of Ouabain detected in cellular supernatant. Expression of adhesion molecules was decreased, and we found profound changes in phenotypic profile of both TAFs and SK-BR3 cells. Based on their anti-angiogenic and
anti-proliferative activity, Na^+/K^+ pump inhibitors could open novel anti-tumoral therapeutic strategies.

P5.20.049
Down-regulation of EMT signal and invasive properties of colon cancer cells by SOCS1

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Growth-stimulatory and inflammatory cytokines promote the proliferation and progression of tumors via invasion and metastasis through multiple signaling pathways in tumor microenvironments. Using colon cancer cells over-expressing or knock-down for suppressors of cytokine signaling (SOCS) isoforms, we have studied the mechanism of anti-tumor effects of SOCS through the regulation of epithelial-mesenchymal transition (EMT) in vitro and metastasis in in vivo animal models. SOCS1 but not SOCS3 promoted radiation-induced G1 arrest and 5-FU-induced apoptosis in p53-sensitive manners. SOCS1 further inhibited the p53+/+ HCT116 cell migration and invasive properties under radiation assessed by wound-healing and Matrigel assays, respectively. The SOCS1-overexpressing cells exhibited upregulation of E-cadherin with reduced beta-catenine and Twist levels, while SOCS1 knock-down cells expressed increased vimentin and Twist levels. Although phosphorylation of STATs were variable, p-Src, p-Akt, p-Erk and p-4EBP and NF-kB levels were suppressed in SOCS1-expressing cells, suggesting a STAT-independent down-regulation of EMT signaling by SOCS1 in this system. In addition, the confocal analysis indicated that SOCS1 helps to retain epithelial morphology by suppressing EMT. Finally in tumor implantation animal models, SOCS1-transduced HCT116 tumor cells exhibited substantial retardation in growth and possible dissemination when introduced in spleen and I v injection into nude mice. The in vivo imaging of tumor cells by PET scanning is being employed to assess the metastatic behavior of SOCS-regulated tumor cells. <Supported by KRF grant #2012-02032067 and #2012-015258>

P5.20.050
B7-H4 modulates anti-tumor responses by limiting immunosuppression in the tumor microenvironment

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B7-H4 belongs to the B7 family of T cell immunomodulatory molecules, and has been shown to inhibit T cell responses and neutrophil expansion. B7-H4 is highly overexpressed in multiple types of human cancers; as such, a pro-tumorigenic role has been implicated for B7-H4. However, its impact on the immune response during tumorigenesis is unknown. Here, we examine the host response in B7-H4-deficient mice upon injection of 4T1 carcinoma cells. We reveal that B7-H4 not only inhibits T helper 1 responses, but that it also inhibits the pro-tumor function of myeloid-derived suppressor cells (MDSC). As such, we observed greater expression of both anti-tumor immune effectors and MDSC-associated transcripts in 4T1 tumors grown in B7-H4 KO mice. Although the rate of 4T1 growth in B7-H4-deficient hosts was similar to that of WT mice, tumors derived from B7-H4 KO hosts grew much slower upon a secondary injection into wild-type recipients. This suggests that tumors grown in the absence of B7-H4 have reduced immune-evasive capacities. Consistently, MDSCs from B7-H4 KO mice suppressed T cell proliferation more effectively than WT MDSCs. Collectively, this implies that MDSCs from B7-H4 KO hosts suppress T cells during tumor growth, leading to greater immunosuppression in the tumor milieu, which allows outgrowth of 4T1 variants with diminished immune evasive capacity. Thus, our data show that B7-H4 has inhibitory roles for both anti-tumor and pro-tumor components of the immune system which may be counterbalanced if B7-H4 blockade is applied during cancer immunotherapy.

P5.20.051
DcR3 gene polymorphisms are associated with sporadic breast infiltrating ductal carcinoma in chinese northeast women:a case control study

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Decoy Receptor 3(DcR3), which gene maps to a chromosomal region (20q13.3) is also called TNFRSF6b, can bind its ligands including FasL, LIGHT and TL1A. The expression of DcR3 is not detectable in normal tissues, but in some malignant tissues its expression is upregulated. In our research, there are 531 female breast IDC cases (age49.5±9.9) and 592 normal controls (age45.4±9.9). We chose five SNPs in DcR3 gene including rs3208008 in 5'-near gene region, rs41309931 in intron, rs2297441 in intron, rs909341 in exon and rs1291207 in intron. The minor genotype frequency of five SNPs was more than 5% in CHB population. From the result, we found that SNPs in rs41309931 and rs909341 had significant statistical differences in genotypes and alleles. The most frequent haplotype appeared in cases and controls were AGGTA (rs3208008 A, rs41309931 G rs2297441 G, rs909341 T, rs1291207 A) and the frequency of it was significant higher in cases. We also analyzed the relationship between the polymorphisms of DcR3 gene and a series of clinicopathologic features, including tumor size, lymph node metastasis, and the statuses of ER, PR, C-erbB2 and P53. In summary, our case-control study was the first report regarding the association between SNPs in DcR3 gene and breast IDC, especially in Chinese females.

P5.20.052
HVEM gene polymorphisms are associated with sporadic breast cancer in Chinese women

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As a costimulatory molecule, Herpesvirus entry mediator (HVEM) can bind with several costimulatory members, so it plays different roles in T cell immunity. HVEM and its ligands have been involved in the pathogenesis of various autoimmune, inflammatory diseases and tumors. In this research, we first investigated a case-control study between polymorphisms of HVEM and breast cancer, including 575 breast cancer females and 604 age-matched healthy controls. Six of HVEM SNPs (rs2281852, rs1886730, rs2234163, rs11573979, rs2234165 and rs2234167) were genotyped by PCR-RFLP. The results showed that rs1886730 and rs2234167 had significant differences in genotypes and alleles (P<0.05). In haplotypes analysis, we found that three haplotypes were associated with breast cancer. The association between polymorphisms of HVEM and clinicopathologic features were shown in our research, including lymph node metastasis, ER, PR and P53. Our results primarily indicated that polymorphisms of HVEM gene were associated with the risk of sporadic breast cancer in northeast Chinese females.

P5.20.053
Association of LTBR gene polymorphisms with sporadic infiltrative duct carcinoma in northeast Chinese Han population

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Background: LTBR signaling plays important roles in the formation of peripheral lymphoid organs, interferon responses to pathogens and dendritic cell regulation. Accumulating evidence indicates that LTBR signaling is also involved in inflammation-induced carcinogenesis and cancer development. Herein, we set out to investigate the association between LTBR gene polymorphisms and sporadic infiltrative duct carcinogenesis and tumor pathology.

Methods: Our research subjects consist of 516 patients with sporadic infiltrative duct carcinoma and 524 age-matched healthy controls. Four SNPs (Rs3759333, Rs11064157, Rs2302256, and Rs2364480) in the LTBR gene were genotyped by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Differences in the genotypic distribution between patients and healthy controls were analyzed using unconditional logistic regression model. In addition, The associations between LTBR polymorphisms and the clinical features of patients including tumor size, clinical grade, lymph node metastasis, human epidermal growth factor receptor 2 (C-erbB2), estrogen receptor (ER), progesterone receptor (PR) and p53 statuses were further analyzed.
Results: In Rs3759333, the frequency of CT genotype and TT genotype were lower in cases than in controls (P = 0.027 and 0.001, respectively). Haplotype TCCA (Rs3759333-Rs11064157-Rs2302256-Rs2364480) has a lower distribution in patients than in controls (corrected P=0.026) . Significant associations were also observed between LTBR polymorphisms and ER, PR, C-erbB-2 statuses in patients.
Conclusion: Our data primarily indicates that LTBR polymorphisms contribute to sporadic infiltrative duct carcinogenesis in a northeast Chinese Han population.

P5.20.054
Association Analysis between HLA-DRB1 Allelic Polymorphisms and Age to Identify Genes Promoting the Early Onset of Solid Cancers

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AIM: To explore relationships between the early-onset solid cancer and HLA-DRB1 allelic polymorphism and develop an efficient research method for identifying early onset solid cancer-related genes. **METHODS:** Patients with different solid cancers, including gastric, liver, and lung cancers, were recruited as the cancer group. On the basis of their age at the time of the first surgery, 70 patients were divided into the following 7 age groups with 5 males and 5 females in each group: < 50 years, 50-54 years, 55-59 years, 60-64 years, 65-69 years, 70-74 years, and 75-79 years. The 70 healthy subjects, who were also divided into 7 age groups as was the cancer group, were served as the control group. The samples were typed using an HLA-DRB1 PCR-SSP typing kit. The mean age of patients and healthy subjects with and without the alleles was calculated respectively. **RESULTS:** In the cancer group, the mean age of patients with HLA-DRB1*01 (52.5 years old) was less than that of patients without this allele (62.1 years old). There was an interaction between the mean age of patients in both groups (cancer and control) and the genes (positive and negative expression; $p < 0.05$). **CONCLUSIONS:** HLA-DRB1 alleles may be involved in the pathogenesis of early-onset solid cancer. The inherent anti-cancer mechanisms may be a common pathway for different cancers and are associated with the immune system and HLA.

P5.20.055
HLA-E is expressed and functional in melanoma but not melanocytes

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Human Leukocyte Antigen E (HLA-E) is a ligand of both inhibitory (e.g. NKG2A) and activating (e.g. NKG2C) Natural Killer (NK) cell receptors. We have previously shown that HLA-E is often expressed in tumors (ovarian and testicular tumors, non-small-cell lung carcinomas and soft tissue tumors) deriving from an HLA-E-negative normal counterpart, and is expressed in a subset of colorectal carcinomas infiltrated by NKG2A+ T cells and characterized by a favorable outcome. Upon immunohistochemical testing of >100 nevomelanocytic lesions (conventional sections and tissue arrays), we now report that HLA-E gradually increases with tumor progression: from weak expression in 15% of nevi (including rare and atypical lesions), to low-intermediate expression in 57% of primaries, to a full range of expression levels (including high homogeneous staining) in 67% of metastatic lesions. Cell surface biotin labeling reveals higher surface HLA-E levels on melanoma than melanocyte cultures. Surface HLA-E partially protects (via NKG2A) most (8/10) melanoma cell lines from lysis by 30% of the tested polyclonal NK cell effectors, and functionally counteracts activating NK receptors. In contrast, all (7/7) the tested melanocyte cultures are fully protected independently of HLA-E, and can be lysed only following IL-2 treatment of NK cell effectors. Tumor-selective usage of HLA-E by NK cells, and the different intrinsic susceptibility of melanoma and melanocytes to NK cell lysis document an unprecedented 'threshold' strategy for the immune discrimination of normal versus neoplastic counterparts. Supported by AIRC.

P5.20.056
Dendritic cell density and HMGB1 expression in non-small cell lung carcinomas

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Lung cancer is the leading cause of cancer death worldwide and non-small cell lung carcinoma (NSCLC) is the most common type of lung carcinomas. In NSCLC, the adenocarcinomas and the squamous cell carcinomas are the most frequent histological types. In NSCLC no studies investigating the role of the alarmin high-mobility group box1 (HMGB1) and the density of intratumoral DCs recruitment have been done. In the present study, a total of 38 cases of advanced stages of NSCLC were included. Tissue samples were obtained from biopsies. DCs were detected by immunohistochemistry, and quantified in high power fields. Also, subcellular distribution of HMGB1 in tumor cells, and in NSCLC cell lines was studied. In addition, HMGB1 produced by NSCLC cell lines was detected by ELISA. Squamous cell carcinomas expressed HMGB1 into nucleus and no or very low infiltrations of DC were observed. In lung adenocarcinomas, high densities of infiltrating DCs were observed and were associated with lung adenocarcinomas expressing cytoplasmic or nuclear-cytoplasmic HMGB1. NSCLC cell lines constitutively produced HMGB1 and the addition of cisplatin (40 μM) induced apoptosis, membrane translocation of calreticulin and an increase of HMGB1 secretion. These results suggest that in adenocarcinoma patients, treatment with conventional chemotherapy may induce immunogenic apoptosis; in addition, the acute release of HMGB1 should attract more DCs, which engulf apoptotic cells to stimulate an antitumor immune response.

P5.20.057
Studies on immunogenetic mechanism of hepatocellular carcinoma caused by Fas gene polymorphism

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Background: To investigate the genetic pathogenesis of hepatocellular carcinoma (HCC) caused by Fas gene polymorphisms and explore the reason of negative expression of Fas in hepatocarcinoma cells. Methods: Forty-five chinese patients with primary HCC and 36 unrelated age matched control patients with benign liver disease participated in this study. One single nucleotide polymorphism (SNP) of Fas at position -670 in the promoter (Fas-670) was genotyped by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Results: Our studies revealed the negative expression of Fas receptor on the surface of HCC cells. Moreover, the Fas-negative HCC cells showed resistance to Fas-mediated apoptosis. In present study, we found that the frequency of AG genotype at position Fas-670 was higher in HCC patients than in control individuals (60.0% vs 30.6%, p=0.013, OR=3.41, 95% Confidence Interval: 1.35-8.61). Meanwhile, there was a decreased frequency of genotype AA in patients compared with

controls (20.0% vs 58.3%, *p*=0.0005, OR=0.18, 95% Confidence Interval: 0.07-0.48). But there was no difference in the frequencies of GG genotype between patients and controls. Moreover, the frequencies of G allele and G allele carrier in Fas-670 site of patients with HCC were all higher significantly than that of controls. Conclusions: Our study suggested that replacement of A to G in Fas-670 site might affect the expression of Fas in hepatocarcinoma cells, thereby may be one of the susceptibility factors for HCC.
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P5.20.058
Identification and characterization of candidate MDSCs in PBMCs of patients with lymphoproliferative malignancies

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Myeloid derived suppressor cells (MDSCs) are a heterogeneous myeloid cell population with the ability to suppress immune responses that include two major subsets, monocytic and granulocytic MDSCs. Aim of this study was to characterize the morphological, immunophenotypical and functional features of myeloid cells in peripheral blood samples from a large cohort (110) of newly diagnosed patients with lymphoproliferative malignancies (LMs) in order to investigate the presence of candidate MDSC subsets. Based on the expression of CD11b, CD66b, CD33, CD16, CD14 and CD45 we evaluated the frequency/number of total myeloid cells (CD11b+ cells), granulocytes (CD11b+ SSChigh CD66b+) and monocytes (CD11b+ SSClow CD33++), as well as their subtypes, within the PBMC fraction of each sample. Our data revealed that the frequency and distribution of myeloid cells were strongly increased in PBMCs of patients with LMs, particularly for the granulocytic lineages. We also found that depletion of CD11b+myeloid cells from PBMCs of patients with LMs restored autologous T lymphocyte proliferation, suggesting the presence of candidate MDSCs within this population. These data reveal the presence of immunosuppressive CD11b+ myeloid cells, most likely of the granulocytc lineages, within the PBMC fraction of patients with active LMs.

P5.20.059
Degree and organization pattern of lymphocyte populations within the active border of human colorectal cancer liver metastases: association between immunological imprint and survival

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Solid tumors represent an extreme example of tissue where immune cells, either resident or infiltrating, might get aberrantly activated and affect the clinical course of disease. We established an algorithm which allows to assess a link between the patient-specific immunological capacity and clinical outcome for patients with colorectal cancer liver metastases (CRCLM). The microscopy-based TissueFAXS system was used for automated scanning of the large-scale tissues sections, followed by identification of single cells in the complex tissue environment and quantification of positively stained immune cell subtypes using image analysis softwares. Tissue sections were analysed for CD45 to detect all classes of infiltrating immune cells; CD20, AID, CD138, and IgM to characterize the B cell populations; and CD68 to detect macrophages. For specimen's comparison, we applied a uniform strategy by defining subregions at tumor-liver border and within liver tissue. Results indicate massive infiltration of $CD45^+$ cells confined to tumor-liver border which showed patient-specific organization patterns. Important observation of the current study describes the formation/presence of ectopic follicular structures. Fully developed ectopic structures were characterized as $CD20^+$/AID^+ surrounded by IgM^+ mantel zone and $CD138^+$ plasma cells. Of importance, the recurrence free survival was found to be significantly better in patients with high CD45 and high CD20 content. The findings emphasize the impact of the B-cell driven local immune response on the CRCLM disease progression. In summary, the established algorithm allows to estimate the prognostic power of infiltrating immune cells within complex malignant tissue as new biomarker for clinical outcome.
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P5.20.060
Melanoma cells produce adenosine through a CD38/CD73 pathway and suppress $CD4^+$ T cell proliferation

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Nucleotide-metabolizing ectoenzymes include CD38/CD157 (ADP ribosyl-cyclases) CD203a/PC-1 (ectonucleotide pyro-phosphatase/phosphodiesterase-1), CD39 (ecto-nucleoside triphosphate diphosphohydrolase-1) and CD73 (ecto-5'-nucleotidase). These ectoenzymes regulate the extra-cellular nucleotide/nucleoside balance. Adenosine (ADO), a purine nucleoside generated by this network, concentrates in different solid tumors and promotes their growth by promoting angiogenesis and inhibiting immune responses. Moreover, inhibition of CD73 in melanoma murine models limits tumor growth and restores anti-tumor immune responses.
We investigated the expression and function of the ectoenzyme network in 3 primary and 3 continuous human melanoma cell lines. CD38 and CD73 were highly expressed by all cell lines. CD39 and PC-1 expression was elevated only in 3 cell lines and low to intermediate in the others. CD157 expression was low in all cell lines. ADO was produced in cell lines from AMP and, to a lesser extent, from ATP and NAD^+. ADO production was increased after pre-treatment of cells with a specific inhibitor of adenosine deaminase (ADA). Three primary melanoma lines were tested for their ability to inhibit proliferation of $CD4^+$ T cells. The proliferation was significantly inhibited, both in co-culture and in transwell systems. The inhibitory effects were reverted when cell lines were pre-treated with specific inhibitors of CD38 or CD73 (but not of PC-1 or CD39).
In conclusion, we have demonstrated that melanoma (primary or established lines) cells induce immunosuppression *in vivo*. This effect is mediated by ADO produced via an ectoenzyme network, which hinges upon CD38 and CD73.

P5.20.061
Nasopharyngeal carcinoma-derived exosomes recruit, expand and up-regulate biological activities of human regulatory T cells

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Exosomes are nano-vesicles found in large quantities in biological fluids and tumors of patients with nasopharyngeal carcinoma (NPC). These tumor exosomes play an important role in tumor progression due to their immunosuppressive properties. In addition, it has been reported that the frequency and suppressor functions of CD4+CD25highFoxP3+CD127low Treg are also higher in NPC patients than healthy donors. As interactions between NPC-derived exosomes and Treg remain unknown, we investigated their ability to induce, expand, activate and recruit human Treg.

Treg were isolated from blood of healthy donors and co-incubated with exosomes purified from (i) culture supernatants of NPC cell lines (C15 exosomes) or (ii) the plasma of healthy donors (HD exosomes). C15 exosomes significantly increased Treg expansion and generated Treg Tim3low insensitive to the suppression induced by exosomes. C15 exosomes also significantly increased the level of expression of CD25high and FoxP3high on Treg and lead to the conversion of CD4+CD25- T cells into CD4+CD25high Treg. Moreover, C15 exosomes induces over expression of the genes associated with Treg phenotype, their suppressive activity and recruitment. These results are correlated with a significant increase in the suppressive activity of Tregs in the presence of C15 exosomes. Finally, the C15 exosomes are able to facilitate the recruitment of Treg cells in CCL-20 chemokine dependent manner.
Our results give new insights about NPC-derived exosomes immunoregulatory properties. Interactions of NPC-exosomes with CD4+ regulatory T cells represent a newly-defined mechanism that might be involved in regulating peripheral tolerance by tumor cells and supporting immune evasion of human NPC.

P5.20.062
Evaluation of the use of the CA-15-3 in monitoring of Breast cancer in A B U teaching hospital, Zaria, northern Nigeria

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Introduction: Breast cancer is a traumatic and pathologically debilitating disease in northern Nigeria. Traditionally, diagnosis of breast cancer is done by clinical examination and histological confirmation. Immunological diagnosis of breast cancer using tumor molecular biomarkers is lacking. This pilot study was conducted to investigate and analyze the use of CA-15-3 marker in relation to disease in patients with breast cancer as a simpler, robust test which could have importance in clinical decision making. **Methodology:** All female patients sent to the Radiotherapy and Oncology center, between January 2009 and January 2013, were purposively and consecutively bled and tested for CA-15-3 in serum using a high sensitivity commercial enzyme immunoassay kit. Informed consent and ethical approval was obtained prior to testing. Levels of CA-15-3 were statistically compared with disease stage and tumor grade. **Results:** A total of 45 patients (33-75yrs) participated. Disease status of the patients included 40 invasive ductal Ca, 4 invasive lobular Ca and 1mixed Ca. There were 31 pre- and 14 post-menopausal women; 36 in non- metastatic and 9 in metastatic disease. There were no patients in disease stage I, 4 in stage II, 32 in stage III and 9 in stage IV (These same 9 patients had primary disease and values of CA-15-3 well above the cut- off limit of positive of 35 units/mL). Values of CA-15-3 correlated positively with disease staging. **Conclusion:** CA-15-3 is clinically relevant in the staging and monitoring of patients with breast cancer.

P5.20.063
Prediction of Radix Astragali immunomodulatory effects of CD80 expression on THP-1 cells from chemical chromatograms by Quantitative Pattern-Activity Relationship (QPAR)

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Our laboratory has previously shown that blood dendritic cells in cancer patients, particularly those in progressive phase, have reduced their CD80 costimulatory capacity. It is known that Chinese Herbal Medicines (CHM) such as Radix Astragali (RA) has a long history of clinical application for immunity enhancement. For quality control to ensure batch-to-batch consistency of CHM due to its complex mixture nature, single component as a representative marker is inadequate. In this Quantitative-Pattern-Associated-Relationship (QPAR) study, our aim is to build a chemometric-bioactivity-chemical predictive model for the CD80 expression due to RA on THP-1 cells. By correlating the chemical and biological data of 72 whole RA extracts, bioactivity of new RA extracts can be predicted by simply providing the chemical fingerprints. Using Elastic-Net-Partial-Least-Square algorithms, both chemical and biological data from 48 RA extracts as a training set were used to establish the CD80 bioactivity of RA predictive model. The remaining one-third (n = 24, test set) was used to validate the predictive power. This model gave a high statistical predictive quality (q2 = 0.92). More importantly, the regions on RA chromatographs with respect to their relative importance in contributing to the CD80 expressions were also identified. In this RA-QPAR study, we successfully explored and exploited the relationship between the chemical and biological fingerprints to successfully establish a predictive model; and revealed the features in the chromatographic profiles responsible for such bioactivities. This may bring novel insights into herbal vaccination-adjuvants preparation and may lead to correct the defective dendritic cell CD80 co-stimulatory capacity.

P5.20.064
Myeloid-derived suppressor cells attenuate Th1 development through IL-6 production to promote tumor progression

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Collaborative action between tumor cells and host-derived suppressor cells leads to peripheral tolerance of T cells to tumor antigens. Currently, IL-6 and a soluble form of IL-6 receptor are increasingly attracting attention as the therapeutic targets because their levels rise in various cancer patients. Here, we demonstrated that in tumor-bearing mice, generation of tumor antigen-specific effector Th1 cells was significantly attenuated, and impaired Th1 differentiation was restored by the temporal blockade of IL-6 activity at the T-cell priming phase. We also found that Gr-1+ myeloid-derived suppressor cells (MDSC) served as a source of IL-6 in tumor-bearing mice. Adoptive transfer approach revealed that MDSC-sensitized effector CD4+ T cells were less potent to mount anti-tumor immune responses, although effectors T cells generated together with Gr-1+ cells from tumor-free mice eradicated established tumors. CD8+ T cells, IFN-γ and MHC-class II expression in host mice were indispensable for CD4+ T cell-mediated anti-tumor activity. Despite comparable suppressive activity of IL-6+/+ and IL-6-/- MDSC on primary T-cell activation, transfer of IL-6+/+ MDSC, but not IL-6-/- MDSC dampened the efficient induction of Th1 cells and counteracted CD4+ T cell-mediated anti-tumor immunity including cognate help for CD8+ T cells in vivo. These findings suggest that, apart from the inhibitory effects on primary T-cell activation, MDSC attenuate functional differentiation of tumor-specific CD4+ T cells into effector Th1 cells through IL-6 production to promote tumor progression. This novel mode of MDSC-induced tolerance of CD4+ T cells should be considered as basis for the rational design of T cell-mediated anti-tumor therapies.

P5.20.065
Evaluation of a hydroalcoholic extract of *Uncaria tomentosa* on dendritic cells and Th1/Th2/Th17 profile, IL-12 in breast cancer patients

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The antitumor immunity uses dendritic cells (DC) activating lymphocytes and immunomodulatory cytokines. **Objective:** Determine the effect of the hydroalcoholic extract of *Uncaria tomentosa* (Cat's Claw-3% oxindole alkaloids) (UG) in subpopulations of DC, its HLA-DR/CD86 molecules and cytokines Th1/Th2/Th17, IL-12 (p70/p40) in mononuclear cells (PBMC) from patients with breast cancer (BC) and healthy women (HW). **Methods:** We isolated PBMC from 07 women with BC stage II (58±12years) and 10 HW (47±9years). Conditions: Baseline Control (CB); Stimulation with lipopolysaccharide (1ug/mL) (LPS) and LPS plus 50, 500 or 1000ug/mL. We cultivate 0.5x106cells/mL with/without UG for 2h and LPS for 24h. PBMC were labeled with anti-CD11c-APC, HLA-DR-

PerCP, CD86-PE and Lin1-FITC for measurements in supernatant by cytokine cytometric bead array (CBA) and ELISA. **Results:** Percentage of mDC (myeloid) in HW decreased at 500 and 1000ug/mL ($p<0.05$) but HLA-DR and CD86 with 1000ug/mL increased ($p<0.05$). Only DCp HLA-DR in HW increased at 1000 µg/mL ($p<0.05$). In both, BC and HW with 500 and/or 1000ug/mL ($p<0.05$) increased IFN-gamma, IL-2, IL-4 and IL-17A. Only in HW, IL-12p70 and IL-12p40 decreased at 1000µg/mL ($p<0.05$). **Conclusions:** BC DC do not respond to UG, but partially in HW had an immunomodulatory opposite effect. UG would encourage Th1/Th2/Th17 antitumor response.

P5.20.066
Allelic variants in promoter region of FOXP3 gene as a protective factor in breast cancer development

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Breast cancer is a heterogeneous disease and normally associated with immune cell infiltration. Regulatory T cells (Tregs) play important role promoting tumor growth and progress by inhibiting the immune response against cancer. One gene involved in various cellular processes on the function of Tregs is FOXP3 (transcription factor forkhead box3). Polymorphisms in FOXP3 promoter region are associated with breast cancer. TGF- β (transforming growth factor β1) is another important molecule that plays a central role in the generation and function of Tregs. An important allelic variant in the TGF-β1, is the +869 C/T (Leu10Pro) that interfere in the cytokine production. For this reason, we investigated the influence of polymorphisms in these genes in susceptibility and progression of human breast cancer. Genomic DNA was obtained from 162 breast cancer patients and from 218 healthy donors and was amplified by allelic specific polymerase chain reactions (ASP-PCR). The two polymorphisms of FOXP3 gene has a protective effect against breast cancer development: FOXP3 rs2232365 (OR = 0.49; 95% CI: 0.30-0.81) FOXP3 rs3761548 (OR= 0.28; 95% CI=0.11-0.77), FOXP3 haplotype (OR=0.08; 95% CI=0.01-0.60), otherwise no association was found with TGF-β polymorphism. A positive correlation between FOXP3 polymorphisms and clinical staging (p=0.011), HER-2 (p=0.033) and nuclear grade (p=0.004) was observed. The presence of allelic variants in promoter region of FOXP3 seems to have a protective effect in breast cancer susceptibility and also that the role of this gene as a marker of tumor progression must be more investigated, particularly in subtypes of breast tumors.

P5.20.067
Modeling leukemia immunoediting in mouse-human chimeras

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Acute Myeloid Leukemia (AML) can be recognized and eliminated by the immune system, as demonstrated by the clinical efficacy of allogeneic hematopoietic stem cell transplantation. Still, immune-resistant variants of AML can outgrow upon the selective pressure of the transplanted immune system and determine clinical relapse, in a process called “leukemia immunoediting”, the biological bases of which remain largely unknown.
In the present study we engrafted primary human AML in immunocompromised NOD/SCID γ-chain null mice and modeled adoptive immunotherapy by serial infusions of human T cells, either autologous or allogeneic to the leukemic cells, with the ultimate aim of analyzing the alterations induced by immune pressure on leukemia gene expression profile.
HLA-mismatched allogeneic T cells eradicated AML from 6/6 treated mice, whereas HLA-identical T cells granted only temporary control in 3/3 mice and autologous T cells were completely inefficacious in 3/3 mice.
We examined the gene expression profile of AML blasts purified from the mice. Leukemic cells not subjected to immune pressure did not significantly differ from primary leukemia. On the contrary, upon escape from immune pressure, leukemic blasts showed a specific gene signature, significantly different from untreated controls. Gene enrichment analysis demonstrated the selective deregulation of genes involved in immune processes. The most importantly deregulated genes were related to proteolysis, to antigen processing and presentation, and to RNA transcription and translation.
Our findings provide a robust model to demonstrate that leukemic cells are able to shape their immunogenicity upon T cell pressure, and a valuable tool to investigate these processes.

P5.20.068
Breast and kidney-expressed chemokine (CXCL14) in breast cancer

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Breast and kidney-expressed chemokine (BRAK), also known as CXC chemokine ligand 14 (CXCL14), was initially identified as a chemokine highly expressed in the kidney and breast. Although CXCL14 was first found in breast, very little is known about the detailed role of it in human breast cancer so far.
In this study, we tried to demonstrate the relationship between CXCL14 and breast cancer. Gene expression of CXCL14 is lower in breast cancer cell lines, and MDA-MB-231HM express lowest levels of CXCL14 mRNA. Overexpression of CXCL14 inhibited cell proliferation and invasion *in vitro* and attenuated xenograft tumor growth and lung metastasis *in vivo*. CXCL14 significantly decreased the intratumor microvessel density (MVD) by immunohistochemistry using the anti-CD34 antibody in the specimens of xenografted mice. In 208 patients with breast cancer, the positive rate of CXCL14 is about 25.5% (53/208). CXCL14 protein level is positively correlated to the overall survival (OS), and negatively correlated to lymph node (LN) metastasis. Our study showed for the first time that CXCL14 is a negative regulator of growth and metastasis in breast cancer, at least partly by inhibition of intratumor angiogenesis.

P5.20.069
Role and potential application of chemokine-binding proteins in breast cancer

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Many chemokines and chemokine receptors, such as CCL2 and CXCR4, participate in the growth, angiogenesis, distal metastasis of tumor. However, the recent failures in the clinical trials of some single target antagonists suggest that intratumor chemokine network is really complicated. Atypical chemokine receptors (ACR) as endogenous and physiological regulators, including Duffy antigen receptor for chemokines (DARC), D6 and ChemoCentryx chemokine receptor (CCX-CKR), may be the powerful candidates for this purpose. Our recent studies have already demonstrated that all of DARC, D6, CCX-CKR and other chemokine-binding proteins play protective role in breast cancer. In total of 558 breast specimens, coexpression of ACR in invasive breast carcinoma (55.9%) was much lower than that of noninvasive breast carcinoma (93.1%) and normal breast tissues (100.0%). The triple positive staining was significantly conversely correlated with lymph node metastasis, disease stage, RFS as well as OS. Interestingly, we also found that Duffy blood group phenotype (DBGP) is correlated with breast cancer incidence, axillary lymph node metastasis and OS. Furthermore, their coexpression is more favorable for the patients, suggesting that multiple ACR may provide better protection against metastasis and relapse by synergistically regulating chemokine network in breast cancer. It is possible that chemokine-binding proteins can be used as novel anti-cancer drugs based on the polypharmacological principle in the future.

P5.20.070
Genetic variation in DNA repair genes: *ERCC4* and *XRCC3* and cervical squamous cell carcinoma risk

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As reduced DNA repair capacity may lead to genetic instability and carcinogenesis, genes involved in DNA repair have been proposed as candidate cancer susceptibility genes including in cervical cancer.
ERCC4 (XPF) is one of central players in the NER, which counteracts the consequences of mutagenic exposure of cell. XRCC3 is known to participate in homologous recombination to maintain chromosome stability and repair DNA damage.
We investigated 4 tagSNPs in 2 DNA repair genes - *ERCC4*: rs3136176, rs1799798 and *XRCC3*: rs3212079, rs3212102 in 132 cervical squamous cell carcinoma (CSCC) patients and 196 healthy controls.
In single SNP analyses, we found that among studied SNPs 3 were directly associated with risk of CSCC: *ERCC4*rs3136176 and in *XRCC3*: rs3212079, rs3212102. With respect to *ERCC4* SNP, we found that carriers of [TT] genotype was 2.82 more prone to CSCC (p=0.01, 95%CI:1.21-6.59).
With respect to *XRCC3* SNPs, presence of rs3212102[CC] genotype 2.18 increased risk of cancer (p=0.04, 95%CI:1.03-4.65) and rs3212079[A] allele (genotype [AA] and/or [GA]) 2.37 increased risk of CSCC (p=0.006, 95%CI:1.27-4.42).
Haplotype *XRCC3*rs3212079[A]/*XRCC3*rs3212102[C] significantly increased risk of CSCC (p=0.0007).
Using multivariate logistic regression analysis, 3 of the investigated SNPs: *ERCC4*rs3136176, *XRCC3*rs3212079 and *XRCC3*rs3212102 showed a significant association with CSCC risk in the over all population.
Moreover, haplotype *XRCC3*rs3212079[A]/*XRCC3*rs3212102[C] showed a tendency to be more frequent in patients with carcimona planoepitheliale akeratodes (Cpa) as compared with keratodes type (Cpk) (p=0.07) and haplotype *XRCC3*rs3212079[A]/*XRCC3*rs3212102[C] showed an opposite tendency (p=0.07). None of studied SNPs were associated with grading or staging.

P5.20.071
Silencing of claudin-10 expression on B-1 cells impaired the increased metastatic behavior of melanoma cells an *in vitro* coculture system

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Recent studies in mice are beginning to reveal important immune – modulating functions of B-1 cells, a subset of B cells found predominantly in pleural and peritoneal cavities. Using an *in vitro* heterotypic coculture system we previously demonstrated that cocultivation of B16 melanoma cells with B-1 cells from C57BL/6 mice (wt), but not with C57BL/6 IL-10 knockout B-1 cells (IL-10KO), increases the metastatic potential of melanoma cells. However, the molecule expressed on B-1 wt cells able to affect the metastatic potential of B16 cells remain to be fully explored. Therefore, the aim of this work was to identify the molecule expressed by B-1 wt, but absent in B-1 IL-10KO cells, which triggers increased metastatic potential of melanoma cells. Three independent experiments of microarrays analyses demonstrated differential mRNA expression of seven (7) genes between wt and IL-10KO B-1 cells. Among these genes, claudin-10, involved with cell communication and cell adhesion, was upregulated in B-1 wt cells. Data was confirmed by western blot analysis and interference RNA assays were performed to evaluate the biological function of claudin-10 in this model. Interestingly, silencing of claudin-10 expression on B-1 wt cells reduced their levels of ERK phosphorilation and prevent their capacity to increase the metastatic behavior of melanoma cells. Together, these findings suggest that claudin-10 expression on B-1 cells is crucial for their commitment to affect the metastatic potential of B16 melanoma cell.

P5.20.072
Role of STAT3 signaling in MDSC-mediated immune suppression

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One of the mechanisms of immune tolerance induced by cancer is due to the expansion of myeloid-derived suppressor cells (MDSCs), a population of immature myeloid cells capable of inhibiting both innate and adaptive immunity. We recently demonstrated that MDSCs can be derived *in vitro* from fresh bone marrow (BM) cells and named these cells BM-MDSCs. As BM-MDSCs are a very heterogeneous population, we further characterized them, and found that only one immature subset (i-BM-MDSC) is responsible for the whole immunosuppression.
To dissect the molecular mechanisms involved in MDSC activity, we focused our attention on signal transducer and activator of transcription 3 (STAT3), a transcription factor known to be involved in immunosuppression. Our results show that STAT3 phosphorylation is present in the suppressive subset of BM-MDSCs and increases after co-culture with activated T cells, thus suggesting that the immunosuppression exerted by MDSCs is the result of a crosstalk with activated T lymphocytes. Since STAT-3 can be activated by the immunosuppressive cytokine IL-10, we measured IL-10 in the culture supernatant and found, accordingly, that its release is significantly enhanced when MDSCs are cultured in the presence of activated lymphocytes. Considering that IL-10 increases B7-H1 in myeloid cells, we investigated the expression of this molecule on i-BM-MDSCs and found that it is significantly increased only when these cells are co-cultured with activated but not with resting T cells. Our results suggest the existence of a loop between IL-10, STAT3 and B7-H1, involved in the immunosuppressive program exerted by MDSCs.

P5.20.073
Inhibition of Th17 cells and induction of regulatory T cells is mediated by SOCS-1 in the tumor microenvironment of breast cancer patients

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Introduction: Suppressors of cytokine signaling (SOCS) are important negative feedback regulators of the JAK/STAT signaling pathway, and have been recently investigated for their role in the development of different cancers. It has been shown that SOCS-1 negatively regulates STAT-3 activation in breast cancer tissues and therefore it may be a regulator of Th17 cells in tumor area. In the preset study, we demonstrated that SOCS-1 is in associated with decrease of Th17 cells and increase of Tregs in breast cancer patients.
Methods and Materials: In this study, we examined the expression of SOCS-1 gene in normal and breast cancer tissue and correlated this with factors related to Th17 cells and Tregs. Human breast cancer tissues from 4 stages and normal tissues were collected. Afterward, mRNA was extracted from the tissues and cDNA was synthesized. Subsequently, the mRNA expression of genes related to Th17 cells (IL-17, RORc, STAT-3) and Tregs (FoxP3, IL-10, CTLA-4) and also SOCS-1 were determined by Quantitative Real-time PCR.
Results: The results showed that the expression of SOCS-1 gene is increased in breast cancer and it negatively associated with IL-17, RORc and STAT-3 gene expression significantly and also factors

related to regulatory T cells such as IL-10, FoxP3 and CTLA-4 is decreased.
Conclusion: We conclude that regulatory T cell responses increase in the tumor microenvironment and they inversely correlate with Th17 cells as a potent anti-tumor immune response. It seems that SOCS-1 inhibits Th17 and also induces Tregs in breast cancer patients with advanced stages.

P5.20.074
Characterization of Th17 cells and regulatory T cells in pleural effusions of lung cancer patients

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Lung cancer is the leading cause of cancer death worldwide. Pleural effusion is a common clinical manifestation of metastatic lung tumors and accumulation of lymphocytes, especially CD4+ T cells, frequently occurs. Th17 cells are a subset of CD4+ T cells that express the transcription factor ROR-γt, induce a variety of proinflammatory mediators and might promote tumor progression. Conversely, Tregs are another subset of CD4+ T cells, which posses immunosuppressor functions. In several types of cancer, Tregs play an important role in inhibiting antitumoral functions. The aim of this study was to investigate the role of Th17 cells and Treg in pleural effusion from lung cancer patients. Treg and Th17 cells were identified in pleural effusion of 30 lung cancer patients, for comparison, 12 tuberculous and 11 non-chronic pleural effusions were included. Th17 cells were identified by flow cytometry using: ROR-γt, CCR6 and CD45RA markers, and production of IL-17A. Tregs were identified using CD25, CD127 and FOXP3 markers. Proportions of Tregs (CD4+FOXP3+CD25+CD127-/low) were decreased whereas Th17 cells were increased in both tuberculous and malignant pleural effusions. The percentage of Th17 cells was significantly higher in malignant pleural effusions with respect to non-chronic pleural effusions; nevertheless, no significant differences were observed between malignant and tuberculous effusions. Our results suggest that the pleural compartment from lung cancer patients is characterized by a proinflammatory, rather than a immunosuppresive, microenvironment which supports the generation or recruitment of Th17 cells.

P5.20.075
Expression of at least one desmoglein is essential for multicellular tumor spheroid (MCTS) formation

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MCTS represent small avascular tumors. They are used as an in vitro model for antitumor drug testing. Not all epithelial cell lines form MCTS. Expression of cadherins is thought to mediate MCTS formation. However, all epithelial cells express cadherins and thus there must be other adhesive contacts which determine MCTS formation. It is known that desmosomal cadherins desmogleins (DSG) and desmocollins (DSC) are responsible for desmosome formation between adjacent epithelial cells. Thus we hypothesized that DSG and DSC are the best candidates to assist MCTS formation. The aim of this work was to identify expression of DSG1,2,3 and DSC1,2,3 in cells forming and nonforming MCTS. MCTS were produced on anti-adhesive film poly-HEMA. DSG and DSC expression was analyzed in flat live cultures by confocal microscopy. We have demonstrated that cell lines expressing DSG2 and DSG3 (HaCaT, A431, BxPC-3, AsPC-1, M3T4, Colo357, Su86.86) form dense spheroids; cells expressing only DSG2 form loose large MCTS which can be easily disrupted into small ones by pipetting (HEK293, MeWo, MDCK); while cells which do not express on their cell surface any DSGs do not form MCTS (PANC-1, A537, MiaPaCa). In this case we found intracellular expression of some DSGs. We have not detected DSG1 membrane expression in any cell lines tested (intracellular in some cells was found). Expression of DSCs in many cases was found intracellular and not membrane associated. Thus, we have concluded that membrane expression of at least one DSG is essential for MCTS formation.
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P5.20.076
Polyomavirus BK large tumor antigen exerts tolerogenic signatures with immunodominant p53-binding regions in prostate cancer

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Polyomavirus BK (BKV) large tumor antigen (L-Tag) has been recently identified as a potential co-factor in the development of prostate cancer (PCa) but its role as target of immune responses in this malignancy remains unexplored. A regulatory profiling elicited by L-Tag peptide-pool stimulation has been observed in BKV seropositive PCa patients bearing BKV positive lesions and evidence of biochemical recurrence. Differently, T cells against p53-binding regions of L-Tag exquisitely belong to effector/memory CD8+ T cells populations in BKV seropositive healthy donors. The ambiguous activity of L-Tag prompted us to investigate the role of functional regions within L-Tag in eliciting peculiar immune responses in PCa. Two peptides, L-Tag406-414 and L-Tag579-587, nested in the p53-binding regions of L-Tag and previously documented to induce pro-inflammatory responses in healthy donors, triggered an immune regulatory response (IL-10- and TGF-β-producing CD4+CD25+CD127- T cells) with suppressive properties in 61% and 54% of PCa patients studied (n=20), respectively. In contrast, four peptides identified within L-Tag regions non specifically required for virus-induced malignant transformation, recalled IFN-γ-producing effector/memory CD8+ and CD4+ T cells with cytotoxic (CD107+) and no-exhausted (PD-1-) phenotype, able to boost immunogenic activities in PCa patients with a BKV-driven tolerogenic signatures. These findings suggest that strategic regions of L-Tag appointed to carry out oncogenic activities might orchestrate tumor-promoting environment. However, it also gives evidence that a systemic boosting of BKV seropositive PCa patients with immunogenic portions of BKV L-Tag would generate potent antigen-specific immune responses and thereby break the tolerogenic potential governed by BKV L-Tag in this disease.

P5.20.077
Role of G-protein coupled receptor 120 in tumor-promotion of human colorectal cancer

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G-protein coupled receptor 120 (GPR120) functions as a receptor for unsaturated long-chain free fatty acids and plays an important role in regulating lipid and glucose metabolism. However, a role for GPR120 in
the development of tumors has not been clarified. Here, we show GPR120 signaling promotes angiogenic switching and motility of human colorectal carcinoma (CRC) cells. We show that the expression of GPR120 is significantly induced in CRC tissues and cell lines, which is associated with tumor progression. Activation of GPR120 signaling in human CRC promotes angiogenesis *in vitro* and *in vivo*, largely by inducing the expression and secretion of proangiogenic mediators such as vascular endothelial growth factor (VEGF), *interleukin-8*, and cyclooxygenase-2-derived prostaglandin E_2. The PI3K/Akt-NF-κB pathway is activated by GPR120 signaling and is required for GPR120
signaling-induced angiogenic switching in CRC cells. Furthermore, GPR120 activation enhances motility of CRC cells and induces epithelial-mesenchymal transition. Furthermore, *in vivo* study shows that activation of GPR120 promotes angiogenesis and tumor growth. Finally, we find GPR120 expression is positively correlated with VEGF expression and inversely correlated with the epithelial marker E-cadherin in CRC tissues. Collectively, our results demonstrate that

GPR120 functions as a tumor promoting receptor in CRC and, therefore, shows promise as a new potential target for cancer therapeutics.

P5.20.078
Role of ectoenzymes in the interaction between myeloma, bone cells and immune effectors: the potential role of adenosine and the oxytocin/oxytocin receptor

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The aim of the study is to test the hypothesis that ectoenzymes exert regulatory effects in the complex interactions that take place in the myeloma microenvironment. The network of ectoenzymes includes molecules that are highly conserved in phylogeny and known to induce activation (e.g., CD38, CD157), that lead to the generation of adenosine (e.g., CD39, CD73) and others involved in the regulation of phosphatases (e.g., CD203a/PC-1).
This peculiar enzymatic chain has been analyzed in terms of surface expression and function in short-term cultures derived from bone biopsies of myeloma patients. The work also included immunohistochemical analyses of osteomedullary biopsies from patients with multiple myeloma (MM) or monoclonal gammopathy of unknown origin (MGUS). The soluble factors analyzed were adenosine (ADO) and oxytocin (OT) (a hormone controlled by CD38 in murine models) and the relative receptors.
The results obtained indicate that the ectoenzyme axis analyzed leads to the generation of ADO through an unconventional pathway, independent from CD39. Preliminary results show that the CD38/CD203a/CD73 axis exists in normal tissues in paraphysiological conditions (e.g., pregnancy) as well as in different disease models (CLL, seroma, recurrent pregnancy loss). CD38 may also represent an escape pathway for myeloma, in conjunction with oxytocin secreted by osteoblasts. This peculiar pathway of ADO generation may also became an appropriate therapeutic target in human myeloma.

P5.20.079
Mesenchymal sterm cells (MSCs) in head and neck squamous cell cancer (HNSCC)

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It is well established that cancer is not simply a disease confined to transformed epithelial cells, but is deeply influenced by the stromal microenvironment. In this contest a major role is played by immune cells, and by cells of mesenchymal origin. One of the solid tumors in which a potential role of MSCs has been proposed, is HNSCC, that is the sixth leading cancer by incidence worldwide.
The aim of this study was to demonstrate MSCs' presence in HNSCC,to evaluate of their immunosuppressive properties *in vitro* and to correlate MSCs frequency tumors extent. 12 HNSCC affected patients has been enrolled in the study. The frequency of epithelial-, endothelial-cells, leucocytes, MSCs and fibroblasts, was determined by flowcytometry on fresh single cell suspensions: of cells in 12 tumors and 6 controls specimens; cells of mesenchymal origin resulting significantly enriched in tumors ($p<0.05$). Form 5 patients we derived homogeneous adherent cells sharing morphology, immunophenotype and, more importantly, *in vitro* differentation potential with bone marrow-MSCs. Interestingly tumor derived MSC (tumor-MSC) showed a significant inhibitory effect on T cell proliferation in in-vitro models, in a dose dependent manner; this activity was, in part, IDO dependent.Finally a positive and significant correlation was observed between tumor extent and the frequency of tumor-MSC.In conclusion we demonstrated that MSCsare enriched in HNSCC and have in vitro immunosuppressive activity on CD4+ T cells.

P5.20.080
Defective IL-23/IL-17 axis protects p47phox-/- mice from colon cancer

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In the colon a sophisticated balance between immune reaction and tolerance is absolutely required. Dysfunction may lead to pathologic phenotypes ranging from chronic inflammatory processes to cancer development. Two prominent modulators of colon inflammation are represented by the closely related cytokines IL-12 and IL-23 which initiate adaptive Th1 and Th17 immune responses, respectively. In this study we investigated the impact of the NADPH oxidase protein p47phox, which negatively regulates IL-12 in dendritic cells, on colon cancer development in a colitis-associated colon cancer model. Initially, we found that mice deficient for IL-12 developed less severe colitis, but are highly susceptible to colon cancer. In contrast, p47phox-/- mice showed lower tumor scores and fewer high grade tumors than WT littermates. Treatment with Toll-like receptor 9 ligand CpG2216 significantly enhanced colitis in p47phox-/- mice, whereas tumor growth was simultaneously reduced. Moreover, in tumor tissue of p47phox-/- mice the IL-23/IL-17 axis was crucially hampered. In tumor tissue increased IL-23p19 protein expression correlated with tumor stage providing an explanation for the difference between p47phox-/- and WT mice. Reconstitution of WT mice with IL-23p19-/- bone marrow protected these mice from colon cancer, whereas transplantation of WT hematopoiesis into IL-23p19-/- mice increased the susceptibility to tumor growth.
In conclusion, our study strengthens the divergent role of IL-12 and IL-23 in colon cancer development. With the characterization of p47phox as a novel modulator of both cytokines our investigation introduces a promising new target for anti-tumor strategies.

P5.20.081
Epigenetic modifications of the long pentraxin PTX3 in human colorectal cancer

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The long pentraxin PTX3 is a fluid-phase pattern recognition receptor that participates in innate immunity and inflammation, by modulating complement activation and leukocyte recruitment, extracellular matrix deposition, and angiogenesis. In the context of cancer, PTX3 can be produced by tumor and stromal cells and is a potential biomarker of cancer. Our *in vivo* studies indicate that PTX3 has a protective role in different murine cancer models. Furthermore, in human esophageal squamous cell carcinoma, PTX3 is epigenetically down-regulated through pro[[Unsupported Character - Codename]]moter hypermethylation. The aim of this study was to investigate the relevance of PTX3 epigenetic modifications in cancer, focusing on human colorectal cancer (CRC). Gene expression analysis showed that PTX3 is not or less expressed in CRC than in epithelial control cells. The demethylating agent 5'aza DC, restored PTX3 expression was restored, whereas trichostatin A, a histone deacetylase inhibitor, was ineffective. PTX3 one CpG island (iCpG) in the promoter region and one ranging from the first to the second exon. By Methylated-CpG Island Recovery Assay, we found that the CRC lines had a

uniform and higher degree of methylation of the promoter compared to healthy epithelium. The second iCpG was differently levels of methylated in the CRC lines analysed, depending on tumor stage and instability status. All together these data suggest that iCpG methylation can be involved in regulation of PTX3 expression in CRC. Furthermore, the site specific methylation could potentially be a biomarker of tumor progression and of instability status.

P5.20.082

Targeting colon cancer cell NF-κB regulates tumor-associated macrophage polarization and enhances anti-tumor immune response

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Interactions between cancer cells and their microenvironment regulate tumourigenesis. The transcription factor NF-κB plays an important role in cancer, and its activation is increased in many tumours, however, its effects on the tumour microenvironment remains to be elucidated. In this study, we developed colon cancer cells expressing the NF-κB super-repressor to investigate the role of NF-κB on tumour infiltrating macrophage phenotype. Using co-culture systems, we show that medium conditioned by NF-κB-deficient CT26 cells induced expression of cytotoxic mediators nitric oxide (NO) (>15 fold) and IL-12 (> 3 fold) in macrophages, indicative of an M1-like phenotype. In contrast, incubation with NF-κB proficient conditioned media induced IL-10 and PGE2 ($p<0.01$), while IL-12p40 and NO was inhibited ($p<0.01$). Using a peritoneal metastasis model, tumour growth by NF-κB-deficient CT26 cells were significantly repressed ($n=16$, $p<0.001$) resulting in prolonged survival. Flow Cytometry analysis of tumours indicated significantly more M1-like inflammatory macrophages ($p<0.05$) and CD4+ and CD8+ CD62L- effector T-cells ($p<0.01$) in NF-κB deficient tumours. Increased numbers of iNOS-expressing mononuclear cells ($n=16$, $p<0.01$) and dramatic levels of intra-tumoural apoptosis ($n=16$, $p<0.001$) were identified by immunohistochemistry in NF-κB deficient tumours. Macrophage depletion in vivo inhibited tumours in mice treated with NF-κB-proficient cells ($n=5$; $p<0.01$) indicating that macrophages increased the metastatic potential of these tumours. This study provides novel evidence that tumour cell NF-κB regulates macrophage phenotype and supports the proposal that targeting an NF-κB regulated soluble mediator(s) that promotes 'alternative' activation of macrophages will enhance the anti-tumour immune response and prevent colon cancer metastasis

P5.20.083

Tumor-specific Th17 cells orchestrate anti-tumoral responses

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Introduction: Th17 cells have been related to the development of inflammatory and autoimmune diseases, but their role in cancer is controversial. The production of angiogenic factors by Th17 cells suggest their involvement in promoting tumor growth however, their inflammatory activity supports the idea they may have a role in anti-tumoral responses as well. We tested the effect of the tumor microenvironment on Th17 cell phenotype and the potential anti-tumor effects of these cells in a murine melanoma model.
Methods: C57BL/6 and RORγt-deficient mice (lacking Th17 cells), were intradermally injected with B16-OVA melanoma cells. In vitro generated, OVA-specific Th17 cells were intravenously injected followed by a strict control over tumor progression. Mice with tumors over 100 mm2 were euthanized and their organs harvested for further analysis.
Results: We show an up-regulation of immunosuppressive markers on Th17 cells present in tumor associated tissues of tumor-bearing mice compared with healthy mice. RORγt-deficient mice present an increased tumor growth rate, which is reverted by the adoptive transfer of Th17 cells. In vitro-generated OVA-specific Th17 cells were able to infiltrate the tumor and associated tissues, secrete IFN-γ within the tumor and reduce the tumor growth rate.
Discussion: Even though the tumor microenvironment induces a regulatory phenotype on endogenous Th17 cells, the adoptively transferred in vitro-generated Th17 cells have the potential to reach the tumor and associated tissues, presenting a clear anti-tumoral effect. These results reveal an unexpected role of Th17 cells in anti-tumor responses and open new possibilities towards the development of new immunotherapeutic strategies.

P5.20.084

The Inhibitory effects of Metformin on tumor cell growth in hypoxia conditions

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Background: Metformin is used as an anti diabetic drug for type II diabetes. Recently, a correlation has been observed between the widely used insulin-lowering drug metformin and decrease in .breast cancer incidence One of the characteristics of solid tumors is its survival ability on hypoxia. To investigate the quantitative response of energy metabolic pathways in human cancer cells to hypoxia we treated these cells with metformin in hypoxia conditions.
Method: The proliferation and apoptosis assay of MCF-7 and HEK293 cells treated with different concentrations of metformin were assessed by MTT assay and Annexin V in normoxic and hypoxic conditions, respectively.
Result: Our preliminary results indicate that metformin has higher inhibitory effects on tumor cell growth in hypoxic compared to normoxic condition.
Conclusion: These data suggest that in real in vivo hypoxic conditions, metformin may be more effective on cancer cells.

P5.20.085

Cooperation between adenosinergic and hypoxic axes in chronic lymphocytic leukemia cells (CLL)

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Extracellular adenosine, produced through the concerted action of the ectoenzymes CD39 and CD73, elicits potent autocrine and paracrine cytoprotective and immunosuppressive effects.
Recent data indicate that $CD39^+/CD73^+$ cells from chronic lymphocytic leukemia (CLL) patients produce adenosine, creating an autocrine adenosinergic axis that favors growth and survival of leukemic cells. These effects are mediated by the A2A adenosine receptor, which inhibits chemotaxis and limits spontaneous and drug-induced apoptosis of CLL cells. We are currently expanding these observations by testing the hypothesis of a functional interplay between the adenosinergic axis and hypoxic signals.
Results indicate that the CLL-like cell line MEC-1 and purified CLL cells significantly increase HIF-1α expression when cultured in hypoxic conditions (1% O_2). RT-PCR analyses and cytofluorimetric data indicate that hypoxia increases expression of the adenosine-generating ectoenzymes CD73 and CD26 and of the A2A adenosine receptor. HPLC analyses confirm that hypoxic cultures are characterized by higher extracellular adenosine levels. Furthermore, activation of the A2A receptor under hypoxic conditions is followed by higher intracellular cAMP concentrations than in a normoxic situation. Conversely, activation of the A2A receptor using adenosine or

pharmacological agonists induces transcriprion of HIF-1a, suggesting the existence of a positive feedback loop.
Together, these results indicate the existence of an interplay between the adenosinergic and hypoxic axes in CLL cells. Future data are needed to understand the functional implications of these observations.

P5.20.086
Oxidative stress induces a temporary initial decrease of HSP70 content in EL4 lymphoma cells

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HSP70 participate in intracellular processes maintaining cell homeostasis. On the other hand, under influence of various stress factors HSP70 are released from many types of cells into extracellular milieu. It was shown that extracellular HSP70 possess essential immunomodulatory properties. Besides, it was demonstrated that serum level of extracellular HSP70 circulating in organism was increased in patients with some types of diseases. Previously we demonstrated stress-induced HSP70 release in EL4 mouse lymphoma cell culture. In the present work we analyzed stress-induced alterations of intracellular level of HSP70 in the same model of EL4 cell culture. The cells were exposed to oxidative stress by using H2O2. Intracellular content of HSP70 was measured by flow cytofluorimetry. The results showed that EL4 cell reaction to oxidative stress had an initial temporary stage with a significant fall of intracellular HSP70 content. This stage was followed by expected increase of the level of intracellular HSP70. Initial decrease of intracellular HSP70 content in this model was registered also by using Western-blot analysis. We suppose that the phenomenon of temporary fall of intracellular HSP70 at the first stage of cell stress response is connected with the protein release to extracellular space. This supposition allows us to consider lymphoid cell populations as a source of soluble extracellular pool of HSP70 in serum of peripheral blood of stressed organisms.
This work was partly supported by RFBR, grants # 12-04-31578 and # 12-04-32065.

P5.20.087
Cancer/testis antigen HCA587 promotes the metastatic behavior of melanoma cells

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HCA587 is a cancer/testis antigen and belongs to MAGE family. It is highly expressed in various types of tumors but not in normal tissues except in male germ-line cells. HCA587 has long been recognized as a tumor specific target for immunotherapy, however, its biological functions have been relatively understudied. In this study, we demonstrate that HCA587 expression correlates with metastatic potential of tumor cells. Suppression of HCA587 by siRNA resulted in decreased migration and invasion capabilities of human melanoma A375 cells *in vitro*, and overexpression of HCA587 in mouse melanoma B16 cells significantly increased the number of metastatic lung nodules upon tail vein injection of tumor cells. Further investigation for exploring the underlying molecular mechanism showed that HCA587 interacts with STAT3 (Signal Transducer and Activator of Transcription 3, STAT3)phosphorylated on Tyr705 in the nucleus, and increases the level of phosphoralated STAT3. Our findings suggested that cancer/testis antigen HCA587 might promote the tumor metastasis with regulating the activity of STAT3. Our study provides an innovative avenue for investigating the biological functions of the member of MAGE family in the progression of tumor.

P5.20.088
Immunophenotypes of Burkitt's lymphoma cell lines alter at different EBV infection latency types

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Epstein-Barr virus (EBV) is involved in the pathogenesis of endemic Burkitt's lymphoma (BL). EBV-positive BL cell lines initially maintain the original tumor phenotype of EBV infection (latency I, LatI), but most of them drift toward a lymphoblast phenotype of EBV latency III (LatIII) during in vitro culturing.
The aim of this study was to characterize the immunophenotypes of BL cell lines and to verify whether particular cell subsets association with the type of EBV infection.
The phenotype analysis of two EBV-negative and eleven EBV-positive (three of LatI and eight of LatIII) BL cell lines was based on determination of CD19, CD10, CD38, CD27, and CD5 expression pattern by means of multicolor flow cytometry (pFC). The same approach was applied for the characterization of B-cell subpopulations in peripheral blood (PB) of 8 healthy adults. The EBV latency type was defined by RT-PCR using EBV transcript specific primers.
In EBV-negative and LatI BL cell lines all cells displayed the CD19+CD10+CD38+ phenotype. In contrast, in three LatIII cell lines that were established by a single cell cloning, more than 90% of the cells were CD19+CD10−CD38+. Four original BL tumor -derived cell lines of LatIII consisted of two cell subsets, CD19+CD10+ and CD19+CD10−, in proportion of 28%-84% and 17%-72% of the cells, respectively. A fraction of the cells in these four LatIII BL cell lines displays the phenotype of PB circulating mature B cells (CD19+CD10−CD38+ CD27+ CD5−). This observation may suggest that expression of the EBV LatIII genes may promote the differentiation of malignant cells.

P5.20.089
CD11b+Ly6G+Ly6Cint myeloid-derived suppressor cells become expanded by medroxiprogesterone acetate in mammary tumor bearing hosts and suppress NK cell effector functions

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The progesterone analogue medroxyprogesterone acetate (MPA) is being widely used in postmenopausal women, for the treatment of endometrial conditions, and as a contraceptive. However, hormone replacement therapy in postmenopausal women has been associated with increased incidence of breast cancer through ill-defined mechanisms. In this work, we explored whether prolonged exposure to MPA restrains immunosurveillance to tumors through mechanisms involving myeloid-derived suppressor cells (MDSCs; $CD11b^+Gr1^+$, composed by $CD11b^+Ly6G^+Ly6C^{int}$ and $CD11b^+Ly6G^-Ly6C^{high}$) and NK cells in mammary tumor-bearing mice. Using the highly metastatic 4T1 breast tumor (which does not express the classical progesterone receptor), we observed that MPA did not affect primary tumor growth but promoted lung metastasis burden. This effect was accompanied by a preferential expansion of spleen and bone marrow $CD11b^+Ly6G^+Ly6C^{int}$ but not $CD11b^+Ly6G^-Ly6C^{high}$ cells. Also, MPA significantly increased the percentage of spleen and lung NK cells in tumor-bearing mice with similar lung infiltration of $CD11b^+Gr1^+$ cells as compared to untreated tumor-bearing mice. However, sorted $CD11b^+Gr1^+$ cells (comprising more than 90% of $CD11b^+Ly6G^+Ly6C^{int}$ cells) from MPA-treated tumor bearing mice displayed a more pronounced suppressive activity on NK cell degranulation in response to YAC-1 cells and a stronger inhibition of IFN- production of NK cells in response to cytokines than those $CD11b^+Gr1^+$ cells isolated from untreated tumor-bearing mice. Thus, in breast cancer-bearing hosts MPA promotes the accumulation of $CD11b^+Ly6G^+Ly6C^{int}$ cells which display a suppressive activity on NK-cell effector functions, potentially contributing to tumor progression and metastasis.

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P5.20.090
IL-12p40, IL-23 and IL-10 production from monocytes of patients with colorectal cancer: effect of inhibition of JNK signaling pathway

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Cytokine production in patients with colorectal cancer (CRC) has an important impact on CRC development. We investigated changes of IL-12p40, IL-23 and IL-10 production from monocytes of CRC patients depending on cancer stages. Isolated monocytes were stimulated with LPS or C3bgp. We also explored molecular regulatory mechanisms focusing on the JNK activation by using selective inhibitor SP600125. The quantity determination of IL-12p40, IL-23 and IL-10 was performed by ELISA.
IL-12p40 production from patients' monocytes was diminished in early and advanced stages of the disease. Similarly, independent of disease stage, patients' monocytes secreted significantly lower IL-23 quantity compared to monocytes from healthy individuals. We observed significantly decreased IL-10 production from monocytes of CRC patients in early stage, but not in monocytes from patients with advanced CRC. Moreover, patients in early stages showed significant lower IL-10 in comparison with advanced stages of CRC.
Inhibition of JNK is clearly upregulating IL-12p40 and IL-23 production from patients' monocytes, unlike healthy donors where inhibition of this kinase is slightly reduced IL-12p40 and IL-23 production is significantly downregulated. Regarding IL-10, the inhibition of JNK did not result in significant changes of its production, while the inhibition of JNK in monocytes from healthy donors led to downregulated IL-10 production.
In conclusion, this study showed altered functional activity of peripheral blood monocytes of CRC patients which are demonstrated through downregulation of IL-12p40 and IL-23, but not of IL-10. This different response of patients' monocytes is mediated partly by changes in involvement of JNK signaling pathway.

P5.20.091
Association of -1082 A/G promoter polymorphism of IL-10 gene with colorectal cancer development

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The cancer-associated inflammation and anti-tumor immune response to colorectal carcinoma (CRC) is regulated by cytokines produced from activated immune cells. IL-10 is a Treg cytokine suppressing both Th1 immune response and anti-tumor immunity in hosts. The role of functional polymorphism of IL-10 gene in CRC development still remains elusive.
This study was designed to compare -1082 A/G *IL10* genotype distribution in 119 CRC patients to a group of 154 matched healthy donors using ARMS-PCR assay. We also investigated serum IL-10 levels in an association to genotype by ELISA. In the study population the slightly enhanced frequency of homozygous genotype GG (18% vs 13%; OR=1.414; 95%CI=0.64÷3.11; p=0.347) was seen in cases versus controls. When CRC patient's group was divided into stages of disease by TNM classification we observed higher risk of advanced CRC (III-IV stages) for individuals with GG genotype: OR=2.229; 95%CI= 0.828÷6.018; p=0.077. G allele was also overrepresented in advanced vs early CRC (OR=1.581; 95% CI=0.912÷2.743; p=0.082). According to the IL-10 serum levels, CRC patients with GG genotype produced higher IL-10 than AA and AG patients in early stages, similar to healthy control. Moreover, advanced cancer patients with AA and AG genotype produced significantly higher IL-10 compared to patients in early stage (p=0.002 for AA; p= 0.007 for AG genotype), whereas for advanced cancer patients with GG genotype this difference does not reach statistical significance. In conclusion, this study demonstrated that -1082 A/G polymorphism of IL-10 gene has a role in CRC progression rather then CRC genetic predisposition.

P5.20.092
Can humoral immunity enhance development of skin tumors?

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The incidence of tumor may be associated with interactions between innate and adaptive immune response. Evidences suggest that antibodies can generate inflammatory conditions facilitating malignant progression in a mouse model of skin cancer. Mice genetically selected for High (H) and Low (L) antibody production treated with 7, 12-dimethylbenzanthracene (DMBA) were used to study the association between humoral immunity and tumor susceptibility. Epicutaneous dorsal applications of DMBA (50 µg) in acetone for 5 days in H and L mice led to an intense initial vascularization in male mice and around 15 days all mice presented a superficial cutaneous inflammation. These lesions regressed in about 30 - 60 days in all mice and skin papillomas started to be detectable in H mice (76%, n= 30) and in L mice (14%, n= 29). Skin tumor multiplicity and malignancy increased with time and was significantly higher in H than in L mice. At 240 days after treatment, few L male presented lung tumors (25%, n=8) whereas all H male animals (100%, n=8) had multiple lung tumors. The High responder mice were more susceptible than low responder mice showing the effect of the genetic selection for humoral response on skin tumor susceptibility and predisposition to lung tumorigenesis.
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P5.20.093
CMTM3 inhibits cell migration and invasion and correlates with favorable prognosis in gastric cancer

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CKLF-like MARVEL transmembrane domain containing 3 (*CMTM3*) is a novel tumor suppressor gene (TSG) with frequent epigenetic inactivation by promoter CpG methylation. In this study, we demonstrated the roles played by CMTM3 in gastric cancer cells as a TSG, and examined the correlation between CMTM3 expression and clinicopathological parameters by immunohistochemistry in gastric cancer patients with different pathological stages (n = 350). We found that CMTM3 expression was reduced or silenced by methylation in several gastric cell lines, and restoration of CMTM3 significantly affected migration and invasion of AGS and SGC-7901 cells (P < 0.001). *In vivo* experiments showed that peritoneal disseminated metastases were significantly suppressed by CMTM3 (P < 0.001). We further showed that the expression of MMP2 and the phosphorylation of ERK1/2 was decreased when CMTM3 restoration, suggesting the potential mechanism of the effect caused by CMTM3 in gastric cancer cells. In addition, the expression of CMTM3 was remarkably weaker in gastric cancer tissues than that in normal mucosa (P = 0.008), and was significantly correlated with Gender (P = 0.033), Tumor Depth (P = 0.049), Stage (P = 0.021), Histologic Grade (P = 0.022). More importantly, CMTM3 expression was associated with prognosis in gastric cancer patients (P = 0.041), and was a significant independent prognostic indicator (HR = 0.704, 95%CI, 0.498 to 0.994; P = 0.046). Our findings indicate that CMTM3 regulates migration and invasion of gastric cancer cells. Moreover, CMTM3 is a candidate marker for gastric cancer prognosis.

P5.20.094
Essential involvement of tumor-derived IDO in ovarian cancer progression

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Immune escape and acquisition of tolerance by tumor cells are essential for cancer growth and progression. Indoleamine 2,3-dioxygenase (IDO) is one of the key molecules to induce tolerance to the host immune surveillance through inhibiting the proliferation and killer function of effector T-cells and NK cells in the tumor microenvironment. In gynecological cancers, high IDO expression was closely correlated with poor clinical outcome through suppressing tumor-infiltrating lymphocytes (TILs) and NK cells. We investigated the functional role of IDO in ovarian cancer progression. We used the mouse ovarian carcinoma cell line, OV2944-HM-1 cells derived from B6C3F1 mice, and established IDO-overexpressed HM-1 (HM-1-IDO) by transfection of mouse IDO-cDNA, and HM-1-mock cells as control. B6C3F1 mice were intraperitoneally injected HM-1-IDO or HM-1-mock cells ($1x10^6$). Tumor size and ascites volume more increased in HM-1-IDO transplanted mice than in HM-1-mock ones. Moreover, HM-1-IDO group significantly impaired survival rate, compared with HM-1-mock one. Immunohistochemically, the accumulation of $CD8^+$ TILs were significantly reduced in HM-1-IDO group, compared with HM-1-mock one. We evaluated tumor-promoting cytokine profiles in ascites. In HM-1-IDO-injected mice, intra-ascitic levels of TGF-β, IL-10 and VEGF were significantly higher than in HM-1-mock-injected ones. However, there was no difference in IL-6 level between HM-1-IDO and HM-1-mock groups. These observations implied that tumor cell-derived IDO would play detrimental roles in the peritoneal dissemination of ovarian cancer through suppressing intratumor $CD8^+$ cell recruitment and reciprocal enhancement of TGF-β, IL-10 and VEGF levels. Collectively, our present results demonstrated that IDO may be a good molecular target for ovarian cancer therapy.

P5.20.095
Immune profiling of lung cancer tissues associated with COPD

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Chronic obstructive pulmonary disease (COPD) is associated with the presence of chronic inflammation which remodels and narrows the small airways, and is strongly related with higher lung cancer risk. Several cellular immune subsets may contribute to the inflammatory processes involved in COPD and lung cancer development; nonetheless, the type and extent of T cell infiltrate in lung cancer is an independent prognostic factor in non-small cell lung cancer (NSCLC). Our aim was to better characterize the immune infiltrate in NSCLC and to evaluate the relationship between the amount and functionality of immune cells and the presence/severity of COPD. We analyzed by flow cytometry the immune infiltrate in tumor samples and in correspondent normal and/or adjacent lung specimens from 56 NSCLC patients with a known GOLD severity grading of COPD. Our data showed an accumulation of T and B lymphocytes and of conventional $CD4^+$ $FoxP3^+$ regulatory T cells, along with a reduction in monocytes, in tumors compared to normal/adjacent specimens. Interestingly, tumors also showed an increased frequency of $HLA\text{-}DR^+$ $CD69^+$ activated $CD8^+$ and $CD4^+$ T cells and a low frequency of $FoxP3^+$ $CD8^+$ T cells. This subset, recently found by us in melanoma, represents an early effector stage of T cell differentiation, consistent with the hypothesis of an ongoing immune response at tumor site. In agreement, preliminary results indicated recognition of autologous tumor by T cells as assessed by CD107a degranulation. Further investigation to evaluate the relationship between the extent/type of NSCLC immune infiltrate and the presence/severity of COPD is ongoing.

P5.20.096
Targeted-Therapy attenuates local and systemic immune suppression in Solitary Fibrous Tumor patients

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Purpose: For Soft Tissue Sarcomas (STS), Targeted-Therapy has evolved as a standard treatment option. The aim of this study is to characterize circulating immunoregulatory cell subsets and tumor immune infiltrate in patients with STS, to assess the immunomodulating potential of sunitinb in this clinical setting. Results observed in the subgroup of Solitary Fibrous Tumor (SFT) are here reported.
Material and Methods: PBMC were collected from patients with malignant SFT receiving sunitinib prior and at different time points during treatment and analyzed by multiparametric Flow Cytometry for the frequency and functional status of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC). Local tumor immunity was evaluated by immunohistochemical characterization in tumor specimens surgically removed before or after drug treatments.
Results: Peripheral Treg (defined as CD4+CD25hiFoxp3+) and MDSC (detected as CD14+CD11b+HLADRlo/neg cells), present at significant higher frequency with respect to healthy donors were down-modulated by treatment. However, disease progression occurring during treatment induced a boost in MDSC that regained or exceed the pretreatment level. Moreover, statistical analysis revealed significant patient-to-patient correlations between improvements in T cell activation and declines in MDSC. Immunohistochemical analysis showed in post-treatment tumor lesions an increase of intratumoral CD3+ lymphocyte, undetectable in untreated samples, and the presence of CD163+ myeloid cells displaying a strong HLADR expression. These phenotypic traits are compatible with the acquisition of an adaptive immune response at tumor site.

P5.20.097
Flow cytometric analysis of the tumour microenvironment during immune mediated tumour rejection or persistence

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The tumour microenvironment as a whole is usually highly immunosuppressive. However, it is not known whether pro-inflammatory elements within tumours exist and can be harnessed or recruited to aid in immune rejection of tumours. We have used several 7-10 colour flow cytometric panels to identify the diverse cell subsets comprising the tumour tissue, preliminary to testing their role in tumour immunity. Within the tumour tissue we were able to identify fibroblastic, endothelial and myeloid cell populations including granulocytes, eosinophils, dendritic cells and monocytes/macrophages. We have also analysed tumour-infiltrating B cells, T cells (CD4 and CD8), regulatory T cells and natural killer cells. Myeloid and immune cells have been isolated for further immunohistochemical analysis and effector cell function using 7-colour 6-way FACS sorting.
To follow changes in the tumour microenvironment during an ongoing anti-tumour immune response, we have used a mouse model of subcutaneous melanoma growth. B16 tumours expressing a Hen Egg Lysozyme-Moth Cytochrome C (HELMCC) fusion protein grew rapidly in immunodeficient RAG-knockout mice but were rejected after adoptive transfer of naive MCC-specific TCR transgenic CD4 T cells. Adoptive co-transfer of MCC-specific regulatory T cells delayed or prevented rejection of established tumours. We are now using a new 12-colour flow cytometric panel to compare the tumour microenvironment during CD4 T cell-mediated tumour rejection versus regulatory T cell-mediated tumour persistence in RAG-knockout mice.

P5.20.098
Antitumor and immunological effects of Nilotinib combined with Interleukin-2 in the B16 melanoma model involve IFN-g producing CD27$^+$ NK cells

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The immune system may contribute to tumor cell killing which can be enhanced by cancer treatments such as tyrosine kinase inhibitors (TKI). Recently, a beneficial effect of NK cells has been reported by adding interleukin-2 to the TKI imatinib. The aim of this study was to address the antitumor and immunological effects of recently approved TKIs. Therefore, we focused on the comparison of imatinib and nilotinib alone or in combination with IL-2 in the murine B16F10 melanoma model. Both TKIs alone or in combination with IL-2 possessed antitumor activity *in vivo*. However, the combination of nilotinib and IL-2 showed a superior outcome. Importantly, not only use of immunodeficient Rag2xFcg$^{-/-}$ mice, which lack T, B and NK cells, but also NK cell-depletion in C57Bl/6 mice reduced the therapeutic effect of nilotinib. Flow cytometry revealed an increase of an IFN-g producing CD27$^+$ NK cell subpopulation by nilotinib and IL-2 and complete loss of the therapeutic effect in IFN-g$^{-/-}$ mice.
In sum, we conclude that nilotinib in combination with IL-2 confers high antitumoral activity involving a subpopulation of IFN-g producing CD27$^+$ NK cells.

P5.20.099
Tumor antigen-specific CD4+ T effectors are highly enriched at ovarian cancer sites and co-exist with, but are distinct from, tumor-associated Treg

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Whereas tumor infiltration by T effectors is generally associated with a more favorable prognosis, the accumulation of CD4$^+$ regulatory T cells (Treg) within tumors is instead often associated with poor disease outcome. Because approaches to improve anti-tumor immunity aim, on one hand, at expanding tumor antigen-specific T cells and, on the other, at eliminating or inactivating Treg, an outstanding question is whether, and to which extent, tumor antigen-specific CD4$^+$ T effectors present at tumor sites overlap with tumor-associated Treg. Here, we used MHC class II/peptide tetramers incorporating an immunodominant peptide from the human tumor-specific antigen NY-ESO-1 to assess antigen-specific CD4$^+$ T cells among conventional CD4$^+$ T effectors and Treg at sites of ovarian cancer. We found that, in patients who spontaneously respond to the antigen, the frequency of NY-ESO-1 tetramer$^+$ cells detected *ex vivo* was highly enriched in tumors as compared to the periphery. At tumor sites, NY-ESO-1 tetramer$^+$ cells were detected concomitant with high proportions of Treg but were distinct from the latter and displayed characteristics of T_H1 effectors. Thus, even in the presence of high proportions of Treg, tumor antigen-specific CD4$^+$ T cells can accumulate in ovarian tumors and maintain an effector phenotype.

P5.20.100
Tumors with processing defects display novel tumor antigens via the non-classical HLA-E

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The non-classical HLA-E accommodates monomorphic leader peptides and functions as a ligand for germ line receptors CD94/NKG2, expressed by natural killer cells and CD8$^+$ T cells. We previously described that the conserved peptides are replaced by a novel peptide repertoire of surprising diversity as a result of impairments in the antigen processing pathway. Whereas the monomorphic peptide/HLA-E complex represents the 'innate face' of HLA-E, this novel peptide repertoire constitutes immunogenic neo-antigens for CD8$^+$ T cells. We found that T cells restricted by the mouse equivalent Qa-1^b dominantly participated in the response to tumors with processing deficiencies. A surprisingly wide spectrum of target cells, irrespective of transformation status, MHC background or type of processing deficiency was recognized by this T cell subset, complying with the conserved nature of Qa-1^b. Target cell recognition depended on T cell receptor and Qa-1^b interaction and immunization with identified peptide-epitopes demonstrated in vivo priming of CD8$^+$ T cells. Current investigations are focused on the involved rearranged TCRs that confer the tumor reactivity Although this CD8 T cell subset does arise in the periphery of TCR-transgenic animals, the thymic selection process seems to be very inefficient. Our data reveal that HLA-E and its mouse homolog Qa-1^b are important for the defense against processing deficient cells by displacing the monomorphic leader peptides, which relieves the inhibition through CD94/NKG2A on lymphocytes and by presenting a novel repertoire of immunogenic peptides, which recruits a subset of cytotoxic CD8$^+$ T cells.

P5.20.101
NLRP3 inflammasome activation is involved in breast cancer cell death in vitro

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Breast cancer is the most prevalent type of cancer diagnosed in women worldwide and their leading cause of cancer death. Chronic inflammation has been described as a risk factor for cancer development. Inflammasomes, which participate in innate and adaptive immune responses, have been described with contrasting roles in cancer development. However, the role of inflammasomes in breast cancer cells is still not understood. To investigate the presence of NLRP3 inflammasome and the phenotypic effect of its activation in breast cancer cell lines, MCF7 (non-invasive) and MDA-MB-231 (invasive) cells were primed with LPS overnight and stimulated with ATP/Nigericin for 4h/1h, respectively. Caspase-1 activation, expression of NLRP3 inflammasome proteins, cytokine secretion as well as cell viability were analyzed. Both cell lines presented caspase-1 activation, as assessed by FAM-YVAD-FMK probe staining, and NLRP3 and ASC expression and colocalization, observed by confocal microscopy. After stimulation, MCF7 cells secreted more IL-6, but neither of cells secreted IL-1β or TNF-α. Inflammasome activation reduced cell viability and increased nuclear fragmentation in MDA-MB-231 cells in early time points and in MCF7 after 48h, evaluated by MTT and PI counterstain, respectively. These results suggest that there is expression and activation of NLPR3 inflammasome in breast cancer cells, which can trigger cell death in a particular way depending on the cell type. Therefore, our data shows that breast cancer cell lines express and activate NLRP3 inflammasome complex, and suggests it could be involved in modulation of cell death in these cells, functioning as a potential pharmacological target. CNPq, FAP-DF.

P5.20.102
Influence of natural killer cells in mammary tumor in female dogs

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Natural Killer cells (NK) induce an innate immune response and they are able to recognize tumor cells and determine the cell lysis. The aim of this study was to investigate the presence of NK cells in mammary tumors in different grades of malignancy (I, II and III). We used 39 samples from mammary glands of female dogs with mammary tumor, 13 samples for each grade malignancy and five samples of normal mammary tissue for the control group, all from the routine diagnosis. The histological classification of the tumors was established according to current World Health Organization for canine mammary tumors. The immunohistochemistry technique was performed on paraffin-embedded sections, using CD56 primary antibody (Serotec). The average NK cells was assessed by counting in five microscopic fields (40x Obj) / per animal /per histological group

(I, II, III and control). The nonparametric statistical methods used were Kruskal-Wallis and Dunn Tests, considering significant differences when P <0.05. The CD56 positive cells had cytoplasmic membrane granular stained. The epithelial cells of tumors had accentuated immunostained, especially those of higher grade malignancy (II and III groups). Positive cells for CD56 were not detected in inflammatory infiltrate. There was no significant difference between groups for the average CD56 immunostained cells. These findings suggested that tumor cells exhibit mimicry immune to CD56, characterizing a mechanism for evasion of the host immune system canine. Financial support: FAPESP (2010/02194-9).

P5.20.103
Antitumor activity of synthetic phosphoethanolamine in experimental colon cancer

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Colon cancer has a long natural history of transition from normal crypts through adenoma to adenocarcinoma, providing a great opportunity for prevention and intervention strategies. In this study, we evaluated the effects of synthetic phosphoethanolamine (PEA) on chemically induced colon cancer. C57BL/6 mice were given four intrarectal deposits of 0.1 ml solution of N-nitroso-N-methylnitroguanidin (5mg/ml) twice a week for two weeks. At 24 weeks after initial carcinogen exposure, animals were treated orally (0.1ml/mouse) with PEA (10 and 40mg/kg) during 28 consecutive days. Treatment of tumor-bearing animals with 10mg/kg of PEA led to a 76% reduction in tumor volume compared to the control group. Increased apoptosis rate together with decreased tumor cell proliferation were also observed. Animals treated with the same dose exhibited increased numbers of CD4 and CD8 T lymphocytes, and macrophages in the intercrypt spaces. Among macrophages we observed decreased numbers of $CD206^+$ cells (M2 macrophage marker), but no differences in the number of $CD11c^+$ cells (M1 macrophage marker). Other markers of tumor progression like COX-2 and VEGF were less expressed in colon cells. In addition, PEA induced higher levels of the pro-inflammatory cytokines IL-1, IL-12, IL-6, TNF-α and IFN-γ, and a lower production of the anti-inflammatory cytokines IL-10 and TGF-β. Increased numbers of CD11c macrophages and decreased MMP9 expression were observed only in response to the highest dose of PEA (40mg/kg). Our results indicate that PEA has an antitumor activity associated with a pro-inflammatory response in colon cancer, and contribute to a better understanding of its immunomodulatory effects.

P5.20.104
CCR5-expressing B-1 cells attract CD8 T cells to melanoma millieu and decrease tumor aggressiveness

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It has been shown by our group that B16F10 murine melanoma cell line, after physical contact with B-1 cells have increased metastatic potential. However the mechanism by which B-1 cells migrate towards melanoma cells is unknown. Objective: The aim of the work was to evaluate the influence of CCR5 expressed in B-1 cells on the course of B16F10 cells melanoma. Results: The present study demonstrated that B16F10 cells release soluble factors that are chemoattractant for B-1 cells. It was demonstrated that 10% of peritoneal B-1 cells express the chemokine receptor CCR5 on their surface. These cells, when inoculated into animals CCR5-/-, leads to in a 10-fold decrease in the number of metastatic nodules resulting from inoculation with B16F10 cells. Moreover, this approach promotes reduced rate of subcutaneous tumor growth, maintaining a stable volume of tumor while control animal presented exponential growth being that last day of measure in treated group show less tumor volume than first day of control group. Besides that, this treatment increased the survival of animals. When mice from treated group started to die, all mice from control groups already came to death. Analyzing leukocyte infiltration in the tumor we found a increased frequency of CD8 T cells in the treated group. Conclusion: Our data suggest that CCR5-expressing B-1 cells can change the development of murine melanoma, controlling it. So, this work contributed to a better understanding of B-1 cells chemotaxis and the influence of migration in evolution of melanoma.

P5.20.105
Devil Facial Tumour Disease, a devil of a cancer that is transmissible and fails to induce an immune response

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Devil facial tumour disease (DFTD) is a transmissible cancer of Schwann cell origin and has caused the death of approximately 80% of the Tasmanian devil population. Once a devil is 'infected' with DFTD cancer cells death always results. One of the remarkable features of this cancer is that the devil's immune system fails to recognise the allogeneic DFTD cancer cells. This is despite evidence that the devil has a fully competent immune system.
Lack of major histocompatibility complex (MHC) diversity may contribute to this lack of allo-recognition. To evaluate the severity of this we conducted mixed lymphocyte reactions and skin grafts. Detectable mixed lymphocyte reactions between some animals were obsered. All successful allografts were rejected within 14 days, even though little or no MHC I and II mismatches were found. Extensive T cell infiltration characterised the immune rejection. There is something unique about the DFTD tumour that allows it to be undetected by the devil's immune system. Preliminary results indicate that the DFTD tumour cells down regulate immune recognition genes and are therefore 'invisible' to the devil's immune system.
The inability of the devil's immune system to reject the transplanted tumours suggests that the tumour cells could be immunologically inert. Immunisation of Tasmanian devils with various formulations of tumour cells and adjuvants has met with limited success. But this limited success has provided evidence that some devils can produce a weak antibody and cytotoxic response. These antibodies are providing useful tools to screen for potential tumour antigens.

P5.20.106
Co-existence of HLA class I expression and CD8+ T cell infiltration is associated with favorable clinical outcomes in Ewing's sarcoma family of tumors (ESFT)

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Ewing's sarcoma family of tumors (ESFT) is one of the most malignant bone and soft tissue tumors in young people. Human leukocyte antigen (HLA) class I displays endogenously processed peptides to CD8+ T lymphocytes and has a key role for host immune surveillance. In ESFT, the investigation concerning both HLA class I expression and T cell infiltration has yet to be reported. This study was designed to examine expression profile of HLA class I and CD8+ T cell infiltration in ESFT. Furthermore, we determined the prognostic significance of HLA class I expression in ESFT. Biopsy specimens from 28 ESFT patients were stained by immunohistochemical (IHC) study with the anti-HLA class I monoclonal antibody (mAb) EMR8-5 and the grade of infiltration of CD8+ T cells was evaluated

semiquantitatively by IHC study using anti-CD8 mAb. Expression of HLA class I was negative (positive cells < 5%) in 10 tumors and down-regulated (positive cells < 50%) in 22 tumors. The status of CD8+ T cell infiltration was closely associated with the expression levels of HLA class I. ESFT patients with down-regulated or negative expression of HLA class I showed significantly poorer survival than the rest of the patients. Our results suggested that CD8+ T cell-mediated immune response restricted by HLA class I might play an important role in immune surveillance of ESFT, and we revealed for the first time that the status of HLA class I expression affects the survival of the patients with ESFT.

P5.20.107
The Immunology of Ovarian Cancer

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Worldwide, more women die from epithelial ovarian cancer (EOC) than any other gynaecological malignancy. The disease is widely held to originate from the ovarian surface epithelium (OSE) and epidemiological evidence implicates recurrent ovulation as a causal factor. As ovulation is an inflammatory process, inflammation-associated damage of the OSE may predispose to malignant transformation. Following ovulation, the OSE undergoes connective tissue remodelling through up-regulation of lysyl oxidase (LOX), an enzyme essential for post-translational collagen cross-linking. In addition there is evidence that LOX may play an important role in progression and metastasis of several cancers. The aim of this study was to assess the pattern of LOX expression in EOC primary cell cultures and genes previously shown to influence LOX expression connective tissue growth factor (CTGF) and bone morphogenetic protein-1 (BMP-1).
IL-1α caused a significant up-regulation of LOX mRNA (4.7-fold induction over untreated control, P<0.01) which was abrogated by coincubation with cortisol. CTGF mRNA was not altered by IL-1α or F alone but in combination IL-1α+F caused a significant down-regulation (0.34-fold over untreated control, P<0.05). Both LOX and BMP-1 were localised by immunohistochemistry to the malignant component of ovarian cancers rather than the supporting stromal cells.
These data suggest that LOX is likely to be active in EOC and inflammation enhances LOX expression. Control of LOX expression appears to be independent of CTGF and BMP-1 in these primary cultures. It follows that LOX and gene products responsible for connective tissue remodelling might be future targets for the detection and treatment of EOC.

P5.20.108
Critical role of interleukin-27 in tumor immunology

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Interleukin-27 (IL-27) plays critical roles in autoimmune diseases and infections; however, the contribution of endogenous, especially the host derived, IL-27 to tumor progression remains elusive. By using IL-27p28 conditional knockout mice, we have demonstrated that IL-27 is critical in protective immune responses against methyl-cholanthrene induced fibrosarcoma and transplanted B16 melanoma, and that dendritic cells are its primary source. DC-derived IL-27 is required for shaping the tumor microenvironment by inducing CXCL-10 expression in myeloid derived suppressor cells and regulating IL-12 production from dendritic cells, leading to the recruitment and functional maintenance of tumor infiltrating NK and NKT cells respectively. Indeed, reconstitution of either IL-27 or CXCL-10 in tumor site significantly restores the recruitment and function of NK and NKT cells and inhibits tumor growth. In summary, our study identifies a previous unknown critical role of DC-derived IL-27 in NK and NKT cells dependent antitumor immune surveillance by shaping the tumor microenvironment, and sheds light on developing novel therapeutic approaches based on IL-27.

P5.20.109
The role of the CXCR3 receptor ligand system in the immune escape of multiple myeloma

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Multiple Myeloma is a clonal B-cell neoplasm that affects terminally differentiated B cells. In this disease defects of the immune system including impairment of dendritic cell functions are well characterized. Tumour-derived chemokines might play a major role in the observed immune deficiency. The role of the CXCR3 receptor-ligand system in immunosurveillance is controversially discussed in different tumour entities. Here we investigated the role of myeloma-derived CXCR3ligands (*CXCL9*, *CXCL10*, *CXCL11*) on the functionality of dendritic cells bearing the appropriate receptor CXCR3 and their ability to stimulate T cells. We found that CXCR3ligand- activated dendritic cells showed a decrease in T-cell stimulatory capacity. Further, chronic stimulation of T cells led to diminished IFN-γ production and reduced CXCR3 receptor expression by T cell-subtypes. Experimental studies in various disease models show that CXCR3 deficiency significantly impairs cell-mediated immunity. Our observations indicate that the presence of CXCR3 ligands in multiple myeloma may lead to T cell exhaustion, possibly contributing to tumour immune escape.

P5.20.110
A Novel Subset of Tumor-infiltrating B7-H3+CD14+HLA-DR-/low Myeloid-derived Suppressor Cells Foster Immune Privilege and Tumor Progression in NSCLC

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Myeloid-derived suppressor cells (MDSCs) are potent immune-suppressive cells promoting tumor progression and metastasis. Although surface markers of Gr-1 and CD11b are widely used to define MDSCs in mice, human MDSCs lack definitive phenotypic markers. Here, we showed that CD14+HLA-DR-/low MDSCs were significantly increased in blood and tumor tissues of non-small cell lung cancer patients. Intriguingly, we found that B7-H3 molecule was exclusively expressed on a subset of tumor-infiltrating CD14+HLA-DR-/low MDSCs but not in peripheral blood or non-tumor tissue-infiltrating MDSCs. Both B7-H3+ and B7-H3- CD14+HLA-DR-/low MDSCs exhibited potent T cell suppressive activity. However, B7-H3+MDSCs, as compared to B7-H3- MDSCs, showed increased tumor-promoting activity and preferentially induced regulatory T cell (Treg) expansion in a manner of depending IL-10 signal. Further analyses revealed that B7-H3+MDSCs, but not B7-H3- MDSCs, were positively correlated with Treg infiltration in tumor. Moreover, in a murine lung cancer model, B7-H3+ MDSCs were found within the tumor microenvironment and increased during tumor progression. Similarly, we found levels of B7-H3+ MDSCs were associated with reduced survival in the patients with NSCLC. Taken together, we identify a novel subset of MDSCs within the tumor microenvironment that preferentially induces Treg expansion leading to tumor progression.

P5.20.111
A novel subset of tumor-infiltrating B7-H3+CD14+HLA-DR-/low myeloid-derived suppressor cells foster immune privilege and tumor progression in NSCLC

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Myeloid-derived suppressor cells (MDSCs) are potent immune-suppressive cells promoting tumor progression and metastasis. Although surface markers of Gr-1 and CD11b are widely used to define MDSCs in mice, human MDSCs lack definitive phenotypic markers. Here, we showed that $CD14^+HLA\text{-}DR^{-/low}$ MDSCs were significantly increased in blood and tumor tissues of non-small cell lung cancer patients. Intriguingly, we found that B7-H3 molecule was exclusively expressed on a subset of tumor-infiltrating $CD14^+HLA\text{-}DR^{-/low}$ MDSCs but not in peripheral blood or non-tumor tissue-infiltrating MDSCs. Both $B7\text{-}H3^+$ and $B7\text{-}H3^-$ $CD14^+HLA\text{-}DR^{-/low}$ MDSCs exhibited potent T cell suppressive activity. However, $B7\text{-}H3^+$MDSCs, as compared to $B7\text{-}H3^-$ MDSCs, showed increased tumor-promoting activity and preferentially induced regulatory T cell (Treg) expansion in a manner of depending IL-10 signal. Further analyses revealed that $B7\text{-}H3^+$MDSCs, but not $B7\text{-}H3^-$ MDSCs, were positively correlated with Treg infiltration in tumor. Moreover, in a murine lung cancer model, $B7\text{-}H3^+$ MDSCs were found within the tumor microenvironment and increased during tumor progression. Similarly, we found levels of $B7\text{-}H3^+$ MDSCs were associated with reduced survival in the patients with NSCLC. Taken together, we identify a novel subset of MDSCs within the tumor microenvironment that preferentially induces Treg expansion leading to tumor progression.

P5.21 Myelo- and lympho-proliferative disorders

P5.21.01
Myeloid cell-derived reactive oxygen species trigger ERK pathway-dependent parthanatos in human lymphocytes

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Reactive oxygen species (ROS) are formed by myeloid cells as a defense strategy against microorganisms. ROS however also trigger poly(ADP-ribose) polymerase 1- (PARP-1) dependent cell death (parthanatos) in adjacent lymphocytes. The ROS-induced lymphocyte parthanatos has been forwarded as a mechanism of immune escape in several forms of cancer. The present study sought to evaluate the role of mitogen-activated protein kinases (MAPKs), in particular the extracellular signal-regulated kinase (ERK), in ROS-induced signal transduction leading to lymphocyte parthanatos. It was found that inhibitors of ERK1/2 phosphorylation rescued human natural killer (NK) cells and $CD8^+$ T lymphocytes from cell death induced by ROS-producing mononuclear myeloid cells. ERK1/2 phosphorylation inhibition also protected lymphocytes from cell death induced by exogenous hydrogen peroxide (H_2O_2) and from ROS generated by xanthine oxidase or glucose oxidase. Phosphorylation of ERK1/2 was observed in lymphocytes shortly after exposure to ROS. ROS-generating myeloid cells and exogenous H_2O_2 triggered PARP 1-dependent accumulation of poly ADP-ribose (PAR), which was prevented by ERK pathway inhibitors. ERK1/2 phosphorylation was induced by ROS independently of PARP-1. These findings imply that ROS-induced lymphocyte parthanatos follows the following scheme of events: ROS:ERK1/2:PARP-1:PAR:parthanatos and suggest that the ERK axis may provide a therapeutic target for the protection of lymphocytes against oxidative stress.

P5.21.02
Notch signalling controls leukemic cells

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Notch signaling and Ikaros transcription factors are implicated in lymphocyte differentiation. Experiments from genetically manipulated mice show that they have a critical role at specific check points of the lymphopoietic pathway. However, how developmental regulated changes are reflected in gene expression programs is still not well understood. The aim of this work is to analyze the genes which regulate differentiation at sequential developmental steps in hematopoietic cells and in leukemic cells with impaired development.
Using the monolayer culture system for T cell development in vitro (OP9-DL 1 cell line), we control and change the conditions of culturing leukemic cells either from cell lines or leukaemia patients. We monitored the influence of Notch and Ikaros family genes on hematopoietic cell differentiation and commitment, by stimulating or inhibiting their activity and measuring downstream gene expression levels in the cells. Our results indicate that Notch signaling is not only a master switch for T lymphocyte commitment, but is also important for survival of leukemic cells. Whether the downstream target genes are part of the HES1 activation pathway has been analyzed in leukemic cell lines and in primary leukemic cells from B CLL patients. We further analyzed whether differentiation and survival of these cells depends on proteins from the Ikaros family, Aiolos and Helios, since they maintain the spatial organization of chromosome structure and regulate cell commitment. Recognition of their expression pattern might help to understand the mechanisms of leukaemia development.

P5.21.03
Vasculitis associated myelodysplasia in a prospective 4 year study.

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The presence of systemic and/or immune manifestations in myelodysplasia is classical, even thought rarely presenting as initial clinical event.
Purpose:
We present a prospective study (2009-2012) with systemic and/or immune manifestations dominating the clinical presentation.
Patients and methods:
341 patients with a formal diagnosis of myelodysplasia were followed-up for 4 years. Among them 36 developped systemic and/or immune manifestations during the follw-up period (2009-2012). The clinical picture in these cases consisted in transient fever, not related to infections (25%), arthralgia or arthritis (17%), and especially cutaneous (purpura 35%, nodular 15%, papular 8%, and necrotic lesions 5%) related to cutaneous lymphocytary and/or leucocytoclasic vasculitis. Lung symptoms were occasional (2% of cases), the same as renal manifestations (3% of cases). Even though infrequently, some rare cases of systemic vasculitis have been reported in our series (one case of Wegener granulomatosis, two cases of microscopic polyangeitis and one case of Churg-Strauss syndrome).
Immune anomalies were recorded in 25% of the cases (antinuclear antibodies 20%, ANCA 10%, rheumatoid factor 12%). A corticosteroid treatment was efficient in 90% of the cases.
Discussion and conclusion: The association between myelodysplasic syndromes with systemic and immune manifestations seems to not be occasional and suggests the eventuality of a potential common primary immune disorder.

P5.21.04
CD137 ligand reverse signalling induces differentiation of primary acute myeloid leukaemia cells

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The ligand for CD137 is expressed on hematopoietic progenitor cells and antigen-presenting cells such as monocytes, dendritic cells and B cells. Reverse signalling of CD137 ligand into the cell delivers a potent activating signal that results in the differentiation of hematopoietic progenitor cells into macrophages, and monocytes into dendritic cells. CD137 ligand is also expressed on acute myeloid leukemia cells, which characteristically possess a maturation block that leads to arrested differentiation and malignancy.
We hypothesize that CD137 ligand reverse signalling via stimulation with CD137 may also induce differentiation of the transformed myeloid cells in acute myeloid leukaemia. Primary acute myeloid leukemia blasts isolated from either the bone marrow or peripheral blood of patients at time of diagnosis were stimulated with a recombinant CD137 protein in vitro. Reverse signalling through CD137 ligand induces differentiation of these leukemic blasts based on morphology, immunophenotyping, cellular functions such as phagocytosis and oxidative burst, and cytokine release. These differentiated cells functionally demonstrate a more potent T cell co-stimulatory capacity as evidenced by up-regulation of co-stimulatory molecules, induction of increased T cell proliferation and cytokine release.
These results suggest that CD137, as a single factor, is able to induce differentiation of the immature blasts in acute myeloid leukemia into more effective antigen-presenting cells with enhanced T cell co-stimulatory potential.
The ability to overcome the block in myeloid maturation and drive differentiation of acute myeloid leukemia cells has implications for the development of differentiation therapies and anti-leukemia vaccines.

P5.21.05
Serum free light chains assays (sFLC) in immunoglobulin light chain monoclonal gammopathies diagnosis and assessment: an Algerian study

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Objectives: the usefulness of serum free light chains assays in the diagnosis and monitoring of monoclonal gammopathies has been reported in numerous papers. In this study, we aim to evaluate the clinical relevance of this assay in a cohort of 65 Algerian patients with immunoglobulin light chains monoclonal gammopathies (LCMG).
Methods: 65 patients (39 males, 26 females) with LCMG were selected from cases of known monoclonal gammopathies, at the immunology department of Beni Messous Teaching Hospital, Algiers, Algeria. The detection, measurement and identification of the monoclonal protein (M-protein) was performed using serum protein electrophoresis (SPEP), urine protein electrophoresis, immunofixation electrophoresis (IFE) and serum free light chains assays. The FLC immunoassay was performed using a nephelometer with the Freelite reagents.
Results: Free light chains ratio was abnormal in 64 patients. The detection of M-protein was not possible in 12 patients using SPEP, UPEP and IFE. sFLC assays enabled the identification of the monoclonal free light chain. The 12 patients were distributed as follows: 8 had multiple myeloma (MM), 1 had amyloidosis, 2 had plasmocytoma and 1 monoclonal gammopathy of undetermined significance (MGUS).
Conclusion: although SPEP and IFE remain the principal methods used to identify M-protein, they proved to be ineffective in some cases of our cohort. sFLC assays allowed the diagnosis of monoclonal gammopathies in patients with “normal” SPEP and IFE results, and thus, an accurate diagnosis and a faster management of the disease.

P5.21.06
Detection of two distinct MLL-AF4 fusion transcripts in a neonatal acute leukemia case undergoing a rapid lineage switch after diagnosis

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Context: The importance of genetic events in the classification, therapy, and prognosis of ALL has gained recently growing credit. Amongst those events, mixed lineage leukemia (MLL) gene rearrangements may be found in leukemia cases of either lymphoid or myeloid phenotypes and translocation t(4;11)(q21;q23) is often associated with pediatric cases.
Objective: To present the clinical-laboratory particularities of a MLL-AF4 positive pediatric leukemia case in which the cell lineage switched early from ALL to AML. Material and Method: In a bone marrow sample of a two months old girl suspected of ALL, the presence of 4 fusion transcripts: BCR-ABL, MLL-AF4, E2A-PBX1, TEL-AML1 was assessed. Flow cytometry analyses were carried out on a FACSCanto-II cytometer.
Results: While at diagnosis a dominant malignant clone displaying a pro-B lymphoid phenotype was described, two weeks later, under corticotherapy, a malignant monocytoid population predominantly expanded. With both occasions, the patient was found to be positive for two distinct MLL-AF4 transcripts: e11-e4 and e10-e4. The bilineage nature of the case suggests that t(4;11) transforms a multipotential progenitor cell. Similar to other reports, this case is predictable to have an aggressive evolution, although expected to benefit from allogeneic HSCT. Two months after the initiation of induction therapy the patient has not achieved remission and has an indication for a more intensive chemotherapy.
Conclusion: Sensitive detection of MLL rearrangements, accurate lineage assignment, and early lineage switch prediction may have a crucial clinical impact, supporting the clinician in treatment making decisions and increasing the precision of minimal residual disease detection.

P5.21.07
Immune reconstitution with subcutaneous immunoglobulin in patients with lymphoproliferative disease and secondary hypogammaglobulinemia after anti-CD20 mAb therapy

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Purpose: Intravenous immunoglobulin replacement therapy (IVIg) represents the standard treatment for hypogammaglobulinaemia secondary to B-cell lymphoproliferative disorders (LPDs). Subcutaneous immunoglobulin infusion (SCIg) is an effective, safe and well-tolerated approach in primary immunodeficiencies but no extensive data are available on their use in LPDs with hypogammaglobulinaemia, a frequent phenomenon after treatment with anti-CD20 mAb.
Methods: In this study we evaluated efficacy (serum IgG levels and number of infections/year) and safety (number of adverse events) of IVIg (250 mg/kg/4weeks) vs SCIg (70 mg/kg/week) replacement therapy in 55 patients with LPDs and hypogammaglobulinemia previously treated with anti-CD20 mAb. In addition, the impact of the infusion methods on the quality of life (QoL) was compared.
Results: Both treatments appeared to be effective in replacing defective Ig production (mean 3,89 g/L ± 1,65 before therapy) although SCIg achieved significantly higher levels of IgG (6,39 g/L ± 1,67) with respect to IVIg (4,71 g/L ± 1,38; p <0,05). Both infusion ways were efficacy in reducing the incidence of infectious events and, specifically, of serious infectious events. Noteworthy, SCIg reached a superior benefit, since SC administration was associated to an increased percentage of patients who did not complain infectious events. As expected, we registered a lower number of adverse events with SCIg with respect to IVIg, with no SAE. Finally, we observed an improvement in health-related QoL parameters after the switch to SCIg.
Conclusion: Our results suggest that SC immunoglobulin is safe and effective in patients with LPDs and hypogammaglobulinemia, confirming our previous report.

P5.21.08
Rituximab in the treatment of EBV-positive low grade B-cell lymphomas. Is there additive benefit regarding EBV infection?

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The vast majority of Epstein-Barr virus (EBV) infections is subclinical, and the virus is capable of establishing a latent infection and causing malignant transformation of B-cells. CD21 is the receptor for EBV on the surface of B-cells, but the virus can also infect T-lymphocytes and monocytes. Following penetration and replication of the viral DNA by cell polymerases, the viral genome remains in the nucleus, and a latency phase is established, during which only a small part of the viral genes is expressed. LMP1 among them is essential for EBV transformation. Low grade B-cell lymphomas are considered non-EBV related although the virus and its gene products are detected in patients with chronic lymphocytic leukemia. Rituximab is a potent factor used in the treatment of these diseases and is also widely used for the treatment of post-transplant lymphoproliferative disorders caused by EBV. The effect of rituximab treatment in non-transplant lymphoma patients has never been studied. We studied the effect of rituximab-based immunochemotherapy in 48 patients with non-EBV related leukemic low grade B-cell lymphomas. Nineteen of the patients were EBV-positive (by qRT-PCR for the BXLF-1 gene). After 3 cycles of rituximab-based treatment, only 1/19 (5.26%) was still positive for EBV. Our results suggests that rituximab used in the treatment of EBV-positive low grade lymphomas is efficient in eradicating the virus from the peripheral blood, a fact that may be important in the course of the disease and the likelihood of recurrences.

P5.21.09
Severe phenotype of IgD multiple myeloma with acute renal insufficiency: report of 2 cases

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Introduction and aim: Despite its rarity, IgD multiple myeloma is known by its acute onset usually at a stage of renal complication. IgD remains the only isotype of immunoglobulin with undetermined function.
Our aim is to report clinical and prognosis features of 2 cases of IgD myeloma.
Observations: We reported two cases of IgD myeloma. One female (F) and one male (M). Their ages are 48 and 63 years respectively. They were admitted in internal medicine department for severe renal impairment of unknown etiology. Serum immunoelectrophoresis of F disclosed increases in IgD and the lambda type light chain and died 3 months after diagnosis. However, immunofixation of M serum showed an IgD with kappa type light chain, he died 5 weeks after diagnosis. Decreased of polyclonal immunoglobulin and high percentage of immature plasma cells was found on the myelogram of both patients.
Conclusion: Important issues of our observation are: - the fact that among the two patients, one is female, which is exceptional in IgD myeloma - Light chain could be Kappa or lambda - No improvement of patient's condition - Both patients had renal failure requiring hemodialysis before the diagnosis of IgD myeloma- The survival period after diagnosis of IgD myeloma is the shortest among all types of myeloma.

P5.21.10
CD27+IgM+IgD+ B cells in Persistent Polyclonal B-cell Lymphocytosis are hyperproliferated memory B cells with a distinctive immunophenotype

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Persistent polyclonal B-cell lymphocytosis (PPBL) is a rare benign disorder characterized by an expansion of CD27+IgM+IgD+ cells. Despite an increasing number of described cases, the nature of the persistent lymphocytosis and its relation to mature B-cell malignancies remain poorly understood. Therefore, we subjected PPBL patients and healthy controls to immunoglobulin (Ig) repertoire and replication history analyses, and gene expression profiling. In addition, we compared the immunophenotype of PPBL cells with B-cell malignancies and B-cell subsets from controls. Replication history analysis revealed that PPBL cells had undergone hyperproliferation in vivo. Still, the IGH gene repertoire of these cells was highly diverse without evidence for stereotypy. Moreover, the *IGHV* genes contained somatic hypermutations (SHM), albeit in low levels and without the selection for replacement mutations in complementarity determining regions that is found in control memory B cells. PPBL cells showed a gene expression profile reminiscent of memory B cells, accompanied by upregulation of Toll-like receptor signaling molecules and heterogeneous deregulation of oncogenes. Furthermore, we identified cell surface markers to discriminate PPBL cells from control B-cell subsets: IgD, CD38, CD73 and CD62L. These combined insights, especially the newly identified cell surface markers, can prove valuable for diagnosis of PPBL.

P5.21.11
Effect of a calcium channel blocker on matrix metalloproteinase-9 activity in human monocytic THP1 cells

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Background: Matrix metalloproteinase-9 (MMP-9) belongs to a big group of enzymes which have essential role in degradation of extracellular matrix and play a key role in inflammation. Verapamil as a calcium channel blocker has been widely used in treatment of cardiovascular diseases. Moreover the anti-inflammatory effects of verapamil have been demonstrated. In this study the effect of verapamil on MMP-9 activity in a human monocytic cell line has been assessed in vitro.
Methods: The human monocytic THP1 cells were cultured in complete RPMI medium. The cells at logarhytmic growth phase were stimulated with phorbol 12-myristate 13-acetate (PMA) at optimum concentration and then incubated with different concentrations of verapamil (0.001-10 µg/ml). Next the MMP-9 activity in cell culture supernates was measured by gelatin zymography.
Results: Verapamil significantly and dose-dependently decreased the MMP-9 activity in human PBMCs after 48 hour incubation time compared with untreated control cells.
Conclusion: In this study verapamil showed a dose- dependent inhibitory effect on the MMP-9 activity in human monocytic THP-1 cells. Thus it seems that the anti-inflammatory properties of verapamil may be in part due to its inhibitory effects on MMP-9 activity.

P5.21.12
Downregulation of IL17 producing T cells is associated with regulatory T cells expansion and disease progression in chronic lymphocytic leukemia

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Little is known about the immunobiology of interleukin-17 (IL17) producing T cells and regulatory T cells (Treg) in chronic lymphocytic leukaemia (CLL). In this study, the frequencies of Th17, Tc17 and CD39+Treg cells were enumerated in peripheral T cells isolated from 40 CLL patients and 15 normal subjects by flow cytometry. Our results showed a lower frequency of Th17 and Tc17 cells in progressive (0.99±0.12% of total CD3+CD4+ cells, 0.44±0.09% of total CD8+ cells) compared to indolent patients (1.57±0.24%, p=0.042, 0.82±0.2%, p=0.09) and normal subjects (1.78±0.2%, p=0.003, 0.71±0.09%, p=0.04). Decrease in IL17 producing T cells was associated with CD39+Treg cells expansion. Variation of IL17 producing cells and Treg cells in indolent and progressive patients was neither associated to the expression levels of Th1 and Th2 specific transcription factors T-bet and GATA-3 nor to the frequencies of IFNγ and IL4 producing CD4+ T cells in a selected number of samples. Additionally, suppressive potential of CD4+ Treg was similar in CLL patients and normal subjects. Our data indicates that progression of CLL is associated with downregulation of IL17 producing T cells and expansion of Treg cells, implying contribution of these subsets of T cells in progression of CLL.

P5.21.13
Common genetic variants of BAFF, APRIL, TACI, BCMA and BAFF-R genes and B-cell chronic lymphocytic leukaemia risk

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TNF superfamily members, B-cell activation factor (BAFF; TNFSF13B) and a proliferation-inducting ligand (APRIL; TNFSF13), may play an important role in B - cell chronic lymphocytic leukaemia (B-CLL) survival. BAFF and APRIL bind with high affinity BCMA (B-cell maturation antigen; TNFRSF17) and TACI (transmembrane activator calcium modulator and cyclophilin ligand interactor; TNFRSF13B) receptors. BAFF additionally interacts with BAFF-R (TNFRS13C) receptor. Abnormalities in the synthesis of these molecules or expression of their receptors have been associated with various B cell malignancies including B-CLL. Therefore, we conducted a case-control study (N=197 vs. N=326) to investigate the possible association of 17 SNPs: 3 SNPs in BAFF, 4 SNPs in APRIL, 3 SNPs in BCMA, 3 SNPs in TACI and 4 SNPs in BAFF-R with susceptibility to B-CLL. Of the 17 SNPs examined in this study, only one SNP rs4985726 of TACI gene was associated with B-CLL. The distribution of the alleles and genotypes of rs4985726 significantly differed between patients and controls (p=0,0004 and p=0,00027, respectively). Individuals possessing allele C had 2.27 times lower risk for B-CLL than homozygous GG (OR=0,44; 95%CI=0.28-0.70; p=0,0004). The features of the BAFF, APRIL, BCMA, TACI and BAFF-R genes were subjected to analysis for correlation with clinical data. Individuals carrying C allele in rs11078355 of TACI showed increased soluble APRIL level compared to TT individuals (p=0.052). Our results suggest that rs4985726 is significantly associated with B-CLL risk and rs11078355 may be involved in clinical course of this disease.

P5.21.14
monoclonal component revealed by a cryoglobulinemia

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Introduction: Cryoglobulins are immunoglobulins that precipitate below 37°C they may be responsible for vasculitis of small vessels. The mixed cryoglobulins of type II and III are associated to auto immune diseases, viral infectious diseases and can be found in some lymphoproliferative syndrome.
Patient, materials and methods: It is a woman aged 59, with rheumatoid arthritis a scleroderma and secondary Sjögren's syndrome, with clinical manifestations suggestive of strong suspicion of cryoglobulinemia such as petechial purpura, ulcers of the legs. A research of cryoglobulinemia and serum electrophoresis were requested.The search for cryoglobulinemia was done in the particular conditions (37 ° C), electrophoresis and immunosubtraction of serum proteins on Cappillarys was made. Electrophoresis, immunofixation on agarose gel (SAS3,SAS4) and assay of immunoglobulins for cryoprecipitate , rheumatoid factor by nephelometry laser (Immage). Quantification of cryoprecipitate (technique pyrogallol red).
Result and Discussion: Serum electrophoresis on capillary, at room temperature objectified a hypogammaglobulinemia. Cryoglobulinemia was positive after 10 days of observation, electrophoresis of serum cryoprécipitant heated at 37 ° C, objectified a monoclonal component isotype IgG lambda.
The agarose gel electrophoresis and immunofixation of cryoprecipitate, were used to classify the cryoglobulin in type IIa "mixed monoclonal".
We would have missed the monoclonal component if we did not search the cryoglobulin.
Conclusion: In front of the precipitating Character of immunoglobulins which occurs preferentially in hematological malignancies and auto immune diseases, the interpretation of serum electrophoresis should be done very carefully when the clinic strongly indicates cryoglobulinemia.

P5.21.15
Evaluation of urokinase Plasminogen Activator Receptor (uPAR), soluble uPAR, and β1 integrin in patients with Hodgkin's lymphoma

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Several studies indicated the role of urokinase plasminogen activator receptor (uPAR), soluble uPAR (suPAR), and β1 integrin in tumor growth and invasion. In this study, the expression levels of β1 integrin and uPAR on lymph node mononuclear cells from 25 Hodgkin's lymphoma (HL) patients were evaluated using two-color flow cytometry. Moreover, the levels of suPAR in the serum samples of HL were measured and compared with 32 healthy controls. Results indicated no significant association of uPAR with tumor size, different stages, or different histological subtypes of HL; however, an increased expression of β1 integrin was detected in advanced stages of HL. Among different subtypes, higher expression of β1 integrin was detected in Nodular sclerosis compared to Lymphocyte Predominant. No significant difference was observed between serum levels of suPAR in patients with different stages of HL and healthy controls. Moreover, the levels of suPAR were significantly higher in Nodular sclerosis in comparison with other subtypes. In conclusion, this study showed that the levels of suPAR and β1 integrin varied between different histological subtypes of HL. Although uPAR may play only a minor role in growth and metastasis of lymphoma, β1 integrin may be important in predicting prognosis and metastasis in HL.

P5.21.16
In vivo imaging of B-cell chronic lymphocytic leukemia extravasation through high endothelial venules in lymph nodes

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B cell chronic lymphocytic leukemia (B-CLL) is characterized by the progressive accumulation of B-CLL cells in the blood, bone marrow, spleen and lymph nodes (LNs). LNs are sites of malignant proliferation and LN enlargement is associated with poor prognosis in the clinics. Although chemotherapy is efficient on circulating B-CLL cells, LN resident B-CLL cells are refractory to treatment and are often the source of relapse. The aim of our study was to provide a better understanding of the mechanism of B-CLL cell entry and retention within LNs in order to reduce B-CLL residency within LNs during chemotherapy treatment. Using intravital microscopy, we studied the behavior of B-CLL patient cells within high endothelial venules (HEVs) of mouse peripheral LNs, the major gateways for lymphocyte entry into LNs (Moussion and Girard, Nature 2011, 479:542-546; Girard et al., Nature Rev Immunol, 2012, 12:762-773). We found that B-CLL cells from patients with clinical lymphadenopathy expressed a higher level of the LN homing receptor CD62-L than B-CLL cells from patients without LN enlargement. High levels of CD62-L expression correlated with an increased proportion of cells that rolled and sticked on LN HEVs. We are now using 2-photon intravital microscopy to visualize B-CLL extravasation through HEVs and B-CLL cell migration within LNs. We are also investigating the effect of drugs on B-CLL cells homing and distribution within LNs.

P5.21.17
The PI3K/ERK dual inhibitor AEZS-136 triggers a potent antitumor activity agains Hodgkin limphoma cells through a ROS-dependent mechanism

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Relapse of disease and resistance to established therapies remains a challenge in the treatment of Hodgkin Lymphoma (HL) and Diffuse Large B-Cell Lymphoma (DLBCL). Previous results from our phase II study in HL indicate that combining the PI3K/AKT inhibitor perifosine with the RAF/MEK/ERK inhibitor sorafenib can achieve remarkable responses in the relapsed/refractory settings. Here we aim to characterize the in vitro and in vivo activity and mechanism(s) of action of the novel dual PI3K/ERK inhibitor AEZS-136 (Æterna Zentaris GmbH, Germany, EU) in HL. AEZS-136 treatment resulted in a time and dose-dependent cell growth inhibition (range, 30% to 80%) and cell cycle arrest in HL cells (L-540, SUP-HD1, KM-H2 and L-428), as well as in germinal center (GCB) (SU-DHL-6, SU-DHL-10, SU-DHL-16) and activated (ABC) (RCK8, RIVA, U2932) DLBCL cells (range, 60% to 90%). Caspase-independent cell death was observed in L-540 and SUP-HD1 cells (62 ± 9% and 46 ± 2%, $P \leq .0001$, respectively) associated with severe mitochondrial dysfunction (up to 40%, $P \leq .001$). Besides downregulating phosphorylation of ERK and PI3K/AKT, AEZS-136 sustained activation of JNK, whose inhibition prevented cell death. These findings were attributed to AEZS-136 time-dependent induction of reactive oxygen species (ROS) and necroptosis. Necrostatin-1 or the ROS inhibitor YCG063 abolished AEZS-136-induced ROS production, mitochondrial injury, JNK activation and cell death. In vivo AEZS-136 dose-dependently inhibited the growth of L-540 nodules (from 22 to 55%, P≤.0001). These results indicate that AEZS-136 induces cytostatic and cytotoxic effects in HL cells and xenografts, suggesting further investigation in future HL trials.

P5.21.18
IgD multiple myeloma: Presenting clinical and biological features Review of 17 cases

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IgD multiple myeloma (MM) is a rare subtype of myeloma≈(2%). To obtain clinical, biological features and prognostic value of serum free light chains (sFLC) analysis, we examined 17 cases of IgD myeloma. From 1998 to 2012, we had 590 multiple myeloma including 17 patients with IgD myeloma. Men predominate with a mean age at diagnosis: 59 ± 12 years. The presenting features: bone pain (75%), lymphadenopathy (16%), hepatomegaly (25%), splenomegaly (8%), associated AL amyloidosis (6%), renal impairment function (82%), infections (47%), hypercalcemia (37%) and anemia (93%). Patients with IgD myelomas have a short median survival (9 months). Serum electrophoretic showed an M-spike which are not high (Mean= 13,22 ± 10 g/L) in all patients associated to an hypogammaglobulinemia. The type of light chain was Lambda (65%), high β2microglobulin in 91% and Bence Jones proteinuria was identified in 71%. The median rate of sFLCs κ was 19.05 mg/L and 296,75 mg/L for sFLCs λ. sFLCR was abnormal in 93% of patients and it showed concordance between baseline sFLCR and the survival (P=0.034). The contribution of FLC assay is crucial for the prognosis of patients with IgD myeloma. However, IgD myeloma has a more aggressive disease course than other myelomas, this is why, a multi-center study is recommended to establish specific median survivals for this entity according to the recent ISS.

P5.21.19
Upregulation of CD200 is associated with Foxp3+ regulatory T-cell expansion and disease progression in acute myeloid leukemia

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Immunosuppression in acute myeloid leukemia (AML) is an important mechanism of tumor escape. CD200, as an immunosuppressive molecule, is over-expressed in some hematological malignancies and it has also been shown to be an independent prognostic factor in AML. In the current study, simultaneous CD200 expression and Foxp3+ regulatory T-cell levels were investigated in Iranian patients with AML by Flow cytometry. We also assessed the effect of CD200-CD200R blockade on Th1 and T-reg cytokine production and T-cell proliferation in autologous AML- and monocyte-DC MLRs. ELISA assay was performed to detect IL-2, IL-12, IFN-γ, IL-10 and TGF-β production in MLR supernatants. Expression of Foxp3, IL-10 and TGF-β mRNAs in MLRs were detected by real-time PCR. Our results demonstrated significant over-expression of CD200 in association with higher frequencies of Foxp3+ T-cells in AML patients. Blocking of CD200-CD200R interaction demonstrated a significant decrease in TGF-β and IL-10 expression in AML-DC MLRs and a significant increase in IL-12 and IFN-γ expression in monocyte-DC MLRs. Elevated T-cell levels with lower Foxp3 intensity was also shown in CD200-CD200R blocked MLRs. Expression of IL-10 mRNA declined significantly only in AML-DC MLRs where CD200-CD200R interaction was blocked and the same result was observed for TGF-β and Foxp3 mRNA in both AML-and monocyte-DC MLRs. These data present a significant role for CD200 in suppressing anti tumor immune response through stimulation of regulatory mechanisms in AML patients and suggest that CD200 may have a prognostic value in this malignancy and its blockade may be used as a target for AML immunotherapy.

P5.21.20
The multiple myeloma in the region of Annaba (Algeria)

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Multiple myeloma (MM) or *Kahler's* disease is a malignant monoclonal proliferation of plasma cells that are actively secreting in inappropriate and exaggerated manner the entire structure of immunoglobulin (Ig) or any segment of it. MM is accounting nearly for 10 % of hematological malignancies. It is slightly more common in men than in women and a peak age at diagnosis is 64 years. The diagnosis of this condition is based on two elements: The detection of a monoclonal component in serum and / or urine, and dystrophic bone marrow plasma cell proliferation.
In our work, we report the clinical and biological and immunochemical characteristics of 165 cases of MM.
165 patients with MM (84M / 81F), aged between 25 and 90 years, 18 of which were age <45 years, the diagnosis occurred by myelogram and the identification of a monoclonal Ig by electrophoresis on agarose gel, immunochemical typing of the monoclonal Ig was held by immune-electrophoresis, immune-fixation and laser-nephelometry. The degree of bone marrow plasma cell proliferation was greater than 30% for all studied patients. The immunochemical study of 165 patients with MM showed that 48.49% have a monoclonal Ig with IgG isotype, 19.4% of samples have a monoclonal IgA and only 5.45% have a monoclonal IgM. Incomplete monoclonal Ig as light chains was found for 19.4% of the cases (14.54% kappa, lambda 12.12%).
Multiple myeloma is a disease affecting the elderly, it can however be observed in young adults, its diagnosis is confirmed by immunochemical study of monoclonal immunoglobulin.

P5.21.21
CXCL12 and TP53 genetic polymorphisms as possible markers of pediatric Acute Lymphoblastic Leukemia (ALL) susceptibility in Brazilian population

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Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy. Genetic polymorphisms in the 3'UTR region of the *CXCL12* (rs1801157) and *TP53* codon 72 (rs1042522) genes may contribute to susceptibility to childhood ALL because they affect some important processes, such as metastasis regulation and tumor suppression. Thus the objective of the present study was to detect the frequency of two genetic polymorphisms in ALL patients and controls and to clarify their impact on genetic susceptibility and prognosis. The *CXCL12* and *TP53* polymorphisms were tested in 54 ALL pediatric patients and in 59 controls by restriction fragment length polymerase chain reaction (RFLP-PCR) and allelic specific chain reaction techniques (ASP-PCR), respectively. The frequencies of both allelic variants were higher in ALL patients than in the controls and indicated a positive association: OR=2.44; CI95%=1.05-5.64 for *CXCL12* and OR=2.20; CI95%=1.03-4.70 for *TP53*. Furthermore, when the two genetic variants were analyzed together, they increased significantly more than 5 fold the risk of this neoplasia development, indicating their potential as susceptibility markers for ALL disease and the relevance of the allelic variant combination to increased risk of developing malignant tumors. Future studies may indicate a larger panel of genes involved in susceptibility and progression of pediatric ALL and other hematological neoplasias. Support: CNPq, CAPES, Fundação Araucaria and PROPPG-UEL.

P5.21.22
Significance of immunoglobulin B-cell receptor features in tumor dynamics of chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is a malignant lymphoproliferative disorder characterized by monoclonal expansion of mature B-cells. Presence/absence of somatic hypermutations in clonal heavy chain immunoglobulin gene (IGH), specifically its variable region IGHV, represents a crucial prognostic marker stratifying patients into subgroups with different clinical outcome. Sporadically, cases with multiple IGHs are observed. From clinical point of view, they preclude conclusive prognostic interpretation. However, they constitute a remarkable model of CLL tumorigenesis.
Thus, we investigated biological cause of the multiple IGHs and over-time clone behavior in 31 CLL patients with respect to molecular features of B-cell receptor.
We identified definite co-existence of two clonal B-cell populations with different light chain restriction (9/31 cases), or highly likely co-existence of two or more independent clones with homogenous phenotype (16/31 cases). In cases with consecutive samples, we performed quantitative evaluation of over-time changes in clone proportions. Interestingly, we observed clonal drifts, mainly diminishing of a clone (18 cases) but also appearing of a new one (3 cases). Clones with specific immunogenetic features of IGH, namely (i) higher IGHV identity to germ-line and/or (i) longer HCDR3, were preferred during the disease evolution. Significantly, these clone characteristics are important markers of clinical outcome.
Our study allowed novel insight into biological causes of multiple IGH detection in CLL, typically monoclonal disease. Importantly, we observed clonal drift favoring specific molecular B cell receptor features, which might eventually provide further insight into disease evolution with implications for patient monitoring and therapy.
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P5.21.23
Prevalence of monoclonal gammopathy detected in the Noor pathobiology lab of Iran

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Background: We assessed the prevalence and isotype distribution of monoclonal gammopathy in the Noor pathobiology laboratory of Iran.
Participants and Methods: From June 21, 2011 to December 20, 2012, a total number of 8525 Iranian patients who referred to Noor pathobiology lab were included in our study. Serum protein electrophoresis was performed on all sera. Serum sample with discrete or localized band was subjected to capillary zone electrophoresis (CZE) and immunosubtraction by Capillarys 2 instrument. Sex- and age-related prevalence rates of monoclonal gammopathy were calculated.
Results: Monoclonal gammopathy was detected in 415 of the 8525 study participants, yielding a prevalence of 4.86% in the total population screened. The prevalence in men was significantly higher than in women. 124 samples of 415 samples were immunotyped. Isotype distribution of immunoglobulin were IgG/Kappa in 46% (n=57) of patients, IgG/Lambda in 23% (n=29), IgA/Kappa in 10% (n=12), IgA/Lambda in 7% (n=9), IgM/Kappa in 6% (n=8), IgM/Lambda in 2% (n=2), Lambda light chain in 5% (n=6) and Kappa light chain in 1% (n=1).
Conclusion: Among patients referred to our clinical lab, monoclonal gammopathy was found in 4.86 percent of persons. Our findings can be the basis of future screening programs and preventive strategies.

P5.21.24
Role of ABCB1 transport activity in the chronic lymphocytic leukemia cells sensitivity to the different anticancer drugs

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Chronic lymphocytic leukemia (CLL) is heterogeneous disease with variegated clinical finding, varying duration of illness and the answer to the therapy, caused by abnormal accumulation of the pathological and immunologically incompetent lymphocytes (usually B-cells) in the bone marrow, nodi lymphatici and peripheral blood. By present time this disease is considered incurable. Unfortunately, the basic barrier during cancer therapy is apoptosis failure in CLL-cells due to different molecular mechanisms. One of which is active drugs elimination from the cell mediated by the integral membrane protein P-glycoprotein (P-gp or ABCB1).
In this study we investigated P-gp transport activity (UIC2-shift assay) in the total population of B-CLL human lymphocytes, $CD19^+$ and $CD5^+CD19^+$ cells *in vitro* after treatment with therapeutic concentrations of anticancer drugs (analogues fludarabine and cladribine, doxorubicin and vincristine) and simultaneously assessed the sensitivity of these cells to used drugs and identified correlations.
It was shown that the percentage of P-gp, which was revealed on the B-CLL cells plasmatic membrane after therapeutic concentrations drugs exposure *in vitro* (during 20h) directly determines the survival of B-CLL lymphocytes total population (r_s=0,81; p=0,00015), $CD19^+$ (r_s=0,77; p=0,00048) and $CD5^+CD19^+$ cells (r_s=0,78; p=0,00034) after 44h incubation with investigated drugs (MTT-assay).
Thus, P-gp transport activity doesn't depend on the investigated anticancer drugs substrate specificity (nucleosides purine analogs are not substrates for P-gp). CLL-lymphocytes survival after 44h drugs exposure is mainly related with P-gp functioning in these cells and doesn't determine its further (after 20h) overexpression and exposition on the plasmatic membrane surface.

P5.21.25
Diffuse large B-cell lymphoma (DLBCL) presenting during maintenance therapy for acquired hemophagocytic lymphohistiocytosis (HLH) of unknown origin: a case report

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Acquired HLH is a rare and challenging disorder characterized by activation of histiocytes in hematopoietic organs. It has been associated with infections, autoimmune disorders and malignancies (lymphomas and leukemias of T-cell lineage). Herein, we describe a late onset DLBCL during HLH treatment.
A Caucasian 58 y-old man presented with persistent fever and severe anemia. Blood tests showed marked anemia, increased LDH and sIL2-R, hyperferritinemia and hypertriglyceridemia; microbiological (included EBV-DNA) and immunological tests were negative. PET-CT and CT scan documented only mild splenomegaly. Bone marrow aspirate showed activated macrophages and hemophagocytosis. The expression level of perforin was decreased; mutational analysis excluded familial HLH. We concluded for acquired idiopathic HLH. During steroid therapy he developed respiratory symptoms and signs of cerebral vasculitis. We started treatment with etoposide and dexamethasone (HLH-94 protocol), with immediate clinical improvement. After induction therapy, anemia and neurological symptoms reappeared; thus etoposide treatment was resumed as a bridge to allogenic HSCT. Four months later, during maintenance therapy, he developed a bulky inguinal lymphadenopathy, consistent with DLBCL, and recurrence of HLH-symptoms. A combined therapy (R-CHOEP21) induced prompt clinical and bio-humoral improvement.
In our patient, there was no clear evidence of lymphoma at presentation. We can hypothesize the existence of smoldering lymphoma at the time of HLH presentation. Etoposide probably controlled the cytokine storm, but did not affected or might even have favoured lymphomagenesis. In conclusion, during work-up of HLH, occult malignancies should be aggressively ruled out as they can manifest at diagnosis or appear during or after treatment

P5.21.26
Defining antibody responses in normal and non-malignant human aging populations

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Understanding immunosenescence in ageing is a pressing need to counter the increased susceptibility to infections, as apparent in the inadequate response to influenza virus that underlies>90% of deaths associated with infection in the elderly. The emergence of non-malignant conditions like Monoclonal Gammopathy of Undetermined Significance (MGUS) in the elderly may further alter this susceptibility, and remain poorly defined.
To investigate this, we examined linked B- and T-cell responses to influenza vaccination in a healthy elderly cohort (n=19) and contrasted these with aged individuals with MGUS (n=19), a benign plasma cell monoclonal expansion in the bone marrow that secretes elevated levels of monoclonal protein. Influenza H1N1- and H3N2-specific IgG levels were measured and the frequencies of H1N1- and H3N2-specific IFN-γ-secreting cells assayed by enzyme-linked immunospot.
Comparable influenza-specific IgG titers were seen in HCs and MGUS at a cohort level, and MGUS patients showed a significant increase in influenza-specific IgG responses post-vaccination. However, significant differences emerged in MGUS in relation to M-protein levels. MGUS with low M-protein showed increased influenza-specific IgG titers post-vaccination but MGUS with high M-protein had lower influenza-specific IgG titers which they failed to expand post-vaccination. Contrary to HCs, MGUS patients revealed impaired T-cell responses, as the number of IFN-γ-secreting cells didnot increase post-vaccination. M-protein concentration inversely correlated with influenza-specific IgG response at d7 (p=0.020) and d28 (p=0.012) as well as with the number of IFN-γ-secreting cells (p=0.018) at d28 post-vaccination.
MGUS, as a non-malignant clonal expansion, depresses immune responses to influenza vaccination that varies strikingly with M-protein levels.

P5.21.27
Oligomeric nucleophosmin forms as a marker of tumor cells and proliferating lymphocytes

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Nucleophosmin plays a critical role in cell proliferation and apoptosis. Malignization of cells leads to overexpression of nucleophosmin and appearance of its abnormal forms. It has been demonstrated that the unique B23 conformation in liver tumor cells likely results from an N-terminal truncation of nucleophosmin that forms SDS-resistant, GB-sensitive oligomers (Ulanet et al., PNAS 2003). We revealed unusual SDS-resistant oligomers of nucleophosmin in human tumor cells of different type (HeLa, Hep G2, K-562, Jurkat, Ramos, U-87, JMR-32). We created antipeptide antibodies which allowed to differentiate monomers and oligomers and to reveal different intracellular localization of them in tumor cells. Immunochemical analysis of nucleophosmin in human peripheral lymphocytes stimulated to proliferation by phytohemagglutinin allowed us to show that during 48-72h treatment (when majority of the cells undergo the S-phase) not only dramatically increasing in nucleophosmin content but also appearance of SDS-resistant oligomers with nucleolar localization occurred. Immunocytochemical analysis of nucleophosmin in

lymphocytes from healthy donors and patients with non-malignant and malignant diseases of the blood revealed a significant nucleolar staining with antibodies specific for nucleophosmin oligomers only in samples of patients with malignant diseases. Staining intensity was correlated with the proportion of proliferating cells (according to Ki-67 staining cells). Thus, SDS-resistant oligomers of nucleophosmin can be used as markers of proliferating activity of lymphocytes, and our antibodies can be used as instruments for their detection. This work was supported by the programm of Basic Sciences to Medicine Foundation (projects 2011-2013) and by the RFBR (grant 13-04-00654).

P5.21.28
High polyunsaturated fatty acids diet promote myelopoiesis through regulating matrix metalproteinases in bone marrow

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N-6 polyunsaturated fatty acids (n-6 PUFAs), which are rich in western high fat diet, promoted obesity and chronic inflammation associated diseases. In contrast, n-3 polyunsaturated fatty acids (n-3 PUFAs) exert beneficial anti-inflammatory effects and widely used in tumor prevention and inflammatory diseses treatment. However, the effects of these PUFAs metabolism on hematopoiesis remain largely unexplored. Here we feed mice with HFD or high n-3 PUFAs diet, and the results showed that both HFD and high n-3 PUFAs diet increased the frequency and total number of CD11b+Gr-1+ myeloid cells in spleen. Compared with HFD mice, high n-3 PUFAs induced more myeloid cells even in early stage. Meanwhile, in high n-3 PUFAs group, more Lin-CD117+Sca-1+ hematopoietic stem cells(HSC) and Lin-CD117+Sca-1- hematopoietic progenitors can be detected in spleen, but only more HSC not hematopoietic progenitors can be found in bone marrow. Mechanically, high n-3 PUFAs diet promoted myelopoiesis tightly associated with high expression of matrix metalproteinase 12(MMP-12) in bone marrow, a protein known to regulate hematopoietic nich. Taken together, our study provides a novel insight into the physiological effects of high PUFAs metabolism on hematopoiesis.

P5.21.29
Effect of garlic consumption on AgNORs (argyrophilic nucleolar organizer regions) pattern in splenocytes and thymocytes of rats

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Nucleolar organizer regions (NORs) are chromosomal segments which contain ribosomal genes and a set of argyrophilic acidic proteins. The number and size of argyrophilic NORs (AgNORs) are often related directly to the cellular demands for ribosome biogenesis and cell proliferation. To test the hypothesis that lymphocytes proliferation associate with garlic consumption, seventeen 6-week-old male rats were randomly separated into two groups. Eight rats were given garlic solution in distilled water (600 mg $kg^{-1}4mL^{-1}$) by gavage and nine received distilled water for 30 days. Silver staining of NORs was performed on the fixed tissues of spleens and thymuses of the animals. The images of 100 analyzable nuclei of lymphocytes from each tissue were transferred by means of a digital camera from light microscope to a computer and nuclear area (NA), nuclear length (NL), total AgNORs area (TAA), total AgNORs length (TAL) and total AgNORs number (TAN) were analyzed by image analyzer software. In spite of a decline in the means of NA and NL in thymocytes of garlic treated rats ($p<0.001$), TAA and TAL were increased significantly ($p<0.001$) in splenocytes and thymocytes of this group. In conclusion, garlic enhanced the lymphocytes proliferation rate in spleen and thymus and this effect was stronger in thymocytes compared with splenocytes.

P5.22 Primary immunodeficiencies

P5.22.01
Identification of B-cell defects using age-defined reference ranges for in-vivo and in-vitro B-cell differentiation

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Background: Primary immunodeficiencies consist to a large extent of B-cell defects, as indicated by inadequate antibody levels or response upon immunization. Many B-cell defects have not yet been well characterized.
Objective: To create reliable in-vivo and in-vitro assays to routinely analyze human B-cell differentiation, proliferation, and immunoglobulin production and to define reference ranges for different age categories. The in-vitro assays were applied to classify the developmental and/or functional B-cell defects in patients previously diagnosed with common variable immunodeficiency (CVID).
Methods: Apart from standard immunophenotyping of circulating human B-cell subsets, an in-vitro CFSE dilution assay was used for the assessment of proliferative capacity comparing T-cell-dependent and T-cell-independent B-cell activation. Plasmablast/plasmacell differentiation was assessed by staining for CD20, CD38 and CD138, and measurement of in-vitro immunoglobulin secretion.
Results: At young age, B-cells proliferate upon in-vitro activation but neither differentiate nor produce IgG. These latter functions reached 'adult' levels at 5 and 10 years of age for T-cell-dependent versus T-cell-independent stimulations, respectively. The capacity of B-cells to differentiate into plasmablasts and to produce IgG appeared to be contained within the switched memory B-cell pool. Using these assays, we could categorize CVID patients into subgroups and identified a class-switch recombination defect caused by an UNG mutation in one of the patients.
Conclusion: We defined age-related reference ranges for human B-cell differentiation. Our findings indicate that in-vivo B-cell functionality can be tested in-vitro and help to diagnose suspected B-cell defects.

P5.22.02
Autoimmune lymphoproliferative syndrome in a patient with homozygous mutation associated to normal Fas expression

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Autoimmune lymphoproliferative syndrome (ALPS) is a primary defect in programmed cell death, characterized by tumoral syndrome, autoimmune manifestations and accumulation of double-negative T cells (DNT). The majority of patients have heterozygous germline mutations in the *FAS* gene associated to a normal Fas expression. Few descriptions of a complete lack of Fas protein expression caused by homozygous mutations in the *FAS* gene have been reported. Here we report a novel homozygous *FAS* gene mutation associated to normal Fas expression.
The patient was 22-year-old Tunisian female. She presented since the age of 7 days with splenomegaly. She developed later multiple adenopathy, anemia and thrombocytopenia. Flow cytometric immunophenotyping showed a high percentage of DNT cells (9%) and a normal expression of the Fas receptor. Very high levels of plasma IL10 (119 pg/ml) and sFasL (>5 ng/ml) were found. Molecular analysis of the *FAS* gene identified a homozygous cytidine to thymidine transition at exon 4 in the second cystein-rich–homology domain (c.C581T/p.R120W). This mutation is probably deleterious as predicted by Polyphen 2 software and was not found in 50 healthy control subjects ruling out the possibility of irrelevant polymorphisms. A previous study describes three affected siblings which carry the (c.C581T) mutation but in a heterozygous state, associated to another missense mutation on the second allele.

This mutation does not affect Fas protein expression but seems to be associated to severe clinical and immunological phenotype. Further molecular and immunological investigations are required in patient and her parents to assess a new autosomal recessive ALPS pattern.

P5.22.03
Evaluation of CD4+CD25+FOXP3+ regulatory T cells function in patients with common variable immunodeficiency

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Common variable immunodeficiency (CVID) is one of the predominant antibody disorders where abnormalities in regulatory T cells (Tregs) may result in autoimmunity and chronic inflammation. To evaluate Tegs frequency and function, 13 CVID patients and 10 age- and sex-matched healthy volunteer were enrolled. The percentages of Tregs were calculated using flow cytomety method. For assessment of Treg function, Tregs were isolated and their suppressive functions were determined using Tregs suppression assay. The levels of immunoregulatory cytokines IL-10 and TGF-β produced by Tregs were also measured. Our results revealed that Tregs frequency ($P<0.001$) and the expression of FOXP3 protein based on its mean fluorescence intensity (MFI) ($P=0.02$) were significantly lower in CVID patients than that in controls. Also, the suppressive functions of Tregs were impaired in CVID patients ($P<0.001$). The level of TGF-β did not differ between CVID patients and controls ($p=0.09$); while the amount of IL-10 was remarkably decreased in CVID patients ($P = 0.007$). Our findings suggest that disturbed Tregs frequency and their functional characteristics might account for aberrant immune responses observed in CVID patients.

P5.22.04
AK2 deficiency in zebrafish recapitulates human reticular dysgenesis

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The adenylate kinase (AK) gene family members are critical players in ensuring cellular energy homeostasis by converting ATP+AMP to 2ADP. The AK2 member is uniquely located in the mitochondrial intermembrane space and is mutated in reticular dysgenesis (RD), an autosomal recessive form of severe combined immunodeficiency characterized by early differentiation arrest in the granulocyte lineage and impaired lymphoid maturation. The mechanisms underlying the pathophysiology of RD remain unclear. No AK2 deficient animals have been reported in the literature, but we have found that murine lines carrying homozygous inactivating retroviral insertions are embryonically lethal. We used zebrafish to perform a comprehensive study of the effects of AK2 deficiency using morpholino oligomer (MO) injections and two different AK2 mutants (a ENU-induced T371C/L124P missense mutant and a null mutant generated using Zinc-finger nuclease technology). In-situ hybridization analyses of AK2-deficient embryos indicated that only erythroid development was affected during primitive hematopoiesis. Conversely, during definitive hematopoiesis, the loss of AK2 function resulted in abnormalities in all hematopoietic lineages suggesting an impairment of hematopoietic stem cell (HSC) development. Moreover, AK2 deficiency induced increased level of reactive oxygen species triggering oxidative stress and consequent apoptosis in primitive hematopoietic progenitor cells and definitive HSCs. Our data suggest a broader role of AK2 during hematopoiesis and provide new insights into the AK2 function. Moreover, they indicate that zebrafish represents a good model for studying the molecular mechanisms involved in RD. To date, our mutant lines represent the first example of animal models of this rare and unique human disease.

P5.22.05
Monoclonal T proliferation associated with a diffuse and invalidant *Sezary-like* skin-infiltration in a patient with Hyper-IgM syndrome

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Hyper-IgM syndromes are a group of primary immunodeficiency disorders characterized by a defective Ig class switch recombination. Signalling through CD40 is the most commonly affected, thus leading to a combined immunodeficiency. Clinical phenotype is dominated by recurrent sinopulmonary and opportunistic infections. An increased risk of malignancies is also observed, including liver and biliary-tract carcinoma and peripheral neuroectodermal tumors. Lymphoproliferative diseases have been rarely described.
We report a case of a 28-years-old man with an X-linked Hyper-IgM syndrome diagnosed at the age of 3, after a meningeal criptococcal infection which led to a complete blindness. Since then, the patient has been totally asymptomatic under Ig replacement therapy. During the latter years the patient developed psoriasiform lesions on the trunk and upper limbs, treated with topical steroids, UVB and Toctino®. In December 2012, the patient experienced heavy worsening of cutaneous lesions, in terms of extension and symptoms. A skin biopsy showed an "atopic-like dermatitis". Blood flow cytometric immunophenotyping showed a population of $CD4^+/CD3^+/CD25^+/CD7^{dim}$ immature T-cells, resulting negative for CD5, CD8, CD10, CD27, TCR^- and class-II MHC. T-cell clonality was confirmed by molecular biology analysis. PET-CT scan showed a moderately increased FDG-uptake in subcutaneous tissues and inguinal lymph nodes. The first chemotherapy administration (according to VACOP-B regimen) was ineffective and complicated by severe and febrile neutropenia. A second-line attempt with cyclosporine was complicated by renal failure and sepsis, which led the patient to death.
The case herein described represents the first report of a peripheral T-cell lymphoma in this rare B-cell immunodeficiency.

P5.22.06
Investigation of ITGB2 gene in 9 Iranian patients with LAD-1 with two new mutations

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Objective: The ITGB2 gene codes for the CD18 antigen (integrin β2 subunit). Any defect in this gene leads to Leukocyte Adhesion Deficiency type-1, an autosomal recessive immunodeficiency disorders. These patients have defects in adherence and migration of leukocytes, in particular of neutrophils, through the intravascular space. In this study, we have evaluated molecular diagnosis of 9 Iranian patients with diagnosis of LAD-1.
Methods: All patients with definite diagnosis of LAD-1 (based on clinical finding, screening tests for primary immunodeficiency diagnosis and flowcytometric analysis) who referred to the Immunology, Asthma and Allergy Research Institute (IAARI) from September 2006 to April 2011 entered this study. After informed consent, the ITGB2 gene was investigated on genomic DNA by PCR and direct sequencing for patients and their parents.
Results: All of the nine patients had significant decreases in expression of CD18 on neutrophils with WBC counts more than 16000 cells/µl. Mutation analysis of ITGB2 gene in nine patients revealed nine different homozygote mutations, including six missense mutations (c.382G>A, c.2146G>C, c.715G>A, c.1777C>T, c,691G>C and novel c.1336G>T), Two frameshift (c.1907delA and novel c. 474dupC) and a splice site mutation (c.1877+2G>A). All of parents were heterozygous for these mutations.

Conclusion: Our findings show two new mutations in the ITGB2 gene. Up to now, over than 60 mutations have been reported for the ITGB2 gene. This report can be used for decisive genetic diagnosis, genetic counseling and also prenatal diagnosis for all patients who are suspected to have LAD-1.

P5.22.07
Adenosine deaminase deficient severe combined immunodeficiency: evidence from three case reports

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Adenosine deaminase (ADA) deficiency is a complex metabolic and immunological disorder, characterized by a severe immunodeficiency due to the accumulation of purine metabolites. Here we report three cases of severe combined immunodeficiency due to ADA deficiency.
Three children 8 days, 50 days and 5,5 months old admitted to our hospital with pulmonary infection. They had severe lymphopenia and decreased IgG, IgM, IgA levels with reduced CD3, CD4, CD8, CD19 and CD16+56 positive lymphocytes. Metabolic findings and mutation analyses were consistent with ADA deficiency.
A matched bone marrow transplant donor could not be found and ADA replacement therapy was initiated for the first and third patients. B lymphocyte and natural killer cell counts reached to normal values in the second week of PEG-ADA therapy in both patients. In follow up the third patient died from pulmonary insufficiency at two month of ADA enzyme replacement treatment. At three years of age, diffuse large B cell lymphoma developed in the first patient and on the twentieth day of treatment the patient died from septic shock and intracranial hemorrhage. The second patient underwent bone marrow transplantation from an HLA identical healthy sister.
Within this report we want to emphasize the importance of screening for adenosine deaminase deficient severe combined immunodeficiency in children with lymphopenia.

P5.22.08
Plasmablasts in peripheral blood after antigen challenge serve as a diagnostic marker for assessing response to vaccination in CVID patients

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Common variable immunodeficiency (CVID) is characterized by hypogammaglobulinaemia and impaired antibody production. Poor vaccination response is essential for the diagnosis of CVID. Determination of antibody production in serum from CVID patients after vaccination and investigation of B cell function in vivo is complicated due to substitution therapy. Therefore we investigated changes in B-cell subpopulations in CVID patients, including plasmablasts, in peripheral blood by flow cytometry after vaccination. Twenty CVID patients and fifteen healthy volunteers were immunized with 23-valent pneumococcal polysaccharide vaccine (PPSV23). Specific antibody levels and B cell subpopulations were measured before vaccination and on day 30 by ELISA assay, and day 7 by flow cytometry after vaccination, respectively.
100% CVID patients lacked detectable changes in the serum level of anti-pneumococcal IgG antibodies against PPSV23 (mean: 122.41±41.94 mU/ml vs. mean: 128.37±52.13 mU/ml, respectively; p=0.24). In none of patients an increase of plasmablasts was noted. In the control group, an increase in circulating plasmablasts on day 7 post immunization corresponded with the appearance of specific antibody levels on day 30 post immunization (r=0.823, p=0.000001) and was statistically significantly higher than before a dose of PPSV23 (before vaccination: 20.12±14.93%, 0.46±0.36x10^3/mm^3; after vaccination: 46.81±26.87%, 1.15±0.77x10^3/mm^3; p=0.01). In contrast, CVID patients failed to increase plasmablasts in peripheral blood after antigen challenge.
Our findings indicate that CVID patients have a block in terminal B-cell differentiation and that flow based assessment of plasmablasts in peripheral blood after vaccination serves as a surrogate diagnostic marker for assessing in vivo antibody responses in patients suspected to have CVID.

P5.22.09
Clinical features and immunological immunophenotyping in Hyper IgE syndrome patients

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Hyper-IgE syndrome (HIES) is a primary immunodeficiency caused, mostly, by autosomal dominant STAT3 mutations; in contrast, DOCK8 mutation is responsible for the less common autosomal recessive form. Both conditions are characterized by eczematoid rash, high serum-IgE levels, recurrent respiratory infections and candidiasis. The dominant form shows prevalence of cold abscesses, recurrent pulmonary pyogenic infections and skeletal malformations, while in the recessive form prevail skin viral infections, neurologic symptoms and T-cell lymphopenia.
We studied the T cell phenotype in 13 HIES patients, including 6 affected with the dominant disease and 7 with the DOCK8-deficient form.
Because Th17 is a subset of CD4+ CD45RO+ T-cells, with CCR6+ and CCR4+ receptor chemokine expression which is essential for immune response to mycotic infections, we evaluated chemokine receptors expression in T cells and generation of Th17 in these patients.
We found that Th17 cells were absent or extremely low in both forms. Analysis of CD4+ subset showed that CCR4 expression, a marker associated with Th2 commitment, was higher in HIES patients with DOCK8 mutations than STAT3-mutated patients. Moreover, a low number of CD4+ memory cells expressing CCR6+ were observed in both groups of Hyper-IgE forms. A further distinctive feature observed in DOCK8 form was a higher incidence of allergies to cow's milk protein airborne allergens. Finally, in STAT3-deficient form a lower IL-10 production in CD4 stimulated cells culture was detected.
Taken together, our results suggest that the detailed analysis of CCR6 and CCR4 expression on T-cells can help to characterize the two forms of HIES.

P5.22.10
Long survival of a patient with IPEX syndrome (Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked)

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We report the case of a 30-year-old man with a history of intractable diarrhea associated to atopic eczema since he was 5-month-old; a diagnosis of autoimmune enteritis associated to autoimmune thrombocytopenia and arthritis was later made. After several years of artificial nutrition and systemic steroids, a partial clinical remission was obtained and maintained with oral cyclosporine. He was admitted to our Hospital in January 2010 presenting a relapse of intestinal symptoms, severe weight loss (BMI 17.9) and renal failure. Laboratory tests showed malabsorption signs and altered inflammation indexes. Cyclosporine was discontinued and intravenous steroid therapy was started associated with enteral nutrition, with partial benefit. No macroscopic lesions were found at endoscopic examination, whereas severe acute and chronic inflammation was documented at histological analysis. Considering all clinical and laboratory findings, an IPEX syndrome was hypothesized. Serological tests confirmed the positivity of antienterocyte IgG antibodies by indirect immunofluorescence on primate jejunum. Peripheral level of CD4+CD25+FOXP3+ T cells was 1.5% (normal value 3%). At genetic analysis the patient resulted hemizygote for c.816+2delT, a new splicing mutation on FOXP3 gene, confirming the diagnosis of IPEX syndrome. Immunosuppressive therapy with sirolimus (0.03 mg/kg/die) was started, with clinical benefit. After 3

months of treatment, the patient developed a persistent not nephrotic proteinuria, that resolved after replacing sirolimus with tacrolimus (0.1 mg/kg bid). During last 3 years of follow-up, the patient was in clinical remission continuing treatment with tacrolimus, without other complications. This represents the most long-lived case of IPEX syndrome reported so far.

P5.22.11
Clinical features and immunological abnormalities of GATA2 deficiency in Japan

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Introduction: GATA2 is a transcription factor involved in the differentiation of hematopoietic precursor cells. Heterozygous GATA2 mutation has been recently reported to cause MonoMAC syndrome, DCML deficiency, and Emberger syndrome in human. GATA2 mutation is also reported to cause familial MDS/AML.
Objective: The purpose of this study is to identify GATA2 mutations in Japan and to clarify their clinical features and immunological abnormalities.
Methods: We search the dendritic deficiency in PIDJ (Primary Immunodeficiency Database in Japan) registered patients.
Results: We identified five different GATA2 mutations in six patients. These mutations include one missense mutation, two nonsense mutations and three frameshift mutations. All mutations affect the zinc finger domain of GATA2. Four Patients with GATA2 mutations manifested MonoMAC syndrome. Three of them suffered from severe or persistent VZV infections. Two patients had severe salmonella enteritis. One patient infected by Mycobacterium kansasii at 27 years old and Mycobacterium intracellulare at 31 years old. One patient manifested Emberger syndrome with lymphedema. Two of six patients (father and son) progressed into familial MDS though they lack DC, B, NK cells.
Conclusions: Clinical manifestations are variable in GATA2 deficiency though the lack of DC, B, NK cells are common features. Analysis to explain this variability is understudy.

P5.22.12
GATA 2 transcription factor deficiency predisposing to severe disseminated Coccidioidomycosis

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Severe disseminated coccidioidomycosis is rare, while localized disease is more commonly seen. We report the case of a 28-year-old man with severe disseminated coccidioidomycosis who was found to have an underlying GATA 2 (3q21.3) transcription factor deficiency. This case illustrates that when a patient presents with invasive fungal disease without clear predisposing factors, the clinician should consider underlying immunodeficiencies so that proper treatment may be initiated to control spread of the disease to other organs and prevent further complications. Interferon-gamma plays a vital role in anti-fungal immunity, and is involved in both innate and active immune responses (1). GATA2, a transcription factor active in myeloid development, has been shown to be crucial in anti-mycobacterial and anti-fungal immunity. Its deficiency has been reported to be associated with a rare immunodeficiency-MonoMAC syndrome (2). Since there are only a handful of cases, no standard guideline is available for the treatment of patients with GATA2 deficiency and disseminated fungal disease. The patient in this report was successfully treated with multiple anti-fungal medications and Interferon-gamma injections. Hematopoietic cell transplantation may serve as a last resort in the management of such patients (3). This report is intended to increase clinician awareness of the possibility of underlying immunodeficiency syndromes in patients who present with disseminated fungal disease with an unclear etiology and may provide insight in diagnosing and further managing patients with similar presentations.

P5.22.13
Common variable immunodeficiency classification by quantifying T-cell receptor and immunoglobulin κ-deleting recombination excision circles

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Introduction: Common variable immunodeficiency (CVID) is the most frequent primary immunodeficiency associated with hypogammaglobulinemia and other various clinical manifestations. Moreover combined immunodeficiency (CID) involving both defective B and T cells is often misdiagnosed as CVID originally reported as defective B cells.
Objective: The aim is to identify novel markers to better classify CVID and distinguish CID from CVID.
Methods: We recently performed real-time PCR based quantification of T-cell receptor excision circles (TREC) and signal joint immunoglobulin κ-deleting recombination excision circles (KREC) for mass screening of severe combined immunodeficiency (SCID) and B-lymphocyte deficiency in neonates. Forty CVID patients referred to our hospital were analyzed. TREC and KREC quantification was performed using DNA extracted from peripheral blood.
Results: Based on TREC and KREC copy numbers, the 40 patients with CVID were classified into 4 groups (A, B, C, D). The cumulative events of complications (opportunistic infections, autoimmune diseases and malignancies) per 10 patient-years were highest in group D (0.98 events/10 patient-years), followed by group C (0.63), group B (0.30), and group A (0.04) (group A vs group D: $P = 0.0022$; C: $P = 0.0092$; B: $P = 0.0692$).
Conclusion: TREC and KREC are good markers to assess the clinical severity and pathogenesis of each patient with CVID and to distinguish CID from CVID. Thus TREC/KREC levels would provide a helpful tool for deciding on an effective treatment plan for each patient with CVID.

P5.22.14
Candida antibiotic resistance in Primary Immunodeficiency Disease

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Infections due to *Candida* spp. are common, especially in patients with primary Immunodeficiency diseases (PID) .The aim of this study was to evaluate susceptibility of candida spp. Isolates from PID patients who referred to Immunology,Asthma and Allergy Research Institute to 4 known antifungal antibiotics (Amphotricin B,Fluconazole,Voriconazole and Itraconazole).
During 2011-2012 from twenty PID patients who had lesion on their body took samples and cultured. Amphotricin B,Fluconazole,Voriconazole and Itraconazole were tested in this assay. Broth microdilution tests were performed according to the CLSI (Clinical and Laboratory Standards Institute).Each well was inoculated with 1 ml of RPMI-1640 Medium, 1 ml of antifungal agent with 10 serial dilution of each antifungal antibiotics and a fungal suspention contains 10^3 Cell/ml. Positive control well contained fungal suspention, RPMI-1640 Medium and negative control well contained RPMI-1640 Medium and distilled water.
During this study, twenty patients with PID were interred the study who had skin lesion with positive culture.Among these PID patients, 9 male and 11female with meadian age of 7years. Seven positive candida were observed in specific culture that they confirmed with Germ tube test and PCR. An antifungal susceptibility test were done with four antibiotics. Candida spp were susceptible to Amphotricin B; but resistant to the Fluconazole,Voriconazole and Itraconazole

respectively. Consideration of PID patients showed that patients who referred with recurrent infection resulted in hospitalized , may be resistance to antibiotic because of excessive prescription of antifungal antibiotics ,so prevalence of antibiotic resistance significantly increased in PID Patients.
Key word : candida ,antibiotic resistance, PID

P5.22.15
Subgroup of Common Variable Immunodeficiency patients with distinct clinical and biological features revealed by B-cell immunophenotyping

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Common Variable Immunodeficiency (CVID) is characterized by low levels of IgG, IgA, and/or IgM, impaired specific antibody response after antigen challenge and the resulting bacterial infections. Investigation of peripheral blood cellular compartments revealed abnormalities in B- and T-cells. Due to high number of analysed parameters it is difficult to define CVID subgroups that possibly share the same ethiopathological mechanisms.
We have compared distribution of cells within all possible immunophenotypes with 8 color flow cytometry and probability binning to reduce CVID heterogeneity. Ninety-eight patients and 47 healthy donors have created hierarchical tree according to B-cell phenotype similarities. The cohort has split into 11 phenotype clusters. B-cell cluster no.5 has been distinguished by aberrant phenotype of T-cells. Cluster 5 CD4+ T-cells have been reduced and presented with decreased proportion of naive cells and increased proportion of intermediate effector memory cells (CD27-CD28+). Increased expression of CD57, PD-1, CD69 and CD70 has suggested a chronic activation but activating cytokine levels have not been elevated.
Moreover cluster 5 patients suffer from autoimmunity and splenomegaly. Similar clinical presentation and phenotypic profile have supported the idea that similar pathological mechanism might be responsible for this phenotypically defined subgroup of patients. Whole-exome sequencing has yielded 23 possibly damaging gene alterations (e.g. single-nucleotide polymorphisms) presented in three cluster 5 patients, but not in control samples that are currently being investigated.
B-cell profiling of large group of CVID patients revealed subcluster with distinct T-cell profile, clinical presentation and shared germline genetic variations.
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P5.22.16
Hax-1 deficiency in Turkish children

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Severe congenital neutropenia (SCN) is characterized by absolute neutrophil count below 0.5 x 10/l. SCN is caused by mutations in the ELA2 and *HAX1* genes.
Purpose: The proposed study aims to analyse neutrophil elastase (ELA2) gene and HAX-1 gene mutations in 11 sporadic cases and four familial cases of SCN and their family members.
Methods: Genomic DNA will be extracted from the patients' and family members' peripheral blood and the coding sequence of the ELA2 gene and HAX1 gene will be amplified by polymerase chain reaction and subjected to direct sequencing. Imaging tests for CNS will be done, if the patient has neurological problem.
Results :W44X mutation in Exon 2 of HAX1 gene was determined in 9 of 15 patients with congenital neutropenia that are in the same family. This mutation was also determined at the same region in five of sporadic cases. We found heterozygous mutations at W44X, 10 of 49 index family members, and an homozygous mutation one of them. ELA 2 mutations were not detected in any of the patients examined. Mild Mental Retardation (MMR) was observed in 5 of the patients with HAX1 mutation. The results of cranial MRI were normal in 9 of the patients with HAX1 mutation. Neurological examinations of HAX1 carriers and of patients without HAX 1 mutation were normal.
Conclusion : At variance with European publications, we suggest that HAX 1 mutations are widespread in our country and may be related to MMR.

P5.22.17
Investigation on frequency of mutation in the gene segments of immunoglobulin in CD40 knockout mice

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Activation-induced cytidine deaminase (AID) is essential for class-switch recombination (CSR) and somatic hypermutation (SHM). AID is selectively expressed in germinal center B cells and its activation is dependent upon CD40 engagement. AID KO mice show defective CSR as well as SHM. Although deficiency of CD40 caused a complete loss in CSR and showed a hyper IgM phenotype as in AID KO mice, the role of CD40 on SHM is still unclear. In the present study, we investigated whether CD40 regulate SHM using CD40 KO mice. WT and CD40 KO mice were immunized with nitrophenylated chicken gammaglobulin (NP-CGG) absorbed to Freund's complete adjuvant (FCA) or alum. In the absence of CD40 gene, SHM was not affected in both spectra and frequency in V_H gene of IgM although the expression of AID was markedly reduced in CD40 KO mice. Based on these results, we are engaged in the investigation of AID-dependent and independent pathway in CD40-mediated SHM in B cells.

P5.22.18
Frequency of lymphomas in a cohort of Common Variable Immunodeficiency (CVID) patients

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Our objective was to evaluate the frequency of lymphomas in a cohort of CVID followed at the PID Clinic. Methods: Analysis of data obtained from medical records. Results: We analyzed 144 CVID patients followed between 1985 and 2012, being 80 men, aged 15-72 years at diagnosis. Seven lymphoma cases were diagnosed (0.5% - 5 men, 2 women) being 1 Hodgkin lymphoma, 6 non-Hodgkin lymphomas. Age of onset of CVID symptoms ranged from 2 - 53y, age at CVID diagnosis from 5 - 55y, age of lymphoma diagnosis from 19 - 53y. The time period between CVID onset of symptoms and lymphoma diagnosis ranged from 7 to 24 years in 4 patients and in 3 the diagnosis of CVID and lymphoma nearly overlapped, preceding CVID diagnosis in only 1 to 6 months. Lymphoma´s manifestations like fever of unknown origin, weight loss, splenomegaly and lymphadenopathy occurred in 100% of patients. Other manifestations observed: renal infiltration (2), hepatic infiltration (4), pulmonary infiltrates (3) and mediastinal mass (1). Three deaths due to lymphoma occurred during the study. Two patients presented lymphocytosis with inversion of CD4/CD8 ratio and one lymphopenia. Increased NK cells were observed in 3. Three patients in whom diagnosis of lymphoma and CVID were simultaneous presented undetectable B lymphocytes while in one, B cells were present at CVID diagnosis but undetectable at lymphoma diagnosis. Conclusions: Patients with absence of B cells in recently diagnosed CVID or vanishing B cells in long term follow-up CVID demands a screening for the presence of lymphomas.

P5.22.19
Genetic analysis of the TNFRSF13B gene in a numerous Italian CVID population

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Introduction: Common variable immunodeficiency (CVID) is characterized by hypogammaglobulinemia, recurrent bacterial infections and defective antibody response. Mutations in ICOS, TACI, CD19, CD20, CD21, CD81 and BAFF-R have been associated with CVID; mutations (homozygous, heterozygous and compound heterozygous) in the gene coding for TACI (TNFRSF13B), are found in 8-10% of CVID cases.
Objective. Analyze TNFRSF13B in Italian CVID patients in order to determine the frequency of TACI mutations and compare the results to the ones reported in literature.
Methods. 115 Italian CVID patients were included in this study. Diagnosis of CVID was made according to ESID criteria. Informed consent was obtained. TNFRSF13B coding regions were amplified through PCR and direct sequencing was performed using an ABI130 sequencer.
Results. The genetic screening evidenced the presence of 14 known variants, 3 synonymous and 11 determining amminoacidic change. **T**he frequencies of A181E and S277S mutations in our cohort are statistically increased when compared to the general population, while C104R and I87N incidences were more frequent, also compared to CVID population. Rare mutations **(**Y164X , L171R and C172Y) normally not found in the general population, were present in our patients although with low frequencies. Regarding compound heterozygous mutations we identified some already documented ones (C104R/C104Y, c204insA/C104R) and two novel ones (C104R/L171R; I87N/C104R).
Conclusions. Our genetic analysis of TNFRSF13B gene reports new compound heterozygous mutations in a large Italian CVID population. The other mutations reported in literature are well represented even if, in some cases, with differences in incidence.

P5.22.20
Hypomorphic mutation in RAG2 gene affects dendritic cell distribution and migration

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Omenn Syndrome (OS) is a severe combined immunodeficiency characterized by erythrodermia and protracted diarrhea due to infiltration of oligoclonal activated T cells, caused by hypomorphic mutations in recombination activating genes (RAGs). $RAG2^{R229Q}$ mouse model fully recapitulates the clinical OS phenotype. We evaluated whether T and B cell defects, together with the abnormal lymphoid structure, could affect dendritic cells (DCs) homeostasis and function. High density of Langerhans cells (LC) was observed in skin biopsies of Omenn patients and in the derma of $RAG2^{R229Q}$ mice, correlating with the presence of erythrodermia. *In vivo* models of cutaneous skin painting and contact hypersensitivity (CHS) demonstrated a decreased migration of $RAG2^{R229Q}$ DCs, in particular LCs, into draining lymph nodes. Interestingly, $RAG2^{R229Q}$ mice showed a reduction in DCs number in all hematopoietic organs, except lymph nodes. Analysis of MHCII marker revealed a diminished expression at steady state and upon LPS driven inflammatory condition. Despite the decreased number of peripheral DCs, conventional DCs bone marrow precursors (pre-cDCs) were present in normal number as compared with $RAG2^{+/+}$ controls, whereas plasmacytoid DCs (pDCs) and monocytes were significantly reduced. Overall, these results point to a secondary defect in DC compartment, which contributes to clinical manifestations and autoimmunity in OS.

P5.22.21
8 patients of ataxia-telangiectasia in Azerbaijan

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Introduction: Ataxia-telangiectasia is pathology of the immune system that occurs as a result of a genetic defect. This disease is autosomal recessive and a large mutant AT (ATM) gene was identified on chromosome 11q22-q23. In these patients the nervous, immune and other systems are damaged and the risk of cancer is high.
Objective: In 2009-2012 8 children with frequent infectious diseases, developed ataxia and dementia, telangiectasia in the eyes were analyzed by immunological parameters.
Methods: We used flow cytometer for leucocyte subset identification in peripheral blood and ELISA for measuring serum IgM, IgG, IgA, IgE levels. Phagocytic activity of neutrophils was performed by NBT. Circulated Immune Complex was examined by photometric method.
Results: It was found that in patients aged 7-16 years the number of CD3+, CD4+, CD8+, CD19+ cells and CD4/CD8 ratio were seriously low than normal, the quantity of CD16/56 + was many times higher. Results of NBT test were low. Especially it should be noted, that only 16 years old patient has normal CD3+, CD4+, CD19+ and CD16|56+, but increase of CD8+ cells. In all patients were determined very low levels of IgA, IgG, IgE, the level of IgM in many patients was much higher than norm. Four of patients had genetic analyses and ATM mutations gene was detected. 6 of patients were died. in ages 5-12 from bacterial complications or cancer.
Conclusion: In the country with many congenerous marriage the frequency of genetic pathology is high. Timely prenatal diagnose and adequate therapy are very important.

P5.22.22
Good's syndrome: First case report in Algeria

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Good's syndrome is a rare adult- onset cause of combined T and B immunodeficiency. Clinically, it is revealed either by a thymoma, or by a recurrent respiratory infection. Patients with Good's syndrome show a high susceptibility to common and opportunistic infections, particularly, respiratory ones which may be life-threatening. We report the case of a 61 years old man, referred to us for the investigation of a mediastinal bulge demonstrated earlier by a chest X- ray. The patient has been healthy until the age of 45, when he developed a pneumococcal chronic otitis. Three years ago, he started to experience recurrent bronchitis. At presentation, the patient had fever, chronic bilateral otitis complicated by a facial palsy, thrush with cutaneous mycosis. High-resolution CT scan showed a rounded mass, bilateral bronchiectasis infected by Pseudomonas aeruginosa and vertebral bodies hemangiomas. Culture of pleural fluid yielded Klebsiella pneumoniae. Serology for HIV was negative; CMV serology came back positive for IgG. Immunological tests revealed a severe hypommaglobulinemia, absence of B cells and T CD4+ lymphopenia. The patient was put under antibiotics according to the antibiogram results. A cyclic IVIG replacement therapy was initiated. After the otitis and bronchitis symptoms improved, the patient was admitted for surgical removal of the thymoma which was labeled type AB upon histological examination, according to the WHO classification. The immunodeficiency found in our patient persisted after the resection of the tumor.
Thymoma association with hypogammaglobulinemia in this patient allowed the diagnosis of Good's syndrome, first case ever described in Algeria.

P5.22.23
Influence of genetic alterations in the WASP gene on the maturation of the B cell compartment

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The Wiskott-Aldrich syndrome protein (WASp) is a multifunctional domain protein expressed in all hematopoietic cell lineages. Mutations in WASp coding sequence are causative of a variety of X-linked disorders, among which Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT) are the most frequent. Both these diseases are characterized by microthrombocytopenia, eczema and immunological defects, affecting also humoral immunity.
Our analysis on 40 XLT/WAS patients showed a general impairment in forming a normal "IgM"-memory compartment, while generation of an adequate number of switched cells correlate with the severity of WASp mutations. Moreover, the flow cytometric measure of WASp expression allowed us to confirmed the presence of this impairment in two different in vivo systems in which wt and mutated cells coexist. In fact, we observed a progressive increase of the ratio between WASp+ and WASp- cells during B cell differentiation both in female carriers of WASP mutations and in WAS patients who display a mixed chimerism in the B cell compartment after hematopoietic cell transplantation.
Despite the inability to form an adequate memory B-cell compartment, we could demonstrate that the percentage of effector B cells (plasmablasts/plasmacells) in our cohort of WAS/XLT patients is in the normal range when not increased. The hypothesis of a normal capacity to generate immunoglobulin secreting cells is supported also by the in vitro generation of an adequate amount of WASp-plasmacells in females carrier of WASP mutations, thus suggesting that differentiation into effector B cells may occur through a WASp independent pathway.

P5.22.24
A normal T cell receptor beta CDR3 length distribution in patients with APECED

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Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a monogenic autoimmune disease caused by mutations in the AIRE gene. AIRE is thought to control the ectopic expression of tissue specific antigens in the thymus. This would lead to generation of autoreactive T cells in APECED patients. Alternative models suggest that AIRE deficiency might lead to more profound changes in thymic architecture or premature activation of T cells in the thymus. Our objective was to assess these possibilities using TCR CDR3β length repertoire analysis. The repertoire is known to shorten during normal thymic development and therefore a comparison between patients and healthy controls should reveal the existence of a profound developmental defect.
TCR repertoire spectratyping and sequencing were used to calculate average CDR3β lengths of thymocytes and peripheral blood T cell populations.
To analyse the normal thymic shortening further, we examined CDR3β repertoires of DP $CD3^{low}$, DP $CD3^{high}$ and CD4 SP thymocytes. Significant shortening was observed between each stage, demonstrating coupling of the shortening to positive and negative selection processes. We then proceeded to compare the CDR3β repertoires of $CD4^+$ and $CD8^+$ T cells of patients and controls and their $CD45^{RA}$ and $CD45^{RO}$ subsets, which represent activated and resting or memory cells, respectively. In each case we observed no significant difference in the length repertoires.
Our results suggest that the defect in T cell development in APECED patients is more likely a subtle one, not affecting the overall CDR3β length repertoire of peripheral T cell populations.

P5.22.25
Clinical and laboratory evaluation of patients with common variable immunodeficiency before and after immunization with polysaccharide and protein antigens

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This study aims to evaluate the clinical response to immunization with protein and polysaccharide antigens (*influenza*, *H1N1* and *pneumococcus*) in CVID patients followed at the Primary Immunodeficiency outpatient clinic of the Division of Clinical Immunology and Allergy, Hospital das Clínicas, FMUSP. CVID patients were diagnosed according the IUIS criteria. Thirty-seven patients were immunized against influenza (H2N3), H1N1 and pneumococcal polysaccharide vaccine while another group with 16 CVID patients were not vaccinated. Clinical evaluation was performed through a *score* with assessment of the following parameters: pneumonia, sinusitis, otitis media, upper respiratory infections (URI), tonsillitis, diarrhea, bronchiectasis, hospitalizations, use of antibiotic therapy, use of prophylactic antibiotics, sepsis and meningitis. Determination of IgG antibodies to pneumococcal serotypes was made by ELISA. H1N1-specific IgG was detected by indirect hemagglutination. The determination of influenza-specific IgG was performed by ELISA. The group of patients who were vaccinated included 37 patients (51% women), aged 20 to 78 years (mean 33 years). We observed a significant reduction in the *score* of URI, sinusitis and pneumonias in the year post administration of the vaccines ($p < 0.001$). Conversely, there was no difference in the infections pre and post supposed vaccination *scores* in the group of CVID patients who were not immunized. There was no significant change in specific antibody titers to *influenza, H1N1* and *pneumococcus* after vaccination. In conclusion, there was a reduction in the number of infections, mainly sinusitis, URIs and pneumonias in patients with CVID vaccinated. The clinical improvement observed in CVID patients reinforces the benefit of vaccination.

P5.22.26
Skin sarcoid-like lesions in CVID with poor outcome

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Common variable immunodeficiency (CVID) is a heterogenous immunodeficiency disorder characterized by different clinical presentations. In about 10% of cases they present with granulomatous lesions occurring in spleen, lymphnodes, liver, lungs, skin or conjunctiva. Most patients belong to group-I CVID classification, either with B cells<1% or absent CD21low. The histopathology is similar to sarcoidosis, but its association with CVID occurs in a limited percentage of cases.
We describe a CVID case, female aged 56yrs observed in November, 2009, but with clinical history dating from before 1988, with recurrent sinopulmonary infections, chronic diarrhea, low IgG (403 mg/dl) with absent IgA and IgM, treated since 2004 with IVIgs (20g every 2 months); she was diagnosed with cutaneous sarcoidosis in 2006 after a biopsy of a lesion on the left leg. Cutaneous granulomatous lesions appeared soon after in the periocular and perioral regions of the face and in both legs. Calcium levels, ACE, G6PDH were normal, Mantoux test neg, no visceral granulomas were detected by HRTC and US.
We started sc Igs which allowed reconstitution of protective levels (to 686 mg/dL serum IgG), and treatments (HCQ, steroids and dapsone) unsuccessful for cutaneous and sinopulmonary new lesions suggesting sarcoidosis. An ocular CMV infection developed, leading to severely impaired sight, lip basal carcinoma and granulomatous lesions of the tongue. Leukopenia was constantly present, with 23% lymphocytes, 88% CD3+, 34% CD4+, 1,3% total and no memory switched B cells. Malignant lymphoma developed late in 2012. Management of this CVID subgroup is difficult and controversial.

P5.22.27
Severe combined deficiency of innate and acquired immunity caused by an IKBKB mutation

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We analyzed the molecular basis of severe combined immunodeficiency (SCID) in four patients from four independent families of a small population of homogenous ethnicity. The patients presented within the first months of life with failure to thrive, oral thrush and invasive bacterial and viral infections.
All patients showed normal numbers of B and T cells and suffered from agammaglobulinemia. Three patients had markedly reduced numbers of NK cells. B and T cells were of naïve phenotype. T cells showed reduced responses to anti-CD3/CD28 treatment. Reactivity of NK cells against K562 target cells was extenuated.
Assuming an autosomal recessively inherited monogenetic defect, we performed homozygosity mapping. We identified a region of interest around the centromere of chromosome 8. We detected a homozygous insertion of one nucleotide in the open reading frame of IKBKB in all patients predicting a frameshift with premature termination of the protein.
Western Blot analysis for the encoded IKKβ protein did not show any residual protein in fibroblasts and PBMC suggesting a null mutation. IKKα and NEMO expression was reduced.
Functional testing of NF-κB pathways was performed in fibroblasts. Phosphorylation of IκBα following stimulation with TNFα was considerably decreased. The binding of NF-κB to DNA after stimulation with TNFα or PMA was markedly reduced. Moreover, TLR4 and TLR5 triggered expression of IL-6 was decreased whereas IL-6 production following stimulation with TNFα or IL-1β was normal.
These data indicate that IKKβ is not necessary for human B and T cell development but it is indispensable for lymphocyte activation.

P5.22.28
Reduced NK cell activity and abnormal expression of CCR7 and CXCR1 by NK cells analysis in patients with DOCK8 deficiency

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DOCK8-deficiency is an autosomal recessive primary immunodeficiency that is characterized by multiple abnormalities of the immune system, including a defect of NK cell cytotoxicity which could not be restored after IL2 stimulation. Nevertheless, unanswered questions remain regarding how the absence of DOCK8 leads to predisposition for malignancy, viral, fungal, and bacterial infections.
To address these questions we have analyzed NK cell phenotype and functions in patients with DOCK8 deficiency. We observed that NK cells derived from five DOCK8-deficient patients displayed dramatically reduced cytotoxicity which was partially restored after IL-2 stimulation.
Analysis of activating and inhibitory NK receptors, including KIRs molecules, chemokine receptors and activation markers on gated CD56+cells by cytofluorimetric analysis showed a substantial defect of CCR7 expression by CD56bright NK cells. Noteworthy, we have also detected the expression of NKG2C and of the chemokine receptor CXCR1 on CD56dull NK cells in DOCK8-deficient cells.
Because CCR7 expression by NK cells can be induced after cell culture with IL18 we stimulated NK cells from DOCK-8 deficient patients with IL18. Despite unstimulated CD56bright NK cells from DOCK8 patients showed reduced CCR7 expression, we could not detect any increase of CCR7 on CD56 dull and bright NK cells of DOCK8-deficient patients, whereas CCR7 expression on NK cells derived from healthy donors significantly increased from 5% to 17%.
Taken together our results suggest that NK cells of DOCK-8 deficient patients show reduced cytotoxicity and abnormal expression of the chemokine receptors CXCR1 and CCR7 suggesting an abnormal recruitment of these cells to secondary lymphoid organs.

P5.22.29
Establishment of newborn screening for SCID / severe T lymphocytopenia in São Paulo

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The aim of newborn screening (NBS) is to identify pre-symptomatic newborns with potentially serious or fatal disorders that can be successfully treated. Since its implementation in 2008 in the USA, SCID NBS has detected many immunodeficiencies others than SCID. We currently develop a pilot for a routine NBS protocol for SCID through the quantification of T-cell receptor excision circles (TRECs) from dried blood spots (DBS) on NBS cards in São Paulo city. Newborns´ samples from 2 hospitals in São Paulo were collected after parents' consent. DNA was extracted from DBS and TRECs were determined by real-time quantitative PCR (RT-qPCR). Until now, 1,495 DBS were collected and TRECs quantification ranged from 70 to 2,181 TRECs/μL of blood. The mean and median numbers of TRECs/μL of blood were 433 and 355 respectively. No TRECs were detected in 3 confirmed SCID controls despite the beta-actin normal amplification.
As already proved in some studies, RT-qPCR is a reliable method to quantitate TRECs and screen for SCID/ T lymphocytopenia. In our study, the estimated cost of reagents used for TRECs determination per sample is R$8.00 (equivalent to US$4 and to the cost of screening for cystic fibrosis). Thus, we are going to implement this technique in large scale on São Paulo state major newborn screening laboratory as a mean to call authorities attention for the problem of undiagnosed SCID patients in Brazil.

P5.22.30
Targeted detection of chronic immunodeficiencies by flow cytometry

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The targeted and rapid identification of immunodeficiencies represents a diagnostic challenge. Based on high biological variance in reference ranges, stepwise approaches starting with a simple immune status have been proofed to be commonly misleading.
We introduced an 8-tube-8-colour based approach including general immune status, B-cell subpopulations, CD4+ and CD8+ subpopulations, regulatory T cells, recent thymic emigrants, and subpopulations as well as activation of NK-cells. Based on age-adjusted samples, calculation of age matched reference values is ongoing.
We collected information about: (i) CD3+,4+,8+,19+,16/56+ and activated CD4/8 cells (ii) immature, naive, non-switched, switched, memory, (activated) CD21low, transitional B-cells, plasma blasts, plasma cells (iii) naïve, central memory, effector, effector memory, TH1, TH2, CCR5+ CD4-cells (iv) naïve, central memory, effector, effector memory, CCR5+ CD8-cells and TH17, (v) regulatory T-cells and CD25+, CD69+ T-cells, (vi) naïve (CD62L) and recent thymic emigrants of CD4/CD8, α/β - and γ/δ -T-cells, (vii) immature, mature, CD56bright, single CD16+ NK-cells, CD94/NKG2D+ (viii) Nkp30, 44, 46 and CD57+ NK-cells.
This screening panel allows quickly detecting and differentiating abnormalities or developmental shifts in subpopulations. This approach can be adapted according to clinical signs, speeds up detection of immunodeficiencies, and supports specific therapeutic decision making.

P5.22.31
A New Mutation associated to Bruton Amammaglobulinemia

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X linked agammaglobulinemia (XLA) or Bruton agammaglobulinemia (OMIM 300300), is an inherited immunodeficiency disease caused by mutations in the gene coding for the Bruton`s tyrosine-kinase (Btk). Btk is critical for the maturation of pre-B cells into mature B lymphocytes. The human BTK gene has been mapped to the long arm of the X chromosome, it comprises 19 exons and an open reading frame of 1980 nucleotides. A database of BTK mutations lists 341 mutations.
A 5 years old male was admitted to the hospital for pneumonia caused by *Haemophilus influenzae* type B and pleural effussion. Before this admission he has suffered recurrent upper and lower respiratory infections and chronic diarrhea. Thomography study showed bronchiectasis. Immunologic study was performed: no B cells was detected (<0.01%) and very low levels of IgG (241 mg/dl), IgM (17 mg/dl) and IgA (<5 mg/dl) were found. Parents were not consanguineous. Clinical and laboratory data raised the suspicion of an XLA. Flow cytometry analysis of Btk on monocytes and genetic study confirmed the diagnosis.
BTK gene messenger RNA was analysed and sequenced in c-DNA (copy-DNA) showing the skipping of exon 16. Genomic DNA sequencing detected a T>C change in the donor splice site of exon 16 (IVS16+2T>C or c.1750+2T>C). The sequence variant found in the patient has not been previously reported, however, we consider it is a pathogenic mutation, as it affects the mRNA splicing and the tirosin-kinase domain of the protein.

P5.22.32
Clinical and Genetic heterogeneity of Chronic Mucocutaneus Candidiasis Disease in four Patients with Heterozygous STAT1 mutations

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We have analyzed four patients with Chronic Mucocutaneus Candidiasis Disease (CMCD) affected by persistent and recurrent infections of nails, skin, oral or genital mucosae caused by Candida albicans. Sanger sequencing of CARD9 and Dectin-1, both genes causative of an increased susceptibily to candida infection did not show any genetic alteration. Therefore in two of them, mother and son, we decided to use a whole-exome approach looking for a relevant heterozygous mutation inherited in a dominant trait. Among different targets we identified a missense heterozygous mutation in STAT1 coiled-coil domain L283M.
Based on this finding we screened two other unrelated patients affected by CMCD with direct sequencing of STAT1. We found other two mutations: R274W in Coiled-Coil domain and L351F in DNA-Binding domain.
In order to investigate how these mutations could affect the ability of the immune system to response against Candida we characterized STAT1 phosphorylation in response to interferon alpha (IFN-alpha) and then the ability of CD4+ T-lymphocytes to polarize to Th17.
These experiments showed a persistent hyper-phosphorylation in response to IFN-alpha stimulation in PBMC of CMCD patients, and the complete loss of polarization toward Th17 in CD4+ T-lymphocytes. These data suggest that heterozygous missense gain of function mutations in Coiled-Coil and in DNA-Binding domain of STAT1 gene can cause CMCD.
Mucocutaneous Candida infections were observed in all the patients. However, one of them did also present Cryptococcal lymphadenopathy and visceral leishmaniasis, suggesting that heterozygous STAT1 mutations can be associated to heterogeneous clinical features.

P5.22.33
T regulatory cells and Helios expression in patients with diGeorge syndrome

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Objective: Syndrome diGeorge is a complex disorder caused by an embryopathy based on del22q11. Due to hypo/aplasia of a thymus resulting immunodeficiency affects mainly T cells. In this study we focused on T regulatory cells, with particular attention to Tregs expressing Helios, previously suggested as a potential marker of natural Tregs arising in thymus.
Patients and Methods: We investigated 26 patients with syndrom diGeorge with confirmed 22q11 deletion, age 9 months to 15 years. Tregs were selected as CD25+Foxp3+ cells. Helios expression was measured in 22 patients in this cohort.
Results: We observed a tendency to higher values of Tregs (1,3-7,5% in diGeorge patients, normal values 1-5%). Helios expressing cells ranged from 60 to 89% in diGeorge cohort. Lowest value 42% of Helios expressing cells was detected only in one patient with complete diGeorge syndrome with a lack of T cells, who, however, underwent hematopoetic stem cell transplantation 1 year prior investigation.
Conclusions: Despite abnormalities in thymic development, patients with diGeorge syndrome present with normal of higher values of Foxp3+ Tregs, including Tregs expressing Helios. We, therefore, confirm recent reports demonstrating that Helios expression does not appear suitable as a marker to distinguish natural and induced Treg cells.
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P5.22.34
B cell dysregulation in autoimmune lymphoproliferative syndrome

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Introduction: Autoimmune lymphoproliferative syndrome (ALPS) is a primary immune deficiency of T cell dysregulation caused by defective Fas-mediated apoptosis witch associated with multiple autoimmune disorders, main of that autoimmune hemolytic anemia (AIHA) and/or immune thrombocytopenia (ITP).
Objective: To analyze B-cell subsets and related cytokines (BAFF, IL10) in a cohort of patients with ALPS in order to characterize B cell differentiation in human model of inborn immunedysregulation.
Methods: Flowcytometric analysis of the following B-cell subset was performed in 11 children from Belarus and Russia, compared to age-matched controls: transitional (CD19+CD38++IgM++), $CD21^{low}$, $CD21^{low}CD38^{low}$, naïve (CD19+CD27-IgM+/IgD+), switched memory (CD19+CD27+IgM-/IgD-), marginal zone B cells (CD19+CD27+IgM+/IgD+), putative self-reactive B cells ($CD19+IgD+IgM^{low}$), putative regulatory B cells (CD19+CD24++CD38++) and BAFF, IL10 serum levels were evaluated by ELISA assay.
Result: Diagnosis of 11 patients was established according to revised NIH criteria and abnormal Fas-apoptosis, 7 pt had mutation in *FAS* gene. The majority of patients had reduced percentage of circulating marginal zone B cells, switched memory B cells and increased percentage of $CD21^{low}$, putative self-reactive and putative regulatory B cells. Serum levels of IL10 and BAFF were increased.
Conclusion: Data show a significant disregulation of B cells subsets in ALPS patients and suggest that intact CD95 signaling is important for normal human B cell differentiation and contribute to breakdown of peripheral tolerance and the entry of autoreactive B cells into the naive B cell compartment.

P5.22.35
Linking the endosome to primary immunodeficiency - a congenital neutrophil defect syndrome associated with mutations in *VPS45*

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Primary immunodeficiencies, affecting the neutrophils, illuminate basic mechanisms of immune protection against infections. These defects involve different steps throughout the development, maturation and function of the neutrophils, including the endocytosis process. Primary endosomal defect, responsible for severe immunodeficiency without associated dysmorphism, has not been yet described.
We now describe a defective endosomal intracellular protein trafficking due to inherited mutations in *VPS45* gene which encodes a protein regulating membrane trafficking through the endosomal system. Our study underlies a new immunodeficiency syndrome involving impaired neutrophil function. 7 members of 5 families with neutropenia, neutrophil dysfunction, bone marrow fibrosis, and nephromegaly, were clinically investigated. To identify the causative gene, we performed homozygosity mapping using single-nucleotide polymorphism arrays and whole exome sequencing. All 7 affected children of two different ethnic origins had homozygous mutations (THR224ASN or GLU238LYS) in the *VPS45* gene. Patient's fibroblasts showed defective motility and increased apoptosis. Both cellular phenotypes were rescued by transfecting mutant fibroblasts with wild type VPS45 gene. In patient cells, VPS45 protein level was reduced, as well as its binding partners Rabenosyn-5 and syntaxin16. Beta-1 integrin, which its recycling is regulated by VPS45, was reduced on the surface of patient's cells. A zebrafish model with reduced vps45 protein exhibited severe neutropenia, resembling that of our patients. The cellular defects in this new disease suggest that other immunodeficiency disorders may also result from impaired vesicle trafficking. This primary immunodeficiency may provide useful insights and complementing experimental approaches to study the endosome function in health and disease.

P5.22.36
Antibody deficiency in Ataxia Telangiectasia is caused by disturbed B and T cell homeostasis and reduced immune repertoire diversity

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Ataxia Telangiectasia (AT) is a multisystem DNA-repair disorder caused by mutations in the *ATM* gene. AT patients have reduced B- and T-cell numbers and a highly variable immunodeficiency. ATM is important for V(D)J recombination and immunoglobulin class switch recombination (CSR), however, little is known about the mechanisms resulting in antibody deficiency severity. The aim of this study was to examine the immunological mechanisms responsible for antibody deficiency
heterogeneity in AT. We included patients with classical AT plus early onset hypogammaglobulinemia (n=3); classical AT (n=8); and variant AT (late onset; n=4). We studied peripheral B- and T-cell subsets, B-cell subset replication history, somatic hypermutation frequencies, CSR patterns, B-cell repertoire and ATM kinase activity. Classical AT patients lacked ATM kinase activity, while variant AT patients showed residual function. Most patients had disturbed naive B-cell and T cell homeostasis as evidenced by low cell numbers, increased proliferation, and decreased antigen receptor repertoire diversity. Impaired formation of T-cell dependent memory B-cells was predominantly found in AT plus hypogammaglobulinemia. These patients had extremely low naive CD4+ T-cell counts, which were more severely reduced compared to classical AT patients without hypogammaglobulinemia. Finally, AT deficiency resulted in defective CSR to distal constant regions that might reflect impaired ability of B-cells to undergo multiple germinal center reactions In conclusion, the severity of the antibody deficiency in AT correlates with disturbances in B and T-cell homeostasis resulting in reduced immune repertoire diversity, which consequently affects the chance of successful antigen-dependent cognate B-T interaction.

P5.22.37
Expression of Bruton's tyrosine kinase gene and endoplasmic reticulum stress markers in X-linked agammaglobulinemia

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Introduction: X-linked agammaglobulinemia (XLA) is characterized by a B lymphocyte differentiation block in the bone marrow, leading to hypogammaglobulinemia with few, or the absence of, peripheral B lymphocytes. Mutations in the *BTK* gene are responsible for XLA and in most cases lead to low protein expression. Misfolded proteins can trigger stress pathways in the endoplasmic reticulum (ER).
Methods: We evaluated eight male Brazilian patients whose diagnosis was based on recurrent infections, markedly reduced levels of IgM, IgG and IgA, and circulating B cell numbers <2%. *BTK* mutations were identified by sequencing and the mRNA expression of *BTK* and ER stress markers was assessed with real-time quantitative PCR (RT-qPCR) technology.
Results: We detected four missense mutations, one nonsense mutation, two frameshifts and one splice site defect. Quantitative real-time detection PCR measurements showed a reduced expression of *BTK* mRNA in patients with mutations that result in a stop codon. However, we found that missense mutations do not affect *BTK* mRNA expression. XLA patients showed an increased level of *XBP-1* mRNA and a reduced expression of *HSP90B1* mRNA, which could be a mechanism to revert cellular ER stress.
Conclusions: The quantification of *BTK* mRNA expression is an interesting tool to identify and differentiate the mutational consequences in XLA patients. It can also contribute to the study of transcripts in other genetic diseases with different types of mutation.
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P5.22.38
Up-regulation of LY6E expression on monocytes attenuates innate immunity by down-regulating CD14 and is associated with HIV disease progression

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Background: Lymphocyte antigen 6 complex locus E (LY6E) is a type I interferon-inducible gene and has been shown to enhance viral replication (Nature 2011, 472:481). However, the underlying mechanism and its role in HIV-1 infection remain to be determined.
Methods: LY6E expression was quantified in HIV-infected subjects and HIV-seronegative individuals, its associations with CD4+ T cell counts and viral loads in HIV-infected subjects were determined. In addition, the roles of LY6E in regulating innate immune responses in HIV-1 infection were further investigated in vitro in monocytes.
Results: LY6E was significantly up-regulated in HIV-infected subjects, its expression was reversely correlated with CD4+ T cell counts and positively with the viral loads; Higher LY6E expression in HIV-1 infected subjects resulted in a more rapid decline of CD4+ T cells than lower LY6E expression individuals during the subsequent 33-month following up visit. LY6E is constitutively expressed on monocytes. Knocking down LY6E expression on both monocyte line and primary monocytes by siRNA profoundly enhanced the production of proinflammatory cytokines and Type 1 interferonand, the enhanced innate responses were suppressed by re-introducing LY6E into monocytes. LY6E down-regulated CD14 expression, accordingly, over-expression of CD14 could overcome the LY6E-mediated suppression. In addition, in HIV-infected subjects, LY6E expression was reversely associated with CD14 expression, successful HAART could reduce LY6E expression.

Conclusion: For the first time we identified that LY6E is an inhibitory molecule on monocytes and is correlated to HIV disease progression; Down-regulation of innate immunity by LY6E was accomplished by reducing CD14.

P5.22.39
Lymphoid Intestitial Pneumonia in CVID -oligoclonal expansion of lymphocytes with preferential CMV-specific immune response and lymphorpoliferative disease promotion. Successful rituximab therapy

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Lymphoid interstitial pneumonia (LIP) is a rare diseases with lymphocytic infiltration of the alveolar interstitium and air spaces, sometimes classified as a lymphoproliferative disease.
In the case report LIP arise primarily from the CVID: at the age of 13 multiple nodules, areas of consolidation was observed and the LIP was confirmed by histological examination. 10 years later the progression to B-lymphoproliferative disease with massive splenomegaly and soft tissue infiltration, coincide with oligoclonal increase of IgM (3340mg/dl), cold agglutinin disease, serum sickness-like reaction.
Immune response polarization was observed in humoral and cellular compartment: high anti-CMV IgM corresponds with high CMV-Quantiferon, terminal CD8 differentiation (75% of CD8 cells was CD57 positive) and clonal expansion: 10, 8% of lymphocytes are CMV pp65-pentamer positive. Surprisingly weak immune response to EBV was observed: specific antibodies did not detected, LMP-1- and BMLF-1-pentamer positive cells show below 0,1%. Interestingly clonality analysis shows oligoclonal serum immunoglobulins (κ/λ=2,84), heavy chain and TCRβ gene rearangation with limited repeitoire.
Taking together this case shows that LIP is consequence of immune deficiency i.e. vigorous immune response to CMV, then asymmetric lymphocyte development after V-D-J rearrangation.
Successful prolonged rituximab therapy with regression of lymphoproliferative disease, nodules and consolidations, unexpected increase FVC (2,59à3,78l) several years after LIP onset prompts the use this monoclonal antibody as a first line therapy.

P5.22.40
Prevalence of Tuberculosis among HIV/AIDS Patients In Obafemi Awolowo University Teaching Hospital Complex (OAUTHC). ILE - IFE

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The prevalence of Tuberculosis (TB) among HIV/AIDS patients in ObafemiAwolowo University TeachingHospital Complex (OAUTHC) Ile-Ife was investigated. In addition, the comparison between microscopical and radiological techniques of TB diagnosis was studied as well as the specimen of most diagnostic value. Out of 86 confirmed Acquired Immunodeficient Syndrome (AIDS) patients examined microscopical with Ziehl Neelson (ZN) cold staining method for Acid Fast Bacilli (AFB) in sputa, were positive for AFB showing a prevalence of 13.8%. the prevalence of TB in AIDS patients with regards to age group and sex distribution showed the highest number of positive cases to be in age group 21-30 yr (5.8%), males made up of 7 (8.1%) and females 5(5.8%). The radiological technique proved more sensitive with 52 (60%) positive cases than the microscopic technique with only 12 (13.9%). In the study of the most valuable specimen, early morning specimens (Collection samples) were of more diagnosis value than others (collected at the clinic between 9am 12 noon) and gave the highest positive result of 13.9% while samples I and III were 10.5% and 9.3% respectively. The high proportion of negative results (86%) obtained from microscopic examination underscores the need for cultural method and clinical diagnosis to accurately identify and confirm TB in HIV/AIDS patients who are sputum negative but may be co-infected. This work confirms that there is a close link existing between active tuberculosis and HIV/AIDS infection in Ile-Ife, Nigeria.

P6.01 Immunomodulation by nutrients and vitamins

P6.01.01
Vitamin D deficiency increases macrophage polarization and inflammatory infiltrates in epicardial adipose tissue of atherosclerotic swine

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Purpose: Patients with obesity and insulin resistance develop accelerated atherosclerosis and pro-inflammatory changes in epicardial adipose tissue (EAT) resulting into increased release of pro-inflammatory mediators that might mediate coronary artery disease, develop endothelial dysfunction and exaggerate intimal hyperplasia following coronary intervention. Here, we examined inflammatory phenotype of epicardial fat and coronary artery function in vitamin D-deficient atherosclerotic swine.
Methods: Yucatan microswine were fed with high fructose high-cholesterol diet. Atherosclerotic lesions were confirmed by coronary angiography and histology. Immunostaining was performed in the adipose tissue and coronary arteries for macrophage phenotype. Contraction and relaxation of coronary arteries was examined in organ bath studies.
Results: In obese swine fed with high-fructose high-cholesterol diet, there was significant infiltration of CD86+ cells (M1 macrophages) with minimal immunostaining to CD206 (M2 macrophages) in adipocytes, which was increased in adipocytes of vitamin D-supplemented swine. Similar pattern was found in coronary arteries. Compared to vitamin D-sufficient high-cholesterol diet, vitamin D-deficient high-cholesterol swine EAT showed dense inflammatory cell infiltrate with significantly decreased expression of SOCS3 protein and marked increase in TNF-α, MCP-1, and IL-6 expression in EAT. The contractile response to serotonin in both carotid and coronary arteries of atherosclerotic swine was much higher in the vitamin D-deficient than in vitamin D-sufficient group. Norepinephrine (NE)-induced contraction in carotid and dilatation in coronary arteries, which was lower in vitamin D-deficient than vitamin D-sufficient swine.
Conclusion: High-fructose high-cholesterol diet enhances pro-inflammatory macrophages, increases vasoconstriction and decrease vasodilatation. Vitamin D supplementation could be beneficial in preventing metabolic effects in atherosclerosis.

P6.01.02
Impact of zinc supplementation on the production of IL-17 in perinatal stages

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The suboptimal contribution of micronutrients may damage the immune response. In the particular case of zinc, this microelement has shown their participation in various immunological processes around perinatal stages. In this regard, it is interesting to know the impact of zinc may have on proinflammatory cytokines such as IL-17 for its role in delayed hypersensitivity and its possible effect in the induction of autoimmunity. This study aimed to evaluate the effects of zinc supplementation on the production of IL-17 over early life stages. For this, we used an experimental model of zinc supplemented mice (500 microgram/mL zinc acetate, F1 Balb/c AnN inbred mice at 21 and 42 days). We measured the microelement concentration in lymphoid organs by Atomic Absorption Spectrometry and also the cytokine production in serum by double antibody immunoenzime assay. Results showed that female group of supplemented mice diminished the cytokine production 13.65% whereas male group of supplemented animals showed a reduction of 28.61 % at 6 weeks of treatment. While at 9 weeks of supplementation female mice

diminished the cytokine production 74.71% and male group of supplemented animals showed a reduction of 76.25%, each group compared with control. Further, zinc concentration showed a noticeable increment in the spleen of female supplemented mice (80%). We concluded that zinc supplementation in neonate mice has the effect to diminish the production of IL-17 by Th17 cells, this means a regulatory response to supplemented individuals and probably effects over thymocytes differentiation and Th17-mediated autoimmune diseases.

P6.01.03
Uncaria tomentosa Willd. DC (Cat's claw) immunostimulatory activity in splenic B lymphocytes

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INTRODUCTION: B lymphocytes play a crucial role in humoral immunity; their activation provides the basis of immunizations against different diseases. This study assessed the immunostimulatory activity of a Cat's claw (Uncaria tomentosa Willd. DC) hydro-alcoholic extract with 5% of pentacyclic oxindole alkaloids (UT) on splenic B cells.
OBJECTIVES: 1. Calculate the UT IC50 value in splenocytes. 2. Evaluate the effect of UT in mononuclear cells activation. 3. Evaluate the effect in splenic B cells.
METHODOLOGY: Spleens were obtained from 7-week-old C57BL/6 mice. Splenocytes were treated with different concentrations of UT. IC50 value was calculated after 72h UT incubation and adding 7AAD. Subsequently, splenocytes were stimulated under the same conditions and the activation of mononuclear cells and B lymphocytes (B220+/CD25+CD69+) was assessed by flow cytometry. Data was analyzed with Graphpad Prism 5.
RESULTS: The IC50 value obtained for UT in splenocytes was 140.9ug/ml. A dose-dependent activation was shown after UT stimulation. Mononuclear cells were significantly activated by UT (18.3% for UT 90ug/ml vs. 2.5% CN). Furthermore, B lymphocytes were also activated (20.45% for UT 90ug/ml vs. 1.8% CN); nevertheless, their population percentage remained the same for all the treated and control groups (65% of total splenocytes in average).
CONCLUSION: These results support the immunostimulatory activity of UT and demonstrate specificity towards B cell activation, suggesting that UT could be used as a potent adjuvant to trigger humoral immunity.

P6.01.04
Leucocytes and Oxidative Stress by Non-Thermal Plasma

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Plasma is ionized gas and is the so-called 4th state of matter. In nature it comes in form of lighting, aurora borealis, or fire. Intensive research has led to "cold" plasma sources operating at room temperature. These plasma sources are already efficiently applied in industry for decontamination of food and heat sensitive materials. The mechanism of action - although not fully understood - is mediated through RONS. Liquid and gas phase plasma chemistry is highly complex but plasma diagnostics revealed dose-dependent levels of hydrogen peroxide, nitric peroxide, superoxide, and others. In plasma medicine, plasma applied to cells and tissues. So far, increased proliferation of fibroblasts in vitro and in vivo anti-tumor activity could be shown. More interestingly, first in vivo and clinical studies revealed a plasma-aided increase in the healing of (chronic) wounds. It is thought that mild oxidative stress may modulate skin and immune cell behavior to resolute chronic inflammation while RONS still being toxic enough to have an anti-microbial activity. The plasma - human-leucocyte interaction has not been elucidated yet. Therefore, we treated human blood PBMC and PMN in vitro with an atmospheric pressure argon plasma jet (kinpen09, CE-marked and safety tested) and monitored viability, proliferation, activation, and cytokine secretion. Differential sensitivity to external oxidative stress of plasma of individual leucocyte subpopulations was observed.

P6.01.05
The sensitivity to different types of milk in patients with Ulcerative colitis

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Objective: Ulcerative colitis (UC) is a form of inflammatory bowel disease, which is treated as an autoimmune disease. The aim of this study was to determine the prevalence of the increased levels of serum antibodies to different types of milk: fresh cow's milk (FCM), fresh goat's milk (FGM), boiled cow's milk (BCM) and boiled goat's milk (BGM) in patients with UC.
Methods: Seventeen patients with UC (15 with proven increased, mainly IgG and IgE immunity to cow's milk proteins (CMP) and 2 without it) were included in this research. Levels of serum IgA, IgG and IgE antibodies to FCM, FGM, BCM and BGM antigens were determined by ELISA test. The statistical analysis of data was performed by Wilcoxon rank sum test.
Results: The levels of serum anti-FCM IgA, IgG and IgE antibodies were significantly higher than levels of serum anti-BCM (p=0.0313; p=0.0256; p=0.0029) respectively. Statistical analysis of IgG and IgE immunoreactivity to BGM and BCM showed that the levels of anti-BCM IgG and IgE antibodies were significantly higher in comparison to levels of anti-BGM IgG and IgE antibodies (p=0.0066; p=0.0005). The level of serum anti-FCM IgG antibody was significantly higher than level of serum anti-FGM (p=0.0125). The level of IgE immunity to FGM is significantly higher than level of IgE immunity to BGM (p=0.0231).
Conclusion: These results indicate that patients with UC with increased immunity to cow's milk proteins, could consider the use of boiled goat's milk as the alternative protein source.

P6.01.06
New therapeutic strategies using xCELLigence system in colon cancer

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The chemo-preventive efficacy was associated to enhanced apoptosis, a cellular "suicide" mechanism that keeps cell number in tissues between normal limits and allows elimination of cells predisposed to malignant transformation. Any therapeutic strategy that specifically triggers apoptosis in cancer cells might have immunotherapeutic value. Anti-cancer drugs exert their cytotoxic action during physiological pathways of apoptosis, leading to tumor cell destruction, which might be added by natural compounds, and apoptosis pathways influenced. Our study focuses on the influence of treatment with drugs and/or natural compounds on antigen *vs.* gene expression of molecules involved in cell proliferation and apoptosis (p53, Bcl-2, Bax) in colon tumour cell lines with different rates of proliferation (LoVo, HT29). The xCELLigence System, a cell-based label-free platform technology that utilizes the inherent morphological and adhesive characteristics of the cell, was used to screen new therapeutic strategies and dose-response models by combining drugs (5-fluorouracyl, cisplatin) with natural compounds (resveratrol, genistein). The system continuously monitored viability/toxicity to pinpoint the optimal time points and concentrations for conducting endpoint assays like flow-cytometry, Western blot for evaluation of nuclear antigen expression, or qRT-PCR for detection of gene expression. In addition, percentages of apoptotic cells were analyzed using Annexin V/ FITC and propidium iodide (PI) double staining, while DNA progression through cell cycle phases was estimated by PI staining, both followed by flow-cytometry analysis. Therefore, new therapeutic approaches could be used for destruction of tumor cells and renewal of the cellular pathways that lead directly to apoptosis, contributing to the immunotherapeutic management of cancer.

P6.01.07
Beta-glucans Uptake by Human Dendritic Cells via a Variety of Endocytotic Mechanisms

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β-glucans are natural polysaccharides carrying β-glycosidic bonds and exist diversely in cell wall of bacteria and fungi. *Ganoderma Lucidum* (*G.lucidum*) is a kind of mushrooms fungus that has been consumed for ages with potent immunomodulatory effects on human immune cells. Since the β-1-3 and 1-6 glycosidic linkages of β-glucans cannot be digested by human, how β-glucans are uptaken and then stimulate dendritic cells (DCs) remains elusive. We therefore explored whether its uptake involves various endocytotic pathways. We utilized human monocyte-derived DCs as *in vitro* models and investigated the involvement of three known endocytosis mechanisms: clathrin-mediated, caveolae-dependent and macropinocytosis. Chlorpromazine (CPZ), genistein (GNS) and cytochalasin D (CCD) were selected as their respective inhibitors. *G.lucidum* β-glucans (GL-PS) were used due to its high potency in stimulating the DCs maturation. Cell colonies were observed by microscopy and selected CD markers expression were assessed by Flow Cytometry. Our results showed that all inhibitors exerted suppressive effects on DCs maturation by downregulating a panel of surface maturation markers expressions. The suppression indirectly suggested that GL-PS was internalized via these 3 mechanisms. Therefore, we hypothesized that clathrin, caveolae and macropinocytosis pathways were all potentially involved in β-glucans uptake into DCs. Further study is ongoing to confirm our findings.

P6.01.08
Nourishment and its relation with immunitary response and oxidative stress

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These last decade's articles were reviewed, all related to the topic and written in English and Spanish, well available at the Infomed intranet.
Background and objectives: It is a systematic bibliographic review about nutrition. Nutrients are classified as proteins, carbohydrates, fat, vitamins and minerals, needed in diet for the well functioning of the human being briefly discuss the design of the study/ program and how it was conducted. We would like to development some projects of investigation to establish a useful therapeutic conduct which allow the patients to take the appropriate diet and prevent lots disorders in the immunological system. **Methods:** We performed a wide and systematic bibliographic revision abaut nutrition immunity and oxidative stress, on the second place we compiled and prosecuted the information.
Results: An analysis is done about the main antioxidants ingested by diet, due to the harmful effect that free radicals cause in the organism ; on the other hand it is explained how nutritional manipulation of immunity may have clinical (cellular immunodeficiency, humoral and phagocitic weakness), biological and therapeutic implications. Besides, it is approached the effect that an adequate nutrition may have on the main defense mechanisms of the organism, present the main results with appropriate statistics/ data analysis and pertinent discussion. **Conclusions:** We analyzed the information of all current studies and in this bibliographic review, it is deductible the importance of an adequate nutrition to maintain a proper physical appearance and to diminish the oxidative stress mechanisms and improve our defenses.

P6.01.09
Inflammatory pattern, vitamin D level and cognitive performance in adults and older adults.

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Age-related and neurodegenerative cognitive decline are associated with increased inflammation, hippocampal atrophy and low serum vitamin D level. The pathological inflammatory mechanisms leading to the neuronal damage and cognitive impairment are direct, secondary to the involvement of various cytotoxic T-lymphocytes activities, or indirect as a result of microglial activation. Vitamin D is an endogenous hormone that depends on adequate sun exposure and food intake. Recently, basic and clinical research studies showed that Vitamin D supplementation up regulates anti-inflammatory cytokine levels. In addition, there is evidence of functional vitamin D receptors found mainly in hippocampal neurons and glia. Also, increased inflammation is positively correlated with hippocampal atrophy. Yet, levels of vitamin D and inflammatory cytokines, known mediators of cognitive performance, have not been fully explored clinically. The aim of this study was to investigate the correlation between serum levels of vitamin D and cytokines (IL10, IL6, IL-1β and IL-1ra) using Luminex technology with cognitive performance in adults (> 30 years) and older adults (> 60 years). Cognitive performance was measured using the Montreal Cognitive Assessment tool, the Rey complex figure test and Symbol digit modalities. Since age-related cognitive decline is associated with increased inflammatory cytokines and low vitamin D levels, we expected that older adults will have more prominent deficits in vitamin D, higher pro-inflammatory cytokines and impaired cognitive function, so they were investigated as a separate group. Preliminary results showed a significant positive correlation between cognitive performance and vitamin D level. The cytokine analysis and patterns are in progress.

P6.01.10
Comparative study of the antiinflammatory effect of Inca Inchi oils (*Plukenetia volubilis* and *Plukenetia huayllabambana*)

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Due to the high content of omega-3 contented in both varieties of Inca Inchi oil, its anti-inflammatory capacity was measured at different concentrations in vivo.
METHODS: It was orally administered 10, 17.4, 30.2, 52.5, 91.2 and 150mL/kg orally of both oil varieties to determine the median lethal dose (LD50). The animals were observed for 7 days. The LD50 value was determined by statistical Probits method.
It was orally administered 5, 10, 20 and 40mL/kg of both oils for 7 days to determine the anti-inflammatory effect. In addition, there were three control groups: negative (no inflammation, no treatment), anti-inflammatory (inflammation + indomethacin) and Positive control (inflammation + no treatment). One hour after administration of the last dose it was applied 0.05ml of λ-carrageenan 1% into the right paw. The inflammation was measured 2 hours after administration of λ-carrageenan, using a micrometer. All mice were taken for histological analysis.
RESULTS: LD50 for *P. volubilis* was 68,723mL/kg, and for *P. huayllabambana* was 80,505 ml/kg, concluding the product as Non-toxic (William´s classification). Histopathological analysis showed villous atrophy and hyperplasia of intestinal epithelium in the last two doses for both oils. In the antiinflammatory assay we found a statistically significant inflammation reduction for both oils. Obtaining the following inhibition percentage: *P. huayllabambana*= 52%, *P. volubilis*= 45% y Indomethacin= 49%. Histopathological study showed fewer inflammatory cells at the last two doses for both oils.
CONCLUSIONS: Both Inca Inchi oils are non-toxic products and reduce inflammation. The variety *P. huayllabambana* appears to be the most effective.

P6.01.11
Iron overload can suppress IL-23 expression from human monocyte-derived dendritic cells and subsequent downstream Th17 expansion

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Introduction: Thalassemia, the most common monogenic disease in the world, has a global carrier rate of 1.5%. The 70,000 infants born with thalassemia major present with severe, transfusion-dependent anemia that, left untreated, is incompatible with life. Iron overload is the most common complication arising from treatment. It is also a

significant risk factor for infection. Infection due to iron overload is a major cause of death in these children. We investigated the role of iron overload on dendritic cells, the most potent antigen-presenting cells.
Methodology: We treated human monocyte-derived dendritic cells (moDC) with various doses of iron citrate in order to investigate the effect of iron overload on moDC maturation, cytokine production and CD4 T cell activation. IL-23 was measured by qRT-PCR and ELISA. Mixed leucocyte reaction was done with iron-treated moDCs; CFSE cell proliferation and immunofluorescent staining of intracellular cytokines IFN-g and IL-17 were measured by flow cytometry.
Results and Conclusion: Addition of iron citrate suppresses IL-23 expression at both mRNA and protein levels whereas it does not affect moDC maturation. Iron treated moDC significantly reduced T helper 17 (Th17) cell differentiation as measured by mixed leukocytes reaction. In conclusion, our study demonstrated that iron-overloaded human moDCs can suppress IL23 production and downstream expansion of Th17 cells, possibly a contributing factor to increased susceptibility to infection in transfusion-dependent thalassemias.

P6.01.12
Antioxidant compounds suppress activity of diamine oxidase (DAO)

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About 1 % of the population presents with histamine intolerance or sensitivity, a suffering characterized by the appearance of various allergy-like symptoms. Histamine is a biogenic amino acid that is contained in many foods, but can also be synthesized and released by a variety of cell types. In healthy people, histamine is degraded by the enzyme diamine oxidase (DAO) that is secreted into circulation upon stimulation. Inefficient degradation gives rise to high intestinal histamine, which is suggested to be responsible for the symptomatology, as histamine is a potent mediator of inflammation and allergic reactions.
Several studies already have discussed the involvement of exogenous antioxidants and “antioxidative stress” in the development of allergies. To analyse the influence of selected compounds on DAO, DAO enzyme activity was examined by using radiolabelled putrescine dihydrochloride as substrate. Plasma samples from healthy human donors or DAO purified from porcine kidney were pre-incubated with curcumin, sodium benzoate or sodium sulphite. After liquid extraction, the reaction product Δ1pyrroline has been measured. The exposure to the compounds resulted in a dose-dependent reduction of enzyme activity. If this in vitro effect of a suppression of DAO can be extrapolated to in vivo, it opens another possibility how overload with antioxidants may promote allergy development.
Although in vitro only, our results emphasize an additional effect to the suppression of Th1-type immune reactions and cytokines by antioxidant compounds, which was demonstrated earlier for food preservatives, colorants, phytochemicals and drugs, thus promoting a shift of the Th1-Th2-type immune balance towards Th2-type immunity.

P6.01.13
Ayurvedic myrobalane extracts inhibit LPS-induced tryptophan breakdown in THP-1 cells

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Triphala, a traditional multicomponent preparation composed of the three fruits amla (Phyllanthus emblicae fructus), chebulic (Terminalia chebula fructus) and beleric myrobalan (Terminalia bellirica fructus), is recognized for its beneficial action in the treatment of intestinal disturbances, impaired liver function and inflammation. The impact of aqueous-ethanolic fruit extracts on cell viability, tryptophan breakdown, neopterin and the activation of the redox-sensitive transcription factor nuclear factor-κB (NF-κB) was determined in human myelomonocytic leukemia THP-1 and THP-1-blue cells.
During human immune response, pro-inflammatory cytokines stimulate the enzyme indoleamine 2,3-dioxygenase (IDO) to convert the essential amino acid tryptophan into kynurenine. In parallel, GTP-cyclohydrolase I (GCH) is induced to produce neopterin. Increased neopterin concentrations and accelerated tryptophan breakdown indicate the activation of the cell-mediated immune system.
THP-1 cell treatment with fruit extracts (25, 50 µg/ml) resulted in a partial reduction of lipopolysaccharide (LPS) induced enhanced neopterin formation and tryptophan breakdown in comparison to non treated stimulated controls. Furthermore, all extracts induced a slight but significant suppression of NF-κB basal activity in unstimulated THP-1-blue cells (NF-κB reporter cells). Interestingly, in LPS-stimulated cells, an adverse effect could be observed for Terminalia chebula and Triphala extract at the highest concentration (200 µg/ml), while lower concentrations (25 - 100 µg/ml) did not achieve significant effects on the expression of NF-κB.
Our in vitro data show immunmodulatory effects of Triphala components that are exerted mainly on the response of stimulated THP-1 cells in a suppressive manner. It could relate to the anti-inflammatory properties of the extracts described from traditional medicine applications.

P6.01.14
Resveratrol enhances TNF-α production of human monocytes upon TLR-stimulation

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Objectives
Resveratrol has been shown to possess anti-oxidant, anti-aging, anti-cancer and anti-inflammatory properties. Although numerous data is available concerning in vitro effects and animal studies, only limited data is available in humans. The purpose of this study was to analyze immunomodulatory properties of the substance in humans and elucidate its molecular action.
Materials and Methods
A pilot study was performed including 10 healthy probands (8=Resveratrol, 2=placebo). Serum cytokine levels (IL-12p40, IL-6, IL-8, IL-10 and TNF-a) were measured over 48 hours after oral application of 5g Resveratrol.
To elucidate the in vivo findings, cytokine release of human PBMCs and monocytes (cytokines as above plus IL-1ß) was measured after incubation with Resveratrol and stimulation with different TLR-agonists. Signaling pathways involved were analyzed utilizing a monocytic NF-κB Reporter cell line and by western blotting of NF-κB pathways components and MAPKs.
Results
Resveratrol treated volunteers presented with significantly elevated levels of TNF-α 24 hours after the application, whereas cytokine levels in the placebo group remained equal. The in vitro analysis of human PBMCs and monocytes showed potently enhanced TNF-α production by Resveratrol treated cells upon LPS stimulation on protein and gene transcription level. Enhanced TNF-α production was also observed upon TLR-2 and TLR-5 targeting. Evaluation of the molecular mechanisms revealed an intense enhancement of alternative p105 dependent NF-κB signaling in Resveratrol treated monocytes, while canonical activation of NF-κB via IκB was unaffected.
Conclusions
Our data suggest not exclusively anti-inflammatory, but differential modulatory effects of Resveratrol on cytokine release of innate immune cells.

P6.01.15
Inhibition of matrix metalloproteinase-9 activity in a leukemic cell line by Mentha spicata extract in vitro

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Introduction: Angiogenesis has an important role in cancer growth and metastasis. Matrix metalloproteinases (MMPs) are a large family of proteases degredate the extracellular matrix and have a significant role in tumor angiogenesis. The essential role of angiogenesis in leukemic patients has been reported. Current therapeutic methods in leukemia have not been very successful. Medicinal plants are widely used in treatment of cancers. Mentha spicata is a herb with known anti-tumor activities. In the present study, the effect of aqueous extract of Mentha spicata on MMP-9 activity in leukemic U937 cells has been assessed in vitro.
Materials and methods: U937 cells were cultured in RPMI with 10% FBS. Then the cells at logarithmic growth phase were incubated with different concentrations of aqueous extract of Mentha spicata (0.1 - 10 mg/ml) in the presence or absence of phorbol 12-myristate 13-acetate (PMA) (25 ng/ml) for 24, 48 and 72 hours. The MMP-9 activity in conditioned medium was evaluated by gelatin zymography.
Results: Mentha spicata aqueous extract significantly decreased the PMA-induced MMP-9 activity in leukemic U937 cells dose and time dependently.
Conclusions: The aqueous extract of Mentha spicata showed inhibitory effect on MMP-9 activity in U937 leukemia cells. So the anti-tumor effects of Mentha spicata may be in part due to its inhibitory effects on MMP-9 activity and consequently angiogenesis. Therefore Mentha spicata might have potential implication in leukemia treatment. Identification the effective element (s) in the Mentha spicata extract with anti-MMP activity could be valuable in designing new natural MMP suppressors.

P6.01.16
Labdane-type diterpene, constituent from myoga which are traditional Japanese food inhibit enzyme activity and gene expression of indoleamine 2,3-dioxygenase 1

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The activity of indoleamine 2,3-dioxygenase 1 (IDO-1), that catalyzes the degradation of tryptophan (Trp) into kynurenine (Kyn) increases after various inflammatory diseases. IDO-1 has an important immunomodulatory function in certain immune-related diseases.
We have developed a cell-based assay to evaluate the suppressive effect of phytochemicals or plant extracts on IDO-1 expression. When stimulated by INF-γ and LPS, profound high expressions of IDO-1 mRNA were detected in human monocyte THP-1 cells and were verified by quantitative real-time PCR.
Twenty two kinds of plants extracts and eleven kinds of phytochemicals were examined by the cell-based assay. As a result, the methanol extracts of Myoga flower buds which are traditional Japanese foods, and labdane-type diterpene galanal derived from the Myoga flowers significantly suppressed IDO-1 expression. The experiments using IDO-1-transfected HEK293 cells clarified that galanal inhibits not only the gene expression but also the enzyme activity. Galanal inhibited IDO-1 enzyme activity in a concentration-dependent manner with an IC_{50} value of 7.7 μM. The Lineweaver-Burk plot analysis indicated competitive inhibition of recombinant IDO-1 by galanal. Galanal decreased the Kyn concentration and recovered the Trp concentration in THP-1 cells stimulated with IFN-γ and LPS. Galanal suppressed the expression levels of pro-inflammatory cytokines. Interestingly, galanal up-regulate IFN-β, IL-2 and IL-3 gene expression levels. Galanal induced apoptosis in T cells by reduction of the Bcl-2: Bax ratio. Because the inhibitory activity of galanal is stronger than 1-methyl Trp which is a Trp analog, galanal may have a great potential as the novel drug for various immune-related diseases.

P6.01.17
HemoHIM, a novel composition of medicinal herbs, restores the lasting imbalance of Th1- and Th2-related immune response in *gamma*-irradiated mice and in aged mice

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Whole body irradiation brings down-regulation of the Th1-like immune response, resulting in an immunological imbalance for the long term. In ageing, one of serious problems is a shift towards a dominance of Th2 related response. HemoHIM, a mixture of 3 edible herbs, had been previously developed to protect the self-renewal tissues and promote the recovery of immune system against acute radiation syndrome. The current study was to evaluate the possibility of HemoHIM to restore the lasting immunological imbalance in γ-irradiated mice and in aged mice. The mice were exposed to γ-rays twice a week for a total dose of 5Gy. 12-month-old mice were used as aged mice. The irradiated mice were administrated orally with HemoHIM (100 mg/kg BW) from 1 week before the first irradiation for 4 or 6 months. The aged mice were intubated with HemoHIM for 3 weeks. HemoHIM ameliorated the lasting imbalance between Th1- and Th2-related immune responses that showed up in irradiated mice and in aged mice. In irradiated mice and in aged mice, HemoHIM restored the NK cell activities despite not changing the NK cell percentages. Moreover, the lasting low levels of IL-12p70 in irradiated mice and in aged mice were ameliorated by administrating HemoHIM. HemoHIM enhanced the phosphorylation of STAT4 that was decreased in the irradiated mice. Our findings showed that HemoHIM can ameliorate the lasting down-regulation of Th1-like immune responses by modulating IL-12p70/pSTAT4 signaling pathway in irradiated mice and in aged mice.

P6.01.18
Implications of the O-GlcNAc modification in the regulation of nuclear apoptosis in T cells

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O-linked β-N-acetylglucosamine (O-GlcNAc) is a nutrient-/stress-sensitive post-translational modification that affects nucleocytoplasmic proteins. Glucose, glucosamine and glutamine are the three major carbon and nitrogen donors for UDP-GlcNAc production. O-GlcNAcylation plays a role in fundamental regulatory mechanisms through the modification of proteins involved in cell division, metabolism, transcription, cell signaling and apoptosis. The aim of our research is to determine the implications of O-GlcNAc on T cell apoptosis. Human T lymphoblastic HPB-ALL cells were treated with an O-N-acetylglucosaminidase inhibitor (PUGNAc) or with glucosamine (GlcN) to increase O-GlcNAcylation. Apoptosis was induced in the presence of tributyltin (TBT) and DNA fragmentation was observed by cell cycle analysis. O-GlcNAcylated proteins were precipitated using succinylated wheat germ agglutinin (sWGA) for further western-blot identification. Our results showed that HPB-ALL cells treated with PUGNAc displayed a significant reduction in DNA fragmentation after TBT-induced apoptosis. DFF45, the protein that inhibits the endonuclease DFF40 in the heteromeric DNA fragmentation factor (DFF) complex, was identified as an O-GlcNAcylated protein. This modification appears to give DFF45 a stronger resistance to caspase cleavage during apoptosis. O-GlcNAcylation of DFF45 may represent a mechanism to control the accidental activation of DFF. Several publications in the last decade have shown a similar protective role of O-GlcNAc against different kinds of stress. Previous work from our laboratory showed that an enteral supplementation of glutamine helped reduce the morbidity and mortality of burn patients. The next step in our research will be to investigate the role of O-GlcNAc modifications in this protection.

P6.01.19

Alliin diminished pro-inflammatory cytokines in supernatants of an in vitro model of adipose tissue.

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Obesity is accompanied by chronic inflammation and is characterized as a disorder having an imbalance between pro-inflammatory and anti-inflammatory mediators. A high -fat diet can increase LPS absorption from gut in to the circulation. On the other hand, different functional foods and phytochemicals have been associated with various health benefits when consumed. The main active component in garlic is the S-allyl cysteine sulfoxide, commonly called alliin, has been reported as a potent anti-diabetic and anti-inflammatory. Pre-adipocyte murine 3T3-L1 cell line has been considered for years, an in vitro model of adipose tissue (AT). We differentiated cells with a hormonal cocktail to mature adipocytes. We added a treatment with alliin (0.1mM) to adipocytes for 24 hours, after that we stimulated cells with LPS (100ng/ml) for an hour. Finally IL-6 and MCP-1 were evaluated in supernatants collected at 6, 12 and 24 hours after LPS stimulus and stored at -20°C until quantification by Luminex technology. Aliin diminished significantly the synthesis of MCP-1and IL-6 in the group Alliin+LPS compared to LPS group. Our recent observations indicated that exposure of 3T3-L1 adipocytes to the phytochemical alliin, is able to suppress LPS-evoked molecular inflammatory signals by causing a decrease in the levels of pro-inflammatory cytokines like IL-6 and MCP-1 on the supernatants of this in vitro model of AT.

P6.01.20

The activity of autoreactive IgG antibodies may be blocked by human serum and breast milk immunoglobulins

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In the immune system of healthy individuals can be found B cells that produce IgG antibodies specific to a variety of self antigens. These natural autoreactive antibodies are found in the serum and in the intravenous immunoglobulin preparations. They are able to interact with antigens which are target of some pathological autoantibodies. The auto reactivity of natural IgG antibodies can be induces by extreme environmental conditions during purification (high and low pH values). This activity is not observed when using non-denaturating methods of purification.
The in vitro interactions between human serum from a healthy individuals, or pooled human IVIg or breast milk immunoglobulins and human liver antigens were investigated. IgG antibodies were purified by two different methods: immunoaffinity elution (using low pH value buffers) and soft salt precipitation (without extreme value buffers). Based on their molecular mass, the purified IgA and IgM molecules were obtained from the different fractions of serum and colostral proteins.
Serum or breast milk IgM and IgA isolated by immunoaffinity elution can block the interaction of eluted IgG antibodies or pooled IVIg preparation to human liver antigens. The fraction containing immunoglobulin F(ab')2 fragments has the same ability.
The results show that binding of auto-reactive IgG antibodies to self-antigens can be inhibited by serum as well as mucosal IgA and IgM. Our study supports previous findings that IgM has a major role in blocking the IgG autoantibodies. We suppose that pooled human IgM and IgA preparations may have a positive immunomodulatory activity in patients with autoimmune diseases.

P6.01.21

Difference in reaction patterns of M1 and M2 macrophages induced by quercetin

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Macrophages show significant heterogeneity in function and are classified into two groups: M1 and M2 macrophages. M1 macrophages mainly produce proinflammatory mediators whereas M2 macrophages produce high amounts of anti-inflammatory mediators. Quercetin, one of the flavonoids, exhibits an anti-inflammatory effect via heme oxygenase (HO)-1-dependent pathway in M1 macrophages stimulated with lipopolysaccharide (LPS), a ligand for toll-like receptor (TLR) 4. Although the anti-inflammatory action of flavonoids in M1 macrophages has been studied elsewhere, less is known about the effect of quercetin in M2 macrophages. In this study, we investigated the difference in reaction patterns between M1 and M2 macrophages stimulated with TLR, especially TLR3, 4, and 7 and the effect of quercetin on these TLR-induced activation of M1 or M2 macrophages. M1 macrophages increased the expression of IL-1β, IL-6, and IFN-β more than M2 macrophages after stimulation with LPS. In contrast, M2 macrophages strongly induced the expression of IL-1β, IL-6, and IFN-β compared to M1 macrophages after stimulation with imiquimod, a ligand for TLR7. The extent of induction of these cytokines were comparable in M1 and M2 macrophages when stimulated with poly I:C, a ligand for TLR3. Quercetin inhibited IL-1β, IL-6, and IFN-β expression induced by TLR3, 4, or 7 via HO-1-dependent manner. Based on these results, M1 and M2 macrophages exhibited different responsiveness to TLRs, and quercetin inhibited macrophage activation by TLR3, 4, or 7 via HO-1-dependent pathway.

P6.01.22

Expression of cytokines that regulate immune responses in alpaca (Vicugna pacos)blood leukocytes stimulated by clostridial antigens and retinoic acid

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Expression levels of cytokines involved in humoral and cellular responses were determined *in vitro* in blood leukocytes from alpacas, stimulated with *Clostridium perfringens* (AgCP) antigens and retinoic acid (ATRA) as adjuvant. Different treatments were tested as follows: 10ng AgCP and 0.25mUI ATRA; 1ng AgCP and 2.5mUI ATRA; 100pg AgCP and 25mUI ATRA; 10pg AgCP and 250UI ATRA. Treated and untreated leukocytes were incubated at 37C for 24 hours after which total mRNA was extracted. We used real time RT-PCR and relative quantification to assess difference in expression of cytokines in leukocytes in comparison with untreated cell controls. We found increasing expression of IFNγ and Interleukin-2 (IL-2) in all treatments up to 100pg of AgPC and 25UI ATRA (8:1 y 7.5:1, respectively). In case of Tumoral Necrosis Factor alfa (TNF-α), increasing expression was observed until 10pg of agCP and 250mUI of ATRA (10.3:1) were used. As for Interleukin 10 (IL-10), it reached maximum expression when 1ng of AgCP and 2.5mUI of ATRA were used (27.4:1). Taken together, our results suggest clostridial antigens and ATRA confer good stimulation of cellular immune responses in alpaca leukocytes; however, lower concentrations of both, antigen and adjuvant, were able to induce maximum expression of IL-10. We thank Consejo Nacional de Ciencia y Tecnología (CONCyTEC - Perú) for financial support.

P6.01.23
Expression of humoral and cellular immune response cytokines in alpacas (*Vicugna pacos*) treated orally with a combination of clostridial antigens and retinoic acid

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Enterotoxemia is a deadly enteric disease caused by *Clostridium perfringens* which can affect up to 80% of newborn alpacas raised extensively in the highlands of Peru. Mucosal immunization could be a feasible alternative to control this disease. We assessed the expression levels of the main cytokines controlling humoral and cellular immune responses in gut tissue collected from animals treated orally with a combination of retinoic acid and clostridial antigens in dose of 0.25mg/Kg of antigen and 0.067mM/Kg of retinoic acid (ATRA). Animals were treated twice with this combination; second dose was given 7 days after the first one as a boost. We extracted total ARNm from gut tissue samples of treated animals and performed real time RT-PCR aided by relative quantification, comparing treated animals with non-treated controls. We observed greater expression of Interferon-γ (IFN-γ) ($p<0.013$), Interleukin-2 (IL-2) ($p<0.027$) and Tumoral Necrosis Factor alfa (TNF-α) ($p<0.007$) in treated animals compared to controls. No difference was observed between these groups in expression of Interleukin-4 (IL-4)or Interleukin-10 (IL-10). Our results suggest this treatment is effective in stimulating cellular immune responses in gut tissue of alpacas. We thanks to Consejo Nacional de Ciencia y Tecnología (CONCyTEC - Perú) by financing support.
Key words: enterotoxemia, retinoic acid, cytokines, alpaca.

P6.01.24
Immune-modulatory properties of cinnamaldehyde are associated with the induction of apoptosis in immune cells

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Background: Cinnamaldehyde (CA) has been reported to have anti-inflammatory, anti-bacterial, anti-fungal, anti-mutagenic, chemoprotective and anticarcinogenic activity. Here, we further investigated the immune-modulating capacity of CA.
Methods: NF-κB activation by CA (0-10µg/ml) alone or in combination with lipopolysaccharide (LPS) was assessed using the THP1XBlue© human monocytic cell line transfected with an alkaline phosphatase reporter. IL10 and nitric oxide (NO) secretion in murine RAW264.7 cells and human peripheral blood mononuclear cells (PBMCs) stimulated with CA alone or in conjunction with LPS were analyzed. Human PBMCS were stimulated with different concentrations of CA alone or in combination with LPS for 24h and subsequently stained with AnnexinV and propidium iodide to assess cell death and apoptosis.
Results: Incubation with CA resulted in a concentration-dependent inhibition to a maximum of 50% of NFκB in LPS-stimulated THP1-cells. Similarly, NO as well as IL10 secretion induced by LPS in RAW264.7 cells were blocked by addition of CA. Induction of apoptosis in human PBMCS was triggered by CA alone or in conjunction with LPS. These results correlated with a dose-dependent decrease in IL10 by addition of CA in LPS-stimulated PBMCS.
Conclusion: Anti-inflammatory properties of CA are likely due to blocking of NFκB-pathway and induction of apoptosis in immune cells.

P6.01.25
The effect of quercetin on the activated alveolar macrophages

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Alveolar macrophages (AMs) belong to M2 type macrophages and play an important role in innate and adaptive immunity in lung by phagocytosing, killing of microorganisms, and activation of other inflammatory cells. We have previously reported that quercetin, one of the flavonoids, exhibited an anti-allergic action via upregulation of heme oxygenase (HO)-1 activity in mast cells. HO-1 is known to exert protective effect against lung injury. In the present study, we determined whether quercetin could lead to an anti-inflammatory effect through induction of HO-1 expression against the oxidative stress in AMs. We showed that quercetin induced the expression of HO-1 in AMJ2-C11 cells, an AM cell line, and mouse bronchoalveolar lavage fluid (BAL) macrophages. When activated by LPS/IFN-γ stimulation, quercetin significantly suppressed nitric oxide (NO) generation and inducible NO synthase (iNOS) expression, and these cytoprotective effects were HO-1-dependent. Furthermore, quercetin inhibited TNF-α, IL-1β, and IL-6 expression induced by LPS/IFN-γ in HO-1-dependent manner in AMJ2-C11 cells. In conclusion, quercetin may well be one of the therapeutic agents to prevent the early stage of lung injury. We are now investigating the signaling mechanisms of quercetin that increase HO-1 expression, focused on Nrf2, Keap1, and Bach1, the molecules that regulate HO-1 expression.

P6.01.26
***Cucumis sativus* aqueous extract protects against different metabolic syndrome- associated pathologies**

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Metabolic syndrome (MS) is an ensemble of disorders including visceral obesity, dyslipidemia, hyperglycemia, and hypertension. Chronic inflammatory alterations and oxidative stress have been identified as major components of MS. Cucumber (*Cucumis sativus*) is a popular vegetable, widely used in traditional medicine as anti-diabetic. This study is aimed to evaluate whether oral treatment with aqueous extracts of *C. sativus* (AqECs) on murine obesity or hypertension models could control the inflammatory response and the pathophysiological damage derived from these two MS-associated pathologies. The protective effects of the daily administration of AqECs were evaluated on obesity or hypertension murine models induced by a hyper-caloric fat intake diet and AgII chronic administration, respectively. Blood pressure, serum glucose levels and tissue IL6, IL10, IL4, and IL1β levels were studied. Tissue damage and immunolocalization of cytokines were also evaluated by histological examination. AqECs were able to reduce systolic and diastolic blood pressure; they controlled weight and Body Mass Index increase, and lowered serum glucose concentrations and tissue pro-inflammatory cytokines (IL6 and IL1β) with respect to non-treated obese or hypertensive mice. Increased levels of the anti-inflammatory IL10 cytokine were also observed in AqECs-treated animals. Also, kidney and liver damage induced by AgII chronic administration was controlled. Overall, this study suggests that AqECs possess anti-inflammatory, antiglycemic and anti-hypertensive effects that may be relevant in controlling the several disorders associated with MS. Project supported by CONACyT CB-169011

P6.01.27
Cytoprotective activity of Georgian Red Grapes Extract in mitogen-activated Jurkat cells

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The aim of the study – establishment of the cytoprotective action of Georgian Red Grapes Extraxt (GRGE) on incubated in oxidative stress conditions and mitogen-activated Jurkat cells (Intensively proliferated Jurkat leukemic T cell line, DSMZ-Deutshe Sammulung von Mikroorganismen und Zellkulturen (Germania)) modeling systems. Experiments were conducted on intact, Hydrogen peroxide(H_2O_2)- and phytohaemagglutinin(PHA)-stimulated Jurkat cells. Cytoprotectiv activity of GRGE was established according viability of the cells (activity of mitochondrial dehidrogenases (MTT test)) and intensity of apoptosis, calculated on the basis of MTT test data.
It was revialed the dose and time-related decrease of viability of Jurkat cells incubated in high oxidative stress conditions. GRGE reveal static activity on viability of Jurkat cells incubated in moderate oxidative stress condition (H_2O_2 25 µl, 50 µl); in intensive oxidative stress condition (H_2O_2 100 µl), the cytoprotective activity of GRGE was rather low.
The viability of Jurkat cells decreases (apoptosis intensity increases) with rising ratio of PHA-activated and nonactivated cells in incubation medium ($[Jurkat]_{PHA}/[Jurkat]_{intact}$). GRGE increases viability of PHA-stimulated Jurkat cells and prevents their apoptosis.

P6.01.28
Chrysin regulates mast cell activation via HO-1-independent pathway

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Flavonoids are a kind of polyphenolic compounds and ubiquitously present in vegetables, fruits, and teas. Flavonoids possess many beneficial effects including anti-oxidant, anti-tumor, anti-inflammatory, anti-allergic, and anti-viral actions. We previously reported that quercetin, one of the flavonoids, exerted anti-allergic effects via heme oxygenase (HO)-1 activity in mast cells. Chrysin, also one of the flavonoids, is contained in propolis, blue passion flower, and fruits. Although several studies have reported that chrysin had anti-allergic activities, the precise mechanism is unclear. In this study, we investigated the mechanism by which chrysin could exhibit an anti-allergic activity in rat basophilic leukemia (RBL-2H3) cells and mouse bone marrow-derived mast cells (BMMC). Our results demonstrated that chrysin did not increase the expression of HO-1 despite the significant inhibition of degranulation and cytokine mRNA expression. Chrysin-induced inhibition of degranulation was unaffected by the addition of HO-1 inhibitor. These findings suggested that chrysin showed anti-allergic activities via HO-1-independent pathway in mast cells. Chrysin inhibited the translocation of stromal interaction molecule (STIM) 1, an endoplasmic reticulum Ca^{2+} sensor controlling store-operated Ca^{2+} entry, to the plasma membrane after stimulation with thapsigargin. These findings suggested that chrysin might regulate calcium mobilization by inhibiting STIM1 activation to exhibit anti-allergic effects in mast cells.

P6.01.29
Methanol extract of *Origanum vulgare* ameliorates autoimmune diabetes in mice

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Type 1 diabetes (T1D), an autoimmune inflammatory disorder, develops as a consequence of pancreatic beta cell destruction mediated by various pro-inflammatory mediators. Since current T1D therapy mainly involves insulin replacement, constant efforts are being directed toward establishing novel therapeutic approaches. Plants or plant extracts are inexhaustible source of medicinal compounds. In this study we have used methanol extract of *Origanum vulgare* (oregano - OE) that has already shown immunomodulatory and cytoprotective properties *in vitro*. OE was prepared from oregano leaves by sequential extraction of four solvents of gradually increasing polarity (hexane, ethyl acetate, dichloromethane and methanol). To evaluate the effect of OE on diabetes development, the extract was administered intraperitoneally to C57BL/6 mice that were subjected to T1D induction by multiple low doses of steptozotocin (MLDS). Results indicate that 10-day OE treatment significantly reduced the incidence and the level of hyperglycemia in MLDS-treated mice and preserved physiological insulin concentration (measured by ELISA). Flow cytometric analysis revealed a reduction in $CD4^+$ cell number within spleen and pancreatic lymph nodes of OE-treated mice. This was accompanied by a significant inhibition of *ex vivo* Th17-related IL-17 secretion, while proto-typical Th1 and Th2 cytokines (IFN-γ and IL-4, respectively) remained unchanged. Further, the presence of $FoxP3^+$ T regulatory cells in lymphoid tissues of OE-treated mice was similar to diabetic mice as well as the percentage of pro-inflammatory $F4/80^+CD40^+$ M1 and anti-inflammatory $F4/80^+CD206^+$ M2 macrophages. In conclusion, our results indicate that OE protected mice from diabetes development through direct blockade of IL-17-mediated lymphocyte response.
Project ON173013

P6.01.30
Evaluation of of crude extracts of sea cucumbers *Holothuria lecospilota* Genus in persion gulf effects on COX-2 and VEGF genes expression and PGE2, VEGF secretion levels in comparison with celecoxib on colorectal cancer cell line

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The sea cucumber is an important food and traditional medicine in Asian countries But The Persion Gulf Sea cucumber *Holothuria lecospilota* is largely un-know for its cytotoxic and anti-inflammatory activities. The aim of this study was determine cytotoxic and anti inflammatory effect of persion sea cucumber and compare with celecoxib.
Colorectal cancer cell line SW 742 were expose to Body wall, Culomic fluide, Internal viscera extraction of sea cucumber, (10, 50, 100 µgr/ml) for 18 h. Following exposure to the sea cucumber, expression levels of COX-2 and VEGF were determined with Real-Time PCR and PGE2 and VEGF secretion levels were determined by ELISA. The capacity of extracts to inhibit SW 742 growth was tested by MTT assay.
Body wall extract of *Holothuria lecospilota* siginificantly inhibited the secretion of VEGF and PGE2 by inhibiting VEGF and COX-2 at their protein and gene levels. All three fractions showed no significant ($p < 0.05$) cytotoxicity on SW 742.
In general, in this study we found that *Holothuria leucospilota* can acts as an anti-inflammatory and anti-cancer agent and no toxicity and could be entering it to food chain and taking advantages of this food.

P6.01.31
In vitro immunomodulatory and cytoprotective effects of Origanum vulgare methanol extract

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In addition to being a spice, Origanum vulgare is recognized as a source of natural antioxidants and exerts anti-fungal, anti-bacterial

and anti-inflammatory actions. In this study we focused on the immunomodulatory effects of methanol fraction of Origanum vulgare (oregano extract - OE) and on its influence on cellular viability. The methanol OE was prepared from ground oregano leaves by sequential extraction of four solvents of gradually increasing polarity (hexane, ethyl acetate, dichloromethane and methanol). Our results indicate that 48-hour treatment of spleen mononuclear cells with OE (25 - 100 μg/ml) did not alter their basic or concanavalin A-triggered proliferation (MTT assay), as well as IFN-γ and IL-4 secretion determined by ELISA. However, OE treatment decreased IL-17 secretion implying a potential inhibitory effect on Th17 cells. Additionally, OE seemed to affect macrophage cytocidal function since it down-regulated production of nitric oxide (NO) in peritoneal cells (PC) (measured by Griess reaction) without an effect on PC viability. However, PC-related IL-1β and TNF-α remained unchanged after OE treatment. Finally, apoptosis measurement by histone-DNA ELISA showed that OE rescued isolated pancreatic islets from cytotoxic effect of cytokines. Furthermore, OE fully preserved beta cell function judging by impaired insulin secretion. The mechanism of OE-exerted protection of pancreatic islets was NO-independent since OE did not change islet NO production. In conclusion, OE alters secretory pattern of immune cells and protects pancreatic islets from cytokine-mediated destruction. The observed immunomodulatory and islet cytoprotective effects of OE are being further explored for potential application for diabetes treatment.
(Project ON173013)

P6.02 Metabolism and immunity interfaces

P6.02.01
Evaluation of the relationship between obesity and pro- and anti-inflammatory cytokines in postmenopausal breast cancer patients

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Aim: This study evaluated the circulating levels of TNF alpha, IL17, IL10 and IL27 in breast cancer patients and their association with obesity and with the clinical-pathological characteristics of the disease.
Method: Seventy-six consented patients, aged 45 to 80 years, were enrolled and classified in two groups based on the anthropometric criteria: body mass index (BMI) plus absence or presence of abdominal fat. Serum samples from both groups were analyzed for TNF alpha, IL17, IL10 and IL27 by Luminex (Millipore). The cytokines levels were also evaluated in relation to clinical-pathological information (TNM staging, estrogen and progesterone receptor status(ER,PR) and HER-2 expression). Data were analyzed using SPSS 16 and the probability value (p) of 0.05 or less was considered significant.
Results: All patients expressed serum TNF alpha and IL27, but only 53.4% and 11.8% presented IL10 and IL17 respectively. The levels of all cytokines were not different between the groups classified by anthropometric measures and they were not associated with TNM staging, RE status or HER-2 expression, except for TNF alpha that showed a tendency (p= 0,0695) to be higher in patients with RP negative tumors. There was also a tendency of direct correlation between TNF alpha and IL27 (p= 0.0540).
Conclusion: The results showed that in the present study there was no association of TNF, IL27, IL17 or IL10 levels with obesity. The absence of detection of IL10 and IL17 in some patients might suggest that the production of these cytokines is down regulated in them.
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P6.02.02
Arginine depletion during ocular autoimmune responses: in vitro study on bovine explants

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During ocular autoimmunity, the local and the infiltrated cells express NOS2, which produce high amounts of nitric oxide (NO). We have previously shown that this reactive molecule has deleterious effects on ocular structures in vitro. Arginase can compete with NOS for their shared substrate, L-arginine. In order to investigate in vitro arginases involvement in the down modulation of ocular NO production in response to ocular auto-antigens, different explants from enucleated bovine eyes were cultured in either MEM or DMEM with FBS (10%) in presence or absence of purified S antigen (ocular auto-antigens) (20μg/ml) with or without hydrocortisone (10μg/ml). After incubation at 37°C in humidified atmosphere with CO2 (5%) during 24h and 48h, NO and urea were assessed in culture supernatants by modified Griess and Berthelot methods respectively. We observed that S antigen increases significantly NO and urea concentrations in all explants culture supernatants ($p<0.01$). However, our results showed different production kinetics depending on the culture medium ($p<0.01$) and the explants' type ($p<0.05$). We noticed with interest that NO production was earlier than urea production. Furthermore, hydrocortisone significantly increased urea production while reduced NO concentrations ($p<0.01$). Our study suggests that arginases may act in NOS inhibition during ocular inflammation by arginine depletion and act as one of the anti-inflammatory molecular mechanism of glucorticoids in situ.

P6.02.03
Respiratory complex III (CIII) or Adenin-Nucloeotide Transporter (ANT) inhibition diminishes human monocyte inflammatory capacity

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Metabolic failure associated with mitochondrial dysfunction has been reported in sepsis, severe sepsis and septic shock. In peripheral blood mononuclear cells, enzimatic activity of respiratory chain is decreased in sepsis, and ATP synthase and oxygen consumption are reduced during septic shock. Circulating monocytes with mitochondrial depolarization is found in septic shock. It remains unknown if mitochondrial dysfunction is related with poor inflammatory response, allowing development of secondary infections. We decided to analyze whether inhibition of mitochondrial function is associated with the inflammatory capacity of circulating monocytes. Peripheral blood obtained from healthy adults, was incubated with or without antimycin A or bongkrekic acid for one hour. Afterwards, lipopolysaccharide (LPS), peptidoglycan (PGN) or polyI:C was added; two hours later CD14, TREM-1, HLA-DR and CD16 was determined. Plasma was collected to measure IL-8, IL-1β, IL-6, TNF and IL-10 at 6hrs. Viability and mitochondrial membrane potential ($\Delta\Psi m$) was verified at all times and conditions. Viability of leukocytes was over 95%. Depolarization induced by antimycin A was associated with decreased expression of CD14, TREM-1 and HLA-DR; after LPS stimulation, we observed no secretion of IL-8, IL-1β, IL-6, IL-10 and TNF, and no secretion of IL-8 and IL-6 after polyI:C treatment. Bongkrekic acid-induced hyperpolarization prevents LPS-induced augmentation of TREM-1 and HLA-DR in monocytes; in LPS condition diminished IL-1β and TNF and no IL-8, IL-6 and IL-10 secretion was observed. Inhibition of CIII or ANT decreases inflammatory capacity of human circulating monocytes in ex vivo stimulation, probably collaborating with so called "immune paralysis".

P6.02.04

The human intermediate monocyte subset can be further subdivided on the basis of HLA-DR expression revealing a distinct abnormality in obesity and diabetes

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Current monocyte classification recognises "classical" ($CD14^{++}/CD16^{-}$), "intermediate" ($CD14^{++}/CD16^{+}$) and "non-classical" ($CD14^{+}/CD16^{++}$) subsets. In this study, proportions and absolute numbers of monocyte subsets were compared by flow cytometry among: Healthy, non-obese adults (n=33); Obese, non-diabetic adults (n=40) and Obese adults with type 2 diabetes (n=53). In addition to CD14 and CD16, monocytes were stained for a range of surface markers and were analysed for uptake of acetylated low density lipoprotein (LDL) using bodipy® labelling.
Among healthy, non-obese adults, intermediate monocytes contained distinct sub-groups with mid- and high-level expression of HLA-DR subsequently referred to as DR^{mid} and DR^{hi} intermediate subpopulations. These represented 14% and 7% of the total monocytes respectively. DR^{mid} intermediates were also distinguishable from DR^{hi} by higher side scatter, higher expression of CCR2, PSGL1, TLR4 and CD36 and lower expression of CD45 and CX3CR1. Obese, non-diabetic adults had significantly lower classical and higher intermediate monocyte numbers compared to non-obese adults, but the increase in intermediates was entirely accounted for by the DR^{mid} subpopulation. A similar abnormality was observed in obese diabetics although total monocyte numbers were lower in this group. In multivariable analyses, DR^{mid} intermediate numbers and proportions correlated significantly with key metabolic indicators (BMI, LDL level, glycosylated haemoglobin). Of all monocyte subpopulations, DR^{mid} intermediates exhibited the highest uptake of acetylated-LDL *in vitro*.
Further subdivision of intermediate monocytes based on HLA-DR expression identifies two distinct subpopulations. Analysis of these subpopulations in the context of obesity and diabetes demonstrates a potentially important clinical relevance to such a subdivision.

P6.02.05

Clinical-immunological assessment of therapy effect of the neuro-sensory-motor integration program of reflex patterns in airway chronic inflammatory diseases

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Airway chronic inflammatory diseases and recurrent exacerbations are mainly caused by damage of immune regulation mechanisms. Treatment of chronic inflammatory diseases of respiratory tract in children and adults was carried out by combining complimentary therapy using the method of neuro-sensory-motor reflex patterns integration (NRI), which works with primary motor system activation and conventional treatment; the parameters of the immune status and the cortisol level in children suffering from of a recurrent obstructive bronchitis are studied, as well as the assessment of therapy efficiency based on NRI method is done. It is established that combining the therapy using primary motor system/reflex integration with the conventional treatment allows correcting the immune system mechanisms' damage to a certain extent mainly to normalize the amount of T lymphocytes (CD3, CD4, CD8) and NK-cells, metabolic function of leukocytes, the level of regulatory and anti-inflammatory cytokines. The therapy based on neuro-sensory-motor reflex patterns integration (NRI) improves the effect of standard therapy on immune system cells and strengthens the polarization of immune response to Th-1 type. It decreases the frequency of airway viral inflammatory diseases, leads to positive dynamics of chronic respiratory diseases and prolongs their remission.

P6.02.06

Diet enriched with small carbohydrate and saturated fat is able to alter immunological parameters in C57BL/6 mice

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The World Health Organization defines obesity as an excess of body fat accumulated in the white adipose tissue with implications for health. Adipose tissue promotes a chronic inflammatory process with progressive infiltration of lymphoid and myeloid cells during his expansion. These features suggest the relevant communication between metabolism and inflammation. This study aimed to evaluate different dietary formulations, using butter and sugar and their effects on metabolic parameters and inflammatory infiltrate in the adipose tissue. At the end of 11 weeks, the percentage of weight gain was 27.36±6.14 (standard commercial diet, SCD), 39.00±3.00 (standard purified diet, SPD), 32.92±3.41 (high sugar, HS) 67.67±2.75 (high fat, HF) 64.00 ± 4.60 (high sugar fat, HSF). We found increasing in epididymal fat tissue of the groups HF and HSF compared to the SCD, SPD and HS. Insulin resistance was observed in the groups HF and HSF. We also observed an increase fasting glucose levels of HSF compared to the SCD, SPD and HS. Animals submitted to HF and HSF had a reduction in frequency of spleen NK. Animals fed with HSF diet showed a reduction in frequency in Foxp3+ regulatory T cells compared to the other groups. We could not find differences in frequency of CD4+, CD8+ and CD19+ cells in all tissue evaluated. In conclusion HF diet and HSF were efficient in inducing obesity, insulin resistance and up regulate the inflammation by reducing the frequency of Foxp3+ cells in C57BL6 animals.
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P6.02.07

Macrophages, but not adipocytes, express TNFa in diet induced visceral obesity

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In this study, the role of macrophages, adipocytes, and cytokines in diet-induced obesity was investigated. Laser scanning confocal microscopy analysis of adipose tissue, from mice fed a high-fat diet or a high-fat/high carbohydrate diet, detected macrophages in inflammatory crown-like structures surrounding adipocytes, and at high magnification many of the macrophages were lipid laden. The majority of macrophages in white adipose tissue expressed TNFa and a few expressed both TNFa and IL-10. Occasionally adipocytes expressed IL-10, but not TNFa. Adipose tissue from obese mice was digested by collagenase treatment and fractionated by density centrifugation into an adipocyte floating layer and a pellet of stromal vascular cells. qPCR analysis revealed a high level of adiponectin mRNA, which is an adipocyte marker, in the floating layer, and no adiponectin in the stromal vascular cells indicating cleanly separated cell populations. The floating adipocyte layer did have a high level of TNFa mRNA, but also contained a high level of F4/80 mRNA indicating the persistent presence of macrophages. Further investigation, by laser scanning confocal microscopy of the floating layer cells, revealed that macrophages either ingest sufficient lipid to be buoyant or remain tightly adherent to adipocytes even after digestion to single adipocytes. The majority of the adherent macrophages expressed TNFa and thus were proinflammatory, while there was no evidence for adipocyte expression of TNFa. Therefore, our data do not support previous reports indicating TNFa expression in adipocytes. We believe that contamination by macrophages in the purified adipocyte floating layer explains the TNFa expression.

P6.02.08
Activation markers show greater expression on adipose tissue resident T-lymphocytes with increased adiposity

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The presence of T-lymphocytes in human adipose tissue has only recently been demonstrated and relatively little is known of their potential relevance in the development of obesity-related diseases. We aimed to further characterise these cells and investigate how they vary with increased adiposity and other markers of metabolic health. Subcutaneous adipose tissue samples were obtained from 17 'healthy' male subjects with waist circumferences ranging from 80.9 to 117.2 cm. Cells comprising the stromovascular fraction were obtained by collagenase digestion of adipose tissue and analysed by flow cytometry. CD4+ and CD8+ T-lymphocyte populations together with macrophages were identified and T-lymphocytes were further characterised by their expression of activation markers CD25 and CD69.
Although the overall proportions of CD4+ and CD8+ T-lymphocytes as a percentage of total cells within the SVF were not correlated with measures of adiposity, there were significant correlations between waist circumference and expression of activation marker CD69 on CD4+ ($R=0.775$, $p<0.001$) and CD8+ cells ($R=0.618$, $p<0.01$) and CD25 on CD4+ ($R=0.602$, $p<0.01$) and CD8+ cells ($R=0.478$, $p<0.05$). In addition, the proportion of macrophages correlated with waist circumference ($R=0.638$, $p<0.005$) and other measures of adiposity. The increased T-lymphocyte activation and proportion of macrophages further correlated with measures of adipocyte size and serum leptin. Our results suggest that T-lymphocyte populations in the SVF are more activated with increased adiposity and we propose that leptin may be important in their regulation as part of metabolic and immune system cross-talk within adipose tissue.

P6.02.09
Galectin-3 deficiency accelerates high-fat diet induced obesity and diabetes by amplifying metaflammation

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Obesity-induced diabetes is associated with low-grade inflammation in adipose tissue and infiltration of macrophages in pancreatic islets. Galectin-3 (Gal-3), a galactoside-binding lectin, has a role in inflammation, uptake and removal of metabolic compounds. We show that ablation of Gal-3 accelerates high-fat diet-induced obesity and diabetes. Wild-type and $LGALS3^{-/-}$ mice on a C57BL/6J background were fed either high-fat (60% fat) or a low-fat diet (3% fat) for 11 or 18 weeks. The increased body weight, amount of total visceral adipose tissue (VAT), fasting blood glucose and insulin levels, homeostasis model assessment of insulin resistance and systemic inflammation were observed in high-fat diet-fed $LGALS3^{-/-}$ mice compared to diet-matched WT animals. Obese $LGALS3^{-/-}$ mice had increased incidence of Type-1 T and NKT lymphocytes and pro-inflammatory $CD11c^{+}CD11b^{+}$ macrophages and decreased $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs and M2 macrophages in VAT. The severe insulitis, increased expression of NLRP3 inflammasome and IL-1ß in macrophages and increased accumulation of advanced glycation endproducts (AGE) and receptor for AGE (RAGE) expression in pancreatic islets of obese $LGALS3^{-/-}$ animals were associated with elevated expression of phospho-NFκB p65 and mature Caspase-1 protein in pancreata and VAT. $LGALS3^{-/-}$ peritoneal macrophages stimulated with lypopolysaccharide (LPS) and saturated fatty acid palmitate *in vitro* produced increased Caspase-1 dependent IL-1β and had increased expression of NLRP3 inflammasome and phospho-NFκB p65 compared to WT macrophages. Transfection of $LGALS3^{-/-}$ macrophages with NLRP3 inflammasome siRNA attenuated IL-1β production in response to palmitate and LPS plus palmitate. Obtained results suggest important protective roles for Gal-3 in obesity-induced inflammation and diabetes.

P6.02.10
P2X7 receptor expression and potential role in T cells in Type 1 Diabetes

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The P2X7 receptor is an ATP-gated ion channel expressed in a variety of cell types. In the immune system, the function of P2X7 has been mostly studied in macrophages and T cells. ATP-mediated P2X7 activation facilitates macrophage maturation and IL-1β secretion. Moreover P2X7 stimulation by high concentration of ATP induces T cell apoptosis.
Although *p2rx7* has been proposed as a type 1 diabetes (T1D) susceptibility gene in NOD mice, its potential pathogenic role has not been directly determined. To test this possibility we investigated *p2rx7* expression in various T cells subsets namely $CD4^{+}CD62L^{high}CD44^{low}$ naive and $CD4^{+}CD62L^{low}CD44^{high}$ effector from pancreatic lymph nodes of healthy , prediabetic and overtly diabetic NOD mice. *p2rx7* expression significantly increases in $CD4^{+}$ T effector cells of prediabetic NOD mice but dramatically decreases in NOD mice with overt disease. Since *p2rx7* is silenced by cognate antigen stimulation these observations underscore the relevance of pancreatic epitope spreading in the development of T1D in NOD mice. Down regulation of *p2rx7* would render effector T cells resistant to apoptosis induction by extracellular ATP generated by inflammatory tissue damage, thereby propagating and sustaining tissue destruction. The role of P2X7 activity in limiting the T cell diabetogenic potential was supported by T1D induction with low-dose of streptozotocin in *p2rx7* knock-out mice, which developed a significantly more severe disease than the wild-type counterpart. Our study suggests that P2X7 could constitute a therapeutic target in the early phases of T1D by promoting apoptosis of potentially diabetogenic effector T cells.

P6.02.11
Insulin resistant obese individuals have increased frequency of CD14++CD16+ monocytes, what is modulated by a bout of aerobic exercise

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The expansion of the CD16+ monocytes has been suggested to occur in human obesity, a low-grade chronic inflammation condition. In this study the frequency of different monocytes subpopulations in obese insulin-sensitive (OB, n=9) (BMI 33.5 ± 2.8 kg/m2, HOMA-IR 0.5 ± 2 mmol.μU/L²) and insulin-resistant individuals (OBR, n=9) (BMI 34.8 ± 2.7 kg/m2, HOMA-IR 4.0 ± 1.0 mmol.μU/L²), before and after a session of aerobic exercise (AE) (3 sets of 20 minutes, at 60% of VO2 peak, in cycle ergometer) was determined. Control eutrophic individuals (CTRL, n=9) (BMI 22.7 ± 2 kg/m2, HOMA-IR 1.0 ± 0.3 mmol.μU/L²) were also enrolled in the study. At baseline, the frequency of intermediate monocytes was higher in the OBR (22.26 ± 12.26%) than in CTRL (7.67 ± 5.37%) ($P = 0.013$, two-way Anova), and AE reduced the frequency of these cells ($P = 0.003$), with a tendency of time versus condition interaction ($P = 0.060$). Additionally, reduced values ($P = 0.051$) were found for OBR (-7.8 ± 8.72%) compared to CTRL (-1.54 ± 1.92%) considering the effect of exercise on the frequency of intermediate monocytes (delta of variation). For classical and nonclassical monocytes, no differences were observed among the groups nor there was effect of exercise. A positive correlation between the frequency of intermediate monocytes and the percentage of body fat, BMI and HOMA1-IR was observed. These data demonstrate that obese insulin resistant individuals, but not insulin sensitive ones, have increased frequency of intermediate monocytes, what is modulated by a single session of exercise.

P6.02.12
The regulatory role of amino acids in the immune response

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L-glutamic acid (L-Glu) plays an important role in maintaining of lymphocytes homeostasis and their function. Metabolic processes occurring in the organism at stresses and diseases lead to use of a large number of L-Glu. The deficiency of L-Glu reduces resistance to infections but additional input of L-Glu enhances the activity of lymphocytes, including increased synthesis of interleukin (IL)-2 and γ-interferon. Further, as an immediate precursor for glutathione synthesis, glutamate plays an important role in the removal of oxidants and regulation of the immune response.
Our research has focused on studying of the additional introduction of L-Glu on the functional activity of T-and B-cell immunity for the actions of the stress and under normal conditions, on the analysis of correlative links between the immune and antioxidant systems, elucidation of the mechanisms involved in this process. Immunological reactivity of the organism was evaluated.
Our results have shown that L-Glu stimulated processes of cell activation, proliferation and differentiation. Total number of T-lymphocytes and rosette forming T lymphocytes with a high density of receptors decreased in animals subjected to stress and which did not received L-Glu. L-Glu affected the functional activity of T-helpers. Introduction of L-Glu lead to the activation of T- and B-cell immunity and receptor structures of lymphocytes. In particular, activation of the immune system led on improvement of parameters antioxidant defense system. L-Glu promotes increasing protective forces of organism, which may indicate a likely increase in T-helper cells with low and medium density of receptors.

P6.02.13
The peptide hormone Insulin-like growth factor-1 affects the immune system during physiological and pathological inflammatory responses

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The peptide hormone Insulin-like growth factor-1 (IGF-1) is an essential regulator of survival, growth and differentiation. It is produced in several isoforms that differ in their N-terminal signal peptide as well as their C-terminal sequences termed extension (E) peptides. In adult mammals, IGF-1 is synthesized in the liver as a systemic growth factor as well as locally in a variety of extrahepatic tissues.
We have demonstrated previously that locally generated IGF-1 accelerates wound healing and enhances regeneration in skeletal muscle and the skin. This effect is partially due to the mitogenic properties of IGF-1 which induces increased proliferation in structural cell types, such as myocytes and keratinocytes. In addition however, accumulating evidence strongly suggests an effect of IGF-1 on a variety of immune cells and the immune system is well established as a crucial factor in orchestrating wound healing and tissue remodelling processes. Both innate cell types, including monocytes and macrophages, as well as adaptive immune cells such as T cells, express the IGF-1 receptor and are therefore able to directly respond to IGF-1 stimulation. Improved regeneration indicates a regulatory effect of IGF-1 on the immune response after tissue injury and in line with this we find that locally acting IGF-1 in the skin dampens the hyper-inflammatory reaction during contact hypersensitivity.
We therefore propose a role of IGF-1 on physiological immune responses during wound healing after tissue trauma as well as a potential therapeutic effect on hyper-inflammatory pathologies.

P6.02.14
Unsuspected relationship between Ferritin Heavy Chain and NK cells tumor susceptibility

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The iron metabolism is crucial for the success of microbial infections. Here, we explored the possible role of iron metabolism in modulating effector immune functions. The ability of pathogens to obtain iron from iron containing proteins of their host is central to whether they live or die. Ferritin is the major intacellular iron storage protein and serves to store iron in a non-toxic form, to deposit it in a safe form, and to transport it to areas where it is required. Ferritin concentrations increase drastically in the presence of ongoing infection or cancer. Previous work have shown that that ferritin plays a regulatory role immune system homeostasis. Here we report an ususpected role of Ferritin Heavy Chain (FHC) on tumor immunogenicity and NK cell susceptibility. By using several cancer cell lines in which FHC was downregulated we have demonstrated an increase in NK tumor cell specific lysis when FHC lack into cells. This correlate with the downregulation of the Major Histocompatibility Complex Class I (MHC class I), and an increased expression of the two DNAM1 NK recepror ligands, PVR and NECTIN 2, that we observe in all the downregulated cell lines.

P6.02.15
Modulation of lipid metabolism and colon cancer cell growth by Cyclosporin A

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Lipid droplets (LD) are inducible organelles that modulate cell signaling, lipid metabolism, membrane trafficking and synthesis and secretion of inflammatory mediators. LD accumulation within non-adipocytic cells is a frequently observed phenotype in inflammatory conditions. Conversely, chronic inflammation, as seen in obesity, is associated with carcinogenesis, being accumulation of LD a common feature of transformed cells and tissues. The Calcium/calcineurin signaling pathway, through activation of NFAT, is essential for effective immunity, and its inhibitors cyclosporin A (CsA) and FK506 are used in the clinics as immunosuppressants. Moreover, NFAT activity is associated with tumorigenesis. We therefore investigated the impact of NFAT inhibition in the expression of genes related to lipid metabolism and growth of four different human colon carcinoma cell lines in vitro. We found that in steady-state conditions, CsA treatment prevented PGE_2 production, slowed cell cycle progression and induced necroptosis in a TGF-β-, NF-κB- and PI3K/mTOR-independent fashion. Interestingly, these effects were calcineurin and NFAT independent since they were not reproduced by FK506 treatment. Upon stimulation with PMA+Iono, the up-regulation of fatty acid synthase, perilipin, ADRP and COX-2, and the accumulation of LD, were abolished by CsA pre-treatment. We conclude that treatment of human colon adenocarcinoma cells with CsA impairs cell cycle progression and necroptosis, besides altering activation-induced changes in lipid metabolism and LD biogenesis. It remains to be shown if the latter events are dependent on NFAT transcriptional activity or an NFAT-independent CsA effect.

P6.02.16
Enhanced glycogen synthase kinase 3beta phosphorylation modulates leukocyte Toll-like receptor-mediated cytokine secretion in obesity

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Obesity is a widespread disease which leads to type II diabetes mellitus and cardiovascular diseases. However, the impacts of obesity on immune function and infectious diseases have not been fully understood. To clarify the role of obesity in the immune responses, we investigated the relationship between insulin-induced signaling and Toll-like Receptors (TLRs)-induced leukocyte cytokine secretion in obese subjects. We found decreased TLR-induced IFN-γ, IL-6 secretion and elevated TLR-induced IL-1β, IL-10 secretion by leukocytes isolated from obese subjects when compared with normal leukocytes. Peripheral blood mononuclear cells (PBMCs) collected from obese subjects showed enhanced basal Akt/Glycogen Synthase Kinase 3β (GSK3β) phosphorylation which was not further increased by insulin and lipopolysaccharide (LPS). We also found that LPS-induced IκBα degradation was inhibited in obese subjects. We further investigated the role of GSK3β on transcription factors NF-κB and CREB activation. We found that insulin plus palmitate stimulation increased GSK3β phosphorylation and IκBα level in THP-1 cells. LPS-induced IκBα degradation was inhibited by GSK3β inhibitor LiCl and insulin plus palmitate stimulation. Moreover, NF-κB nuclear translocation was reversed by insulin plus palmitate stimulation and by GSK3β knockdown using lentiviral delivery of shRNA in THP-1 cells. GSK3β knockdown also enhanced CREB phosphorylation. These findings indicate that GSK3β is important in the regulation of NF-κB and CREB activation in cells under abnormal metabolic environment. Our study provides a possible mechanism of how metabolic abnormalities compromise leukocyte functions in humans with obesity.

P6.03 Pathogenesis and immunointervention in diabetes

P6.03.01
B-1 cells produce a insulin-like factor and regulate blood glucose levels in STZ-induced diabetic mice

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A regulatory role of CD5+ B cells was described in diabetes model, although the role of these cells in diabetes is discordant. This study aims to investigate the involvement of B-1 cells in murine streptozotocin (STZ)-induced diabetes. C57/BL, BALB/c and BALB/xid (B-1 cells-deficient mice) male mice strains were treated intraperitoneally (i.p.) with STZ (40mg/kg) for 5 days. BALB/c mice do not develop diabetes when this dose of STZ was used. Animals were evaluated for blood glucose levels (BGL) and cell populations in the pancreas by flow citometry. Our findings show that in BALB/xid mice BGL were higher than C57/BL or BALB/c mice. To evaluate the role of B-1 cells in diabetes induction, peritoneal B-1 cells obtained from BALB/c mice were purified based on expression of CD19+ CD23- and adoptively transferred i.p. to BALB/xid mice before or after the STZ-treatment. BALB/xid mice adoptively transferred were not diabetic after STZ-treatment and B-1 cells infiltrate pancreatic islets in these animals. To investigate the mechanism that B-1 cells protect BALB/xid of STZ-diabetes, we treated BALB/xid diabetic mice with B-1 cells culture supernatants. BGL decrease in these animals 2 hours after injection and begins to return 4 hours after injection. In conclusion, our data show that B-1 cell-deficient mice showed higher reactivity to STZ-treatment. In addition, B-1 cells migrate to pancreatic islets of STZ-treated mice and are able to regulate BGL by secretion of a insulin-like factor.

P6.03.02
Regulation of inflammasome activation and its implication in diabetes-induced microangiopathies

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Sterile inflammation and activation of the inflammasome are implicated in the development of diabetes complications. Oxidative stress has been shown to be a key pathogenic factor in the development and progression of diabetic microangiopathies such as diabetic retinopathy (DR). In this study we have analyzed the specific relationship between altered endogenous antioxidant activities and induction of sterile inflammation in diabetic patients and human post-mortem retinas from diabetic donors and normoglycemic control donors. Western blotting, immunohistochemistry, flow cytometry assay along with proteomic-based analysis were used to analyze protein level and activity of components of the thioredoxin (Trx) system in correlation with the expression pattern and protein-protein interaction of TLR4, NLRP3, and the thioredoxin-interacting protein (TXNIP). TLR4 expression was significantly increased in monocytes and lymphocytes of diabetic patients presenting clinical signs of DR. Analysis of human post-mortem diabetic retinas revealed impaired thioredoxin activity in diabetic retinas which correlated with increased expression of TXNIP, TLR4 and NLRP3. Proteomic analysis of TXNIP interactome revealed that TXNIP interacted with NLRP3 and TLR4 in the human diabetic retinas and that TXNIP was phosphorylated at specific serine residues. In conclusion our results show that sterile inflammation through activation of the NLRP3-inflammasome is directly involved in the pathogenesis of DR. In addition, our data reveal for the first time that TXNIP-induction of the inflammasome activation could be regulated by post-translational modifications such as serine phosphorylation.

P6.03.03
Autoimmune Anti-prothrombin Antibodies in Type 1 Diabetes Mellitus

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Type 1 diabetes mellitus is a chronic autoimmune disease caused by specific immune destruction of the insulin producing pancreatic β-cells. The thrombotic factors and hypercoagulable state may be the cause of morbidity and mortality in diabetes mellitus. In the last decades, antiphosphoslipid (aPL) antibodies have been identified in various autoimmune diseases and prothrombin (PT) is recognized as one of the major antigenic target for aPL antibodies. Some studies have reported an association of anti-prothrombin (aPT) antibodies with thrombosis. The objective of this study is to evaluate the prevalence of autoimmune aPT antibodies in patients with Type 1 diabetes mellitus.
This study was performed with 125 patients with type 1 diabetes and 92 healthy volunteers were formed our control group. The quantitative determination of aPT IgG, IgM antibodies were performed by enzyme-linked immunosorbent assay.
aPT IgG antibodies were present at 50.57% of type 1 diabetic group whereas it was 2.16% for controls. The circulating levels of aPT IgG was significantly higher in type 1 diabetics (22.75±9.88 AU/ml) when compared to controls (4.65±2.05 AU/ml, $p<0.001$). We found elevated aPT IgM levels at 85.40% of type 1 diabetic patients.
In conclusion, we found high prevalence of aPT antibodies in type 1 diabetic patients. These findings may indicate the presence of high thrombotic risk in type 1 diabetes.

P6.03.04
Ursolic acid effect on proinflammatory cytokines in response to diet-induced obesity in rats

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BACKGROUND. Obesity represents a major metabolic alteration of worlwide impact. Inflammation constitutes a molecular feature in obesity mediated by the expression of TNF-α, IL-1 and MCP1, among other proinflammatory cytokines, which contributes to the development of associated morbidities. Antiinflammatory activity of Ursolic acid (UA), a pentacyclic triterpenoid, has been evidenced both *in vivo* and *in vitro* models. OBJECTIVE. The aim of this work was to evaluate the antiinflammatory effect of UA on diet-induced obese rats. MATERIAL AND METHODS. Antiinflammatory preventive effect of AU was assayed by intragastrically administering UA (50 mg/kg/day) together with a high-fat diet (HFD) to Wistar rats weighing 150-180 g during 3, 6, or 9 weeks. Antiinflammatory reversal effect of AU was evaluated by administering AU (50 mg/kg/day) during 3 weeks to previously HFD fed animals during 6 weeks. Control group received only HFD. ELISA and QPCR to quantify proteins and cytokines expression respectively, were performed. RESULTS. Comparisons were always assessed between experimental and control group. Adipose tissue relative expression of TNF-α was statistically lower ($p<0.05$) after 9 weeks of preventive treatment. Significantly ($p<0.05$) lower expression of IL-1 and MCP-1 was appreciated after 6 and 9 weeks of preventive treatment and also after reversal treatment. Circulating levels of TNF-α and IL-1 were not detectable. CONCLUSIONS. AU is capable to counteract obesity-induced inflammatory cytokine expression in animals fed HFD.

P6.03.05
Syngenic anti-idiotypic and anti-anti-idiotypic monoclonal antibodies raised against the rat monoclonal autoantibody IC2 and their role in prevention of autoimmune diabetes

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A pancreatic beta-cell surface specific monoclonal autoantibody IC2 has earlier been developed by hybridoma fusion of unimmunized diabetic BioBreeding (BB)-rat spleen cells with the HAT-sensitive rat myeloma cell line Y3-Ag123. We have now reinjected this IgM monoclonal autoantibody back into syngenic diabetes resistant BB rats and created new hybridomas from spleen cell fusion with the rat HAT-sensitive myeloma cell line YB2/O. We have thereby obtained both anti-idiotypic and anti-anti-idiotypic monoclonal antibodies against IC2. Eleven such anti-Id and 6 anti-anti-Id monoclonal antibodies are now under further investigation and preparative production for in vivo therapeutic trials. The monoclonal autoantibody IC2 has in vitro shown an almost 100% inhibition of Type-I NKT cell cytokine release most likely through binding to CD1d, which has recently been found on pancreatic beta-cells. Studies in animal models of autoimmune diabetes as well as diabetic children show that NKT functionality and number is affected. The in vitro observed inhibitory effect of IC2 on NKT cells is therefore interesting in vivo as well. We are currently purifying the newly developed anti-idiotypic antibodies for therapeutic trials in BB-rats to investigate further if some of our new monoclonal anti-idiotypic antibodies might have a preventive effect on diabetes. Futhermore, we have isolated anti-anti-idiotypic antibodies with similar specificity to the beta-cell surface as seen with the original IC2 autoantibody from the same fusion, leading us to believe that the idiotypic network could have an important role in diabetes prevention.

P6.03.06
Differential expression of microRNAs in type 1, type 2 and gestational diabetes pinpointed miR-142-5p as potential biomarker for diabetes mellitus

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MicroRNAs are involved in several biological functions and processes following a tissue-specific expression, and control gene expression of one third of the genome. Several studies have been conducted to evaluate specifically expressed microRNAs in the diverse types of diabetes. In the present study we performed a meta-analysis of microRNA profiles of peripheral blood mononuclear cells (PBMC) from patients with type 1 (T1D), or type 2 (T2D), or gestational (GDM) diabetes mellitus using the microarray technology and *in silico* analyses for pinpointing specifically biomarkers for diabetes. Human microRNA microarray kits (v3) 8x15K were used to perform hybridizations of samples from T1D (n=7), T2D (n=7), and GDM (n=6) patients. Differentially expressed microRNAs were identified using the Rank products non-parametric method by the R package RankProd. Nine microRNA (hsa-miR-126, hsa-miR-1307, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-144, hsa-miR-199a-5p, hsa-miR-27a, hsa-miR-29b, and hsa-miR-342-3p) were differentially expressed between T1D, T2D and GDM. Between them, the hsa-miR-142-5p had the best score of area under curve for roc curve in all comparisons. This microRNA was demonstrated to be a negative regulator of CD4 T cells, and was overexpressed in systemic lupus erythematosus CD4 T cells, which may reverse T cells hyperactivity and inhibit antibody production. No study described miR-142-5p in association with diabetes yet and we observed an increase expression of miR-142-5p from GDM to T1D to T2D. Taken together all data and literature information, we may suggest that miR-142-5p may be a good candidate for further studies aiming to find a biomarker for diabetes mellitus.

P6.03.07
TCRγδ T cells in human type 1 diabetes: evidence or clue ?

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TCRγδ T cells share features of both innate and adaptive immune responses. In humans, two main subsets of TCRγδ T cells have been described so far: Vδ2 cells, mainly circulating in the peripheral blood and Vδ1 cells, mainly tissue-resident. While the function of this (often neglected) cell subset in anti-tumor and anti-pathogen responses is well-established, their role in human autoimmune pathologies, including autoimmune type 1 diabetes (T1D), remains controversial. In this study we analysed the peripheral blood of paediatric patients with T1D at onset, paediatric subjects at-risk of developing T1D, and age-matched healthy donors. TCRγδ-cell frequency was decreased in patients with T1D (mainly due to Vδ1-cell reduction) while it increased in at-risk subjects (mainly due to Vδ2-cell expansion) as compared to those in healthy donors. The expression by TCRγδ T cells of membrane receptors such as NKG2D, CCR5, CXCR3, CXCR4, CD31 and CD161 was similar between all the groups of donors tested. Murine TCRγδ T cells $CD27^{neg}$ have been recently shown to produce high levels of IL-17 and infiltrate the pancreas of non-obese diabetic (NOD) mice, worsening the disease. We found that Vδ1 $CD27^{neg}$ T cells were present only in healthy donors and not in patients with T1D nor in at-risk subjects, suggesting a possible role for this subset also in human T1D. It is still unclear whether our findings corroborate a role for TCRγδ T cells in the pathogenesis of T1D or if they represent only an indirect and early marker of disease progression.

P6.03.08

Resistance of Diabetes in Aged NOD Mice is Mediated by $CD4^+CD25^+Foxp3^+$ Regulatory T Cells

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Some female NOD mice do not develop diabetes in pathogen-free housing facilities. We investigated the role of $CD4^+CD25^+$FoxP3+ regulatory T cells in diabetes resistance in aged NOD mice. At 60 weeks age, diabetes was developed between 74% and 79% NOD mice in 6 groups (n=600). Adoptive transfer of lymphocytes from diabetic NOD mice to aged nondiabetic NOD mice did not significantly increase the onset of diabetes. When $1x10^7$ lymphocytes from age NOD mice were co-transferred with $5x10^6$ lymphocytes from diabetes NOD mice into NOD.scid mice, all mice developed diabetes at 6 weeks. When $2x10^7$ lymphocytes from aged NOD mice were co-transferred, none developed diabetes at 6 weeks and only 50% NOD.scid mice developed diabetes at 12 weeks. When $1x10^6$ $CD4^+CD25^-$ T cells was co-transferred with $5x10^6$ lymphocytes from diabetic NOD mice, diabetes was developed in all NOD.scid mice. When $1x10^6$ $CD4^+CD25^+$ T cells were used, only 17% mice developed diabetes at 12 weeks. The percentages of $CD4^+CD25^+Foxp3^+$ T cells in the pancreatic lymph nodes, spleen and peripheral blood were 20.1±5.3%, 14.6±4.1% and 9.8±3.5% in aged NOD mice; and 5.8±1.5%, 8.6±0.7% and 6.4±1.7% in control diabetic NOD mice. When a low dose of cyclosphamiade was given, diabetes was developed in 75% young NOD mice and in 18% aged NOD mice. However, when the high dose was given, 58% aged NOD mice had diabetes and $CD4^+CD25^+Foxp3^+$ T cells in pancreatic lymph nodes were 7.0±1.7%. Our data demonstrated that immunoregulation through $CD4^+CD25^+Foxp3^+$ T cells mediated the resistance of diabetes in aged NOD mice.

P6.03.09

The effect of induced hyperglycemia on the expression levels of TLR2 and TLR4 genes in the hippocampus of male Wistar rats during a time course induction of diabetes type 1

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The relationship between inflammation and diabetic neuropathy progression included the processes and complex molecular networks. In this study the time course expression of toll like receptors (TLR2 and TLR4 (in hippocampal brain tissue of diabetic male Wistar rats were studied. Hyperglycemia was induced in male wistar rats with intraperitoneal (I.P.) injection of Streptozotocin. In different time points (4, 6, 8 and 20 weeks) post diabetes type 1 induction, rats were euthanized and hippocampal brain tissues was removed for further analysis. RNA was extracted from hippocampal brain tissues samples followed by cDNA synthesization using oligo-dT primers. Exon specific TLR2 and TLR4 primers were used to amplify rat TLR2 and TLR4 cDNA. After performing semiquantitative RT-PCR, the expression level of TLR4 mRNA was quantified by real time quatitative PCR (qPCR). Up-regulation of TLR2 and TLR4 transcripts during the time course after diabetes induction as compared to the control group was shown. Our results demonstrate that the expression of TLRs may play a decisive role in the pathogenesis and expansion of diabetes. It is possible that the expression of TLRs can eventually lead to neurodegenerative disease such as Alzheimer. Therefore, studies on the precise role of TLRs in neurodegenerative disease may yield potential molecular targets for developing therapeutics for control and prevention of diabetic neurodegenerative disorders.

P6.03.10

Appearance of beta cell autoantigen primed CD4+ T cells is synchronous to circulating islet autoantibodies in early pre-type 1 diabetes

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Islet autoantibody seroconversion is the first detectable evidence of ongoing autoimmunity in the process leading to type 1 diabetes. The incidence of islet autoantibody seroconverion is highest at around 1 year of age in genetically at risk children. Autoantibodies are markers of pre-diabetes, whereas beta cell destruction is mediated by T cells. Little is known of the timing of autoreactive T cell activation to beta cell antigens in the prediabetic period.
The BABYDIET study followed children with a strong genetic predisposition for type 1 diabetes frequently during infancy and offered the possibility to search for T cell activation against islet antigens. Samples were tested for CD4+ T cell responsiveness to GAD65 and Proinsulin before, at and after islet autoantibody seroconversion in antibody positive children and matched islet autoantibody negative children. CD4+ T cells responsive to GAD65 or Proinsulin with characteristics of memory in vivo primed cells were restricted to samples from children who had islet autoantibodies. Moreover, for both GAD65 and Proinsulin, these responsive cells were rare in samples taken prior to islet autoantibody seroconversion but appeared in significant numbers from the time of seroconversion. Cytokine gene expression in autoantigen responsive cells from one child was tested and showed a shift from IL-10 producing cells toward IFNγ producing cells with progression of autoimmunity.
Activation of T and B cell islet autoimmunity is synchronous in children. This implies that the peak incidence of islet autoimmunity around one year of age reflects a period of susceptibility to beta cell immunization.

P6.03.11

Relationship between BK_{Ca} channel and the apoptosis of NIT-1 cells induced by IFN-γ/IL-1β treatment

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Increased evidence has suggested that apoptosis is likely to be the main form of β-cell death in early type 1 diabetes in rodents and human beings. Sub-cellular and mitochondria regulation of β-cell apoptosis has been extensively studied, while little attention has been paid to the ionic regulation mechanisms. The current density of high voltage-activated Ca^{2+} currents and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in NIT-1 cells were increased by IFN-γ/IL-1β treatment. Blockade of L-type Ca^{2+} channel by nifedipine abrogated apoptosis of NIT-1 cells induced by cytokines. Chronic treatment with cytokines also increased the current density of K^+ currents in NIT-1 cells and the steady-state open probability (NPo) of the large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca} channels). Low concentration TEA and IBTX (BK_{Ca} channels blockers) attenuates apoptosis of NIT-1 cells by IFN-γ/IL-1β. These results indicate that L-type Ca^{2+} channels and BK_{Ca} channels may play important roles in the apoptosis of NIT-1 cells and suggest new therapeutic strategies in type 1 diabetes.

P6.03.12

Anti-inflammatory and anti-diabetic activities of the methanolic fraction of *Centratherum anthelminticum* seed in STZ-nicotinamide-induced type 2 diabetic rat

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This study aimed to ascertain the potential of *Centratherum anthelminticum* seeds methanolic fraction (CAMF) for the management of type 2 diabetes and its associated complications. CAMFs was initially tested on β-TC6 cells for H_2O_2-induced nuclear factor-κB (NF-κB) translocation effects. The results showed that

CAMFs significantly inhibited NF-κB translocation from cytoplasm into the nucleus, dose-dependently. Furthermore, a 12-week sub-chronic CAMFs study was carried out on streptozotocin (STZ)-nicotinamide-induced type 2 diabetic rat model to evaluate glycemia, essential biochemical parameters, lipid levels, oxidative stress markers, and pro-inflammatory cytokines level. Our study result showed that CAMF reduced hyperglycemia while significantly increasing serum insulin, C-peptide, total protein, and albumin levels. Blood glucose, glycated hemoglobin, lipids and enzyme activities were restored to near normal levels. CAMF confirmed its antioxidant potential by elevating glutathione (GSH) and reducing malondialdehyde (MDA) levels in diabetic rats. Interestingly, CAMF down-regulated elevated tumor necrosis factor α (TNF-α), interleukin (IL)-1β, and IL-6 levels in tissues and serum of diabetic rats. We conclude that CAMF exerts apparent antidiabetic effects and represents a valuable nutraceutical candidate for insulin-resistant type 2 diabetes and its associated complications, such as dyslipidemia, oxidative stress, and inflammation.

P6.03.13
Can dietary intervention with whole grain decrease risk of type 2 diabetes by reducing inflammasome assembly and IL1β secretion by circulating human neutrophils?

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Central obesity, hypertension, dyslipidemia, and dysglycaemia are all common risk factors for development of type 2 diabetes (T2D) and have been suggested to induce a state of chronic low-grade systemic inflammation (CLGSI), driven by the proinflammatory cytokine interleukin 1β (IL1β), released by the predominant leukocyte in the blood, the neutrophils. Cell and animal studies suggest secretion of IL1β can be reduced by binding of short chain fatty acids (SCFAs) to the free fatty acid receptor 2 (FFAR2/GPR43) on the surface of neutrophils. In the human gut, fermentation of dietary fibers results in formation of SCFAs. These are capable of crossing the epithelial barrier and enter the blood stream. In the current study, we hypothesize that a diet rich in whole grain will increase SCFA-levels in the blood, reduce proinflammatory cytokine-levels (incl. IL1β) in the blood, and thus alleviate the state of CLGSI. Sixty adult study participants (SPs), harboring at least two of the risk factors for T2D development, are subjected to a cross-over dietary intervention. SPs will consume >75 g of whole grain (WG) daily for 8 weeks, and <10 g of WG daily for 8 weeks, separated by 6 weeks of wash-out. Flanking each 8-week period, a fasting blood sample will be taken and the immunological state will be evaluated by measuring blood cytokines levels (e.g. IL1β, TNFα, IL6, IL18, and INFγ), surface availability of IL1β-receptors on neutrophils, and expression levels of proinflammatory cytokines and regulatory components of IL1β, such as NLRP3 and caspase-1.

P6.03.14
Carbon monoxide-releasing molecule CORM-A1 attenuates the development of autoimmune diabetes in mice induced by multiple low doses of streptozotocin

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Recent studies identified carbon monoxide (CO) as a potential therapeutic molecule due to its anti-inflammatory and anti-apoptotic abilities. CO-releasing molecule CORM-A1 is a compound designed to liberate controlled quantities of CO in the cellular systems. However, its biological activity in autoimmune type 1 diabetes (T1D) has not been examined so far. Therefore, in the present study we investigated possible therapeutic value of CORM-A1 in the model of diabetes induced in C57BL/6 mice by multiple low doses of streptozotocin. Administration of CORM-A1 during diabetes induction, or even after the induction of the disease, improved clinical and histological signs of the disease. The interference with the disease was accompanied with reduced proinflammatory cytokine (IFN-γ, IL-2, IL-17 and TNF-α) production concurrent with increased IL-4 and TGF-β secretion. In addition to anti-inflammatory properties, *in vitro* studies revealed that CORM-A1 reduced cytokine-induced cell death of pancreatic islets, as well as beta cells (MIN and RINm5F insulinoma cells). However, a cytoprotective effect was lost when inactive CORM-A1 form, that does not liberate CO, or hemoglobin, a scavenger of CO, was employed. Although the molecular mechanisms involved in the drug action remain to be established, our results suggest that the observed beneficial effect of CORM-A1 in the disease process could be attributed, at least partly, to the interference of CORM-A1-released CO with cytokine-mediated pro-apoptotic stimuli within endocrine pancreas. CORM-A1 may thus represent a novel treatment strategy that would operate through interfering with an islet-directed autoimmune response.
(Project ON173013)

P6.03.15
Activation of Natural Killer T Cells Promotes Th2 Immune Response in Adipose Tissue of Obese Galectin-3 Deficient Mice and Improves Systemic Glucose Homeostasis

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Diet induced obesity leads to inflammation in adipose tissue and insulin resistance. After shorter high-fat diet feeding (15 weeks) these effects, accompanied with an increase in IFN-γ expressing NKT cells, were seen in Galectin-3 deficient (LGALS3$^{-/-}$) mice but not in wild type C57BL/6 mice. To elucidate the role of NKT cells activation and the role of Galectin-3 in this process we treated mice with lipid agonist α-galactosylceramide (α-GalCer). We found that activation of NKT cells by α-GalCer significantly decreased fasting blood glucose and insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) in obese LGALS3$^{-/-}$ mice fed high-fat diet for 15 weeks. After treatment, visceral adipose tissue (VAT) of obese LGALS3$^{-/-}$ mice exhibited increased incidence of CD3^{+}NK1.1^{+} NKT, NK1.1^{+} and CD11c^{+} dendritic cells, while F4/80^{+} macrophages were decreased. In addition, the number of IL-4 and IL-5 expressing NKT cells were increased in VAT and was associated with higher levels of IL-4 and IL-13, and lower IL-1β in sera of obese LGALS3$^{-/-}$ mice. α-GalCer increased NK1.1^{+} and CD11c^{+} dendritic cells and significantly reduced pro-inflammatory F4/80^{+}CD11c^{+}CD206^{+} and F4/80^{+}IL-6^{+} macrophages in livers of obese LGALS3$^{-/-}$ mice. *In vitro* stimulated splenocytes by anti-CD3 and anti-CD28 antibodies from obese LGALS3$^{-/-}$ mice treated with α-GalCer produced significantly higher amounts of IL-4 and IL-10 compared to saline-treated diet-matched controls. These findings suggest that activated NKT cells skew the immune response to a Th2 phenotype where Th2 cytokines and anti-inflammatory IL-10 could be responsible for the improvement of insulin resistance in obese Gal-3 deficient mice.

P6.03.16
IL-2 and rapamycin in immunotherapy of type 1 diabetes: friends or foes ?

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Administration of low-dose IL-2 alone or combined with rapamycin (RAPA) prevents type 1 diabetes (T1D). Also, low-dose IL-2 cures

recent onset T1D in NOD mice, partially by boosting pancreatic regulatory T cells (Treg cells). These approaches are currently being evaluated in humans. Here, we studied the mechanism of action of higher IL-2 doses and low-dose IL-2 and RAPA (RAPA/IL-2) combination. We assessed the effect of high doses of IL-2 or the combined treatment in NOD T1D evolution, including flow cytometric analysis of immune-competent cells and glucose metabolism assessment by glucose tolerance tests and microarray analysis of liver response to glucose. We show that high doses of IL-2, despite further boosting Treg cells, rapidly precipitated T1D in pre-diabetic female and also male mice and induced a striking increase in pancreatic myeloid cells. RAPA counteracted IL-2 effects on Treg cells, failed to control IL-2-boosted NK cells and broke IL-2-induced tolerance in a reversible way. Notably, RAPA/IL-2 combination failure to cure T1D was associated to an unexpected deleterious effect on glucose homeostasis at multiple levels. Our data help understand the therapeutic limitations of IL-2 alone or RAPA/IL-2 combination and could lead to the design of improved therapies for T1D.

P6.03.17
Treg CD39+ and Th17 cells in Type 2 Diabetes Mellitus Patients

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Type 2 diabetes mellitus (T2D) represents a chronic and progressive syndrome characterized by hyperglycemia which results from insulin resistance and pancreatic β-cell dysfunction. It is clear that inflammation and cytokine production by the immune system and adipose tissue plays an important role in the pathogenesis of T2D. On the other hand, naturally occurring regulatory T (Treg) cells maintain the tolerance to self-antigens. A dysregulation in the number or function in Treg cells contributes to autoimmune diseases, chronic inflammatory diseases, and cancer. CD39, an ectonucleotidase which hydrolyzes ATP is expressed on a subset of human natural Treg cells. IL-17-producing $CD4^+$ T cells (Th17 cells) could be pathogenic in many diseases and are resistant to suppression by human $Foxp3^+$ Treg cells. The aim of this work was to evaluate the expression of Treg CD39+ and Th17 cells in peripheral blood mononuclear cells from T2D patients. We found similar levels of CD4+Foxp3+CD39+ and CD4+Foxp3+CD39- Treg, in healthy subjects (n=24) and T2D patients (n=24). In contrast, Th17 cells were found significantly diminished in T2D patients compared to healthy subjects. T2D patients were classified according to the WHO body mass index categories and patients with overweight and obesity showed the lowest levels of CD4+IL-17+ T cells, compared to T2D patients with normal weight. These data are in agreement with the levels of mRNA IL-17. In conclusion, our results indicate that decreased expression of Th17 cells could be important in understanding the defective regulation of inflammation in diabetes.

P6.03.18
Immunohistochemical study of the pancreas of patients with long termed diabetes mellitus type 1

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Pathological findings in the pancreases of diabetes mellitus type1 (DM1) patients include peri-and intra-insular inflammatory infiltrates, namely insulitis. Although considered to be pathognomonic for recent onset disease, insulitis has only been described in approximately 150 cases over the past century.
We have studied the autopsy samples of the pancreas of 5 adult patients with long termed DM1 and of 9 adults not suffering from disorders of carbohydrate metabolism as a control. Antibodies to insulin and glucagon were used for the detection of hormones of the endocrine cells. Antibodies to CD8, CD16, CD20, CD25 and CD71 were used as immunological markers.
In the control samples of the pancreas as well as in the pancreas of two patients with DM1 CD16-, CD25-, CD8-, CD20- and CD71-positive cells were observed only in a small number among the blood cells.
We have identified the inflammation in the acinar part of three patients with DM1. CD16+ and CD25+ cells have made the largest contribution to this reaction. The number of cells positive for antibodies to the transferrin receptor (CD71) was increased compared to the control. The amount of cells positive for antibodies to CD8 and CD20 in these samples was small.
It is believed that insulitis is characteristic for the islets, which have retained a significant part of the b-cells. We have detected the inflammation in exocrine part of the pancreas in 2 patients with DM1 with absolutely absence of b-cells. Therefore, this reaction can hardly be regarded as directed only against insulin-containing cells.

P6.03.19
ST2 deficiency enhances diet-induced inflammation in visceral adipose tissue and obesity in mice

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Obesity associated low-grade chronic inflammation in adipose tissue and may contribute to type 2 diabetes. Although interleukin (IL)-33 may have protective role in obesity and atherosclerosis, the contribution of IL-33/ST2 axis in metabolic disorders needs to be elucidated. We investigated the role of ST2 in high-fat diet (HFD)-induced obesity using ST2-deficient ($ST2^{-/-}$) and wild type mice on BALB/c background relatively resistant to HFD-induced obesity. The deletion of ST2 enhanced systemic and visceral adipose tissue (VAT) inflammation and was associated with significantly higher weight gain and amount of total VAT in $ST2^{-/-}$ mice fed HFD for 18 weeks. More numerous M1 macrophages and markedly decreased M2 macrophages were observed in VAT of HFD-fed $ST2^{-/-}$ mice. Additionally, VAT of $ST2^{-/-}$ mice fed HFD had increased percentage of $CD3^+$ T cells with lower incidence of $CD4^+CD25^+FoxP3^+$ T regulatory cells and $CD4^+PD\text{-}1^+$ T cells in comparison with low-fat diet fed controls and a pronounced increase of the percentage of $CD19^+$ B cells. The incidence of IL-5 and IL-17 expressing stromal vascular fraction cells were significantly lower in HFD-fed $ST2^{-/-}$ mice. Serum levels of pro-inflammatory cytokines IL-1β and IFN-γ were also increased in HFD-fed $ST2^{-/-}$ mice, while the levels of IL-6 and CRP did not differ among groups. Importantly, the levels of anti-inflammatory IL-10 and IL-13 were significantly lower in the sera of $ST2^{-/-}$ mice compared with wild type controls. Our findings suggest that protective role of IL33/ST2 signaling in diet-induced adipose tissue inflammation is mediated by downregulating M1 macrophages and Th1 cell induction.

P6.03.20
Oral administration of L. Lactis secreting $hGAD65_{370\text{-}575}$ and hIL-10 can revert diabetes in recent-onset NOD mice when combined with low-dose anti-CD3

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In type 1 diabetic patients insulin-producing pancreatic beta-cells are destroyed by self-reactive autoantigen-specific T cells. As a therapy, reinstalling antigen (Ag)-specific tolerance is considered the favorable approach. Previous studies show that orally-administered genetically-modified *lactococcus lactis* (*L. lactis)* secreting human pro-insulin and human IL10 (hIL10) induce diabetes remission in NOD mice when combined with low-doses of anti-CD3. Using *L. lactis* as an innovative protein carrier this study aimed to determine whether another islet autoAg, GAD65, could establish or increase this diabetes reversal rate in new-onset NOD mice. Based on epitope spreading, bacterial secretion and growth profile, a bacterial strain secreting hIL10 and $hGAD65_{370\text{-}575}$ was evaluated *in vivo*. New-onset diabetic NOD mice were given a subtherapeutic dose of anti-CD3 for 5 consecutive days (clone 145-2C11, 2.5 µg/d, iv.) and *L. lactis* 5 times weekly for 6 weeks (10^9 CFU/d, by gavage). Treatment with low-dose anti-CD3 and $hGAD65_{370\text{-}575}$ plus hIL10 (n=36) significantly induced 67% diabetes reversal in new-onset diabetic NOD mice compared to 31% and 37% using anti-CD3 alone (n=42) or with empty vector (n=32),

respectively. In addition, $CD4^+CD25^+$ T-cells isolated from combi-GAD cured animals could counter the proliferation and IFNy secretion of polyclonally stimulated $CD4^+CD25^-$ responder T-cells. In conclusion, like pro-insulin also GAD65 when combined with hIL-10 and a systemic low-dose of anti-CD3 stably reverses diabetes in recent-onset NOD mice.

P6.03.21
Transfer of type 1 diabetes by bone marrow transplantation

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Transfer of autoimmune type 1 diabetes (T1D) between HLA-identical siblings by bone marrow transplantation (BMT) has been previously described (Lampeter et al. Lancet 1993). However, the mechanism at the basis of disease transfer has never been investigated. Here we report a case in which T1D was developed following allogeneic BMT in a patient affected by mycosis fungoides. The donor was an HLA-identical brother with T1D. Full donor engraftment was reported 1 year after BMT and confirmed annually up to 7 years after transplantation. Nine years after BMT, the recipient was diagnosed with T1D. Anti-GAD and anti-IAA autoAbs were negative in the recipient before transplantation, while 9 year after transplant autoAb levels were high and almost superimposable to those found in the donor. The frequency and functional activity of T cells specific for GAD, Pro-insulin and tetanus toxoid (TT) (as control) were tested. Donor and recipient had similar frequencies of circulating diabetogenic T cells and Ag specific T-cell clones had similar cytokine profile. On the contrary, the frequency of TT-specific T cells was very different between donor and recipient. Data from TCR deep sequencing are being generated and will help to understanding whether auto-reactive T cells isolated from the donor and the recipient originate from the same clone or not. This rare phenomenon of T1D transfer after BMT represents a unique opportunity for better understanding the origin and, potentially, the cure of autoimmune T1D.

P6.03.22
Characterization of monoclonal ZnT8-specific antibody

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Immune-mediated destruction of insulin-producing beta cells of pancreas leads to autoimmune type 1 diabetes mellitus (T1D). Recently, ZnT8 (zinc transporter 8) has been identified as an autoimmune target in T1D. ZnT8 is expressed in the pancreatic tissue and regulates Zn metabolism in beta cells. Zn is critical for normal storage of insulin in the secretory vesicles of islet beta cells. ZnT8 autoantibodies are directed mostly against the C terminal domain. Molecular biological tools for basic research of ZnT8 role in T1D are needed. In this report, we characterize a new monoclonal antibody to the ZnT8 C-terminus. Mice were immunized with Semliki Forest virus-like particles expressing full-length ZnT8 and hybridomas were obtained and cloned. Hybridomas were tested using LIPS (Luciferase Immunoprecipitation system) and ELISA using purified recombinant C-terminal domain of ZnT8. One of the hybridomas giving a very high signal was selected for further analysis. Protein A purified Mab was used to characterize binding epitope using 18 overlapping peptides designed according to C-terminal sequence of ZnT8. Binding epitope was identified using four methods: LIPS assay, ELISA, biosensor binding test, and immunohistochemistry assay with monkey pancreatic iselets. In conclusion, we have characterized a new monoclonal antibody usable as a molecular biological tool for ZnT8 research.

P6.03.23
Autocrine CCL2, CXCL4, CXCL9 and CXCL10 signaling in retinal endothelial cells is enhanced in diabetic retinopathy

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Chemokines influence many biological processes, including immunological reactions and angiogenesis, both physiological and pathological. Different chemokines contribute to either side of the angiogenic/angiostatic balance. CXCR3 ligands for instance favour angiostasis, inhibiting endothelial cell proliferation and chemotaxis. This study aimed at examining the production of chemokines (angiogenic CCL2/MCP-1 and angiostatic CXCL4/PF-4, CXCL9/Mig and CXCL10/IP-10) in proliferative diabetic retinopathy (PDR), an inflammatory condition characterized by exaggerated retinal neovascularisation leading to blindness. Chemokine production in human retinal microvascular endothelial cells (HRMEC) and chemokine levels in vitreous samples from PDR patients were analyzed. CCL2, CXCL4, CXCL9 and CXCL10 as well as the angiogenic factor VEGF were elevated in vitreous fluid from PDR patients. Additionally, we found HRMEC to produce CCL2, CXCL9 and CXCL10 *in vitro*, namely after IFN-γ, IL-1β or lipopolysaccharide stimulation. IFN-γ synergistically enhanced CXCL9 and CXCL10 production in response to IL-1β or lipopolysaccharide. CCL2 was also produced in response to VEGF. Further we wanted to improve our understanding of the contradictory role of angiostatic chemokines produced in angiogenic pathologies. Interaction of angiostatic chemokines and angiogenic factors (VEGF and the chemokines CCL2 and CXCL12) was investigated at the level of signal transduction. The MAPK family was shown to be a link between angiogenic and angiostatic signaling. Phosphorylation of ERK induced by angiogenic factors was inhibited by CXCL4, CXCL9 and CXCL10. Finally, in accordance with inhibition of angiogenic signaling, CXCL4 inhibited *in vitro* migration of HRMEC. Our study thus reveals enhanced autocrine signaling of CCL2, CXCL4, CXCL9 and CXCL10 in HRMEC in PDR.

P6.03.24
Protein S attenuates streptozotocin-induced diabetes mellitus

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OBJECTIVE: Protein S (PS) is an important cofactor of activated protein C (APC) in the inhibition of coagulation cofactors Va and VIIIa. PS can also regulate the inflammatory response and apoptosis. The effect of PS on glucose tolerance is unknown. In this study, we evaluated the effect of PS on streptozotocin (STZ)-induced diabetes mellitus.
METHODS: PS transgenic mice were used in the experiments. Diabetes was induced in PS transgenic and wild type mice by intraperitoneal (i.p.) injection of STZ for five consecutive days. Mice were categorized into four groups: wild type/saline (WT/SAL), PS/saline (PS/SAL), wild type/STZ (WT/STZ) and PS/STZ (PS/STZ) groups. Blood glucose was measured weekly. Four weeks after STZ or saline treatment, glucose tolerance test and glucose-stimulated insulin secretion test were performed and mice were sacrificed. Serum insulin level at sacrifice and islet area in hematoxilin-eosin stained pancreatic tissue section were measured.
RESULTS: The blood glucose levels were significantly lower on days 14 and 21 in PS/STZ mice compared with WT/STZ mice. Improved glucose tolerance and insulin secretion were observed in PS/STZ mice compared with WT/STZ mice. The islet area was significantly decreased in WT/STZ mice compared with other groups.
CONCLUSION: PS attenuates diabetes probably by inhibiting the effect of STZ on pancreatic islets.

P6.03.25
Effects of OPN and its cleavage products on human monocyte differentiation and survival

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Passive immunization against osteopontin (OPN), an inflammatory mediator increased in adipose tissue by obesity, counteracts obesity-associated adipose tissue inflammation and insulin resistance by reducing macrophage abundance in metabolic tissues, in part due to augmented macrophage apoptosis. Protease cleaved forms (cOPN) occur after thrombin or matrix metalloprotease cleavage at inflammatory sites and expose specific cryptic epitopes. Here we show that presence of OPN for 2 days in vitro increased the number of metabolically active human primary monocytes by 400%. TUNEL assays revealed that the percentage of apoptotic monocytes was reduced to 20% with OPN compared to untreated cells 24 hours after isolation. Furthermore, OPN induced a massive cytokine response of IL-1β, IL-10, TNF-α, RANTES and MCP-1. Monocytes differentiated for 8 days in presence of OPN up-regulated CD206 and CD163 but also CD40 and CD80. Cytokine analysis of supernatants taken on day 8 after isolation following 24 hours of stimulation revealed an increase in IL-1β and MCP-1 production but a strong down-regulation of IL-10 secretion mediated by OPN. The effects of cOPN were qualitatively very similar, but quantitatively less pronounced. In conclusion, OPN is a survival factor for human monocytes that may, independently of protease cleavage, considerably contribute to obesity-induced adipose tissue inflammation. Moreover, OPN may contribute to the peculiar phenotype of human adipose tissue macrophages with M2-like surface marker expression but M1-like cytokine production.
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P6.04 Treatment of rheumatoid arthritis and other inflammatory joint diseases

P6.04.01
Modulation of the oxido-inflammatory and immunomodulatory cascade contributes to the anti-arthritic activity of curcumin loaded solid lipid nanoparticles in CFA-induced arthritis in rats

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Rheumatoid arthritis (RA) is a systemic inflammatory disorder which is characterized by chronic inflammation of the joints and destruction of cartilage. Effectiveness of curcumin has been established in a wide variety of human diseases including inflammatory disorders, but it is yet to be approved as a therapeutic agent due to its poor absorption, instability at physiological pH, rapid metabolism and systemic elimination. To overcome the compromised physicochemical properties of curcumin, we proposed to evaluate curcumin loaded solid lipid nanoparticles (C-SLNs), on mobility scores, biochemical, hematological and radiological alterations in complete Freund's adjuvant (CFA)-induced arthritis in rats. CFA injected rats exhibited marked decrease in reaction time (thermal hyperalgesia) in hot plate test along with significantly decreased paw withdrawal threshold in Randall-Sellito test (mechanical hyperalgesia) and von-Frey hair test (mechanical allodynia). Arthritic rats also showed a significant joint hyperalgesia, joint stiffness and increased paw volume along with marked decrease in mobility score. CFA-induced arthritis was associated with significantly enhanced blood leukocyte count, oxidative-nitrosative stress, tumor necrosis factor-α (TNF-α), C-reactive protein and radiological alterations in tibio-tarsal joint. All these alterations were significantly and dose-dependently ameliorated by Curcumin-SLNs administration (10 and 30 mg/kg). Plain curcumin 30 mg/kg showed some protection whereas plain curcumin 10 mg/kg failed to show any significant effect. In conclusion, the current findings suggest the protective potential of curcumin in ameliorating CFA-induced arthritis in rats through modulation of oxido-inflammatory & immunomodulatory cascade. Further, the results emphasize that SLNs are a novel approach to deliver curcumin into the inflamed joints.

P6.04.02
Efficacy and safety of biologic agents in adult-onset Still's disease: a long-term follow-up of 19 patients at a single referral Center

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Background: Long-term outcome of biological drugs treatment in Adult-Onset Still's Disease (AOSD) is undetermined. We evaluated efficacy and safety of different biological agents in a large cohort of AOSD patients.
Methods: Nineteen AOSD patients treated with biological agents were followed-up between 1998 and 2013. We evaluated disease courses, efficacy of treatments, and adverse effects. Efficacy was evaluated as 'Complete response' (CR: absence of disease manifestations/inflammatory indexes;>50% reduction in corticosteroid dosage), 'Partial response' (PR: clinical improvement without normalization of inflammatory markers, nor>50% reduction in corticosteroid dosage), or 'Treatment failure' (TF: persistence/worsening of disease manifestations/markers; increased corticosteroid dosage).
Results: Average follow-up was 5 years. Overall, biologic drugs induced an improvement in 17(89%) patients. Anakinra was used in all patients; etanercept, tocilizumab and adalimumab in 6, 4, and 1, respectively. Fifteen patients responded to anakinra (79%, with, 68% CR and 11% PR). Four patients (21%) did not respond to anakinra; 3 (16%) responded to tocilizumab, and 1 (5%) to adalimumab. Etanercept proved unsuccessful in six patients. Corticosteroids dose was decreased in all patients; immunosuppressants dose in 14 (74%). Three patients experienced herpes zoster reactivation.
Conclusion: Biological agents represent an effective and safe therapeutic resource for AOSD refractory to conventional treatment. Anakinra represented the mainstay of treatment. Both anakinra and tocilizumab were more effective than TNF-α blockers. IL-6 blockade may be particularly effective in patients with chronic articular involvement. Patients unsuccessfully treated with biologics had already developed irreversible structural damages prior to initiation of treatment. Thus, a more prompt initiation of biological agents could be beneficial in severe AOSD.

P6.04.03
Modulations in the Expression of Leukocyte Complement Receptors (CR1 and CR2) in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder of unknown etiology. The complement proteins have been known to play complex roles in the pathogenesis of RA. The growing importance of complement receptor 1 (CR1) and complement receptor 2 (CR2) not only in modulation of complement activation at several levels but also in B-cell activation and immune complex localization, suggests that its expression may have significant effects on health and disease. We conducted a case-control study to explore the role of Leukocyte CR1 and CR2 ((L- CR1& L-CR2) in human RA. The L-CR1 and L-CR2 expression in 57 healthy controls and 57 RA patients was evaluated at mRNA levels by RT-PCR. Disease activity scores (DAS28) were monitored in RA patients using swollen and tender joint counts and the ESR. The circulating immune complex (CIC) levels were spectrophotometrically determined and levels of C3 were measured by nephlometry in both controls and patients. The correlations of L- CR1 and L-CR2 expression with clinical parameters (DAS28, CIC and C3) were evaluated. The L-CR1 and L-CR2 transcripts declined significantly in patients. A significant negative correlation of CR1 and significant positive correlation of CR2 transcript were observed with CIC only in patients. C3 was correlated

positively with CR2 transcript in both patients and controls. In essence, our findings suggest a close relationship of CR1 and CR2 with the pathophysiology and disease activity of RA. The findings also may have important diagnostic, prognostic and therapeutic implications.

P6.04.04
APL-1, an altered peptide ligand derived from HSP60 in combination with methotrexate, attenuates murine collagen induced arthritis associated with an increment of regulatory T cells

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Activation of autoreactive T cells induced by autoantigens play an important role in the pathogenesis of rheumatoid arthritis (RA). Recent studies have identified defects in the number or activity of regulatory T cells (Treg) in patients with this disease. In this sense, induction of peripheral tolerance has been considered a promising approach to the treatment of RA. Previously, we demonstrated that an Altered Peptide Ligand (APL) derived from Hsp60, an autoantigen involved in the pathogenesis of RA, efficiently inhibited the course of adjuvant induced arthritis model. This effect was associated with an increase of the proportions of Treg. In this work, we aimed to evaluate the therapeutic effect of this APL in combination with metotrexate (MTX) in collagen induced arthritis (CIA). MTX is considered the gold standard treatment for RA. CIA was induced in male DBA/1 mice at eight weeks of age by immunization with chicken collagen. APL, MTX or both were administrated beginning from arthritis onset. Therapeutic effect was evaluated by arthritis and joint pathologic scores. In addition, TNFα in sera was measured with ELISA and Treg induction was assessed by FACS analysis. Therapy with APL, MTX or the combination reduced arthritis scores and histological lesion. This effect was associated with a decrease of TNFα levels. However, only the combination of APL and MTX increases the proportions of the Treg. These results indicate a therapeutic potentiality of APL with MTX and support further investigation of this combination for treatment of RA.

P6.04.05
Interplay between TWEAK and IL-17 biology: a novel therapeutic concept for treatment of autoimmune diseases (e.g. RA and Lupus)

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Rheumatoid arthritis is a systemic autoimmune disease characterized by synovial inflammation and neovascularization, resulting in cartilage/bone damage and joint destruction. There remains a high unmet medical need for novel therapeutics to achieve sustained remission as there is a high rate of non-responders to standard therapies. One approach could be the targeting of synergistic and/or complementary disease pathways. In this study, we assessed the impact of TWEAK and Interleukin-17 in several pre-clinical models as these two cytokines are found at elevated levels in the serum/inflamed tissue of patients suffering from autoimmune diseases such as RA and Lupus. Interestingly, in addition to effects of the single cytokines, combination of TWEAK and IL-17 clearly demonstrated additive or synergistic effects on cytokine and MMP production by different RA-relevant cell types (synovial fibroblast and chondrocytes), osteoclast activation and reduced osteoblast differentiation in vitro, whereas ICAM-1 expression and FLS proliferation was more TWEAK-dependent. Parallel blockade of both pathways in an in vivo RA-model demonstrated beneficial impact on osteoclast and osteoblast numbers and bone formation markers (in the range of TNFα blockade), but lower effects in the reduction of inflammation, bone erosion ad cartilage damage.
Finally, inhouse generated bispecific antibodies that efficiently neutralized both cytokines showed higher inhibitory potential also upon combinational cytokine treatment in vitro.
In summary, our data demonstrate that TWEAK and IL-17 are crucial players in RA pathogenesis. We hypothesize that dual targeting of these cytokines will result in complementary anti-inflammatory and bone/cartilage regenerative activity, representing a novel and attractive treatment strategy for RA.

P6.04.06
Generation of recombinant antibodies with TCR-like specificity directed toward autoimmune epitopes

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Rheumatoid arthritis (RA) is a chronic inflammatory process of the joints that progressively leads to functional impairment and bone erosions. This autoimmune disease occurs with relative high frequency in individuals expressing Human Leukocyte Antigen (HLA) DR4 (DRB1*0401 allele). While presented by HLA-DR4 molecule, residues 261-273 of Collagen Type 2 (CII) represent an RA immunodominant T cell epitope. By using phage display technique and antibody engineering approaches we aim to isolate recombinant antibodies (rAbs) that recognize HLA-DR4/CII-261-273 autoreactive epitope. These rAbs, termed T Cell Receptor-Like (TCRL) rAbs, bind the autoimmune-associated peptide only in the context of the MHC class II in a TCR-Like specificity pattern. A major advantage of TCRLs is that while mimicking the specificity of the TCR they have significantly higher binding affinity. TCRL rAbs against autoantigen bound to its HLA-DR4 molecule can be significance for two major research directions: as a tool to study autoantigen presentation during the autoimmune disease with the ability to detect and image antigen-presenting cells that present the autoantigen. Second, for translational-clinical applications, where TCRL rAbs potentially will be able to regulate the proliferation of autoreactive T cells in a highly selective manner. By establishing our TCRL technology, we intend to generate TCRL rAbs against HLA-DR4/CII-261-273 epitope. These TCRL rAbs will represent a valuable research tool for studying autoantigen presentation during RA. Additionally, by blocking presentation of this epitope, TCRL rAbs potentially will be able to regulate pathogenic T cells responses in a highly selective manner and may constitute novel RA therapeutic agents.

P6.04.07
Therapeutic effects of adiponectin-mediated trimeric sTNFRII on collagen-induced arthritis

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We have developed a novel trimeric sTNFRII, named sTNFRII-gAD fusion protein, which exhibits a higher in vitro antagonistic efficacy for TNFα compared with sTNFRII-Fc. This study was aimed to investigate the anti-arthritic capacity of sTNFRII-gAD in a rat collage-induced arthritis model (CIA). The rats were injected intradermally with 200μg collagen type II (CII) at day 0 and day 7. Three days post the second CII injection (day 10), the rats were intraperitoneally given 200μg of sTNFRII-gAD or sTNFRII-Fc, or sterile saline. Effects of sTNFRII-gAD treatment were evaluated by CIA incidence, severity and pathological changes. Serum TNFα, IL-17A and regulatory T cell (Treg) in periphery were examined at day 10 and 16, respectively. Our results showed that sTNFRII-gAD significantly decreased CIA incidence and severity ($p<0.05$), and that sTNFRII-gAD treatment led to a dramatic reduction in cartilage and bone damage. Moreover, the increase in serum anti-CII and IL-17A, and the reduction in Treg population were suppressed ($p<0.05$) by the administration of sTNFRII-gAD or sTNFRII-Fc. TNFα was found to be accumulated in serum in the groups treated with sTNFRII-gAD or sTNFRII-Fc compared with the group treated with saline ($p<0.05$). Noteworthily, sTNFRII-gAD showed a better efficacy than sTNFRII-Fc in CIA

incidence, pathological changes in cartilage and the elevation of anti-CII antibody, indicating that sTNFRII-gAD is potentially a more efficacious TNFαantagonist for rheumatoid arthritis.

P6.04.09
TRAIL reduced joint inflammation, osteoclast activation and bone loss in experimental arthritis

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TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in diverse tumor cells. Recent studies have shown TRAIL also regulate osteoclast activation and inflammation, suggesting that it may be involved in the pathogenesis of rheumatoid arthritis (RA). We investigated whether treatment with TRAIL would modulate the severity of the disease in a collagen-induced arthritis (CIA) rat model. Rats with CIA were treated subcutaneously with recombinant TRAIL, with the tumor necrosis factor (TNF) blocker etanercept, or with TRAIL in combination with etanercept. Arthritis severity was determined according to the hind paw thickness, arthritis severity score, degree of cartilage damage, bone mineral density, and cytokine production, which were evaluated using microfocal computed tomography, and enzyme-linked immunosorbent assay. Our results demonstrated that treatment with TRAIL alone or in combination with etanercept significantly reduced the severity of arthritis by decreasing the hind paw thickness and swelling, preventing cartilage damage and bone loss, and reducing the expression of IL-1β, IL-6 and TNF in synovial tissue. In vitro, TRAIL inhibited RANKL-induced osteoclast activation. Taken together, treatment with TRAIL inhibited inflammation and bone loss in rats with CIA. Treatment with TRAIL combined with etanercept protected rats from CIA better than treatment with etanercept alone. Our findings provide evidence that TRAIL may be a potential therapeutic agent for RA

P6.04.10
Diacerein ameliorates the evolution of inflammatory periodontal bone loss by inhibiting RANKL-induced osteoclastogenesis

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Diacerein (DAR) a purified anthraquinone derivative, which is found originally in plants with anti-inflammatory and analgesic activities, therefore it also known as “disease-modifying osteoarthritis drugs (DMOAD)” or “chondroprotective agents”. Both in vitro and in vivo experiments have also revealed that DAR possesses anti-inflammatory properties. Therefore, in the present study, we investigated the therapeutic effects of DAR in a rat model of periodontal disease induced by ligature placed around the maxilla second molars of each animal. Male S-D rats were divided into three groups: 1) animals without ligature placement receiving administration of empty vehicle (control); 2) animals with ligature receiving administration of empty vehicle; 3) animals with ligature receiving administration with oral DAR (100 mg/kg/day). The animals were sacrificed after 3, 7, 10 14 days after induction of periodontal disease, and maxillae and gingival tissues were removed for further analysis. An in vitro assay was also employed to test the inhibitory effects of DAR on osteoclastogenesis. Histomorphological analyses of periodontal tissue demonstrated that DAR-treated animals presented ameliorated alveolar bone destruction, compared to those animals with ligature, but treated with empty vehicle. Corresponding to such results obtained from in vivo experiments, DAR also suppressed in vitro osteoclast differentiation in the presence of RANKL in osteoclast precursor cells, along with the down-regulation of the expression of RANKL-induced TRAP mRNA. These data indicated that DAR may suppress the bone resorption by inhibiting RANKL-mediated osteoclastogenesis elicited during the course of experimental periodontitis in rats.

P6.04.11
Therapeutic effect of proteoglycan/vitamin D association in experimental arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory systemic autoimmune disease that compromises the joints. Current RA treatment consists in general suppression of immune response which increases susceptibility to infections. Considering the immunomodulatory properties and tolerogenic effects of active vitamin D (VitD3), this study aimed to evaluate if the association of the specific antigen (proteoglycan) with VitD3 has a therapeutic effect on experimental arthritis. For disease induction, female BALB/c retired breeder mice were intraperitoneally injected three times (21 days interval) with 100µg of bovine proteoglycan (PG) and 1mg of dimethyldioctadecyl ammonium bromide adjuvant. Ten days after the second injection, mice received 0,1µg of VitD3 (i.p.) every other day during 15 days. PG (50µg or 100µg) was co-administered on second and on eleventh day of treatment. After the third injection, disease severity was daily assessed by score evaluation. Although there was no difference on RA incidence, the association of PG and VitD3 determined lower scores and later disease onset. However, similar disease amelioration was also observed in animals treated with PG or VitD3 alone. Spleen cells from all groups produced similar levels of IL-6 and IL-5 and no detectable levels of TNF-alpha and IL-17 in response to stimulation with the specific antigen. Mice treated with PG/VitD3 association produced similar levels of IFN-gamma comparing to the untreated arthritic group. However, IL-10 levels were significantly increased in those treated animals, suggesting the contribution of regulatory T cells to the tolerogenic effect triggered by PG/VitD3 association.

P6.04.12
Metformin downregulates Th17 cells differentiation and attenuates murine autoimmune arthritis

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Introduction: This study was undertaken to determine whether metformin has anti-inflammatory effects in the collagen antibody-induced arthritis (CAIA) murine model. The effect of metformin on Th17 cell differentiation was also investigated.
Methods: CAIA mice were treated with 100 and 150 mg/kg i.p. metformin (low- and high-dose groups, respectively). Arthritis activity and histological joint destruction were studied. Flow cytometry was used to (i) determine RORγt-expressing CD4+ percentages in draining axillary lymph nodes (ALNs) from metformin-treated and untreated mice with CAIA, (ii) determine Th17 percentages in splenic CD4+ T cells cultured *ex vivo* for 3 days in Th17-differentiation-inducing conditions, and (iii) determine the percentages of RORγt+ CD4+ T cells when normal splenic T cells from DBA/1 mice were cultured in Th17-differentiation-inducing conditions together with various metformin doses. Western blot analysis was used to assess the intracellular signaling of the metformin-treated splenocytes.
Results: Metformin attenuated both arthritis scores and bone destruction in CAIA mice, decreased the serum levels of the pro-inflammatory cytokines, TNF-α and IL-1, and reduced the number of RORγt+ CD4+ T cells in the ALNs. Splenocytes from metformin-treated CAIA mice differentiated less readily into Th17 cells upon *ex vivo* stimulation. Metformin treatment of normal cells cultured in Th17-differentiation-inducing conditions decreased the number of RORγt-expressing CD4+ cells in a dose-dependent manner and downregulated STAT3 phosphorylation *via* the AMPK pathway.

Conclusions: Metformin had an anti-inflammatory effect on murine autoimmune arthritis due to the inhibition of Th17 cell differentiation. Metformin may have a possible therapeutic value for treatment of rheumatoid arthritis.

P6.04.13
Sinomenine inhibits osteoclastogenesis and attenuates LPS-induced osteolysis

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Sinomenine (SIN) is an anti-inflammatory and anti-arthritic alkaloid derived from the Chinese medicinal plant Sinomenioumacutum. Osteoclasts can resorp bone and are involved in the bone destruction in rheumatoid arthritis (RA). SIN is an active compound to treat RA, but its effect on osteoclasts has been hitherto unknown. In the present study, we observed that SIN suppressed RANKL and LPS-induced osteoclast formation and survival in vitro. The osteoclastic specific marker genes in RAW264.7 cells and osteoclast-like cells (OCLs), including TRACP, MMP-9, c-Src, integrin αVβ3 and cathepsin K, were also inhibited by SIN dose dependently. Furthermore, SIN inhibited LPS-induced TNF-α production in vitro. Mechanistic studies revealed that SIN obviously suppressed LPS-induced NF-κB activation, as well as the expression of TLR4 and TRAF6. In addition, SIN could selectively block LPS-induced phosphorylation of the MAPK including p38, and suppress the intracellular Ca2+ influx. Moreover, SIN abrogated LPS-induced over activation of AP-1 and NFATc1. Later, the therapeutic effects and molecular mechanisms of SIN on lipopolysaccharide (LPS)-induced osteolysis was investigated. We found that SIN could block LPS-induced osteolysis and TNF-α production in mouse calvarial model. Taken together, it demonstrated that SIN could inhibit osteoclastogenesis and relative signaling pathways to reduce LPS induced osteolysis. SIN might potentially treat bacterial or inflammation induced osteolysis, and might ameliorate RA partly via attenuating the inflammation-induced bone destruction.

P6.04.14
Carbon monoxide exposure improve immune function in lupus prone mice

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Systemic Lupus Erithematosus (SLE) is an autoimmune disease characterized by multiple alterations affecting the normal function of immune cells, such as lymphocytes, dendritic cells (DCs) and monocytes. Although the understanding of autoimmunity has been significantly increased recently, the breakthrough in effective therapies has been modest, making necessary the development of new therapeutic strategies. Here we propose that a new potential target for therapy is hemeoxygenase-1 (HO-1), an enzyme that catalyzes the degradation of the heme group into biliverdin, carbon monoxide (CO) and Fe+2. These products exhibit immunosuppressive and anti-inflammatory effects, which can contribute to improving tolerance during organ transplantation. The aim of this study was to evaluate whether CO administration could ameliorate disease in the FcγRIIb KO mouse model for SLE. We found that CO administration decreased the expansion of CD11b+ cells and prevented the decline of regulatory T cells (Tregs) observed in untreated FcγRIIb KO mice. Furthermore, CO-treated animals showed reduced anti-histone antibodies and less kidney damage as compared to untreated mice. These data suggest that CO administration can ameliorate autoimmunity and prevent the lupus symptoms shown by FcγRIIb KO mice, underscoring HO-1 as a potential new target for autoimmune therapy.

P6.04.15
Prostaglandin D2 in inflammatory arthritis and synovial myeloid dendritic cells as a source of hematopoietic prostaglandin D-synthase

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Prostaglandin (PG)D_2 has been shown to be an active agent in the resolution of experimentally induced inflammation. This study was undertaken to determine the presence of PGD_2 in chronic joint effusions and to explore the potential contributions of myeloid dendritic cells (mDC) and monocytes to the intra-articular synthesis of PGD_2.
Synovial fluid (SF) was obtained from patients with inflammatory arthritis and knee effusions. PGD_2 and PGE_2 were detected in SF by ultrahigh performance-tandem mass spectrometry. Cellular fractions in SF were separated by density-gradient centrifugation and flow cytometry. The expression of hematopoietic prostaglandin D synthase (hPGDS) and PGE synthase (PGES) mRNA was determined by RT-PCR.
Both PGD_2 and PGE_2 were detected in blood and SF, with PGD_2 being more abundant than PGE_2 in SF. mRNA for hPGDS was more abundant in SF mDC than SF monocytes (p<0.01) or PB monocytes (p<0.001). SF PGD_2 and hPGDS mRNA in SF mDC were inversely associated with serum C-reactive protein (p<0.01) and erythrocyte sedimentation rate (p<0.01) and a positive correlation was found between SF PGD_2 and expression of hPGDS by SF mDC (p<0.02).
The findings suggest synovial mDC may be an important source of hPGDS and that systemic disease activity may be influenced by actions of PGD_2 in RA and other arthropathies.

P6.04.16
Immunomodulatory activity of vitamin D on dendritic cells in patients with inflammatory arthritis

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Excessive activity of myeloid dendritic cells (DC) is postulated to be a central disease mechanism in rheumatoid arthritis (RA). *In vitro* studies were undertaken to explore a possible influence of 1α,25-dihydroxyvitamin-D3 (1,25OHD) on expression of mRNA encoding the synthase for the pro-resolution mediator prostaglandin D2 (PGDS) and certain inflammatory mediators in synovial fluid (SF) DC and peripheral blood monocyte-derived DC (MDDC) in patients with inflammatory arthritis.
SF aspirated from knees with effusions. DC in SF were separated by flow cytometry. DC were activated by LPS and the expression of mRNA was determined by RT-PCR.
The presence of 1,25OHD during *in vitro* differentiation of MDDC inhibited markedly expression of co-stimulatory molecule CD80, maturation marker CD83 and pro-inflammatory cytokine TNFα. During LPS activation of MDDC, 1,25OHD enhanced expression of hPGDS and CD14 mRNA and suppressed expression of RelB, a subunit of NFkb. Combined treatment with 1,25OHD and LPS had a synergistic effect on expression of IL1β and IL6 mRNA in SF mDC and MDDC compared stimulation with LPS alone. There were no significant changes in expression of prostaglanidin E synthase, IL23 and CD86 mRNA.
These *in vitro* studies suggest that 1,25OHD has complex effects on inflammation and may enhance resolution of chronic inflammation through increased expression of hPGDS but may also enhance expression of effector molecules. These latter effects may enhance innate defence against acute infection.

P6.04.17
Diagnostic value of Immunoblotting assay for determination of anti nuclear antibody (ANA) concentration in rheumatologic diseases

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Background: Antinuclear antibodies (ANAs) are common features of autoimmune connective tissue diseases. Various detection methods are used and there are newer techniques that are continuously put forward to facilitate diagnosis and therapeutic monitoring in connective tissue disease (CTD) patients. Immunofluorescence (IF) or Fluorescent Antibody Technique the most used and "gold standard" test for diagnosis. Enzyme-Linked Immunosorbent Assay (ELISA) is another routine test. For Immunoblotting (IB) assay, autoimmune ANA profiles are used which provide a qualitative in vitro assay for human autoantibodies to 15 different antigens. This study was conducted to compare three techniques (IF, IB, ELISA) in detection of ANA. If the sensitivity and specificity of IB superior to other methods, we can replace IF and ELISA with IB.
Materials and Methods: An analytical cross-sectional study of 85 sera from patients with Systemic lupus erythematous (SLE), Systemic sclerosis (SSc) and Dermatomyosities(DM) was undertaken at rheumatology and nephrology department and clinic of Emam Reza hospital from 89/11/1 to 90/10/30. Sera collected and stored at -80°c. Then they were used to detection of ANA with three techniques.
Results: Of all sera 63 (74.1%) were ELISA positive and 22 (25.9%) had negative ELISA.74 (87.1 %) IF and IB positive and 11 (12.9%) IF and IB negative were observed. The sensitivity and specificity of IB in comparison with IF was 98.65% and 90.91%, respectively. In comparison, with ELISA we found 93.65% and 31.82% of sensitivity and specificity.
Conclusion: Immunoblotting has high sensitivity and specificity, and it can be used in the screening of ANA.

P6.04.18
In vitro evaluation of infliximab-specific T cells in patients with acute infusion reaction

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Infliximab (IFX), a chimeric anti-TNFα monoclonal antibody, is an established targeted therapy for immuno-mediated inflammatory diseases. As other biotherapeutics, it carries potential risks of immunogenicity, with the production of specific anti-IFX antibodies (ATI) that can lead to loss of response or acute infusion reactions. Our study was aimed to analyse the memory T-cell response to IFX in patients who developed ATI.
We investigated the presence of IFX-specific T cells in 50 subjects (16 reactive ATI+, 8 non responder ATI+, 17 tolerant ATI- and 9 non exposed healthy donors), using different T-cell proliferation assays. Drug-specific T cell clones (TCC) were also generated from PBMC of 2 reactive patients.
PBMC obtained from 11 (9 reactive and 2 non responder) out of 24 (45.8%) ATI+ patients displayed a proliferative to IFX, while no proliferation was detectable in both ATI- patients and healthy donors. Cytokine (IL-13, IFNγ, IL-17) production by PBMC, as well as by TCC, upon in vitro re-stimulation with IFX, was highly variable between the patients. The analysis of Vβ usage analysis of drug-specific TCC revealed an oligoclonal expansion. An high proportion of IFX-specific TCC, showed a regulatory phenotype and function. Cross-reactivity of IFX-specific TCC with other chimeric mAbs and murine IgG was also detected.
Overall, our study provide evidence of both effector and regulatory memory T cells specific for IFX and contributes to the understanding of pathogenic mechanisms of immunogenicity of biological agents.

P6.04.19
Immunological mechanism of IL-7R antibody on collagen II-induced arthritis

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In this study, we used collagen II-induced arthritis (CIA) model in DBA-1 mice to investigate the potential effects of IL-7Rα antibody on rheumatoid arthritis. Our results showed that in vivo treatment of CIA mice with IL-7Rα antibody had great protective benefit at both clinical and histological levels. Disease suppression was associated with inhibition of CII-specific $CD4^+$ T cell proliferation and decrease of inflammatory cytokine secretion (such as IFN-γ, IL-17 and TNF-α). We further analyzed the mechanism of IL-7Rα antibody on $CD4^+$ T cell subsets. The results showed that the amount of Th1 and Th17 subset from treatment group were decreased, accompanied by the down-regulation of transcriptional factor T-bet and RORγt. In conclusion, this study provided evidence that IL-7Rα antibody had great therapeutic effect on CIA through its inhibition actions on pathogenic Th1 and Th17 cells. These results suggest that IL-7Rα antibody may be of potential therapeutic value in rheumatoid arthritis and provide a basis for further research on mechanism of IL-7Rα antagonists for rheumatoid arthritis therapy. (This work was supported by grants from National Natural Science Foundation of China 81273307 and 81072470).

P6.04.20
Regulatory T cells are essential for the anti-arthritic effects of Methotrexate in experimental arthritis

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints. The first line pharmacotherapy for RA comprises the use of methotrexate (MTX), an anti-metabolic drug. Moreover, it's suggested that anti-arthritic effects of MTX in RA may be related with number/suppressive activity of regulatory T (Treg) cells. Our aim was to investigate the role of Treg cells on the anti-arthritic effects of MTX in antigen-induced arthritis (AIA) model. mBSA-immunized C57BL/6 mice were pretreated with MTX (2 mg/kg-orally) weekly for 5 weeks before challenge with mBSA (30 µg/cavity) at day 21 after first immunization. Articular hyperalgesia, neutrophil migration to the joint, plasma titers of specific IgG and Treg cells frequency in the spleen and draining lymph nodes (DLNs) were evaluated 7h after challenge with mBSA. For Treg cells depletion, mBSA-immunized C57BL/6 mice pretreated or not with MTX were undergoing administration of anti-CD25 (250 µg/ml) three times per week during the immunization protocol. MTX-treated mice showed a reduction of arthritis development, evidenced by reduction of neutrophil migration into the joint and mechanical hyperalgesia. The effect of MTX-treatment was not due to impaired mBSA priming, since the plasma titers of specific anti-mBSA IgG were similar between the groups. Interestingly, while the frequency of CD4+FoxP3+ cells in the DLNs was similar, MTX-treated mice had an increase of this population frequency in the spleen. Furthermore, Treg cells depletion with anti-CD25 treatment reversed anti-arthritic effects triggered by MTX. These findings show that the anti-arthritic effects of MTX are Treg-dependent in AIA.

P6.04.21
Development and kinetic of anti-Infliximab antibodies: their high levels are related to adverse infusion reactions

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Infliximab (IFX), a chimeric anti-TNFα monoclonal antibody, carries potential risk of immunogenicity with the production of specific anti-IFX antibodies (ATI), leading to potential loss of response or acute infusion reactions.
In this study a panel of 187 patients suffering from immuno-mediated inflammatory diseases treated with IFX were enrolled to analyse the development of ATI. They were categorized into 5 groups: responder (I), non responder [totally (II) or partially (III)], reactive (IV) and reactive/non responder (V) patients. Non isotype-specific ATI were measured by Elisa.
Decreased percentages of positivity were shown in different groups of patient: 100% in V, 82.16% in IV, 38.% in II, 22.2% III and 9.9% in I. When evaluated for their titres, the reactive patients exhibited the highest amounts of ATI. A longitudinal study of more than 30 patients showed that the development of ATI occurred after the initial infusions of IFX in all groups of patients, even after the interruption of therapy. In the group of patients who interrupted IFX for adverse infusion reactions or unresponsiveness we evaluated the kinetics of ATI in the absence of therapy or in the presence of alternative regimens. The presence of cross-reactive ATI before the treatment with IFX and their correlation with relapse of chronic viral infections have been also evaluated.
The direct correlation between the titre of ATI and the adverse drug reactions, as well as their onset during the first infusions or different cycles of therapy, are relevant issues for prevention and management of such patients.

P6.04.22
Interleukin-6 targeting strategy for immune-mediated diseases

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Interleukin (IL)-6, a typical cytokine featuring redundancy and pleiotropic activity, when produced transiently, contributes to host defense by inducing immune responses and acute phase reactions. However, dysregulated persistent IL-6 production of mostly unknown etiology has been demonstrated to play a significant pathological role in various immune-mediated diseases, so that tocilizumab, a humanized anti-IL-6 receptor antibody, was developed. Clinical trials have proved the outstanding efficacy and tolerable safety of tocilizumab for patients with rheumatoid arthritis, resulting in its approval in more than 100 countries worldwide. It is likely to be broadly applicable for the treatment of various other immune-mediated diseases. Indeed, our experience of off-label use with tocilizumab has suggested this possibility. The skin sclerosis of two patients with systemic sclerosis softened in association with the reduction of the number of activated myofibroblasts in the dermis after 6 injections of tocilizumab. The prominent effects of tocilizumab were also observed in several intractable diseases such as polymyositis, relapsing polychondritis, polymyalgia rheumatica, reactive arthritis, amyloid A amyloidosis or Behcet's disease. The mechanism(s) through which IL-6 blockade is effective for various phenotypically different diseases remain to be determined. It is suggested that IL-6 blockade can repair the imbalance of Th17 over Treg and/or inhibit autoantibody production. Further clinical trials to evaluate the efficacy of tocilizumab on immune-mediated diseases, as well as research to elucidate the therapeutic mechanisms of tocilizumab and to clarify the cell source of IL-6 and the mechanisms through which dysregulated continuous IL-6 synthesis is induced are important issues for future studies.

P6.04.23
Rheumatoid arthritis: physical activity and disease-activity scores

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Regular moderate intensity Physical Activity (PA) has significant benefits for health. The present study aimed to explore the role of PA in Rheumatoid Arthritis (RA). Thirty RA patients matched for therapy (mean disease-duration 90.87±66.73 months) and thirty age-, sex-, BMI-matched controls were enrolled. PA -including daily occupation and physical exercise- was assessed through validated self-complete questionnaire. Metabolic Equivalents (METs) and Energy Expenditure (EE) were calculated. Disease Activity Score (DAS-28), Functional Status (HAQ), Pain Visual Analogue Scale (VAS), Fatigue VAS, Stiffness VAS, Disease-activity VAS, Short-Form-36 Health Status (SF-36), General Health Assessment, Arthritis Impact Measurement Scales were measured. Biochemical markers included erythrocyte sedimentation rate, C reactive protein, rheumatoid factor, anti-CCP antibodies. Proportion of occupations was significantly lower in patients compared with controls ($p=0.0006$); nevertheless sedentariness (MET 1-1,5) did not significantly differ. A lower number of patients performed high intensity physical exercise ($p<0.05$) compared with controls. However the same proportion performed regular moderate intensity exercise (MET 3-5.9) and the difference of EE for physical exercise between the two groups was not statistically significant (388.54 kcal/day/patient, 584.12 kcal/day/control). RA patients performing physical exercise showed significantly lower DAS-28 ($p=0.004$), Stiffness VAS ($p=0.005$), Disease-activity VAS ($p=0.003$), SF-36 ($p=0.01$) than other patients. There was no statistically significant difference in biochemical markers and in other disease-activity scores. Despite the reduced number of patients, this study represents a proof of concept for the most recent evidences concerning the association between PA and disease-activity scores in RA suggesting that physical exercise may represent a favourable therapeutic strategy.

P6.04.24
Combination therapy of methotrexate with ellagic acid attenuates the over expression of Pro-inflammatory Cytokines and modulates antioxidant status in collagen induced arthritis

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The inclusion of antioxidant for the treatment of arthritis, especially under the therapy with immune-suppressant, is motivated because antioxidant plays an essential role in disease progression and moreover, immunosuppressive treatment suffers redox homeostasis balance of the organism. The present study was designed to evaluate the combinatory effect of methotrexate (MTX) and ellagic acid (EA) on the progression of collagen induced arthritis. Arthritis was induced in Wistar rats by collagen induced arthritis (CIA) method. CIA rats were treated with methotrexate (1 mg / kg / week) and EGCG (60 mg / kg) daily, and combination of MTX and EGCG for a period of 28 days. Paw swelling changes, scoring and histopathological was assessed to evaluate the antiarthritic effect. The effects of treatment in the rats were assessed by biochemical (articular elastase, MPO, LPO, GSH, Catalase, SOD and NO) in joint tissue homogenate to observe the modulation of antioxidant and inflammatory status, inflammatory mediators (IL-1β, TNF-α, IL-10, and PGE_2) and histological studies in joints. MTX and EA combination potentiated the antiarthritic (decrease of hind paw volume and scoring) and the antioxidant effect (GSH, Catalase, SOD) as well as suppression of lipid peroxidation. Combination therapy of MTX and EA significantly inhibited the development phase of arthritis, which is supported by histopathological and attenuation of pro inflammatory cytokines. EA act as potent antioxidant and immunomodulator, suggesting that combined administration of MTX along with EA suppressed the development of arthritic progression in rats.
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P6.05 Prevention and intervention in allergy

P6.05.01
The Role of Bioinformatics in Allergenicity Assessment of Recombinant Proteins

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Allergy is an important health issue, which is caused by certain food proteins, including recombinant proteins. The prevalence of allergic diseases has grown in many industrialized and urbanized countries during the last 50 years and almost 30 to 40 percent of the world's populations suffer from allergic diseases. Accordingly, an international regulation evaluating the allergenicity of recombinant proteins is necessary. Bioinformatics analysis has been considered a main section for the safety assessment of recombinant proteins expressed in food plants, food animals, food mushrooms, drugs, etc. Use of bioinformatics screening in allergenicity assessment of recombinant proteins in allergen databases is recommended by the World Health Organization, the European Food Safety Authority and the US Environmental Protection Agency. Bioinformatics allows two questions to be asked: Is the recombinant protein an existing allergen? Is the recombinant protein likely to cross-react with an existing allergen? A weight of evidence approach is recommended by the Codex Alimentarius Guidelines to assess the risk of allergenicity of any recombinant proteins, including bioinformatics analysis, digestibility and animal models. Bioinformatics analysis for allergenicity assessment of proteins is carried out via allergen databases and other in silico tools. Until now many allergen databases have approved for identification of allergens and bioinformatics allergenicity assessment of recombinant proteins in many countries. Thus, bioinformatics has an important role in allergenicity assessment of recombinant proteins. This role will increase with additional studies of 3d structure of proteins, proportion of amino acids of allergen proteins, and sequencing of allergen epitopes.

P6.05.02
A hypoallergenic vaccine based on a hepatitis PreS fusion protein incorporating peptides from the major allergen, Der p 23, for immunotherapy of house dust mite allergy

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Background: We have recently identified Der p 23 as new, major house dust mite (HDM) allergen with an IgE-binding frequency of more than 70% and high allergenic activity. Therefore, Der p 23 is an essential component in a vaccine for HDM immunotherapy.
Objective: To construct and characterize a hypoallergenic vaccine for Der p 23-sensitized patients.
Method: Non-allergenic Der p 23 peptides were identified from a panel of synthetic Der p 23 peptides by IgE serology and basophil activation testing. A synthetic gene coding for a fusion protein consisting of non-allergenic Der p 23 derived peptides with reduced capacity to induce T-cell proliferation and the hepatitis B virus-derived PreS domain for providing T cell help was expressed in E. coli and purified to homogeneity (Der p 23/P4P5). The IgE-reactivity and allergenic activity of the Der p 23/P4P5 was compared with rDer p 23 in IgE dot blot assays and basophil activation tests using blood from HDM allergic patients. Rabbits were immunized with Der p 23 or Der p 23/P4P5 to investigate whether the molecules induce specific IgG antibodies which are able to inhibit patients' IgE binding to Der p 23.
Result: A recombinant Der p 23/P4P5 fusion protein lacking allergenic activity was expressed and purified. Upon immunization of rabbits, Der p 23/P4P5 induced IgG antibodies that inhibited the binding of patients' IgE to Der p 23 comparable to IgG antibodies induced with Der p 23.
Conclusion: Der p 23/P4P5 represents a promising candidate for the treatment of sensitized HDM allergic patients.

P6.05.03
Assessment of physiochemotoxic reactions after the injection of contrast media

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Although contrast media (CM) is known to cause adverse reactions, the actual reason for adverse reactions remains unknown. There are two common theories that are used to describe adverse reactions to intravenous iodinated contrast media. Adverse reactions are classified as physiochemotoxic or idiosyncratic in nature. The cardiovascular system is most commonly affected by physiological changes that can be produced by CM.
Physiochemotoxic reactions are commonly related to the following: 1. Physical properties of CM; 2. Iodine concentration of CM; 3. Volume of the CM injected; 4. Rate or injection speed.
-The physical properties of the CM include the ions or other particles. The chemical composition of ionic and non-ionic CM contains iodine. CM is primarily divided into two categories, high osmolar contrast media (HOCM) and low osmolar contrast media (LOCM).
-The iodine concentration is determined by the number of iodine molecules in mg/ml. The higher the iodine concentrations are more radiopaque than comparative low iodine concentrated agent and greater the risk of an adverse reaction.
-Increasing the injected volume increases the possibility of an adverse reaction occurring.
-Increasing the speed or rate of injection may increase the risk of an adverse reaction occurring.
Anytime CM is introduced into the body there exists a possibility of a reaction. The radiologic technologist's immediate recognition and response to an adverse reaction is very important because it can save a patient's life. Technologists must become familiar with radiology department protocols regarding adverse reactions to CM as well as all emergency situations that may arise.

P6.05.04
Microwave irradiation of bovine milk reduces allergic response in mouse model of food allergy

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Introduction: Cow's milk (CM) contains many proteins that are considered allergenic and capable of inducing immune responses in allergic subjects. Heating, a commonly used method in dairy technology, are likely to induce changes in milk allergens. Microwave irradiation (MWI) is an alternative method to a conventional heating. The purpose of this study was to determine MWI effects on bovine milk protein allergenicity .
Methods: Cow's milk was irradiated by Microwave or heated by conventional method. Whey samples were analysed by Poly-Acrylamide Gel Electrophoresis in the presence of Sodium Dodecyl Sulfate. Immunoreactivity was estimated by indirect enzyme-linked immunosorbent assay (ELISA) using murine sera. Allergenicity was studied in vitro in chamber Ussing by intestine challenge in a murine model of β-lactoglobulin allergy.
Results: The electrophoretic patterns of heated and irradiated samples revealed a considerable decrease in intensity of the bands corresponding to whey proteins. Bovine whey antigenicity was clearly reduced by MWI as well as by conventional heating. The allergenic potential of bovine whey was markedly reduced after MWI as shown by short circuit current (Isc) and conductance (G) which remained unchanged after intestine challenge with irradiated whey. However, whey allergenicity was enhanced by heating whey at 102°C.
Conclusion: MWI as well as conventional heating may reduce whey proteins antigenicity. Moreover, bovine whey allergenicity was significantly increased by conventional heating but markedly reduced by MWI.

P6.05.05
Effect of static magnetic field on pollen-induced allergic airway inflammation in a murine model

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It has been demonstrated that pollen NAD(P)H oxidases generate oxidative stress in the airway epithelium and this immediate oxidative insult is crucial for the development of allergen-driven airway inflammation. In this study we aimed to define the effect of inhomogeneous static magnetic field (iSMF) on pollen-induced allergic airway inflammation since several lines of evidence suggest that iSMF is able to trigger biological responses at least partly through free radical reactions. BALB/c mice were sensitized by two intraperitoneal injection of ragweed pollen extract (RWE) and challenged with RWE intranasally. Inflammation was evaluated by determining inflammatory cell accumulation and mucin levels, as well as histological analysis of the airways. iSMF was generated with an apparatus optimized to small experimental animals. We found that iSMF did not affect the sensitization phase of the allergic responses; however, even a single 30-min exposure to iSMF after i.n. RWE challenge was able to reduce the airway inflammation. In addition, prolonged exposure to iSMF after RWE challenge decreased more effectively the severity of inflammation. In animals exposed to iSMF immediately after challenge, RWE induced a lower increase in the total antioxidant capacity of the airways suggesting that effects of iSMF on allergic inflammation were mediated at least partly by modulation of ROS levels. These data indicate that iSMF is able to reduce the inflammation in an *in vivo* system, despite the fact that it does not interfere with ROS-production of RWE NAD(P)H oxidases directly in cell free conditions.
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P6.05.06
Allergy-protective activation of dendritic cells by the cowshed bacteria *L. lactis* G121 requires endosomal acidification *in vitro* and *in vivo*

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It is well established that farming environment in early childhood reduces the occurrence of allergic reactions later in life. The Gram-positive cowshed isolate *Lactococcus lactis* G121 has been shown to prevent allergic immune responses in a mouse model of acute allergic inflammation. However, the molecular mechanisms by which this allergy-protective immune response is achieved are mainly unknown. Experiments with HEK293 cells indicated no involvement for TLR2 in *L.lactis*-mediated cell activation but rather for intracellular receptors such as Nod2 and TLR8. Stimulation of DCs with *L.lactis* led to release of several cytokines including IL-12p70, IL-23, IL-10, and IFN-β. DCs preincubated with the inhibitors Cytochalasin D and Bafilomycin A1 (Baf) showed a strong decrease of overall cytokine production, indicating the importance of not only the uptake of bacteria but also of the endosomal acidification. Moreover, Baf-treatment of DCs inhibited the *L.lactis*-induced upregulation of costimulatory molecules as well as IFN-γ and IL-10 release when these DCs were co-cultured with T-cells. To investigate the importance of endosomal acidification in *L.lactis*-mediated allergy protection *in vivo,* we transferred *in vitro*-derived BMDCs stimulated with *L.lactis* in presence or absence of Baf intranasally into naïve mouse recipients. While *L.lactis*-stimulated BMDCs prevented mice from the development of allergic inflammation, pretreatment with Baf significantly reduced the protective effect, as indicated by eosinophils in BAL and goblet cell number. Overall, we found that uptake and endosomal acidification are essential for the allergy-protective effect of the cowshed bacteria *L.lactis* G121 (supported by DFG, TR22-project A2).

P6.05.07
Chitosan-alginate core-shell caged vaccines for allergy Treatment

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Type I allergy is manifested by the release of vasoactive factors induced by IgE contact with full-length allergen. IgE usually do not recognize linear epitopes of allergens. We used this property to produce a caged vaccine against house dust mite (HDM) allergy constructed from full-length Dermatophagoides farina (Der) allergens hidden inside the polymeric core particle and Der peptides exposed on the polymeric shell. Proteins carry both T and B cell epitopes making vaccine immunogenic; while peptides represent linear B-cell epitopes which cannot trigger IgE-dependent degranulation of must cells but can collect B-cells from circulation during immune synapse formation. Hydrophobic laurylchitosan with immobilized Der f 1 and Der f 2 (ChDer) was used to form core particles and alginate conjugated to Der f 1 and Der f 2 hydrophilic peptides (AlDerp) to form a shell layer. Slow mixture of ChDer with AlDerp induced polyelectrolyte complex formation consisting of Der proteins caged inside the particle, and Der peptides exposed to the environment. As a control we also produced particles with core Der proteins and shell irrelevant (Alir) peptides to study IgE binding. We demonstrated that sera from patients with HDM allergy bound pure Der proteins and did not bind: i) pooled Der peptides; ii) ChDer-AlDerp particles; iii) ChDer-Alir particles showing that neither Der peptides (free or immobilized) nor caged Der proteins were recognized by IgE specific to HDM. Such constructs can be used to conduct specific immunotherapy of HDM allergy in a rush protocol.
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P6.05.08
Selective elimination of allergen-specific B lymphocytes with chimeric protein-engineered molecules

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Background: DerP1 is one of the major allergenic molecules of *Dermatophagoides pteronyssinus* which causes the widespread house dust allergy. The pathological DerP1-specific B cells produce allergen-specific IgE antibodies that mediate most of the hypersensitivity allergic reactions.
Aim: The selective elimination of allergen-specific B cells by artificial protein molecules which inhibit Dpt-recognizing IgE antibodies production is a legitimate therapeutic goal for allergy. Co-crosslinking of the immunoglobulin receptor and the inhibitory B cell receptor CR1 (complement receptor 1) on the DerP1-specific B cells by this molecule have the potential to deliver suppressive signal, selectively silencing these B-cells only.
Methods: A synthetic peptide, DerP1 p52-71, containing a B and T cell epitopes and anti-CR1 monoclonal antibody were used for the construction of DerP1 chimera. We analysed the effects of the chimeric molecule on PBMC from allergy patients.
Suppression of DerP1-specific IgE antibody production was measured by ELISpot.
Apoptosis assay was performed by FACS using AnnexinV-FITC/PI staining.
Results: We observed significant inhibition of allergen-specific proliferation and reduction of specific IgE antibodies after treatment of PBMC from allergic patients with DerP1-peptide chimera. Culturing of these PBMC in the presence of the same chimera increased the percentage of apoptotic (Annexin V-positive) B lymphocytes, but not T lymphocytes.
Conclusion: The constructed protein-engineered chimeric molecule binds DerP1 specific B lymphocytes via their BCR and suppresses selectively the production of anti-DerP1 IgE antibodies by co-crosslinking of the BCR and CR1. This way we could alter the allergic immune response towards a milder outcome.

P6.05.09
Bet v 1 and homologous food allergens are similarly processed by antigen-presenting cells but differ in T cell reactivity

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Background: Various plant foods, e.g. hazelnut, apple and celery, express proteins that are homologues of the major birch-pollen allergen Bet v 1, e.g Cor a 1, Mal d 1 and Api g 1. These proteins share a common 3-dimensional structure. Despite the great molecular similarity, Bet v 1 is the only one among its homologues with the ability to sensitise atopic individuals.
Aim: To assess whether differences in the uptake and processing by antigen-presenting cells and in the presentation to T cells could be responsible for the ability to sensitise.
Methods: Uptake of allergens by PBMC, surface binding to and degradation by monocyte-derived dendritic cells (mdDC) were assessed. Peptides derived from digestion of Bet v 1, Cor a 1, Mal d 1 and Api g 1 by endo-lysosomal extracts were analysed by mass spectrometry. Epitope-specificity of allergen-specific T cell lines from birch pollen-allergic individuals with associated food-allergies was mapped using synthetic 12-mer peptides. Binding of allergen-derived peptides by HLA class II molecules was analysed in silico.
Results: Significant differences were found neither in surface binding, in the kinetics of uptake by PBMC, the intacellular degradation by mdDC nor in the degradation by endo-lysosomal extracts. An immunodominant T cell epitope was found only in Bet v 1, but could not be referred to preferential binding to the most common HLA class II molecules.
Conclusion: The ability of Bet v 1 to sensitise is not conferred by differential antigen-processing but might stem from differences in T cell reactivity.

P6.05.10
Influence of administration route and polysaccharide addition on adjuvanticity of AFPL1 to allergens of Dermatophagoides siboney

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Background. AFPL1 is a pro Th1 adjuvant containing proteoliposome of Neisseria meningitidis and alum. Previous work has shown its anti-allergic effect in a murine model of respiratory allergy. Aim. To determine the influence of Polysaccharide C (PsC) and immunization route on to the antibody response to Dermatophagoides siboney allergen.
Materials and Methods. Balb/C mice were administered s.c or i.m with two doses of three variants: AFPL1, Der s1 (5 µg) and PsC (50 or 25 µg). The second dose was injected 14 days afterward. The antibody response was assessed starting from 21 days. D. siboney specific IgG, IgG1, IgG2a, and IgE were measured by ELISA.
Results: No significant differences (p=0.05) were obtained between the three variants by i.m route, i.e., in absence of PsC, or containing 25 or 50 µg of it. In contrast, the addition of PsC by s.c route induced a marked and significant raise of allergen-specific and total IgE, as well as, IgG1 and IgG, and a decrease of IgG2a, as compared to i.m route. This increase was dose- dependent with respect to PsC content. The s.c route in absence of PsC showed the highest value of IgG2a/IgG1 ratio indicating a higher pro-Th1 effect.
Conclusion. PsC is able to modulate and partially counteract the AFLP1 pro-Th1 adjuvant effect, raising the IgE response, although, only when is administered by s.c route. Since this route is preferred for allergen vaccines due to safety reasons, an optimal formulation of an AFLP1-adjuvanted antiallergic vaccine should avoid the presence of PsC.

P6.05.11
Is allergy to peanut important in Cuba?

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Introduction: Peanut allergy is alarmingly increasing in developed countries. Roasted peanut (Arachis hypogaea) is a common food in Cuba. Nevertheless, reported values of sensitization to peanut are usually Objective: To perform a preliminary assessment of allergic sensitization and IgE specificity profile to peanut allergens in Cuban allergic patients Methods: The Skin Prick Test was performed to each patient/subject, using two glycerinated allergenic extracts, prepared from raw or roasted peanuts, BIOCEN, Cuba. Two groups of subjects were studied: a cohort of general adult population (N=292), and patients attending allergy services at 4 hospitals in Havana, comprising 16 adults (above 16 years old) and 66 children (2-16 years). The IgE binding profile of 10 selected SPT positive patients was further analyzed by Western Blotting Results: In general adult cohort the prevalence of sensitization was 2.7% and 4.7%, to raw and roasted peanut, respectively ($p<0.05$, McNemars). Unexpectedly, among patients suspecting food allergy, the positivity rate was higher to raw peanut (62%) versus roasted (51%). Interestingly, sensitization to roasted, but not to raw peanut, increased from 45% in children, to 75% in food allergic adults. IgE binding was shown mostly by the 60-64 KDa bands, tentatively identified as (Ara h1 and Ara h3), known as clinically relevant allergens Conclusions: Allergic sensitization to peanut seems not to be as important in Cuba as in developed countries, although it should be taken into account for improving the specific diagnosis of food allergy. Differences between sensitization to raw or roasted peanut deserve further investigation.

P6.05.12
Tamoxifen a new therapeutic approach for allergy airway disease

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Recurrent airway obstruction (RAO) is an asthma-like condition that develops in mature horses following stabling and exposure to dusty hay and straw. The hallmark of this disease is that hay/straw exposure induces clinical airway obstruction, airway neutrophilia and increased airway mucous production in. Our previous study indicated that tamoxifen has the ability to induce apoptosis in vitro in granulocytic cells from peripheral blood and broncheoalveolar lavage fluid (BALF). Therefore, we propose that tamoxifen, when used as a treatment in RAO-affected horses, induces a resolution of allergy airway inflammation through action on polymorphonuclear neutrophils. For this purpose, nine RAO-susceptible horses sensitized to Aspergillus fumigatus (RAO herd) will be selected for use in this study. The animals will be exposed to dusty/moldy hay, and once the signs of the disease appear. Later, the horses will be treated with 100 mg PO of tamoxifen every other day in a remission environment. During treatment and after drug administration has ended, periodical evolution of RAO clinical signs will be conducted to evaluate the effect of tamoxifen on airway inflammation (clinical score, mucus score, endoscopic examination and BALF for apoptosis evaluation). Our results indicate that RAO-affected horses treated with tamoxifen displayed a significant reduction of neutrophils in BALF and a concomitant improvement in their clinical status. This reduction in neutrophils BALF is related to the percentage of cells positive Anexin-V. These results indicate that the apoptotic mechanisms under these experimental conditions would affect blood and BALF granulocytic cells, particularly in early apoptosis (positive Anexin–V).

P6.05.13
Effect of depletion of 33D1+ dendritic cells on allergic airway sensitization in mouse model

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Th2 dominance in Th1/Th2 balance in the acquired arm plays a central role in the induction of allergic sensitization as well as in the chronic inflammatory cascades of allergic diseases. It has been shown that two major distinct subsets of dendritic cells (DCs) in the innate arm are arranged to regulate the immune responses in vivo; DEC 205+ DCs having the capacity to establish the Th1 polarization and 33D1+ DC to establish Th2 dominance.
We have previously reported that 33D1+ DCs could be successfully depleted from mice by anti-33D1-specific monoclonal antibody treatment [Cancer Immunol. Immunother., 59:1083-1095,2010] and recently demonstrated that the fetal loss was induced by the depletion of 33D1+ DCs during perinatal period mediated through Th1 up-regulation via transient IL-12 secretion [Immnobiol., 217:951-961, 2012]. Based on these findings, we studied the effect of 33D1+ DC depletion on allergic airway sensitization against ovalbumin (OVA) in vivo mice model. Mice were sensitized i.p. with OVA plus alum, and were intranasal challenged with OVA solution. The results showed that, when 33D1+ DCs were depleted, anti-OVA IgE level in the serum and allergic symptoms such as sneezing or nasal scrubbing times were apparently decreased as compared with control mice . These results suggest that innate 33D1+ DCs may induce Th2 polarization on allergic airway sensitization in acquired immunity and modulation of DC subsets may offer a new therapeutic strategies for various allergic diseases by altering Th2 polarization.

P6.05.14
Characterization of airway inflammation and hyperreactivity induced by sole aeroallergen exposure in humanized allergy mice expressing a human mugwort-specific T-cell receptor and HLA-DR1

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T-cells play a key role in the development of allergic diseases thus representing a promising target for therapeutic interventions. While mice expressing an I-A^d restricted murine T-cell receptor (TCR) specific for chicken ovalbumin (Ova) are frequently used to mimic allergic disease induced by aeroallergens *in vivo,* implications for human allergic diseases remain questionable, since Ova is not a human-relevant aeroallergen. We here created double tg mice expressing a human TCR specific for the major mugwort (*Artemisia vulgaris*) pollen allergen Art v 1 and HLA-DR1. In tg mice ninety percent of peripheral blood $CD4^+$ T-lymphocytes expressed the Art v 1-specific TCR, while $CD14^+$ monocytes and $B220^+$ B-lymphocytes revealed HLA-DR1 expression. Splenocytes of tg mice specifically proliferated upon incubation with the human-relevant immuno-dominant Art v 1_{25-36} peptide or whole Art v 1 protein when compared to control mice. *In vivo*, after allergen-specific sensitization, only tg mice showed enhanced airway-hyperreactivity (AHR) as assessed by methacholine or allergen-specific challenge. Three consecutive exposures to nebulized allergen were found to be sufficient for induction of altered airway function, as mirrored by airway and lung inflammation, due to massive peribronchiolar and perivascular infiltration with immune cells. The detailed results of bronchoalveolar lavage (BAL) fluids, lung histology and specific antibody titers will be described and discussed. Humanized allergy models are amenable to the evaluation of human-relevant allergen formulations or cellular intervention protocols and will contribute to the further understanding of allergic diseases and their cure.

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P6.05.15
Development and affinity maturation of human scFvs against IL-4 and IL-13 by phage display

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Interleukin-4 and Interleukin-13 shared receptor chain IL-4Ra, and the intracellular signal transducer and activator of transcription, STAT6, promote the IL-4 and IL-13 responsive genes, such as those associated with, T-helper type 2 (Th2) cell differentiation. IL-4/IL-13 pathway is key components in the development of air way inflammation, mucus roduction, and airway hyper responsiveness in asthma. The human scFv 68 against IL-4 and scFv 101 against IL-13 were selected from a large non-immune scFv library by phage display. Subsequently, by in vitro mutagenesis of a gene encoding for the scFv 68 and scFv 101, two new libraries was established, and new scFv antibodies with improved affinity towards the IL-4 or IL-13 were selected and characterized. The scFv 68 against IL-4 was affinity-maturated by in vitro mutagenesis and the new scFv clone M3 was isolated. And the scFv 101 against IL-13 was affinity-maturated by in vitro mutagenesis and the new scFv clone M46 was isolated. The Biacore results showed that the M3 and M46 have over 10 times higher affinity with the antigen IL-4 or IL-13, compared to scFv 3 or scFv 68. And the western blot with serial dilutions IL-4 or IL-13 showed that the intensity of eveloped color was increase when affinity-maturated scFvs was used for staining.

P6.05.16
Induction of Th1 immune response and suppression of IgE via immunotherapy with recombinant hybrid molecule encapsulated in Liposome-protamine-DNA nanoparticles in a model of experimental allergy

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Background: Liposome-protamine-DNA nanoparticles (LPD) are safe, effective, and non-toxic adjuvant that induce Th1-like immune responses. We hypothesize that encapsulation of allergens into liposomes could be an appropriate option for immunotherapy. The present study evaluated the immunotherapeutic potential of a recombinant hybrid molecule (rHM) encapsulated in the LPD nanoparticles in a murine model of Chenopodium album allergy.
Methods: BALB/c mice sensitized with allergen in alum and immunotherapy procedure was performed with two subcutaneous injections of either the LPD-rHM or rHM or emty LPD with weekly interval. Humoral immune responses were examined through specific antibodies measurement in serum. The splenocytes of immunized mice were stimulated in vitro and cell proliferative responses along with cytokine secretion determined. Expression of genes involved in immunotherapy was examined by real-time PCR.
Results: Sensitized mice developed a Th2-biased immune response that characterized with strong specific IgG1 and IgE production, and presence of IL-4 as well as GATA3 transcription factor in the culture of spleen cells. Treatment with the LPD-rHM was led to reduction of IgE level and markedly increased ratio of IgG2a/IgG1 antibody level. The LPD-rHM induced strictly Th1 allergic responses with strong IFN-γ production as well as T-bet expression in stimulated splenocytes. In addition, LPD-rHM treated mice induced higher lymphoproliferative

responses than other groups. Removal of nanoparticle from the rHM resulted in decreased allergen immunogenicity.
Conclusion: These results indicate that the rHM complexed with LPD nanoparticles has a marked suppressive effect on the allergic response and causes a shift towards a Th1 pathway.

P6.05.17
IgE epitopes of Tri a 37, a new wheat food allergen, are not of the conformational type

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Wheat is an important staple food that can cause IgE-mediated food allergy. In-vitro diagnostic tests using wheat allergen extracts frequently give false positive test results. Aim of this study was the expression of alpha purothionin (rTri a 37) as a recombinant protein in two different expression systems, to compare their IgE reactivity and to evaluate their usefulness in the diagnosis of wheat-dependent food allergy.
Recombinant alpha purothionin was expressed in a prokaryotic system using E.coli cells (EcTri a 37) and in a eukaryotic system (i.e. in baculovirus infected insect cells - BvTri a 37) producing rTri a 37 as a secreted protein. The purified recombinant allergens were characterized regarding molecular, structural and immunological properties.
Alpha purothionin was identified as a new wheat food allergen. Structural analysis showed that EcTri a 37 is unfolded whereas BvTri a 37 is an α-helical protein. EcTri a 37 was recognized by approximately 20% of wheat food allergic patients (n=103). When we compared the IgE binding capacity of both recombinant allergens, comparable results were observed. Clinical data indicated that the risk of developing wheat-induced anaphylaxis is more than four-fold higher in the rTri a 37- reactive patients.
Tri a 37, a new wheat food allergen, comprises non-conformational epitopes and may be a new tool for in-vitro diagnosis of potentially severe wheat food allergy.

P6.05.18
Gene expression of innate cytokines in response to probiotic treatment in allergen-sensitized mice as an experimental model

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Chenopodium album is one of the main sources of pollen allergy in desert and semi-desert areas and contains three identified allergens. Respiratory effects of Lactococcus lactis, as probiotics, in animal models have included attenuating allergic airway responses. IL-33 and IL-25 are type 2 cytokines that promote airway allergic responses. After allergen insult these two cytokines are produced and released from lung epithelial cells and trigger the downstream responses that aggravate allergic symptoms.
We used a protocol previously established in our lab for inducing airway allergy using recombinant form of C. album allergen number 2, rChe a2,. Three groups of BALB/C mice were sensitized and two of them were treated orally with Lactococcus lactis (L.L) and L.L plus rChe a2 respectively. Animal were sacrificed and RNA extracted from lung tissues. cDNA synthesis and real-time PCR for IL-33 and IL-25 were performed.
Relative quantity based on dR in two treated groups (L.L and L.L+ rChe a2) compared with sensitized but not-treated group, are 0.745 and 0.880 for IL-33 and 0.191 and 0.257 for IL-25. Fold relative quantity based on dR are -0.425 and -0.184 for IL-33 and -2.380 and -1.960 for IL-25.
In our study, probiotic therapy caused to a reduction in IL-33 and IL-25 gene expression. Lactococcus lactis therapy was more effective when it was used alone than mixed with rChe a2.

P6.05.19
Preventative and therapeutic anti-allergic effect of a novel House-Dust-Mite vaccine based on a combination adjuvant.

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Current allergen-specific immunotherapy is based on repeated allergen injections. The adjuvant effect of the outer membrane proteoliposome (PL) of *Neisseria meningitides* is attractive for designing novel vaccines, with fewer administrations. The aim was to assess the immunomodulatory effect of a novel vaccine based on *Dermatophagoides siboney* allergens and a combination adjuvant, containing PL and alum, in prophylactic and therapeutic models of respiratory allergy. Balb/C or C57/BL6 mice were administered with 3 doses of the vaccine (2µg Der s1) by subcutaneous route. In the therapeutic model, mice were previously sensitized to *D.siboney* by ip injection plus exposure to aerosolized allergen. A challenge test was used after receiving the vaccine in the prophylactic or therapeutic models. Allergen-specific antibody response was assessed by ELISA, and the cytokine response, by FACS. The vaccine induced IgG2a and IgG1 antibodies in both, naïve and sensitized animals, and prevented the development of systemic (IgE) and local allergic response in mice subjected to allergen challenge, regarding histological signs of lung inflammation. In sensitized mice, it was noted a significant increase of the IgG/IgE ratio, and a decrease of blood eosinophils. Moderate levels of IFN-γ were induced in Der s1-stimulated lymphocyte cultures, whereas IL-13 levels decreased as compared to non-treated controls. IL-10 levels showed no significant ($p>0.05$) differences. The adjuvanted vaccine does not exacerbate the allergic response, nor promote Th1 inflammation, supporting a satisfactory safety profile for further clinical trials. This immunomodulatory effect suggests clinical benefits in cellular and blocking antibody responses for treatment or prevention of respiratory allergy.

P6.05.20
Differences in the IgE- and IgG-reactivity profile of asthmatic and non-asthmatic house dust mite-allergic patients revealed with micro-arrayed HDM allergens

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Background: House dust mites (HDM) represent one of the most important inducers for respiratory allergies worldwide.
Objective: To investigate the IgE- and IgG-reactivity profiles of HDM-allergic individuals suffering only from allergic rhinitis or from allergic asthma.
Methods: This study included sera from clinically well characterized asthmatic (n=105) and non-asthmatic (n=53) HDM-allergic patients. IgE- and IgG-reactivity to seven HDM-allergens (nDer p 1, rDer p 2, rDer p 5, rDer p 7, rDer p 10, rDer p 21 and rDer p 23) were measured using a customized allergen microarray (i.e., ISAC chip, Thermofisher, Vienna, Austria).
Results: HDM-allergic individuals suffering from asthma showed striking differences regarding their IgE reactivity profiles compared to the non-asthmatic group. First, the frequency of IgE reactivity to the tested HDM allergens was up to 3-fold higher in the asthmatic than in the non-asthmatic group. Seventy percent of the asthmatics reacted with 3 to 6 of the tested allergens compared to only 45% in the non-

asthmatic group. Furthermore, IgE-levels to nDer p 1, rDer p 2, rDer p 5 and rDer p 23 were significantly higher in the asthmatic group. In contrast, HDM-allergic asthma patients showed a lower IgG-binding frequency to the seven HDM allergens than HDM-allergic patients without asthma.
Conclusion: The IgE and IgG reactivity profiles to HDM allergens differ considerably in patients with mild (i.e., rhinitis) and severe (i.e., asthma) respiratory symptoms due to HDM allergy.

P6.05.21
Naproxen alleviates anaphylactoid reactions to N-acetylcysteine in healthy volunteers

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A relationship has been demonstrated between a low serum paracetamol at the time of treatment and the development of anaphilactoid adverse reactions to N-acetylcysteine (NAC). Paracetamol itself might actually offer some protection against the development of adverse effects to NAC. Naproxen inhibits the production of thromboxane by 95% and platelet aggregation by 88%. To inhibit the cyclo-oxygenase activity before starting an NAC trial on healthy volunteers, at least 7g paracetamol would be need. Since administration of this dose to healthy volunteers was not feasible, an alternative option was to use a NSAID instead of paracetamol before the trial. Therefore, we decided to use a cyclo-oxygenase inhibitor (naproxen) for this purpose. Ten healthy male volunteers, age between 23-40 (median 32) and weight between 63-87kg (median 78.5) were recruited from the general population after fully informed and written consent and after ethical approval. Each volunteer was asked to participate on two separate occasions: pre-treatment with naproxen prior to infusion of NAC using the recommended UK regimen and without naproxen pre-treatment. Data were analysed by using Excel, Prism, and WinNonlin software packages as appropriate. Plasma histamine concentration was determined during intravenous administration by an ELISA method. A paired t-test showed that plasma histamine was significantly higher at 10 and 15min in the control phase compared with the naproxen pre-treatment phase ($P<0.05$). In conclusion, histamine release may not be the only mechanism of adverse events associated with NAC, but also the release of some other factors e.g. prostaglandines or tromboxanes could be involved.

P6.05.22
Oxygen therapy of a murine model of atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory disease of skin resulting from excessive stimulation of immune cells by external antigens due to disruption of epidermal barrier. Most cases of AD are managed by corticosteroid or calcineurin inhibitors, but complications and intractable cases still remain as problems. Recently, many evidences are accumulating on the protective role of reactive oxygen species (ROS) in autoimmunity. There are also many reports on the therapeutic effects of hyperbaric oxygen therapy (HBOT) in chronic inflammatory or autoimmune diseases. We hypothesized that HBOT may improve AD symptoms by increasing tissue ROS level. We also applied another measure to increase tissue ROS level, an oxygen-carrying chemical, perfluorodecalin (PFD). Dihydroethidum staining demonstrated both HBOT (100% O_2, 3 atm for 90 min daily) and PFD increased tissue ROS level. Immunohistochemistry showed indoleamine 2,3-dioxygenase (IDO) was also increased. Mouse model of AD is developed by mechanical stripping of an ear followed by repeated application of 1% 2,4-dinitrochlorobenzene and *Dermatophagoide farinae* extract. The results showed both HBOT and PFD significantly decreased ear swelling of AD as much as 0.1% prednicarbate. Histological examination showed severe epithelial hyperplasia, exocytosis, hyperkeratosis, parakeratosis, perivascular inflammatory cell infiltration and dermal fibrosis in the AD model. Meanwhile, HBOT or PFD-treated group showed only mild epithelial hyperplasia and perivascular inflammatory cell infiltration without telangiectasia. Increased expression of IDO and decreased level of hypoxia-inducible factor-1α, demonstrated by immunohistochemistry and RT-PCR, might be involved in the molecular mechanisms of oxygen therapy in AD.

P6.05.23
Effects of JSP-TCM on IL-13 and IL-17 levels in a murine model of asthma

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Jade Screen Powder (JSP), a traditional Chinese medicinal prescription, has been widely used about 7 centuries to treat asthma in China. Its
efficacy and action mechanisms in asthma attack prevention remain nonetheless to be further explored. In this study, asthma was induced exposing ovalbumin (OVA) sensitized mice to repeated OVA challenges for 4 weeks. Mice were treated with JSP for 1 week just after the final challenge. In this murine model of asthma, were accompanied with suppression of the IFN-gamma level and increase of IL-13 and IL-17 levels in the lung homogenized thick liquids (LHTLs) even four weeks after the final challenge, indicating that the airway structural changes continued to develop even after interruption of OVA challenges. However, after JPS treatment, the airway hyperresponsiveness (AHR) was sharply relieved, accompanied by IL-13 and IL-17 levels were decreased, meanwhile the inflammatory cells were decreased but the IFN-gamma level increased in LHTLs. In conclusion, JSP could prevent the development of asthma, thus reducing asthma attacks. Our results indicated that it should be used as a supplementary therapy on preventing asthma attacks from asthma patients.

P6.05.24
Efficacy of experimental allergen-specific immunotherapy in mouse model of atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease. In this study we examined whether allergen-specific immunotherapy (ASIT) is effective in a mouse model of AD.
Methods: Female BALB/c mice were epicutaneously (e.c.) sensitized by three applications (lasted 7 days each) with 1 mg/ml of ovalbumin (OVA). Between 1st and 2nd applications mice were subcutaneously (s.c.) treated with increasing doses of: non-modified OVA (group 1 «ASIT OVA»); OVA modified with succinilation (85 % of modification) (group 2 «ASIT sOVA») or sham treated with PBS (control group 3). The levels of anti-OVA IgE, IgG1, IgG2a antibodies in sera obtained before, during and after ASIT were detected by ELISA. After the last application IL-4, IL-5, IL-17 and IFN-γ were detected in the spleen cell culture by ELISA and skin samples from patch areas were removed for histological examination.
Results: Anti-OVA IgE and IgG1 levels in treated groups 1 and 2 were gradually decreased during ASIT, while the level of anti-OVA IgG2a antibodies increased during ASIT in compare to control group 3. IL-4, IL-5 were decreased after ASIT in both treated groups while IL-17 decreased in group 1 only. The level of IFN-γ was significantly higher in group 2 compared with other groups. The ratios of IgG1/IgG2a and IL-4/IFN-γ support idea that ASIT with sOVA can shift response to allergen towards Th1-like. Histological picture of skin in all ASIT groups demonstrated suppressing of allergic inflammation.
Conclusion: ASIT is effective treatment in experimental AD that may be relevant for the clinical practice.

P6.05.25
Maternal allergen immunization in mice: prevention of offspring allergy is possible mediated by Breg and Th17 cells

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Background:Previously we have been observed that pre-conceptional immunization with Ovalbumin (OVA) is able to prevent the development of allergy in offspring.
Methods: Femalemice immunized with OVA were mated with non-immunized males. Offspring were evaluated at 3 and 20 days-old (d.o.) or immunized with OVA at 3 d.o. and assessed at 20 d.o for B and TCD4+ cytokines secreting cells.
Results: Maternal immunization up-regulates the expression of FcγRIIb on offspring B cells at 3 and 20 d.o. This effect was maintained even when neonates were immunized. It was in parallel to decreased sera IL-6 levels. Although offspring from immune mothers showed similar levels of IL-10+, IL-4+ and IFN-γ+ B cell frequency, but median fluorescence intensity analysis revealed higher IL-10 intensity, regardless neonatal immunization. Moreover, newborns from immune mothers showed an increased number of TCD4+-secreting IL-17 as well as IFN-γ. After OVA-immunization only IFN-γ levels were augmented on offspring from immune mothers.
Conclusion: Up-regulation of FcγRIIb on offspring B cells occurred concomitantly to the B-cell IL-10 response and TCD4-secreting IL-17 suggesting that maternal immunization could induce the generation of B regulatory and Th17 cells in offspring. The role of these populations on offspring in the allergy development must be investigated.

P6.05.26
***H. pylori* induced immune suppression**

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Helicobacter pylori are the dominant members of the gastric microbiota and have persistently colonized the stomach in humans since our early evolution out of Africa. There is now emerging evidence that *H. pylori* could be beneficial to the host with increasing evidence of *H. pylori* protection against esophageal and cardial pathologies, childhood asthma and allergies and inflammatory bowel disease. More recently, partial protection by *H. pylori* has been confirmed in mouse models of acute allergic asthma and colitis. The major outcome of these studies identified regulation of the host immune system by regulatory T (Treg) cells and a contribution of NKT cells. Our data complements these studies by identifying a systemic immune suppression based on decreased CD8 T cell and antibody responses towards a superinfection in *H. pylori*-infected mice. Protection from asthma in mice could be reproduced using a cagPAI negative VacA positive strain that is very well tolerated by human volunteers. Our result opens up the possibility to apply *H. pylori* in treatment of asthmatic and allergic patients.

P6.05.27
Construction of a naive human phage scFv library and selection, identification of human scFvs

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The IL-4, IL-13 and IL-33 are key components in the development of allergic disease such as asthma. To construction of human non-immune library and selection of human scFvs against the three cytokines, the mRNA of human peripheral blood cells (PBMC) was extracted from healthy volunteer, and then the cDNA library was constructed. The variable region of heavy chain (V_H) and light chain (V_L, including Vκ and V_λ) were amplified from full length cDNA library. The V_H and V_L were ligated with linker and the DNA library of single-chain Fv fragments (scFvs) was developed. After transforming into *E.coli* TG1, the phage library was constructed. The antigen IL-4, IL-13 and IL-33 were cloned, expressed and identified. The scFv library was enriched for three rounds by phage display for selection of human scFvs against IL-4, IL-13 and IL-33. By PCR, forty two bands of V_H, sixteen bands of Vκ and eighteen bands of V_λ were got. And the scFv library was constructed successfully with $2.5x10^8$ development clones with full length scFv inserts. Through three rounds of phage display, 30% clones showed positive using ELISA for detection. Four to five scFvs with high affinity against IL-4, IL-13 and IL-33 respectively were screened, expressed, and identified by Biacore, western blot and sequencing. The results showed the size of proteins of identified scFvs were about 32 kDa and the sequences were correct. The human non-immune library was constructed and the scFvs specific to IL-4, IL-13 and IL-33 respectively were selected successfully.

P6.05.28
Establishment of a simple method for Japanese cedar pollinosis and sublingual immunotherapy in mice

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Japanese cedar (*Cryptomeria japonica)* pollinosis (JCP) is one of the most common allergic diseases in Japan. A number of therapeutic strategies are currently being developed to induce allergen-specific immune tolerance. Sublingual immunotherapy (SLIT) has been performed as a safer and more beneficial treatment. However, immunological mechanisms and therapeutic biomarkers for SLIT remain unclear. In this study, we have established a new murine model of JCP that resembles human JCP using crude pollen grains in the absence of adjuvant and examined the effects of SLIT. SLIT was performed by the graded doses of cedar pollen extract painting onto the ventral surface of tongue for 28 days. [Results] The frequency of sneezing and the duration of rubbing for 10 min after the final challenge, serum IgE and pollen-specific IgG1titers, thickness of nasal mucosae, infiltrated eosinophils in BALF and nasal mucosae were significantly elevated in the pollinosis mice. However, SLIT efficiently reduced the above manifestations. SLIT significantly inhibited Th2 cytokines and pollen-specific Th1 & Th2 cytokines production by splenic T cells as well as APC-abilities in DC like MHC class II, CD86 expression in the regional lymph nodes. Histological examination revealed that repeated pollen extract painting onto the sublingual mucosae replaced sublingual resident DCs with newly recruited $CD11b^+$ DCs. These results suggest that our new pollinosis model brings benefit to develop a new immunotherapy and to evaluate therapeutic biomarkers.

P6.06 Cancer immunotherapy and anti-tumor vaccines

P6.06.001
Inhibitory effects of single chain fragment variable (scFv) antibodies against prostate stem cell antigen (PSCA) on prostate and pancreatic cancer cell lines

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Background: PSCA is a cell surface antigen with limited expression in normal tissues and overexpression in some human malignancies. In this study, we evaluated inhibitory effects of a PSCA specific scFv on proliferation of PSCA expressing as well as PSCA negative cells.
Materials and Methods : Specific scFvs against an immunodominant epitope of PSCA were isolated from phage-displayed library of scFv antibodies. Panning followed by PCR and DNA fingerprinting were applied for isolating scFvs and one clone (C9 scFv) was selected and used as source of specific scFv. Phage ELISA determined specific binding of C9 to its corresponding peptide. DU-145and AsPC-1 cells (as PSCA expressing) and LNCaP (as PSCA negative cells) were treated with different concentrations of C9 for 24 and 48h. Inhibitory and apoptosis inducing effects of C9 were assessed by MTT and Annexin-V assays, respectively.
Results: After 24h and 48h treatment, all concentrations of C9 resulted in significant inhibition of proliferation of DU-145 and AsPC-1

cells, whereas it had no inhibitory effect on LNCaP cells. The results of Annexin-V showed that C9 significantly induced apoptosis in DU-145 cells but not inAsPC-1 cells.
Discussion: The results showed that C9 was effective against the growth of PSCA expressing cells, while the remaining PSCA negative cells were unaffected. It was also shown that inhibitory effects of C9 on DU-145 cells resulted from apoptosis induction rather than decreased cell proliferation. These results provide remarkable evidence for usefulness of PSCA targeting with scFvs for the treatment of PSCA-expressing cancers, including prostate and pancreatic cancers.

P6.06.002
Application of palm-tocotrienol adjuvanted dendritic cell in cancer therapy

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We have reported previously that murine bone marrow-derived dendritic cells (DC) pulsed with whole tumour lysate and with combination of tocotrienol rich-fraction (TRF) can mediate potent anti-tumour immune response in in-vivo as well as in-vitro studies.
In our present study, we demonstrate that a combination treatment of tumour lysate-pulsed DC and TRF (DC+TL+TRF) are able to mediate regressions of pre-established s.c. tumours, leading to significant cure rates and prolonged survival of treated mice. Systemic administration of one milligram TRF daily is capable of mediating significant increases in DC based immunizations. The efficacy of TRF supplementation in tumour lysate-pulsed DC induced protective immunity to lethal tumour challenge as well as enhanced splenic cytotoxic T lymphocyte activity in treated mice. Furthermore, interferon gamma (IFN-γ) and interleukin 12 (IL-12) increased in TRF group compared to others in splenic culture. In addition, the same treatments were used in microarray study to look at regulation of related genes to cancer. We found one interesting gene called special AT rich binding protein 1 (SATB1) from microarray analysis. This gene was reported to have dual function in different cells and ability to induce aggressive breast cancer cells. Validation of this SATB1 was done through Real-Time RT-PCR and currently we are in progress of silencing the gene expression in order to look at the clear functions of SATB1 in both 4T1 mammary cancer cells and dendritic cells.

P6.06.003
GMP-production of an allogenic DC-based cancer vaccine (COMBIG-DC) for treatment of patients with metastatic renal cancer using the ELUTRA Platform

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The aim of this Phase I/II study in patients with metastatic renal cancer is to evaluate adverse effects and anti-tumor effects of a therapeutic vaccine-concept that is based on intratumoral injections of activated allogenic dendritic cells (DCs) produced from healthy donors.The strong allogenicity of such cells and their sustained production of pro-inflammatory mediators is expected to create a local inflammatory environment that promotes recruitment, antigen-loading and activation of endogenous DCs, As clinical studies require protocols where a sufficient number of well characterized dendritic cells are produced according to Good Manufacturing Practice (GMP) guidelines, we validated the COMBIG-DC GMP platform using ELUTRA® -enriched monocytes from leukapheresis products obtained from healthy donors as starting material.Collected fractions were phenotypically analysed for cell content by flow cytometry. The enriched monocyte fractions were differentiated into immature DC in culture bags using GM-CSF and IL-4 and matured for 18 hours using a cocktail of Poly-IC, R 848 and IFNγ. DC-generation was determined using phenotypic markers and expression of cytokines. Our results show that the COMBIG-DC vaccine production platform is very robust and reproducible resulting in high initial monocyte purity, high recovery and efficient differentiation/maturation of phenotypically and functionally mature DCs. Delivered frozen vaccine cells show a high and reproducible quality after thawing. This work was supported by Immunicum AB, Gothenburg, Sweden

P6.06.004
Breast cancer immunopathology to immunotherapy in Northern Emirates of the United Arab Emirates

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Retrospective study of 81 cases of breast cancer identified in northern emirates of the United Arab Emirates (UAE). Female to male ratio was 80:1. These cases came from several hospitals, but the final diagnosis was established in our referral hospital. The sections were stained with monoclonal antibodies for estrogen receptors, progesterone receptors, and ERBB2 (HER2/neu), and to be stained for SATB1, basal cytokeratins, p53, androgen receptor, E-cadherin, P-cadherin, EGFR, Bcl2, Cathepsin D, and cyclin E.
Breast cancer was more commonly seen in UAE nationals 14 cases (17%), Philippinos 14 cases (17%), Egyptian 9 cases (11%) and Indians 7 cases (9%). It was more common in age group 30-50 (50 cases). Invasive ductal carcinoma NST (no special type) was seen in 51 cases (62%), 11 cases (14%) were invasive lobular carcinoma, 12 cases (15%) were mixed invasive ductal and lobular, and 7 cases (9%) were ductal carcinoma insitu. Majority of cases (51 cases; 63%) were staged as stage II, 17 cases (21%) as stage I and 13 cases (16%) as stage III. Lymph node involvement was noted in 29 cases (36%). Estrogen receptors (ER) were positive in 11 cases (13%) and 5 cases (6%) overexpressed ERBB2 (HER2/neu).
Overall breast cancer in Northern Emirates presented at earlier age and its incidence is less in nationals than in non-nationals. Most cases presented with breast lump. Invasive ductal carcinoma, NST was more commonly seen and showed more aggressive behavior. This behooves us to individualize the diagnosis and use specific biotherapy such as trastuzumab.

P6.06.005
Generation and characterization of a new chimeric antibody against Her2

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Her2 proto-oncogene encodes a receptor tyrosine kinase overexpressed in a variety of solid tumors. Amplification of this gene has been shown to correlate with poor prognosis in breast cancer patients. Immunotherapy with anti-Her2 antibody has shown promising results in patients with Her2-positive breast cancer. We have recently reported characterization of a mouse monoclonal antibody (1T0) directed against Her2, which binds to an epitope different from that recognized by Trastuzumab, and specifically inhibits proliferation of tumor cells overexpressing Her2. In the present study, we report chimerization of this antibody. The VH and VL genes of 1T0 hybridoma cells were amplified and inserted into a mammalian expression vector containing cDNA of human gamma-1 and kappa constant regions using Splice Overlap Extension (SOE) PCR. The construct was subsequently transfected in the mammalian CHO cell line and the chimeric antibody was characterized by ELISA, Western blot and flow cytometry. The purified chimeric antibody specifically binds to recombinant Her2 and Her2 positive tumor cells. Binding of this antibody to tumor cells inhibited proliferation of these cells. The affinity of the chimeric Ab was comparable to the parental mouse mAb. This chimeric anti-Her2 mAb is potentially a valuable tool for targeted immunotherapy of Her2 positive malignancies.

P6.06.006
Annexin A2 is a Glioma Damage-Associated Molecular Pattern Enriched in Physiologic Oxygen

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Cross priming remains a major hurdle for the majority of brain tumor patients on cell-based vaccine immunotherapy. Through the culture of glioblastoma cells in 5% (physiologic) oxygen, we enhanced the adjuvant cross priming ability of whole cell lysate vaccines from these cells. 5% oxygen-derived lysate vaccines lack efficacy in mice deficient in toll-like receptor 2 (TLR2), suggesting enrichment of a TLR2-binding damage associated molecular pattern (DAMP) under 5% oxygen. Through subtractive proteomics, we identified annexin A2 (ANXA2) as a TLR2-binding DAMP. ANXA2 monomer induced TLR2 signaling and TLR2-dependent expansion of surrogate antigen-specific CD8 T cells. ANXA2 was also capable of robust cross presentation and cross priming to surrogate antigens. These mechanisms likely underly the extension of median survival and increased cure rate seen when ANXA2 is combined with poorly immunogenic lysate vaccines from atmospheric oxygen. Many gene expression differences exist due to oxygen tension, with potentially many molecules contributing to the cross priming activity of lysates from 5% oxygen. However, surrogate antigen-specific CD8 T cell expansion was no longer enhanced if cells for lysate vaccination express a short hairpin RNA construct targeting ANXA2, indicating its requirement for the adjuvant cross priming activity of lysates from 5% oxygen. Lastly, ANXA2 enrichment occurs in 5% oxygen cultures of primary human glioma cells, and ANXA2 can enhance cross presentation of the pp65 cytomegalovirus antigen to peripheral blood mononuclear cells of serum-positive donors. These data indicate an important role for oxygen and ANXA2 in glioblastoma cells cultured for vaccine production.

P6.06.007
MHC I multimer assay: Optimized double staining for improved sensitivity in detection of low affine CD8+ antigen-specific T cells by flow cytometry

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Sensitive and reliable monitoring of cellular immune responses is highly important in cancer vaccine and immunotherapeutic development. Flow analysis using conventional fluorescent MHC multimers like Tetramers and Pentamers has made a great impact in this field, enabling visualization, enumeration and phenotypic characterization of antigen-specific T cells. However, difficulties in detecting low-affinity interactions such as in tumor-specific T-cell responses, and low reproducibility and stability are still prominent problems. MHC-Dextramer® reagents, the next generation of fluorescent MHC multimers, can minimize these issues due to a higher number of MHC-molecules and fluorochromes than conventional MHC multimers.
Here, we show how a double-color staining method can increase the sensitivity of the Dextramer even further, by improving resolution and separation between the antigen-specific T-cell population and the negative population. A staining protocol for the use of PE- and APC-labeled MHC Dextramers of the same specificity in the same tube has been developed. Using this approach the Dextramer population will be “double positive”, allowing for detection of positive cell populations that are not bright enough to be identified with a single color due to poor separation from background staining.
The double staining method makes it easier to identify and enumerate low-affinity antigen-specific T-cell populations such as cancer-specific T cells, and opens up for more accurate measurements than previously seen. It is useful in the improvement of immune monitoring of cancer immunotherapeutics, and together with the high sensitivity of the MHC Dextramers, it makes a valuable tool in cancer research.

P6.06.008
Increased efficacy of dendritic cells by protein components compared to total extract of *Listeria monocytogenes* in experimental tumor Immunotherapy

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Using dendritic cells (DCs) loaded with tumor antigens as a therapeutic strategy against tumors has been proposed. In order to increase the efficacy of DCs, a variety of factors such as TLR ligand molecules and bacterial products are used. In this study, the protein components of the bacteria *Listeria monocytogeneses* (LM) for induction of dendritic cell maturation were used.Bone marrow cells of Balb/c mice in the presence of IL-4 and GM-CSF were cultured for 5 days. On day 5, tumor lysate and then protein components or total extract of LM was added to immature DCs. In order to survey the maturation status of DCs, on day 7,the expression of CD80, CD86 AND MHC-II on the cell surface was evaluated. After induction of tumors in mice using WEHI-164 cell line, 10^6 mature dendritic cells subcutaneously injected. Tumor growth rate,survival rate and cytotoxic activity of spleen cells were evaluated in the studied groups.In mice vaccinated with protein components matured-DCs,delayed tumor growth rate and increased survival were seen. In addition,in these mice, the cytotoxic activity of spleen cells was higher compared to other groups. In all groups receiving protein components or total extract mature-DCs, increased cytotoxic activity and decreased tumor growth rate were seen compared to controls. *Listeria monocytogenes* protein components compared to total extract have higher ability to increase the efficacy of DCs for tumor immunotherapy in mouse model.

P6.06.009
Green tea polyphenols modulate certain indices of nonspecific anticancer resistance

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Introduction. Green tea polyphenols are of increasing interest in relation to their anticancer and antioxidant properties. Our previous investigationd have demonstrated significant anticancer activity of green tea polyphenolic extract (GTE) as to different experimental tumors and it's ability to diminish anticancer drugs' toxicity.
Aim of the work was to investigate GTE effect on certain immunity indices in healthy and tumor-bearing animals.
Methods. Experiments were performed in adult hybrid CDF mice, healthy and grafted with P388 or L1210 lymphatic leukemia. GTE were applied as 0.1% solutions in drinking water. Peritoneal macrophages cellularity (M), their metabolic activity (MAM) and splenocytes' cytolytic activity (CTL) were measured.
Results. GTE-treated healthy mice, after 24 hours, were shown to have 2.5 times higher M value versus control; at 7-21 days this value was some lower. Also, both CTL and MAM values was increased in these mice, with a maximum at 1-st and 21-th day (CTL) and 7-th day (MAM). GTE significantly (for 25%-30%) retarded development of L1210 and P-388 leukemias. In GTE-treated leukemias-bearing mice, M value at 8-th and 11-th days after leukemia have been grafted was 3-6 times higher and CTL value _ for 25-35% higher versus nontreated animals.
Conclusion. The data have been obtained show immunomodulating GTE effect to be one of the it's possible anticancer pathways.

P6.06.010
Polysaccharides of the Lentinus edodes modulate certain indices of specific anticancer resistance

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Polysaccharides isolated from fruiting bodies and mycelia of higher fungi are increasingly being used in immunotherapy of cancer. In our

previous studies have shown that the polysaccharide fraction isolated from the fruiting body of the fungus Lentinus edodes (PSFLE) has strong antitumor activity. The aim was to investigate the effect of PSFLE on some parameters of the immune system. Research carried out on intact outbred mice males and animals with transplanted sarcoma 37 (S 37). The study found that PSFLE significantly increases metabolic and phagocytic activity of macrophages in vivo. It also has a positive effect on splenocytes and thymocytes CLA. The study DTH and CLA serum as indicators of specific answers revealed statistically significant increase after the introduction DTH PSFLE doses 250mkh/kh and 5 mg / kg. The animals of these groups did not affect tumor growth as the value index DTH (I DTH) as in control animals, and even at the end of the experiment DTH level in animals with experimental groups was significantly higher (P <0.01) than in control Although the indicator showed a downward trend when compared with previous values. In addition, mice immunized cytolytic index (CLi) was significantly increased compared with the control. The most pronounced effect was obtained at 21 days of the experiment. Conclusions. Polysaccharide fraction isolated from the fruiting body of the fungus Lentinus edodes effect on tumor growth indirectly through activation of specific and non-specific components of the immune system.

P6.06.011
Anti-Metastatic Effect of Anti-HER2 scFv Antibodies on Breast Cancer Cells

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Introduction: Breast cancer is the most common malignancy and the second leading cause of cancer death in women. In addition, 70% of patients with advanced breast cancer develop metastases in bone marrow and bone. Since the expression of CXCR4/CXCL12, CXCR3/CXCL10, and MMP2 in breast tumors has been correlated with increased metastasis, we assessed the effect of three specific single-chain antibodies against HER2, a tumor antigen which overexpression is associated with markedly aggressive forms of cancer, on the expression of these factors in vitro.
Methods: HER2 overexpressed breast cancer cell line, SKBR3, treated with three specific and high affinity anti-HER2 scFv antibodies and quantification of CXCR4, CXCL12, CXCR3, CXCL10, and MMP2 transcripts was performed by RealTime-PCR. Furthermore, western blot was done to evaluate the expression level of CXCR4 and CXCL12 proteins as the key pro-metastatic axis in breast cancer.
Results: After 48h treatment of cells with anti-HER2 scFvs all three scFvs significantly down-regulated the expression level of CXCR4, CXCR3, CXCL10, and MMP2 transcripts, individually and in combination. Moreover, Western blot outcomes confirmed this down-regulation and demonstrated a reduction in CXCR4 protein expression. Furthermore, scFv-II and combination of all three scFvs significantly decreased the expression of CXCL12 at both the mRNA and protein levels.
Conclusion: The expression of CXCR4/CXCL12, CXCR3/CXCL10, and MMP2 in breast tumors is associated with poor prognosis and increased metastasis. However, effective anti-metastatic therapy remains a challenge. Regarding our results, three anti-HER2 scFvs have shown a good promise to be considered for anti-metastatic therapy in HER2-positive breast cancer.

P6.06.012
ImmTACs: bi-specific TCR-anti-CD3 fusions for potent re-directed killing of cancer cells

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The human immune system has the potential to clear tumours but fails to do so in many patients, as thymic selection removes high avidity T cell responses to self-antigens. This problem is compounded by the immunosuppressive tumour microenvironment, involving a complex array of mechanisms including Treg cells and MHC down-regulation. To address the issues of low T cell avidity, tumour tolerance and absence of tumour specific target antigens, we have engineered bi-specific ImmTAC reagents comprising soluble, pico-Molar affinity T cell Receptors (TCRs) fused to an anti-CD3 specific scFv. The TCRs target peptide epitopes derived from cancer-associated antigens presented on the cell surface by Class I MHC, and the anti-CD3 moiety re-directs a potent anti-tumour T cell response. Target epitopes are selected to meet several criteria including a) Mass Spectrometry validated tumour presentation, b) highly restricted expression in normal tissues, and c) frequent expression in common tumour types. We have demonstrated that ImmTACs are very sensitive, recognising as few as 10 epitopes per cell; this sensitivity far exceeds that of tumour-specific T cell clones isolated from cancer patients. ImmTACs demonstrate potent (pico-Molar EC_{50}) activity *in vitro* and *in vivo*, re-directing T cell killing of cancer cells with very low levels of cell surface MHC whilst sparing normal, antigen negative tissue cells. IMC-gp100, an ImmTAC specific for the HLA-A2 presented $gp100_{280-288}$ epitope, is undergoing Phase I/IIa clinical testing in melanoma patients; it is well tolerated and induces T cell mobilisation and tumour shrinkage.

P6.06.013
Oroxylin A affects frequency and function of myeloid-derived suppressor cells in experimental model of cancer

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Background: Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of myeloid progenitors. These cells have essential role in tumor development that specially related to chronic inflammation. Different studies characterized MDSC as immature myeloid cells that are precursors of DCs, macrophages, and granulocytes. They can suppress innate and adaptive immune response that would otherwise eliminate abnormal cells.
There are many herbal products with anti-inflammatory properties which could interfere with tumor progression. Oroxylin A is one of these products as a natural flavonoid isolated from Scutellariae radix, which has been indicated to possess antioxidant, anti-inflammatory and immunomodulatory properties. We sought to determine Oroxylin A effects on the frequency and function of MDSC population in tumor bearing hosts.
Methods: MDSCs were isolated from the spleen of tumor bearing mice, and then the frequency of MDSCs was evaluated using flow cytometry and compared with untreated group. Moreover, the Oroxylin A's effect on NO production was analyzed using Griess reagent.
Results: Based on our experiments, Oroxylin A had a modulatory effect on MDSCs.
Conclusion: Oroxylin A, as an anti-inflammatory drug, may exert beneficial effects on immune responses against tumors and should be considered as an agent that affects the frequency and function of MDSCs.

P6.06.014
T cell tolerance as function of tumor progression

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Peripheral tolerance to tumor antigens is a major obstacle in antitumor immunity. Among the possible mechanisms are presentation of tissue-derived proteins by quiescent dendritic cells, suppression by regulatory immune cells and secretion of factors that suppress T cells leading to anergy or deletion. To investigate anti-tumor immune responses, Tumor (B16 transfected with ovalbumin) bearing mice were transferred with admixed naïve splenocytes from pmel-1 and OT-I.GFP mice. At several time points, cells were isolated from mice and tested. Tumor growth sufficiently induce proliferation and effector phenotype acquisition of pmel-1 and OT-I.GFP cells in the draining lymph nodes (T-DLNs). Pmel-1 cells were unable to mediate tumor regression and show impaired capacity of killing target cells or secretion of IFNγ. Within the tumor, pmel-1 cells proliferated extensively while their frequencies were decreasing rapidly. In contrast, OT-I.GFP cells were able to mediate tumor regression and

killing capacity both in the tumor and in the periphery. While OT-I.GFP cells in the DLNs proliferated at low rates, extensive proliferation was detected in the tumor. Suggesting that tolerance followed by elimination of pmel-1 T cells develops already in the T-DLNs; indicating that lack of anti-tumor response efficacies are not solely due to general immunosuppressive mechanisms in the tumor microenvironment. Recently, we tested the involvement of the lymph node stroma in mediating CD8 T cell peripheral tolerance. We demonstrated that LN-resident lymphatic endothelial cells express peripheral tissue antigens and directly present the melanocyte protein PMEL17/gp100, to pmel-1 cells, resulting in their deletion, thus preventing a protective immune response.

P6.06.015
TLR3/BATF3 axis in CD8α+DC induces anti-tumor CTLs by dsRNA

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Many clinical trials using TLR ligands for tumor immunotherapy are in progress. The use of TLR3 ligand, e.g. Poly(I:C), has been examined in various experimental models in mice, which results in promising. Poly(I:C) is recognized by TLR3 in endosome and MDA5 and RIG-I in cytoplasm, and recruits TICAM-1/TRIF and MAVS/IPS-1 as an adaptor protein, respectively. Both signaling pathways activate three types of transcription factors, IRFs, NF-kB and AP-1, that participate in the induction of type I IFN which promotes the formation of Th1 immunity and CTL activation. Alternatively, it has been reported that BATF3, one of the AP-1 family molecules, functions as a master transcription factor for CD8α+DC in 129S6/SvEv mice, but not in C57BL/6J mice. We previously reported that poly(I:C) could induce anti-tumor CTL through the TICAM-1/IRF-3/7 pathway and retard tumor growth in vivo. However, it is currently unknown whether BATF3 is activated under TLR3/TICAM-1 signaling and involved in CTL activation. Therefore, we tried to investigate the role of BATF3 in dsRNA-induced tumor immunity. In this study, we found that the hallmarks of CD8α+DC, such as TLR3 expression and IL-12p40 production, were markedly decreased in Batf3-/- CD8α+DC. Furthermore, tumor retardation by poly(I:C) was abolished in Batf3-/- C57BL/6J, and the adoptive transfer of WT CD8α+DC to Batf3-/- mice successfully recovered tumor retardation activity in Batf3-/- mice. Collectively, our data revealed that BATF3 is important for not only the development but also the function of CD8α+DC. Moreover, these data underscored the importance of TLR3/BATF3 signaling in CD8α+DC in dsRNA-induced tumor immunity.

P6.06.016
Anti-tumor effects of heat shock proteins, Bacillus Calmette-Guérin, angiogenesis inhibitor, Bifidobacterium and Immuno-Modulator Drug on fibrosarcoma in mice

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Introduction: Despite the major advances in conventional forms of treatment (surgery, radiotherapy and chemotherapy), there are still many patients that are resistant to standard treatments. Immunotherapy is one of the best strategies in cancer therapy. Five important current methods include: using of HSP, BCG, angiogenesis inhibitors, Bifidobacterium and IMOD.
Materials and Methods: For this study, Balb/c mice (male, age, 6-8 weeks) were provided and were divided to 12 groups.WEHI-164 tumoral cells were used for creation of fibrosarcoma tumor. First, 1×106 cells\100µl of WEHI-164 cells were injected to mice and after 11 days, tumors were created. Then, surveys were carried out on the study protocol.
Results: Nevertheless injection of certain amount of tumoral cells to all of mice, tumor was not created in 5 mice and the size of tumors was different. The size of tumors was measured each week in special day and the volume of tumor was yielded by the formula: length×width2×π/6 in mm3. The survey of tumors was continued until end of 14th week. In some of groups, drugs showed synergistic effects and inhibited tumoral growth significantly. Mice in group 10 (IMOD, Thalidomide) had the least lifetime and the group 11 (BCG, HSP, Thalidomide) had the longest lifetimes.
Conclusion: In some groups, drugs showed synergistic effects and inhibited tumor growth significantly. The HSP, BCG, Thalidomide Group showed very good results. In this group, Thalidomide inhibited angiogenesis and HSP had role in presentation of tumoral antigens to immune system and BCG had a role in enhancing of immune system.

P6.06.017
Maternal immunization: transfer of a protective anti-tumor immunity from mothers to the offspring; a first evidence

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Recent studies involving maternal immunization against neonatal tetanus and influenza virus have shown that this approach was very effective in providing newborns with protection from these pathogens. We studied the effect of an anti-tumor DNA vaccine on the progression of carcinogenesis in cancer prone Her-2/neu transgenic (BALB-neuT) mice born from mothers immunized against the extracellular and transmembrane domain of neu. High levels of specific anti-neu IgGs were detected in both milk from vaccinated mothers and in sera of their offspring. The progression of spontaneous mammary carcinogenesis was hampered in BALB-neuT female offspring born and fed by vaccinated mothers as compared to controls. Immunized mothers KO for the µIg chain, thus unable to produce antibodies, failed to transfer protection to their offspring. Likewise the protection was nil when the newborns were KO for FcγRI/III, thus unable to develop antibody-dependent cytotoxicity. Furthermore, in neu-vaccinated mothers' pups we observed the expansion of low avidity $CD8^+$ T cells bearing a TCR rearrangement reacting with neu dominant peptide (p63-71) and usually not expanded in neu-vaccinated BALB-neuT mice. Moreover a T-cell-mediated cytotoxic response against p63-71 was found. This phenomenon could be due to the transfer of immune complexes of IgGs together with part of the neu protein shed from transfected cells after DNA vaccination. Taken together our results show for the first time that maternal immunization against a tumor associated antigen could offer effective protection to the offspring genetically predestined to develop mammary carcinoma, indicating the potential application of this approach in neonatal cancer diseases.

P6.06.018
The peripheral blood compartment of patients with Diffuse Large B Cell Lymphoma (DLBCL) at diagnosis is characterized by distinct phenotypic and functional Immunological alterations

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Tumor ability to modify the immune competence of the host may be particularly relevant in the case of hematological malignancies, such as B-cell lymphoma. This phenomenon might hamper the efficacy of chemo-immunotherapy.
We analyzed the phenotypic and functional asset of PBMC in 30 DLBCL at diagnosis (as compared with 23 healthy, age- and sex-matched controls), and the possible correlation of the immunological profile with pathological and clinical traits.
Patients showed a higher absolute monocyte number, and a lower lymphocyte count, mostly due to the selective diminution of CD4+ T cells (but not of Treg cells) and B lymphocytes. Accordingly, lymphocyte/monocyte and CD4/CD8 ratios decreased, while NK percentage increased.
The phenotypically skewed profile of PBL was associated with functional alterations: 1) IFN-γ producing cells were increased among

CD4+ and CD8+ T cell subsets, but not in CD56+ T and NK cell populations; 2) a higher frequency of Granzyme B-expressing cells characterized T and NK subsets; noteworthy, natural and CD16-dependent NK cytotoxic activities were unchanged. IL-6 and IL-10 plasma levels were significantly higher in DLBCL.
Selected alterations differently correlated with the DLBCL Germinal Genter B (GCB) vs non GCB-type and with the presence of bulky disease or extranodal involvement.
Our findings show a deeply altered phenotypic and functional profile in DLBCL PBMC populations, with the lymphocyte compartment showing traits of chronic activation; moreover, distinct immunological alterations correlated with histological subtypes and other clinical features. SB and RLS equally contributed to the study; MCC and GP equally contributed to the study.

P6.06.019
Adenosine receptor blockade potently suppresses the metastasis of CD73$^+$ tumours

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It is now accepted that tumours utilize multiple mechanisms to suppress anti-tumour immunity. One such mechanism is the generation of adenosine by CD73 which inhibits anti-tumour immunity through the activation of adenosine receptors expressed on multiple immune subsets. CD73 has previously been shown to enhance tumor metastasis, although the immune subsets and adenosine receptor subtypes involved are unknown. In this study, we revealed that both adenosine receptor 2_A (A_{2A}) and A_{2B} antagonists significantly reduced the metastasis of CD73$^+$ tumors. A_{2A}/A_{2B} receptor antagonists were effective in reducing the metastasis of tumours expressing CD73 endogenously (4T1.2) and when CD73 was ectopically expressed (B16F10). CD73-generated adenosine promoted metastasis, in part, through A_{2A}-mediated suppression of NK cell cytotoxicity. Treatment with an A_{2A} antagonist increased the expression of granzyme B in NK cells isolated from tumour-bearing mice and significantly reduced the metastasis of CD73$^+$ tumours in a perforin-dependent manner. A_{2B} blockade had no effect on NK cell cytotoxicity indicating that an NK cell-independent mechanism also contributed to the increased metastasis of CD73$^+$ tumours. Preliminary data suggests that A_{2B} blockade enhances the expression of MHCII on CD11b$^+$CD11c$^+$ cells within the metastatic site. Our data indicates that CD73 promotes tumour metastasis through multiple mechanisms including suppression of NK cell cytotoxicity and that A_{2A} or A_{2B} antagonists may be useful for treatment of tumour metastases. This data has potential therapeutic implications given that A_{2A}/A_{2B} receptor antagonists have already entered clinical trials for other therapeutic settings whereas there are no anti-CD73 mAbs currently in clinical development.

P6.06.020
Development of a DC-based therapeutic vaccine for AML patients: characterization of GMP-grade TLR-agonist matured 3-day DCs expressing the leukemia-associated antigens WT1 and PRAME

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We have designed a new generation of dendritic cells (DCs) optimized for the use in cell-based immunotherapy of cancer patients. Our goal was to tailor these DCs to be used for vaccination in acute myeloid leukemia (AML) patients with a high risk of relapse after intense induction/consolidation therapy in order to eradicate minimal residual disease (MRD).
We have developed a three-day manufacturing protocol using a cytokine cocktail containing a synthetic TLR7/8-agonist for generation of monocyte-derived mature DCs (mDCs) with improved immunogenicity. For induction of a specific T cell-based anti-AML response against residual tumor cells, our mDCs are loaded with RNA encoding the leukemia-associated antigens WT1 and PRAME. Additionally, DCs transfected with RNA encoding CMV-pp65 will be included as a surrogate antigen.
In this study, we present the careful evaluation of our 3d mDCs generated from healthy donors following RNA electroporation and cryopreservation, ensuring a fully functional phenotype of the autologous vaccine formulation. Our studies demonstrate a high and controllable expression of all three antigens following RNA loading. To ensure functional integrity of our DCs, the ability to secrete the critical cytokine IL12p70 upon T cell encounter was analyzed.
Furthermore, cryopreserved DCs expressing the different antigens also displayed a high capacity both for reactivation of antigen-specific pre-primed effector cells and for priming of naïve T cells in vitro, showing proper processing and presentation of the introduced antigens. These studies demonstrate that our manufacturing protocol yields improved DCs with a high potential to initiate long-lasting anti-leukemic responses in patients with AML.

P6.06.021
Development of new therapeutic antibodies formats directed against the folate receptor alpha.

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In our laboratory two mAbs directed against the folate receptor alpha (FRα), MOv18 and MOv19 were discovered, providing the foundation upon which to build new human Abs. Using phage display technology were developed fully human Fab fragments with the same specificity of the murine Ab MOv19 and two of them, AFRA4 and AFRA5.3 were later engrafted in complete immunoglobulins. These new reagents were assessed for binding properties and specificity on FRα and epithelial ovarian cancer cell lines. AFRA complete antibodies of IgG1 and IgE isotype were compared with ChiMOv19 for their binding properties and the IgG1 for the ability to enhance the effectors functions, as evaluated by antibody dependent cell-mediated cytotoxicity (ADCC) assays. Enhancement of ADCC on high FRα expressing cells, comparable to that of ChiMOv19, was observed for AFRA Abs despite their reduced binding affinity and different binding kinetics. The complete human structure could be probably more efficient in inducing ADCC, balancing the reduced binding activity. In order to assess the effects *in vivo* in immunocompetent mouse models we built “reverse chimeric” (RC) forms of AFRA, composed of human Fabs and murine Fc and we assessed the RC AFRA5.3 IgG2A in two immunocompentent mouse models. In the first model C26 cells expressing human FRα were injected subcutaneusly but, although we noticed a trend in reducing tumor growth by AFRA, we encountered problems with control mice. Therefore we shifted to a transgenic immunocompetent mouse model which expresses FRα on breast cancer cells and experiments are ongoing.

P6.06.022
Toxoplasma gondii protein fractions for the generation of dendritic cell based cancer vaccine

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Dendritic cell (DC) based immunotherapy appears to be a promising approach to successfully induce an antitumor immune response. Therefore, DC vaccines frequently used in many cell based therapy protocols. DCs are the most potent antigen-presenting cells and up on maturation with different stimulators exhibit an increased ability to activate Th1-polarized immune responses. Several protocols have

been developed to generate mature DCs. In our previous studies it has been shown that Toxoplasma gondii (T.G) can induce competent mature DCs. In this study we have investigated that fractionated protein components of T.G stimulated DC maturation. Materials and Methods: T.G was sonicated and exposed to stepwise precipitation to increased amount of ammonium sulfate to obtain four distinct protein fractions. Fractionation was followed by ion exchange chromatography method. DCs were generated from C57/BL6 mouse bone marrow in the presence of IL-4 and GM-CSF, immature DCs were exposed to T.G protein fractions. After 24 hours DC maturation markers CD80, CD86 and MHC II and IL-12p70 production were evaluated. Mouse splenic cell proliferation assay also were performed and compared with immature DCs. Tumor growth rates and vaccinated mice survival were followed. Results and Conclusion: The results indicate that maturation of DCs with certain T.G protein fractions induce higher expression of CD86, CD80, MHC II and production of IL-12p70 by DCs. In addition these mature DCs significantly increased the splenic cell proliferation and survival in vaccinated animals. Further fractionation may lead to identification of some protein components of TG with strong DC maturing properties.

P6.06.023
Comparison of DNA-based vaccine and non-pathogenic live vaccine strategies using the mini-chaperones for the prevention of HPV16 E7-overexpressing cancers

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Immunotherapy potentiating host immunity against human papillomavirus (HPV) is a promising strategy for the prevention and treatment of cervical cancer. The efficient antigen delivery systems including biological carriers (e.g., the attenuated or non-pathogenic live vectors) and physical carriers (e.g., electroporation) are the key issue of developing an effective cancer vaccine. Furthermore, heat shock proteins (HSPs) including Gp96 act as potent immuno-adjuvant to enhance antigen-specific tumor immunity. Recently, we generated DNA- and live *Leishmania tarentolae* (*L.tar*)-based vaccines containing the N-terminal and C-terminal domains of gp96 fused to HPV16 E7 and compared their immuno-stimulatory activity in C57BL/6 tumor mice model. We found that subcutaneous DNA injection with E7-CT (gp96) followed by electroporation generates the significant E7-specific IFN-γ immune responses as well as the best protective effects as compared to E7 or E7-NT (gp96) DNA vaccines. In addition, we observed that subcutaneous injection with *L.tar*-E7-CT (gp96) produces similar IFN-γ immune responses as well as insignificant protective effect in comparison with *L.tar*-E7-NT (gp96). Both recombinant *L.tar*-E7-CT (gp96) and *L.tar*-E7-NT (gp96) could not induce complete protective effects against HPV16 E7-overexpressing cancers. Our studies indicated that subcutaneous administration of E7 DNA linked to CT (gp96) fragment followed by electroporation can significantly enhance the cellular immune responses and the protective effects in comparison with non-pathogenic live *L.tar*-E7-CT (gp96). Briefly, DNA-based vaccine was more efficient than non-pathogenic live *leishmania tarentolae*-based vaccine. Moreover, the structural domains of immuno-chaperones show the potential of generating effective immune responses against different clinical disorders such as cancer.

P6.06.024
Antitumor potential of novel betulin derivative on human A375 melanoma cells

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There is increasing evidence that chronic inflammation causes most of chronic diseases, including cancer and that one out of seven malignant tumors today is a result of chronic inflammation. Betulinic acid is a betulin derivative, pentacyclic triterpene with lupane skeleton, which is one of the first natural product isolated from birch bark. It has a number of pharmacological effects including anti-inflamatory, antitumor and anti-HIV. Betulinic acid induces mitochondrial pathway of apoptosis by directly interacting with permeability transition pore complex and through production of ROS which facilitates opening of the pore. It downregulates NF-κB controlled proinflammatory genes such as COX-2, MMP9 and *i*NOS. In this study we evaluated antimelanoma effect of novel betulin derivative, 3-O-ethylcarbamate-28-O-acetyl-betulin. We found that novel compound significantly reduced viability of *i*NOS$^+$ A375 human melanoma cells, with IC50 value four times lower than betulinic acid. Cell cycle analysis demonstrated considerable accumulation of cells in *sub*G phase after two days of treatment with IC50 dose, while Ann/PI staining demonstrated that cells undergo apoptosis much faster under treatment with novel compound compared to betulinic acid. Real-time PCR analysis revealed enhanced caspase 9 and pro-apoptotic Bax gene expression accompanied with general caspase activation confirmed by apostat staining. DHR and DAF-FM staining demonstrated massive production of reactive oxygen and nitrogen species indicating that mechanism of cell death induction is not related with inhibition of *i*NOS. In conclusion, novel betulin compound has a much stronger antimelanoma effect realized through mitochondrial dependent apoptosis synchronized with production of reactive oxygen and nitrogen species.

P6.06.025
Early growth response gene 2 acts as an intrinsic negative regulator of DC immunogenicity

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Early growth response gene 2 (Egr2) is an immediate early gene encoding a zinc finger transcription factor. Egr2 is rapidly and transiently induced in various cell types independent of *de novo* protein synthesis. Although Egr2 is well established in T cell development, Egr2 expression and its biological function in dendritic cells (DCs) have not yet been described. Here we demonstrate Egr2 expression during DC development, and its role in DC-mediated immune responses. Egr2 is expressed in the later stage of DC development from bone marrow (BM) precursor cells. Even in a steady state, Egr2 is highly expressed in mouse spelnic DCs. Egr2-knockdown DCs showed increased levels of major histocompatability complex (MHC) I & II and co-stimulatory molecules and enhanced antigen-uptake and migratory capacities. Furthermore, Egr2-knockdown abolished SOCS1 expression and STAT5 activation during DC development, probably resulting in the enhancement of IL-12 expression and Th1 immunogenicity of the DC vaccine. DC-mediated cytotoxic T lymphocyte (CTL) activation and anti-tumor immunity were significantly enhanced by Egr2-knockdown, while impaired by Egr2-overexpression of antigen-pulsed DC vaccines. These data suggest that Egr2 acts as an intrinsic negative regulator of DC immunogenicity and can be an attractive molecular target for better DC vaccine development.

P6.06.026
ENO1-DNA vaccine significantly delays pancreatic tumor progression by eliciting complement-dependent cytotoxicity

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Pancreatic ductal adenocarcinoma (PDA) is a very aggressive malignancy characterized by rapid progression, invasiveness, and resistance to treatment. By SERological Proteome Analysis we have identified novel PDA-associated antigens recognisable by either CD4 and CD8 T cells. We have also in vitro characterized one of these antigens, alpha-enolase (ENO1). PDA patients with serum IgG against ENO1 showed T cells secreting IFN-γ in response to ENO1 and Ag-specific T cells were able to inhibit the in vivo growth of HLA matched PDA cells in immunocompromised mice. By using genetically engineered mice (GEM) developing spontaneously PDAC, we investigated the potential of ENO1 as therapeutic vaccine. In GEM

vaccinated with a vector expressing cDNA for human ENO1, a specific ENO1 immune response improves antitumor responses and survival. The immune response is associated with an increased level of anti-ENO1-specific IgG, an augmented frequency of ENO1-specific Th1/Th17 cells, and a decrease of suppressor cells. The induced anti-ENO1 IgG are able to recognize and bind surface of murine PDA cells and elicit complement-dependent cytotoxicity, which has been proposed as an effector mechanism of anti-tumor immunity (e.g rituximab and ofatunumab). Moreover, ENO1 vaccine elicited a Th1/Th17 response that had a limited effector function but an important helper role in driving autoantibody isotype switching. To elucidate the role of Th17 in PDA progression and DNA-vaccine-induced responses we are breeding GEM with IL17 KO mice.
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P6.06.027
Enrichment of KIR+CD57+ highly cytotoxic NK cells in sentinel lymph nodes of melanoma patients

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NK cells contribute to melanoma cell recognition and anti-tumor immunity, which is traditionally analyzed using human peripheral blood NK cells1. An important checkpoint in the progression of malignant melanoma is the metastasis to lymph nodes. To investigate the role of lymph node NK cells in disease progression, we analyzed frequency, phenotype and functions of NK cells purified from either tumor infiltrated lymph nodes or tumor-free ipsilateral lymph nodes of the same patients. Lymph node NK cells were compared to peripheral blood NK cells from either melanoma patients or healthy donors. The data showed an expansion of CD56dimCD57+CD69+CCR7+KIR+ NK cells in tumor infiltrated lymph nodes. This phenotype corresponds to a recently described fully mature and highly cytotoxic NK cell population2,3, and indeed we found that these lymph node NK cells displayed robust anti-tumor activity against autologous melanoma cells. Moreover, a high proportion of KIR+CD57+CD56dim in the infiltrated lymph nodes was associated with improved patients' survival. Our data suggest that NK cells from tumor infiltrated lymph nodes are attractive candidates to improve current NK cell-based immunotherapy of melanoma.

P6.06.028
Multiple Myeloma cells in Vk*MYC mice are highly susceptible to WT1 and MAGE-A specific cytotoxic T lymphocytes

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The need to improve the clinical outcome in multiple myeloma (MM) patients has led to a renewed interest in immunotherapy. The identification of primary models of the human disease in which novel immunotherapeutic approaches can be tested is pivotal. As the Wilms tumor (WT1) and the MAGE-A antigens are overexpressed in MM cells, we investigated their presence and immunogenicity in Vk*Myc mice, a primary model of autochthonous MM. We demonstrated that both antigens are overexpressed in neoplastic plasma cells from Vk*Myc mice. We have also found that upon injection with dendritic cells either pulsed with the WT1 or the MAGE-A CTL epitope, wild-type C57BL/6 mice were able to mount a consistent antigen-specific cytolytic response. WT-1- and MAGE-A-specific CTL were able to kill Vk*Myc bone marrow plasmacells in an in-vivo cytotoxicity assay. These findings prompted us to investigate in Vk*Myc mice the efficacy of a protocol that combines vascular targeting and adoptive immunotherapy. Indeed, we reported that systemic administration of picograms of the Cys-Asn-Gly-Arg-Cys (NGR)-TNF conjugate, which targets TNF to an aminopeptidase N (CD13) isoform selectively expressed by endothelial cells in tumor vessels, increases the penetration of tumor specific CTL (Calcinotto et al. 2012). Thus, Vk*Myc mice affected by MM, stage at which neoangiogenesis is active, have been treated with NGT-TNF and splenocytes from WT-1-MAGE-A-sensitized C57BL/6 mice. This experiment will be concluded in a few months. So far, our data demonstrate that WT-1 and MAGE-A are optimal targets of immunotherapy in Vk*Myc mice, and support further investigation in MM patients.

P6.06.029
Vaccination with human CSPG4 DNA induces an anti-tumor antibody response and prolongs survival in dogs with oral malignant melanoma

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Chondroitin sulfate proteoglycan-4 (CSPG4) is a membrane-bound chondroitin sulfate proteoglycan with a restricted distribution in both human and canine normal tissues, but highly expressed by human melanoma cells and by about 60% of canine malignant melanomas (CMM). Human and canine CSPG4 amino acid sequences have 82% homology, and 88% similarity. CMM is a spontaneous, aggressive, and metastatic neoplasm, sharing many characteristics with its human counterpart, thus representing a valuable clinical model to assess the efficacy of novel anti-cancer strategies.
The capability of DNA electroporation of a plasmid coding for the human CSPG4 (hCSPG4) in inducing an effective immune response in dogs with stage II-IV surgically resected CSPG4-positive oral CMM was evaluated. Vaccination did not cause any clinically relevant local or systemic side effects. All vaccinated dogs developed antibodies against hCSPG4 cross-reacting with the canine orthologue, demonstrating the ability of this vaccine to overcome host immune tolerance to dog CSPG4. The overall survival time of vaccinated dogs (n=12) was significantly longer as compared to that of non vaccinated dogs with either CSPG4-positive (n=13) or CSPG4-negative (n=7) stage II-IV surgically resected oral CMM, thus suggesting that vaccination against hCSPG4 by DNA electroporation is efficacious against CMM when used in an adjuvant setting.

P6.06.030
Correlation between the high expression of cancer testis antigens and the prognosis in patients with esophageal carcinoma in China

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Background: Survival rates after esophageal cancer diagnosis still remain poor, despite standardization of surgery and adjuvant treatment. Cancer testis (CT) antigens as targets for immunotherapy in cancer patients have been heavily investigated. We aim to evaluate the expression of CT antigens in Chinese esophageal carcinoma and the correlation with the prognosis. **Methods:** The expression of MAGE-A3, MAGE-A4, MAGE-C2 and NY-ESO-1 in fresh cancer tissues from 103 patients with esophageal carcinoma were analysed by RT-PCR. The expression of HLA-A2 was detected by flow cytometry. **Results:** 59.8% esophageal cancer samples were HLA-A2 positive. CT antigens were frequently expressed in esophageal carcinoma. The expression percentage of each gene in cancer tissues samples were as follows: MAGE-A3, 87.9%; MAGE-A4, 57.1%; MAGE-C2, 67.0%; and NY-ESO-1, 22.0%. In all, 93.4% of samples expressed at least one of CT genes analysed. The percentage of samples co-expression HLA-A2 and CT genes were: MAGE-A3, 51.6%; MAGE-A4, 36.3%; MAGE-C2, 44.0%; and NY-ESO-1, 12.1%. MAGE-A3 expression was associated with age, tumor stage and tumor differentiation ($P<0.05$); MAGE-C2 was associated with tumor stage ($P<0.05$). There was no correlation MAGE-A4 or

NY-ESO-1 expression with clinical characteristics such as gender, age, HLA-A2 positive, clinical stage, grade of differentiation and lymph node metastasis (P>0.05). **Conclusion:** CT antigens might be prognostic markers and factors related to the progress of esophageal carcinoma, and also be served as the immunotherapeutic targets of esophageal carcinoma, especially in multi-antigen vaccine preparations and tumor antigen specific lymphocytes infusion.

P6.06.031
CD38 immunotherapy: killing two birds with one stone

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CD38 is a suitable cell surface target in the design of monoclonal antibody (mAb)-mediated therapy. CD38 is an appealing target in virtue of its bi-functional roles of receptor and ectoenzyme. This work presents a range of perspectives and caveats in the challenges encountered while conducting a pre-clinical studies for anti-CD38 mAb therapy. The disease models chosen for in vitro analysis were multiple myeloma (MM) and chronic lymphocytic leukemia (CLL). The first step was to study events occurring at the cell membrane, where antigen-antibody complexes were internalized. The second step was the analysis of the effects triggered by mAb ligation on the trascriptome and on miRNAs (33a-5p and 340-5p are up-modulated, while 193b-5p and 629-3p are decreased). The next possibility was to increase CD38 surface expression by using retinoids. CD38 was increased in both MM and CLL, where only the CD38+ samples responded to retinoids. The last hypothesis tested was that CD38 may generate adenosine, inducing local immunosuppression. This was confirmed by using an in vitro bone marrow model with stromal cells, osteoblasts, osteoclasts and lymphocytes. This model consented the identification of an ectoenzyme (CD38/CD203a/CD73) network which generates adenosine, explaining survival of myeloma cells in closed systems. The role of oxytocin (controlled by CD38 in mouse models) is under analysis. The data obtained allow to define the safety of pre-clinical anti-CD38 mAb therapy plans. Added values are that antibody-mediated therapy may be used directly, as a drug carrier or to redirect nanovescicles, and could be applied in association with other drugs.

P6.06.032
Combination immunotherapy with tumor-specific CD4+ and CD8+ T cells decreases CD8+ T cell exhaustion and augments therapeutic efficacy

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Adoptive immunotherapy with tumor-specific TRP-1-CD4$^+$ TCR-Tg T-cells (TRP-1-CD4$^+$) and pmel-CD8$^+$ TCR-Tg T-cells (pmel-CD8$^+$) cured mice with established experimental metastases of the poorly immunogenic B16BL6-D5 melanoma. However, when the same dose of either TRP-1-CD4$^+$ or pmel-CD8$^+$ were transferred alone they were non-therapeutic. Characterization of T cells post adoptive transfer found that TRP-1-CD4$^+$ significantly ($p<0.001$) increased the total number and function of memory pmel-CD8$^+$. We examined whether TRP-1-CD4$^+$ maintained effector pmel-CD8$^+$ by sorting CD62L^{lo}CD44$^+$ (effector) pmel-CD8$^+$ and combining the sorted or total pmel-CD8$^+$ with TRP-1-CD4$^+$ for adoptive immunotherapy. Both were equally therapeutic suggesting CD4$^+$ T cells are maintaining activated effector pmel-CD8$^+$. We examined when CD4$^+$ T cells were necessary for enhancing therapeutic efficacy of CD8$^+$ T cells by administering anti-CD4 Mab at different times (day 3 and 10) following adoptive transfer. Depletion of CD4$^+$ cells 3 days after adoptive transfer eliminated therapeutic efficacy and correlated with loss of function and increased expression of the exhaustion marker PD-1 on pmel-CD8$^+$ ($p<0.05$). Depletion of CD4$^+$ cells 10 days after transfer led to elimination of metastases at day 18 but animals did not survive long-term. Since blocking PD-1 may increase survival of pmel-CD8$^+$, mice were given combination treatment with pmel-CD8$^+$ and anti-PD-1. PD-1 blockade significantly ($p<0.05$) increased total and memory pmel-CD8$^+$ compared to treatment with pmel-CD8$^+$ alone, however, this did not improve therapeutic efficacy. These data show that tumor-specific CD4$^+$ T cells are helping to reduce CD8$^+$ T cell exhaustion and underscores the clinical relevance of generating both tumor-specific CD4$^+$ and CD8$^+$ T cells.

P6.06.033
A new pathway of tumor antigen loading of human dendritic cells via intercellular communication

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Most cancer cells down-regulate gap junctions resulting in loss of communication with their surrounding microenvironment.
Recent data obtained in a mouse melanoma model (Saccheri et al., Sci TM 2010) showed that infection of tumors with Salmonella could induce, in tumor cells, the up-regulation of connexin 43 (Cx43), a ubiquitous protein that forms gap junctions. This up-regulation leads to the formation of functional gap junctions between tumor cells and dendritic cells. Herein, we show that the formation of this intercellular way of communication could be used, in vitro, to load human autologous dendritic cells with tumor antigens and generate a cancer vaccine. Indeed, human dendritic cells cocultured with salmonella-infected SKmel24 melanoma cell line were able to elicit a tumor-specific CD8 T-cell response in vitro. The transfer of tumor antigens was gap junction dependent because abolished by the presence of specific inhibitor. Tumor-specific CTLs recognized other melanoma cell lines indicating that dendritic cell were loaded with tumor-associated antigens shared among melanoma cell lines. Moreover, the in vitro generated CTL were melanoma specific because they were unable to lyse colorectal adenocarcinoma cell lines.
We exploited an antimicrobial response present in tumour cells to activate cytotoxic CD8 T cells specific for tumour-peptides through a new pathway of antigen loading of human dendritic cells via intercellular communication channels that can be used clinically as therapeutic vaccine.

P6.06.034
A phase Ib clinical trial of cisplatin and pemetrexed in combination with the CD40 activating antibody CP-870,893

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CD40 is a co-stimulatory molecule expressed on antigen presenting cells. Pre-clinical data supports synergy between cytotoxic chemotherapies and CD40 activation in mouse tumor models however, little is known regarding their combination in human disease. Here we investigate the safety profile, maximum tolerated dose (MTD) and immunological activity of the CD40 activating antibody, CP-870,893 combined with pemetrexed and cisplatin in a Phase 1b study. Patients with advanced malignant pleural mesothelioma (MPM) received standard does of cisplatin and pemetrexed on day 1 and CP-870,893 in escalating doses from 0.1 mg/kg on day 8 of a 21 day cycle for a maximum of 6 cycles. Fifteen patients received combination therapy at three dose levels of CP-870,893. Three patients were treated at dose level 1 (0.1mg/kg) with no dose limiting toxicities (DLT). Three patients were treated at dose level 2 (0.2mg/kg), with 2 DLTs (hyponatremia and confusion; splenic infarction). Three patients were treated at dose level 1.5 (0.15 mg/kg) with no DLTs observed, and an additional 6 patients were accrued to an expansion cohort at this dose level. The best radiological response was partial response in 6 patients (40%), and stable disease in 8

patients (53%). Two patients showed progression followed by subsequent prolonged stabilisation of disease (>24 months). The proportion of Tregs, $CD4^+$ and $CD8^+$ T cells remained consistent throughout treatment with updated DC and B cell analysis to be presented. A combination of cisplatin, pemetrexed, and CD40 activation with CP-870,893 can be safely administered in patients with MPM with clinical responses observed.

P6.06.035

Optimising regulatory T cell (Treg) depletion in combination with chemotherapy for enhanced anti-tumour immunity

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Cytotoxic chemotherapy, widely used to palliate many malignancies, can enhance anti-cancer immunotherapy as shown by abundant preclinical data. Tregs are immunosuppressive $CD4^+$ T cells thought to inhibit anti-tumour immune responses; murine data suggests that Treg eradication may augment existing anti-tumour immunity. Cyclophosphamide (CTX) is immunostimulatory and at low doses selectively depletes Tregs in mice and humans.
In this phase 1b study we investigate an optimum dose and schedule of iterative low dose oral CTX for Treg depletion, in the context of pemetrexed-based chemotherapy, and how treatment affects the function and phenotype of the cellular immune response. Thirty-one patients with advanced malignant pleural mesothelioma (MM) or non-small cell lung cancer (NSCLC) received standard doses of pemetrexed ± cisplatin or carboplatin on a 21 day schedule (6 cycles max.). From the second cycle, escalating doses of oral CTX were administered, initially with 50 mg daily. Weekly peripheral blood samples were collected, and the proportion of Tregs within the $CD4^+$ population (Treg%) determined, amongst other immunological parameters.
Following commencement of CTX the peripheral blood Treg% decreased (maximum Treg depletion occurred during Cycle 3), then increased back toward baseline. Optimal Treg depletion was achieved with alternating 50/100 mg per day, with increasing doses up to alternating 100/150 mg daily not resulting in further depletion. Analysis of PBMC T cell populations shows cell proliferation (Ki67) and activation (inducible co-stimulator; ICOS) to peak and trough with each cycle of chemotherapy. Combining CTX with routine cytotoxic chemotherapy is feasible with no additional haematological or other toxicities.

P6.06.036

Radiation therapy plus IL-2 in patients with metastatic melanoma is associated with release of danger associated molecular patterns when compared to IL-2 alone but not an increase in circulating inflammatory cytokines

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We completed a Phase I study combining high-dose per fraction radiation therapy (SBRT) to metastatic lesions in the lung and liver of patients undergoing high dose IL-2 treatment for metastatic melanoma or renal cell carcinoma. This study demonstrated a response rate of 66%. We have an ongoing randomized Phase II study of SBRT followed by IL-2 versus IL-2 alone in patients with metastatic melanoma. To understand the higher response rate associated with radiation therapy we undertook analysis of serum markers of inflammation and adjuvant release. We analyzed the serum of the first 16 patients enrolled in our phase II study. We demonstrated a significant increase in the release of danger associated molecular patterns (DAMPS), including Uric Acid and HMGB-1, in the serum of patients undergoing radiation and IL-2 when compared to IL-2 alone. Conversely, 24 hours following IL-2 administration patients receiving IL-2 alone showed a significantly higher level of circulating pro-calcitonin levels when compared to patients receiving combined therapy as well as significantly higher levels of circulating IL-12 and IL-15. On day 8, circulating RANTES was significantly higher in patients receiving combined therapy when compared to patients receiving IL-2 alone.
We conclude that radiation therapy in combination with IL-2 is associated with an increase in DAMPS. Yet, combined therapy results in a dampening of systemic inflammatory cytokines. We hypothesize that this reflects a change in location of the immune response from the blood to the tumors and is supported by the higher circulating RANTES level in patients receiving combined treatment.

P6.06.037

RIL-21 combined with CD4-T cell depletion reprograms CD4+ T cells from an immune regulatory to an anti-tumor phenotype in syngeneic neuroblastoma

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Tumors sustain the expansion of immune suppressive cells, including CD4+CD25+FoxP3+ regulatory T (Treg), through factors such as TGF-beta, PGE2 or IL-10. IL-21 is an immune enhancing cytokine showing promising results in pre-clinical and clinical cancer immunotherapy. We previously observed that CD4+CD25+ FoxP3+ Treg cells increased in a syngeneic model of disseminated neuroblastoma (NB). In addition, the administration of anti-CD4 cell-depleting antibody strongly enhanced the anti-tumor effects of an IL-21-engineered NB cell vaccine, by inducing depletion of Treg cells. In this study we addressed the effects of a therapeutic combination of recombinant (r)IL-21 and anti-CD4 antibodies in disseminated NB. rIL-21 administered s.c. at two dose levels had a limited effect, while the co-administration of a cell-depleting anti-CD4 mAb allowed to cure 28% to 70% of mice receiving 0.5 or 1 µg/dose levels, respectively. Anti-CD4 antibody treatment completely depleted CD4+CD25+FoxP3+ Treg cells and their CD25- precursors. The co-administration of rIL-21 by-passed the requirement of CD4+ T helper cells and allowed the development of a CD8+ T cell response, which was required for tumor eradication and for long-lasting immunity. Nonetheless reconstitution of the CD4+ T cell pool after combined immunotherapy was also required for immunity to NB antigens, as indicated by CD4+ T cell depletion and re-challenge experiments in cured mice. In conclusion, our data support a role of immune suppressive CD4+ T cells in a syngenic NB model and suggest that rIL-21 combined with CD4-T cell depletion reprogams CD4+ T cells from immune regulatory to an anti-tumor memory phenotype.

P6.06.038

Pre-clinical development of an agonistic CD40 antibody (ADC-1013) for local immunotherapy of cancer

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Local administration of immune activating antibodies may increase the efficacy and reduce the immune-related adverse events associated with systemic immunotherapy of cancer. Here we report the development of a fully human agonistic CD40 antibody, ADC-1013, which has been optimized for local immunotherapy by FIND® recombination, thereby increasing potency and tumor retention.
ADC-1013 activates CD40 receptors on antigen-presenting cells such as dendritic cells, resulting in up-regulation of the co-stimulatory molecules CD80 and CD86, and induction of IL-12. In addition, ADC-1013 induces direct tumor killing of $CD40^+$ tumors. The anti-tumor effects of ADC-1013 have been assessed in a bladder cancer model (EJ) in immunodeficient NSG mice. Significant anti-tumor responses were demonstrated, and further augmented in mice repopulated with human monocyte-derived dendritic cells and T cells.
To our knowledge, ADC-1013 represents the first immunomodulatory antibody optimized for local immunotherapy of cancer. It is currently in late pre-clinical development and will enter clinical trials in 2014.

P6.06.039
Th1/Th2/Th17 profile and T cell phenotype of an in vitro antigen presentation system in response to DC functionality, IL-2 and IGF-1 presence

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T cells play an important role in controlling tumor growth and mediating tumor regression. The aim of this study was the generation of tumor-specific CD4+ and CD8+ lymphocytes by the use of combined MHC class I and class II epitopes of the tumor antigen MAGE-3. The construct that has been used consists of a CD8/MHCI epitope and a CD4/MHCII epitope, joined on an oligopeptide carrier. This construct has been used as a model, dual MHC, foreign antigen to assess the dynamics of antigen presentation.
Monocytes have been isolated from healthy donors, have been cultured in the presence or absence of IGF-1 and have been transformed to dendritic cells. The peptide has been added to the DC culture, and the pulsed cells have been transferred to a co-culture with lymphocytes from the same donor, either as irradiated feeders or untreated, and were cultured in the presence or absence of IL-2. Several rounds of restimulation followed. The lymphocytes were analyzed by Flow Cytometry, ELISA and Cytometric Bead Array for Th1/Th2/Th17 profiling. The results showed that the lymphocytes in culture upregulated their activation markers and produced Th1 proinflammatory cytokines in response to the peptide, optimally when it was presented by non-irradiated dendritic cells in the presence of IL-2. After prolonged stimulation the culture displayed a Th17 polarization but only in the presence of the peptide. IGF-1 displayed a “rescue” potential for the dendritic cells after the irradiation and also acted as upregulator of co-expression molecules, resulting in improved antigen presentation.

P6.06.040
The predictive role of αβ-double negative T cells in clinical outcome of patients with lymphoma: preliminary data

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Numerous aspects of lymphoma pathophysiology indicate mutual interactions between the host immune system and lymphoma cells.An unconventional subset of CD4−CD8− double-negative T cells (DNTs) has been described to specifically contribute to anti-tumor immunity,acting as both regulatory T cells and/or cytotoxic T cells.No data are available on their role in human anti-lymphoma immunity,their interaction with other immune cells, functional attitude and on their prognostic significance in lymphomas.
The aim of study is to assess the frequency and the functional attitude of circulating DNTs in Lymphoma patients (pts) in order to evaluate their predictive role on clinical outcome.
Peripheral blood of 25 Lymphoma patients and healthy donors age as controls were prospectively collected to perform phenotypic and functional characterization of circulating DNTs.For functional studies DNTs were purified from PBMCs using specific MACS microbeads.
A significantly reduction of circulating (TCRαβ) DNTs in Lymphoma patients compared with healthy controls (1,482%±2,60, p<0,01) was observed and they correlate with disease relapse/progression.Moreover,Hodgkin's Lymphoma showed higher number of αβ−DNTs as compared with other histotypes.Furthermore in preliminary data was observed a characteristic cytokines profile with a cytotoxic potential in ex-vivo expanded DNTs, mainly with an increase of IFN-γ and granzyme B secretion,known to be the main immune effectors for anti-tumor activity.
This study suggest that the reduction of αβ−DNTs in untreated lymphoma patients may correlate with the suppression of tumor-specific immune response and the clinical outcome.However ex-vivo expanded DNTs likely possess a cytotoxic attitude, suggesting their possible use as new strategy for immune therapy

P6.06.041
Deciphering adaptive anti-tumor immunity induced by anti-CD20 mAb treatment

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We have previously demonstrated that anti-CD20 monoclonal antibody (mAb) exerts a long-lasting anti-tumor protection in tumor-bearing mice through the induction of an adaptive immune response (1). Using a mouse model in which immune competent mice are injected i.v. with human $CD20^+$ tumor cells and then treated with an anti-huCD20 mAb, we have shown that $CD4^+$ T cells are central to tumor protection by depletion and adoptive transfer experiments. We have then investigated whether anti-CD20 mAb treatment modifies the balance between $CD4^+$ T cell subsets. Strikingly, analyses of spleen T cells at different time points after tumor injection revealed that the initial expansion of Treg ($FoxP3^+$ $CD4^+$) observed in both untreated and mAb-treated mice is reversed in anti-CD20-treated mice that exhibit an increase of the Th1 cell subset (IFN-γ^+ $CD4^+$). Concomitantly, anti-CD20-treated mice showed an increased in IL-12 serum level. IL-12 neutralization abolished the protection induced by anti-CD20 mab and decreased the number of Th1 cells, demonstrating the critical role of this cytokine. Neutralization of IL-10 or TGF-β or depletion of Treg with an anti-CD25 mAb that ameliorate protection of tumor-bearing animals per se, did not lead to an increase in the survival rate of mAb-treated mice, indicating no synergy when combining anti-immunosupressive and anti-CD20 treatments. Altogether, these data demonstrate that the balance between Treg and Th1 compartments, essential in the control of tumor progression, can be fine-tuned by therapeutic mAbs to switch the immune response from a pro- to an anti-tumor response.
1. Abès et al., Blood, 2010, 116: 926-34.

P6.06.042
The tumor microenvironment can vary with anatomical site to affect responses to therapy

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Besides cancer cells, tumors comprise a complex assortment of molecular and cellular components called the microenvironment. How surrounding normal tissues at different anatomical sites affect tumor microenvironments and their subsequent response to therapy is not known. We studied the microenvironments of the same type of tumor developed in different tissues.
We found that established tumors in the skin could be eradicated in the majority of mice using a combination of three antibodies, whereas the same tumors developing in visceral organs responded much less. Analyses using flow cytometry together with gene and protein arrays demonstrated that tumors in visceral locations had a microenvironment with a higher frequency of immunosuppressive alternatively activated macrophages (AAM) and increased levels of their associated molecules (such as CD206, IL-10 or Arginase) relative to tumors growing in the skin. Indeed, we demonstrated that the therapeutic effect of antibodies on visceral tumors could be improved by targeting some key AAM-associated molecules using a CCL2-neutralizing antibody and IL13-KO mice. Importantly, a much reduced therapeutic response of subcutaneous tumors was observed when kidney tumors were simultaneously present in the same mice, suggesting a negative impact of immunosuppressive-tumors on distant responsive-tumors.
The tissue-specific response observed in this system suggests that the anatomical location of tumors can affect the tumor microenvironment and highlights the importance of normal tissues in sculpting the tumor microenvironment. Also, the observation that distant tumors are able to influence each others’ response to therapy may be an important clinical consideration when in the design of treatment strategy.

P6.06.043
Antitumor activity of PEGylated Fab-fragments of GD2-specific antibody

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Monoclonal antibodies against tumor-associated ganglioside GD2 are attractive tools for researchers and clinicians as an agent for anti-cancer immunotherapy. Despite some success of their application, there are a number of side effects associated with Fc-fragment of GD2-mAb. Recently, it was shown that the GD2-mAb has direct strong cytotoxic activity against tumor cells expressing GD2. The fact that these effects did not associated with antibody's Fc-fragment allows the use of active antibody fragments without Fc-region (Fab-fragments). Purpose of the study is to evaluate the possibility of applying Fab-fragments of GD2-mAb for tumor therapy.
In our work, we have obtained purified fraction of Fab-fragments from GD2-mAbs. Using flow cytometry assay, we showed their antitumor effects on GD2-positive tumor cell lines. The main drawback of smaller Fab-fragments in comparison to whole antibodies is their drastically shorter elimination half-life. In order to increase the half-life of Fab-fragment GD2-mAb we produced their site-specific PEGylation. Maleimide-based reaction allows to covalently attach PEG (from 1 to 3 molecules per Fab) to cysteines of Fab-fragment far away from the epitope binding region. Using ELISA, flow cytometry assays and confocal microscopy, we have shown that PEGylation does not lead to significant changes in the binding of GD2 compared to unmodified Fab-fragments. Thus the use of this method has allowed us to maintain the activity of Fab-fragment GD2-mAb.
We have established a syngeneic mouse model of GD2-positive tumor. Using different criteria for evaluating of tumor progression, we analyzed antitumor effects of full-size GD2-mAb, their Fab-fragments and PEGylated Fab-fragments of GD2-mAb.

P6.06.044
Generating Stronger T cells for Adoptive Immunotherapy

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Adoptive immunotherapy mediates effective regression of advanced metastatic melanoma in patients. To target other cancers, we engineered T cells to express chimeric antigen receptors (CARs) empowering them with anti-tumour activity, specifically to the tumor-associated antigen, erbB2. Our current CAR, utilising CD28-CD3ζ signaling domains, although promising, mediates lower activity compared to endogenous TCR responses against foreign antigens. Therefore, we hypothesized that incorporating multiple alternate signaling domains into CARs could result in significantly enhanced anti-tumor responses. To investigate this, we generated a library of approximately 30,000 CARs that incorporated 14 different signaling molecules randomly ligated together directionally and in-frame flanked by SfiI sites and inserted into an anti-erbB2 CAR retroviral vector. The Jurkat T cell line was then retrovirally transduced to express the library and screened for increased CD69 expression or IL-2 secretion in response to antigen specific stimulation. Genomic DNA of selected clones was then extracted, sequenced and cloned into the pSAMEN vector to transduce human peripheral blood mononuclear cells. A diverse range of CARs containing different signaling chains were identified. A particular CAR, containing the tripartite signaling domains DAP10-CD3ζ-CD27 endowed human T cells with the ability to mediate significantly enhanced killing of erbB2+ tumor cells in vitro compared to the CD28-CD3ζ CAR ($p<0.05$). Strikingly, adoptive transfer of T cells expressing this novel receptor induced significantly increased anti-tumor activity in vivo ($p<0.01$). In conclusion, it is anticipated that the generation of CARs with novel combinations of signaling domains will lead to stronger T cells for the treatment of cancer.

P6.06.045
Chimeric protein of IL-2 linked to light chain of anti-IL-2 mAb S4B6 that structurally mimics IL-2/S4B6 immunocomplexes: design and production

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IL-2/anti-IL-2 mAb immunocomplexes were reported to have significantly higher biological activity than free IL-2 *in vivo*. Depending on the mAb used they show different selectivity of their stimulatory activity. However, possible dissociation of IL-2 from mAb or excess of either substance in prepared immunocomplexes are general disadvantage. In order to overcome such problems, we designed and produced protein chimera consisting of IL-2 linked to light chain of anti-IL-2 mAb S4B6 through flexible oligopeptide spacer $(Gly_4Ser)_3$. The intramolecular interaction of IL-2 and mAb part in our protein chimera is similar to that in immunocomplexes of IL-2 and anti-IL-2 mAb S4B6 (henceforth IL-2/S4B6), but dissociation of IL-2 too far away from mAb is prevented by covalent attachment to S4B6 mAb.
We showed the produced protein chimera has predicted molecular weight slightly higher than to IL-2/S4B6, contains both IL-2 and S4B6 mAb in one molecule and IL-2 interacts with binding site of S4B6 mAb in *cis*.
Thus, we conclude that IL-2-S4B6 protein chimera mimics IL-2/S4B6 immunocomplexes in terms of structure and cytokine-mAb interactions.
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P6.06.046
Enhanced efficacy of a cancer vaccine by blocking TGF-β-dependent induction of regulatory T cells with a retinoic acid receptor-α antagonist

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A major goal of cancer immunotherapy is to direct effector immune responses against tumour cells, leading to tumour eradication and enhancing survival. However, tumours have evolved immunosuppressive mechanisms to overcome effector immune responses. For example, TGF-β has been shown to induce the conversion of naïve $CD4^+$ T cells into regulatory T (Treg) cells and this is enhanced by retinoic acid (RA), a Vitamin A metabolite. In the present study we investigated the capacity of a RA receptor-alpha antagonist (RARi) to block the induction of Treg cells and thus to enhance anti-tumour immunity. We found that RA and TGF-β induced conversion of naïve $CD4^+$ T cells into Treg cells could be blocked by RARi. TGF-β in combination with RA converted committed $CD4^+$ T cells into Treg cells, decreasing the effector T cell population and IFN-γ production. Furthermore, RARi suppressed the secretion of the anti-inflammatory cytokine IL-10 and enhanced IL-12 production from TLR-activated dendritic cells (DCs). Using a B16 melanoma murine tumour model, we found that RARi enhanced the therapeutic efficacy of a TLR-activated antigen-pulsed DC vaccine, significantly attenuating tumour growth and enhancing survival. Furthermore, treatment with RARi reduced the frequency of $Foxp3^+$ and $IL\text{-}10^+$ Treg cells, while enhancing IFN-γ-secreting $CD4^+$ and $CD8^+$ T cells infiltrating the tumours. Our findings demonstrate that blocking RARα is promising approach to enhance the anti-tumour effect of cancer vaccines and immunotherapeutics.

P6.06.047
Lymphocyte imprinting with melanoma antigens acquired by trogocytosis facilitates identification of tumor-reactive T cells

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Trogocytosis is a contact-dependent inter-cellular transfer of membrane fragments and associated molecules from antigen presenting cells to effector lymphocytes. We previously demonstrated that trogocytosis also occurs between tumor target and cognate melanoma antigen-specific cytotoxic T cells (CTL). Here we show that, following trogocytosis, immune effector cells acquire molecular components of the tumor, including surface antigens, which are detectable by specific monoclonal antibodies. We demonstrate that CD8+ and CD4+ T cells from melanoma patients' PBMC and tumor infiltrating lymphocytes (TIL) capture melanoma antigens, enabling identification of trogocytosing lymphocytes by staining with antigen-specific antibodies. This finding circumvents the necessity of tumor pre-labeling, which in the past was mandatory to detect membrane-capturing T cells. Through the detection of melanoma antigens on TIL, we sorted trogocytosing T cells and verified their preferential reactivity and cytotoxicity. Furthermore, tumor-antigen imprinted T cells were detected in fresh TIL cultures, shortly after extraction from the tumor. This feature is of special relevance regarding cell-based immunotherapy, as it may provide an efficient way to identify and isolate tumor-reactive lymphocytes at the initial stages of TIL propagation. Thus, T cell imprinting by tumor antigens may serve as a tool to detect and isolate melanoma antigen-specific T cells for immunotherapy of cancer.

P6.06.048
Inhibition of intercellular communication between prostate cancer cells by anti-STEAP-1 single chain antibody

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Background: Six-transmembrane epithelial antigen of the prostate-1 (STEAP-1) located at the cell-cell junction on the secretory epithelium of prostate cancer, acts as a transporter protein in intercellular communication and has been introduced as appropriate tumor antigen for immunotherapy. scFv with properties such as human origin, high affinity and small size provides a suitable tool for immunotherapy .In this study we selected specific single chain antibodies against STEAP-1 and showed role of this antibody to block gap junction between the prostate cancer cells.
Materials and Methods: Phage rescue was performed on a diverse non immune library of single chain Fv. Phage supernatant was used to enrich the library against immunodominant epitope of STEAP-1 using panning process. PCR and DNA fingerprinting were done to select the specific clones against the epitope. Phage ELISA was performed to show the specificity of the selected clone. Intercellular communication assay was done to show the role of scFv in inhibition of dye transferring between the cancer cells.
Results: One predominant pattern with frequency 35% selected for intercellular communication assay in PC3 and LNCaP cell lines. Results showed the concentration of 1000scFv/cell could inhibit intercellular communication between cells.Anti-STEAP-1 scFv in LNCaP more than PC3 in comparison of controls.
Conclusions: In the current study we used intercellular communication assay to show the role of this scFv in inhibition of dye transferring between the cells. Blockage of this channel that implicated in progress of prostate cancer suggests that scFv can be helpful to prevent from advance prostate cancer.

P6.06.049
Soluble MICA serum levels as predictive biomarkers of anti-CTLA-4 melanoma immunotherapy

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Ipilimumab, which blocks CTLA-4, has demonstrated an improvement in overall survival (OS) in patients with advanced melanoma. Cinical responses responses and disease control rate are reported in 15%-30% of patients, but major adverse events are frequent.
This study aimed to find possible correlations between positive outcome under anti-CTLA-4 and the MHC class-I polypeptide-related sequence A (MICA) molecule, broadly expressed on tumors and whose shedding from tumor cells promotes tumor escape.
41 consecutive patients with an inoperable state III or IV melanoma and receiving 3 mg/kg of Ipilimumab were included in a non-randomized prospective study. Ipilimumab was administered every 3 weeks until week 12. Sera levels of soluble MICA (sMICA) and anti-MICA antibodies (Abs) were determined longitudinally and clinical evaluation was performed at week 16, assessing response to treatment (from complete response to progressive disease). Levels of sMICA and anti-MICA Abs were analyzed according to response to Ipilimumab and overall survival. Positive outcomes or stable disease were reported in 46% of cases. No association between either sMICA or anti-MICA and OS was found. Levels of anti-MICA Abs were similar in all groups of response. Serum concentrations of sMICA were significantly lower in patients responding to Ipilimumab and were particularly high in the group of progressors. This difference was observed at each time point and even before the beginning of treatment (P = .017).
Our data suggest that sMICA may be an early predictive marker in the context of anti-CTLA-4 immunotherapy with a significant inverse correlation between serum levels and clinical response.

P6.06.050
Bispecific single-chain diabodies for T cell retargeting to solid tumors

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Current research in the field of cancer immunotherapy focuses on the development of strategies inducing a powerful, target-dependent immune response at the tumor site. This effect can be achieved with bispecific antibody formats that act as adaptor between tumor cells and cytotoxic T lymphocytes by simultaneously binding to a tumor-associated antigen and the T cell specific receptor CD3. We have developed different bispecific single-chain diabodies targeting the EGF receptor family members EGFR and HER2 or the cancer stem cell marker EpCAM as well as CD3. ScDbEGFRxCD3, scDbHER2xCD3 and scDbEpCAMxCD3 were produced in HEK293 cells and showed highly specific binding to their respective recombinant antigen as well as antigen-expressing human cancer cell lines. Functional activity of all single-chain diabodies was shown by IL-2 release of activated PBMCs upon cross-linking with tumor cells. Remarkably, the use of scDbEpCAMxCD3 resulted in significantly better T cell activation compared to scDbEGFRxCD3 and scDbHER2xCD3. Our results indicate that bispecific single-chain diabodies, especially in combination with EpCAM targeting, are suitable tools for initiation of tumor-directed T cell responses and are therefore further investigated.

P6.06.051

Natural Killer adoptive cell transfer targeting bladder cancer stem cells: preliminary results for fine-tuning strategy

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Bladder cancer (BC) is characterized by an aggressive phenotype with high propensity for recurrence and/or metastasis, probably related with the presence of Cancer Stem Cells (CSC). Natural Killer (NK) cells are lymphocytes able to kill a wide range of cancer cells due to its powerful cytolytic activity, being considered suitable candidates for adoptive immunotherapy.
We aim to explore the role of BC-CSC in the susceptibility to NK cell mediated-based immunotherapy.
Two human BC cell lines (HT-1376 and UM-UC3) were assayed for their susceptibility to NK cells-induced lysis, using the CD107a-based degranulation assay. The presence of CSC was analyzed using the sphere-forming assay. Cells' chemosensitivity cisplatin (CIS) and methotrexate (MTX) was determined using the MTT-colorimetric assay.
A subset of CSCs was identified in the HT-1376 cell line. Surface expression of CD107a in NK cells increased significantly ($p<0.05$) compared to the baseline activity (17.44±2.17%) when exposed to UM-UC3 cells (59.51±8.17%) and the sphere forming HT-1376 cells (43.81±8.65%). MTT results showed that HT-1376 cells are more resistant to CIS and MTX than the UM-UC3 cells. Drugs concentration required to inhibit cell viability in 50% (IC50) for HT-1376 cells was of 7.45±1.20µM (CIS) and 0.18±0.03µM (MTX), significantly higher ($p<0.05$) as compared with the UM-UC3 cell line (CIS:IC50=3.98±0.70µM; MTX:IC50=0.04±0.01µM).
The sphere-forming HT-1376 cells are more chemoresistant than the UM-UC3 cells, probably due to the presence of CSC. Both BC cell lines are susceptible to NK cell-mediated cytotoxicity, independently of the presence of CSC.

P6.06.052

A Dendritic Cell (DC) microRNA signature predicts clinical outcome in Melanoma Patients treated with DC vaccine

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Background: MicroRNAs (miRNAs) are short non-coding RNAs with gene regulatory functions, whose expression is de-regulated in melanoma. It has been reported that patients treated with DC vaccine have a better clinical outcome and a longer overall survival if positive to the delayed-type hypersensitivity (DTH) cutaneous test. The present preliminary study aims to observe whether a different expression of miRNAs occurs in iDC and mDC of metastatic melanoma patients, and whether this expression correlates with the response to treatment, evaluated by the DTH test.
Methods: iDC and mDC were collected from 10 patients. DC maturation was obtained with a standard cocktail of cytokines (PGE2, IL-6, TNFa, IL-1b). Total RNA was extracted and hybridized with the 4.0 version of the non-coding RNA array developed at the Ohio State University, which is able to determine the expression of 476 different human miRNAs.
Results: We identified a signature of 24 de-regulated miRNAs (10 down- and 14 up-regulated) in iDC of DTH-positive patients (better prognosis) versus DTH-negative patients (worse prognosis), and a signature of 31 de-regulated miRNAs (9 down- and 22 up-regulated) in mDC of DTH-positive versus DTH-negative patients. Interestingly, 5 miRNAs (miR-182, miR-150, miR-330-5p, miR-548-3p, miR-556-3p) were common to the two signatures.
Conclusions: Our study indicates that miRNome aberrations at the DC level may account for a different response to treatment, and that miRNA expression in iDC and mDC can suggest which patients will benefit of a DC vaccination.

P6.06.053

Anti folate receptor Ab conjugated iron-oxide nanoparticles for tumor localization in an ovarian cancer models

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The European project Magnifyco aimed at producing multifunctional magnetic nanoparticles (MNP) to perform antitumor therapy with focal hyperthermia and controlled drug release. MNP were functionalized with human Fab recognizing folate receptor α (FRα) that is overexpressed in ovarian carcinoma. MNP-Fab were used for *in vivo* studies in two different mouse models. Mice were either inoculated subcutaneously with FRα+/- cells and MNP given intravenously or, in order to allow ascites formation and better mimic the human disease, injected intra-peritoneum (IP) with OVCAR3 cells followed by IP administration of MNP. Accumulation of MNP was assessed by using analysis of elemental iron. The first *in vivo* model verified the specific localization of MNP-Fab to the FRα-positive tumor, indicating that functionalized MNP may reach and penetrate tumor mass. Likewise, in IP model, the MNP-Fab , but not the control, specifically localized in tumor cells and in regions usually infiltrated by the tumor, such as mesenteric tissue and the genito-urinary tract. The iron levels increased over time in target organs and tumor cells while a lower iron amount was concomitantly detected in the ascitic fluid, suggesting that, from the peritoneal fluid, targeted MNP were selectively uptaken by FRα+ cells and maintained inside the target. Our experiments are a proof of principle of the MNP-Fab ability to target specifically FRα positive cells *in vivo*. Since MNP are superparamagnetic and retain heat-generation capability, we may possibly design and study a system capable of releasing drugs *in situ* by increasing temperature after magnetic field application.

P6.06.054

Combining prime-boost anti-tumour vaccination with debulking surgery for the treatment of solid tumours

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The development of effective anti-cancer immunotherapies is of critical importance. While many anti-cancer immunotherapies have had limited success as monotherapies, their efficacy may be enhanced when combined with conventional therapies such as surgery or chemotherapy. Here we describe the development of a prime-boost (P/B) anti-tumour vaccination protocol that significantly improved survival outcome when combined with partial debulking surgery and targeted suppression of regulatory T cells (Treg).
Using our well established AB1-HA mouse tumour model, tumour bearing mice received prime (influenza A PR/8/34/H1N1; PR8) and boost (HA expressing recombinant modified Vaccinia Ankara; rMVAHA) vaccinations either before (neoadjuvant) or after (adjuvant) 75% debulking surgery. Only neoadjuvant P/B vaccination induced tumourspecific immunity that resulted in significantly delayed tumour growth when combined with debulking surgery; although this was not sufficient to prevent tumour outgrowth. Depletion studies demonstrated that CD8 T cells were essential for the delay in tumour growth. Interestingly, depletion of CD4 T cells and more specifically Treg during neoadjuvant P/B vaccination lead to cures in greater than 60% of treated mice. These surviving mice also resisted tumour rechallenged indicating the establishment of long term anti-tumour immunological memory.
Taken together, these data suggest that vaccine induced anti-tumuor immunity is "restrained" by Treg and can be enhanced by the targeted removal of Treg. Based on these findings we are actively

investigating whether combining novel immunotherapies with conventional treatments in the absence of "immunological restrainers" such as Treg and myeloid derived suppressor cells (MDSC) may generate effective therapy for MM and other solid cancers.

P6.06.055
Anti-PSMA scFv as theranostic tool for prostate cancer

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Prostate carcinoma (PCa) is the most common malignant tumor diagnosed in men and the second leading cause of cancer related mortality. As a result of the limited efficacy of conventional treatments for advanced PCa, other strategies are needed. Antibody-based reagents represent a promising approach to provide the clinic with potentially effective diagnostic and therapeutic tools. To date, the most extensively tumor associate antigen studied for PCa immunotherapy is the Prostate-Specific Membrane Antigen (PSMA). PSMA expression and enzymatic activity are increased in PCa and are also correlated with aggressive disease.
The D2B monoclonal antibody directed against h-PSMA extracellular domain was produced by hybridoma technology. Preliminary diagnostic specificity of the IgGD2B has been investigated in xenograft murine models by imaging. Overall, the low penetration rate from vasculature into solid tumor mass and their prolonged residence in circulation limit the application of whole antibody for imaging or radio immunotherapy.
For these reasons we re-shaped the IgGD2B into scFv format. Due to its smaller size, scFv represents the ideal candidate as diagnostic reagent because shows a faster blood clearance and a better penetrability in the tissue. ScFvD2B functionality was successfully tested on PC3 cell line expressing h-PSMA and BIAcore showed that the antibody fragment retains a good strength of binding despite its monovalent binding.
For the potential use of scFvD2B for radioimaging we have firstly set the radiolabelling procedure with ^{131}I and then we have performed the pre-clinical studies in animals bearing tumors expressing or non expressing PSMA with conventional radio localization experiments.

P6.06.056
Generation of EBV/CMV bi-specific T cells using retroviral T cell receptor (TCR) gene transduction for adoptive immunotherapy

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Immunocompromised patients after allogeneic haematopoietic stem cell transplantation are prone to reactivation of latent CMV infection, resulting in pathogenesis, because they lack CMV-specific T cells. This complication is particularly frequent when CMV-positive patients receive transplants from CMV-negative donors. Transfer of CMV-specific T cells can control viral reactivation, however CMV-specific T cells usually cannot be isolated from a CMV-negative donor. Alternatively, TCR gene transfer can be performed to generate CMV-specific T cells. Previously, polyclonal T cell populations with irrelevant specificities were used for this approach. As the majority of donors are EBV-positive, gene transfer of CMV-specific TCRs into EBV-specific T cells promises to be a reliable improvement. Hypothetically, the adoptive transfer of CMV/EBV bi-specific T cells has several advantages. The risk of stimulating alloreactive TCRs co-expressed with the CMV TCR in the same cell is reduced. Moreover, EBV/CMV bispecificity will limit the probability of TCR mispairing to two possibilities that can be analyzed for alloreactivity. Furthermore, activation of bi-specific cells by either pathogen will sustain these cells and thus maintain immunity against both pathogens. We compared different methods to generate CMV/EBV bi-specific T cells, with a view to minimize generation time and the number of required technical steps. T cells specific for different lytic and latent EBV antigens were enriched according to different protocols and transduced at different time points in order to optimize bi-specific TCR expression. Transduced cells were stimulated and thus selectively expanded using B cells presenting CMV antigens. Functional analyses of the cellular products are ongoing.

P6.06.057
Sub-lethal irradiation of human colorectal carcinoma cells imparts enhanced and sustained expression of important modulators of effector CTL activity

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Sub-lethal doses of radiation can alter the phenotype of target tissue by modulating gene products that may make tumor cells more susceptible to T-cell-mediated immune attack. Previously, we demonstrated that colorectal cancer lines responded to radiation by up-regulating surface expression of CTL relevant proteins including numerous death receptors, cell adhesion molecules and tumor-associated antigens. The present study was designed to determine the extent of CTL relevant changes induced by radiation in human carcinoma cells. Here, several tumor cell lines (SW620, HCT116, Caco-2, Colo205 and WiDr) were examined for their response to various sub-lethal doses of radiation (0-10 Gy). Experiments quantified changes in the expression of genes that could result in enhanced effector CTL activity (OX40L, 41BBL, ICOSL and CD70) against irradiated tumor cells. One to 7 d post-irradiation, changes in expression of effector costimulatory molecules was examined. All cell lines altered expression of one or more of these molecules post-irradiation. Increased expression could be observed as long as 7-days post-irradiation. In some tumor cell lines, altered expression of these gene products correlated with enhanced killing of irradiated tumor cells by both TAA-specific CTLs in an in vitro cytotoxicity assay. This lysis was reversed by gene-knockdown. Furthermore we saw enhanced activation and survival of CTLs exposed to irradiated tumor cells. Overall, the results of this study suggest that non-lethal doses of radiation can be used to make human tumors more amenable to immune system attack even in the absence of innate immune response to 'danger' from dying cells.

P6.06.058
Differential regulation of B-cell proliferation by IL21 in different subsets of chronic lymphocytic leukemia

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Interleukin-21 (IL-21) plays an important role in B-cell proliferation, survival and differentiation. Contrary to its stimulatory effect in normal B cells, it has been shown to induce proapoptotic effect in leukemic B cells from CLL patients. Little is known regarding the biological function of IL-21 in leukemic B cells from progressive and non-progressive CLL patients. In the present study, the proliferative effect of IL-21 in combination with TLR9 agonist (CpG) was investigated in B cells isolated from 24 CLL patients and 8 normal subjects by radioactive thymidine incorporation assay. Patients were broadly classified into either progressive (n=6) or non-progressive (n=18) and immunoglobulin heavy chain variable region (IGHV) gene mutated (n=16) or unmutated (n=8) subtypes. B cells were enriched from peripheral blood mononuclear cells by negative selection using magnetic beads (MACS) and immunophenotyped by flow cytometry. Our results showed that IL-21 enhanced the proliferative effects of CpG in both normal and leukemic B cells, though no significant differences were observed between CLL patients and healthy controls. Comparison between different subsets of patients revealed that while the combination of IL-21 and CpG significantly inhibited the proliferation of B cells from progressive compared to non-progressive

patients (p=0.001), it enhanced proliferation of leukemic B cells from IGHV mutated compared to unmutated patients (p=0.001). Our findings suggest differential effects of IL-21 in different subsets of CLL patients and suggest its potential therapeutic implication in patients with a more progressive disease.

P6.06.059
Lysis-on-chip of single cells after programmed interaction with cytotoxic T-lymphocytes (CTLs) or natural killer (NK) cells on a dielectrophoresis (DEP) based array

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Guiding single cells acting as partners in heterotypic interactions (e.g. effectors and targets of immune lysis), and monitoring the outcome of these interactions may be regarded as crucial bio-medical achievements. Using a dielectrophoresis (DEP)-based Lab-on-a-chip platform (the DEP array) we generated closed DEP cages entrapping cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells as either single cells or clusters. A single virus-presenting or tumor cell can be reversibly immobilized within the chip at a pre-determined position. Cages and their content are moved to pre-determined spatial coordinates by software-guided routing, and a cytotoxic effector is forced to physically interact with a putative target within a secluded area by merging their respective cages. Alternatively, cages can be generated containing effector and target cells at pre-determined effector:target ratios. Cytotoxicity is accurately assessed by real-time quantitation of the release kinetics of the fluorescent dye calcein from single target cells. More than 50 lytic events may be simultaneously tested, and assay endpoint is reached within 16 minutes of initial effector:target cell contact. These observations provide the proof of principle that antibody-based phenotyping and on-chip lysis can be simultaneously assessed, that lytic and non-lytic effector:target combinations can be discriminated on the single-cell level, and that non-lytic effector phenotypes can be resolved from target refractoriness to immune lysis. Thus, highly cytotoxic, virus-specific CTL clones, as well as tumor cells resistant to NK cell lysis can now be directly identified. These features are of primary interest in targeted immunotherapy. Supported by EU FP6 COCHISE and AIRC.

P6.06.060
Anticancerogenic and immunotropic properties of GA-40 preparation

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The nontoxic natural substance with antitumor and immunotropic properties GA-40 preparation has been derived from Georgian endemic medical plant. GA-40 is a standardized polypeptide complex. Preclinical and clinical tests have shown that GA-40 possesses anticarcinogenic and immunotropic properties. It shows direct necrotic action on malignant tumor cells by stimulating apoptosis. GA-40 has no negative effect in the normal cells. GA-40 has direct action on human myeloleukemic cells; it stimulates transformation of myeloleukemic cells into a normal cell. GA-40 stimulates expression of CD11b antigens on the surface of a leukemia cell (HL-60 strain). GA-40 causes activation of immune status of the organism and restores the quantitative distribution of T and B lymphocytes, T-helper, T-cytotoxic, T-killers cells, macrophages and granulocytes. GA-40 causes activation of mononuclear cells and stimulates production of cytokines, Tumor Necrosis Factor-α and Interferon-γ). GA-40 provides normalization of the blood biochemical indicators. GA-40 has positive influence on the dynamic of carcinoembryonic antigens, alphafetoprotein and prostate-specific antigen; it inhibits the development of tumor cells growth and stimulates regression of the metastases.

P6.06.061
Chronic exhaustion of tumor-specific CD4+ T cells leads to recurrence of melanoma.

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Although many strides have been made in treating primary tumor occurrence, treatment of cancer recurrence has remained elusive. We developed a B16 melanoma mouse model in which ~50% of mice receiving adoptive cellular therapy (i.e. cytolytic CD4+ T cells recognizing the tumor/self antigen, TRP-1) demonstrate tumor relapse following initial tumor regressions. Upon analysis of TRP-1-specific CD4+ T cells from mice with relapsing tumors, levels of the exhaustion markers, PD-1, LAG-3, Tim3 and TIGIT were markedly increased, compared to CD4+ T cells from naïve and cured mice. The numbers of TRP-1-specific Foxp3+ CD4+ regulatory T (Treg) cells (comprising 5-15% of the original CD4+ T cell pool at the time of transfer) were also elevated in tumor relapse mice. By taking advantage of the diphtheria toxin receptor (DTR) expressed on these Foxp3+ Treg cells, it was evident that Treg cell ablation, with DT injection, was not sufficient in treating tumor recurrence. In addition, targeting exhaustion pathways, i.e. with anti-PD-L1 (the ligand for PD-1) or anti-LAG3 antibodies, provided no therapeutic benefit against the recurring tumor. However, when single modality therapies were combined, i.e. Treg depletion/anti-PD-L1 or anti-LAG-3/anti-PD-L1, relapsing tumors regressed. The regression of advanced-stage recurrent tumors through dual therapy resulted from decreases in PD-1, LAG-3, Tim3 and TIGIT, and increases in IL-7Rα expression and dual production of IFNγ/TNFα, in TRP-1-specific CD4+ T cells compared to cells from monotherapy- and non-treated mice. Taken together, these data clearly illustrate the reinvigoration of exhausted tumor-antigen-specific CD4+ T cells in the setting of recurring B16 melanoma treatment.

P6.06.062
Effect of polychemotherapy on leukocytes under acute lymphoblast leukosis in children

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The aim of investigation was the search for toxicity criteria of cytotoxic preparations while using the analysis of blood values' dynamics following the chemotherapy with cytostatics' inclusion.
The analysis of hematological parameters was carried out in 24 patients at different terms of treatment during the chemotherapy according to AII-MB-2005 protocol.
On completion the first course of treatment the sharp decrease in leukocyte number as observed in a day that corresponds to 2-grade hematotoxicity according to WHO's classification.
Thereafter, the parameter elevation was detected that retained to the end of first therapy cycle. During the treatment it was found that the increment in neutrophil content was realized at the expense of the increase in the number of their mature forms. The initial level of peripheral blood blast cells comprised 25% prior to therapy, 7% (3rd day), and 1% (8th day), then all of patients demonstrated 0% that maintained further. Meanwhile the efficient bone marrow hemopoiesis suppression occurred as well. Given that 4% is a standard value and initial background is 89% in average, the amount of bone marrow blast forms was retained at 1,7% level for the 36 days of therapy. All the patients examined after the first course of polychemotherapy (induction of remission) demonstrated the state of hematological remission.
Thus, the suppression of bone marrow hemopoiesis under cytostatic therapy according to AII-MB-2005 protocol is not excessive and is maintained at the level that allows achieving remission.
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P6.06.063
Identification and validation of hypoxia-specific tumor associated antigens in prostate cancer

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Prostate cancer (CaP) is characterized by unique prostate–associated antigens; hence, it has been considered a prime candidate for immunotherapy. Despite numerous laboratory advances, clinical outcomes have been partial and transient. The overall goal of this study is to optimize the effectiveness of therapeutic whole–cell CaP vaccines by taking into consideration tumor–associated hypoxia as a relevant determinant of tumor antigenicity. We propagated CaP cells at pO_2 = 2 kPa and 20 kPa. To identify potential tumor-associated antigens (TAAs), we prepared CaP cell lysates, resolved them by 2D electrophoresis and immunoblotting using spontaneous antibodies from plasma derived from CaP patients and control subjects. Antibody-labeled spots were analyzed by MALDI-TOF mass spectrometry and validated by ELISA. Three potential TAAs; two hypoxia-specific (HSP70 and hnRNP L) and one hypoxia-nonspecific (HSP60) were selected for validation. The frequency of HSP60 autoantibodies was elevated in the CaP patient plasma; however the frequency of HSP70 autoantibodies was not elevated in CaP patients relative to healthy controls; even more, higher autoantibodies frequencies were found in other tumors and in rheumatoid arthritis. Protein expression for hnRNP L (detected by Western Blot technique) was elevated in CaP tissue. Our results suggest the value of selected candidates (i.e. HSP60 and hnRNP L) as potential TAAs and immunotherapeutic targets for CaP. Additionally, their overexpression in tumors suggests their role in tumor growth and progression. Hypoxia may be used as a tool in the development of cellular vaccines and to identify novel diagnostic and prognostic tools for CaP.

P6.06.064
A melanoma cell lysate induces CCR7 expression on human dendritic cells and its in vivo migration to draining lymph nodes

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Recently, we developed an original method for production of therapeutic dendritic-like cells named Tumor Antigen Presenting Cells (TAPCells) using an allogeneic melanoma-derived cell lysate (TRIMEL) as activation factor and antigen provider. TAPCells-based immunotherapy induced T cell-mediated immune responses and improved long-term survival of stage IV patients in studies involving more than one hundred individuals. Dendritic cell (DC) migration from the injected site to the lymph nodes is an important requirement for an effective antitumor immunization. Herein, we investigated if the TRIMEL was capable to induce the expression of the C-C chemokine receptor type 7 (CCR7) on human therapeutic DCs and enhance their migration to the lymph nodes in an animal model. Our results confirmed that TRIMEL induced the maturation and increased expression of surface CCR7 on the monocytic/macrophagic line THP-1 and also on TAPCells derived from melanoma patients. Moreover, in vitro assays showed that TRIMEL stimulated THP-1 cells and patients derived TAPCells were capable to specifically migrate in the presence of CCL19. Finally, we demonstrated by flow cytometry and immunohistochemical methods that TAPCells could migrate in vivo from the injection site into draining lymph nodes in an ectopic xenograft mouse model. This work contributes to understand the biology of ex vivo produced DCs allowing the study of new strategies for a more effective DC-based vaccine design.

P6.06.065
Low-dose conventional chemotherapeutics synergizes with dendritic cell - based immunotherapy in antitumor activity

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Background. The benefit of combining low-dose chemotherapeutics with cancer vaccine therapy has not been established. Here we investigated the antitumor effect of conventional chemotherapeutics cyclophosphamide (CP) or doxorubicin (DOX), or cisplatin (CIS) in combination with DC-based immunotherapy in murine tumor models. Methods. Studies have been performed with 100 C57Bl/6 and 213 CBA mice with lethal dose of Lewis lung carcinoma (LLC) or sarcoma 37 (S37) respectively. DCs loaded with mechanoactivated lyophilized tumor cells have been used as immunotherapy agent. DCs were administered i/v three times with two-day interval. Low-dose chemotherapeutics were administered in “metronomic” regimen before DC-immunotherapy. Results. Addition of CP improved DC-immunotherapy leading to a significant decrease in the number and volume of metastases in lungs. Combination of DC-immunotherapy with DOX or CIS significantly improved median survival time of animals with S37 in comparison with control group. Metronomic chemotherapy resulted in a significant reduction in the proportions of T-reg cells and mRNA levels of immunosuppressive cytokines in the peripheral blood and lymph nodes lymphocytes. Addition of chemotherapeutics to DC-immunotherapy also augments Th-1 immune response, increases number of activated CD69+, CD54+, CD25+ splenocytes, enhance spleen macrophages phagocyte activity and reactive oxygen species (ROS) production, cytotoxic activity of NK-cells and CTL ($p<0,05$). Antitumor effectiveness of DC-immunotherapy combined with low-dose chemotherapeutics are associated with significant decrease in mRNA levels of TGF-β, VEGF, IL-10 in the residual tumor sites. Conclusions. Low-dose chemotherapeutics enhance the antitumor effect of DC-based immunotherapy. These findings form the basis to a new multimodality treatment in cancer patients.

P6.06.066
Targeting effector CD8 T cells to the tumor using high dose radiation therapy in mice

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Adoptive transfer of T cells and tumor-targeted vaccine therapies are effective means to introduce tumor antigen-specific cells into tumor-bearing hosts. However, tumor-specific T cells have a limited ability to traffic to and function in tumors due to the suppressive tumor immune environment. We hypothesize that radiation therapy provides both a means to prime tumor-specific T cell responses through release of tumor antigens and adjuvants, and a means to target these T cells to the tumor environment. To test this hypothesis, we treat transplantable and spontaneous tumors in immunocompetent mice with targeted radiation, using both clinical linear accelerators and an advanced treatment platform to deliver conformal radiation to tumors in mice. We demonstrate that high dose radiation therapy can be targeted to tumors in mice and can change the immune and inflammatory environment of tumors. Radiation therapy results in production of inflammatory chemokines and recruitment of activated T cells to the treatment site. We demonstrate that the efficacy of radiation depends in part on these T cells, and that immunotherapy to increase T cell activity improves tumor control by radiation therapy. These studies demonstrate that radiation therapy synergizes with immunotherapy, acting to both prime and target effector cells to control residual cancer cells.

P6.06.067
Vaccination with autologous glioma lysate induces tumor-reactive IgG antibody response and increases survival in dogs with glioma

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Gliomas are invasive primary brain tumors for which there is currently no cure or very effective therapy. In spite of widespread and intensive ongoing investigations for improved glioma treatments, there is disappointingly little progress in translational medicine. Pet dogs can serve as an outstanding comparative animal model for study of novel therapies because they develop spontaneous brain tumors that faithfully recapitulate human disease.
We have undertaken a clinical trial using immunotherapy with autologous glioma cell lysate vaccines, combined with the TLR9 agonist (CpG oligodeoxynucleotides) to treat canine patients with glioma. The objective of this trial is to induce specific anti-tumor immunity directed at residual tumor and to reduce recurrent tumor after resection. Treatment groups consist of surgery and vaccination with or without gene therapy (intracranial injection of ad-INF-γ vector). Dogs treated with palliative therapy alone (historical controls) or with surgery and chemotherapy (temozolomide) served as control groups. No severe toxicities have been observed, validating the safety of the treatment. Our preliminary results demonstrate the ability of this vaccine to activate specific humoral antitumor responses with concurrent extension of survival times in the treated dogs compared to controls. Tumor-reactive IgG antibody responses were detected post vaccination in all dogs.
Examination of immune responses in therapeutic trials of dogs with glioma can greatly aid in predicting outcomes of similar treatments in humans. Data from these trials also provide an opportunity to accelerate the development of more efficient immunotherapeutic strategies for treatment of intracranial malignances.

P6.06.068
"First AID" for T cell adoptive transfer: TCR affinity maturation by somatic hypermutation.

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Adoptive cell transfer of tumor specific T lymphocytes, was shown to mediate objective cancer regression of metastatic melanoma. Effective T cell activation depends, among other factors, on the functional avidity of the peptide-MHC complex to the T cell receptor (TCR), i.e. on the affinity and the number of pMHC-TCR contacts. Since the T cell repertoire is controlled by negative and positive selection in the thymus, naturally occurring TCRs have mostly low affinities. Moreover, unlike antibodies whose affinities improve over time by somatic hypermutation (SHM), TCR do not undergo SHM. In our research we developed a system that can increase the affinity of a TCR to its ligand by subjecting TCR genes to SHM, directed by the mutator enzyme Activation Induced cytidine Deaminase (AID). Affinity maturation reactions are performed in easy transfectable cells and affinity maturated TCRs are selected by tetramer staining followed by FACS sorting. The affinity maturation system is designed to be modular so the maturation can be done in several cycles in order to optimize TCR affinity. The affinity maturated TCRs are used to create anti-tumor reactive T cells by means of gene transfer into naïve lymphocytes. These transformed T cells are then functionally tested in-vitro and in-vivo with murine melanoma models. Using this system we are trying to define the parameters that govern the changes in affinity as well as the biological consequences of these changes. Such systems can potentially be used to augment T cell responses to Tumor Associated Antigen peptides, for the treatment of cancer.

P6.06.069
HPV-derived T-helper epitopes for therapeutic HPV vaccine design

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Persistent infection with high-risk types of human papillomavirus (HPV) can lead to malignant transformation and the development of various human malignancies, such as cervical cancer. Fortunately, most HPV infections are successfully eradicated by the immune system. Accumulating data show that both cytotoxic T cell and T helper cell responses play a pivotal role in the clearance of HPV infection. To date, therapeutic HPV vaccines have mostly been developed to elicit tumor-specific CTL responses. The exclusive targeting of HLA class I-restricted HPV epitopes might be insufficient, as HLA class I surface expression can be reduced as a result of HPV immune evasion strategies. In contrast, HLA class II molecules are expressed in high-grade cervical lesions and cervical cancer. Therefore in this study, with the help of immune-bioinformatic approaches, we aim to identify novel E2-, E5-, E6- and E7-derived CD4+ T cell epitopes that bind to multiple HLA-DR alleles, are naturally processed and presented, and immunogenic. Promiscuous 15-mer candidate epitopes from HPV16 E2, E5, E6 and E7 proteins were analysed in silico for their affinity to bind seven different HLA-DR molecules. Candidate peptides were evaluated in vitro for their immunogenicity in a panel of immunoassays. T cells isolated from the majority of healthy donors responded to at least one of these HPV-derived peptides. We conclude that the identified CD4+ T-helper epitopes most likely represent a memory response that correlated with viral clearance in healthy donors. They could complement the development of epitope-specific HPV immunotherapy approaches.

P6.06.070
CpG-activated pDCs contribute as APCs in the generation of Th17 cells that control tumor growth

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Plasmacytoid dendritic cells (pDCs) rapidly and massively produce type I interferon and other inflammatory cytokines in response to foreign nucleic acids. Consequently, pDCs indirectly influence adaptive T cell responses by modulating the immune functions of other professional antigen (Ag) presenting cells (APCs). In addition, pDCs can present Ag to naïve T cells and directly regulate T cell differentiation. Depending on the immune environment, pDCs exhibit either tolerogenic or immunogenic properties. Here we show that CpG-activated pDCs promote efficient Th17 differentiation in vivo. Th17 responses are impaired in mice selectively lacking MHCII on pDCs upon antigenic challenge. Furthermore, TLR-triggered production of inflammatory cytokines is not altered in MHCII deficient pDCs, demonstrating that MHCII sufficient pDCs function as APCs and directly modulate T cell outcome. Importantly, upon vaccination with tumor Ag in the presence of CpG, the absence of MHCII expression by pDCs leads to impaired number of tumor infiltrating TH17 cells, and consequently, increased tumor growth. Our results highlight a new role for tumor Ag presentation by CpG-activated pDCs to CD4+ T cells in inducing potent anti-tumor Th17 cells, and suggest that pDCs could be used not only as inflammatory cytokines producers, but also as efficient APCs, to improve tumor vaccine efficacy.
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P6.06.071
Role of B cells in anti-tumour immunity

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The role of B cells in anti-tumour immunity remains controversial, with positive and negative effects reported in animal models. In humans, B cell infiltration of tumour samples correlates with a better prognosis in some cancers.
We have developed a transgenic animal model to study the interaction of naïve tumour-specific B cells and CD4 T cells during an anti-tumour response. The model utilises B16.F10 melanoma cells engineered to express the model antigen Hen Egg Lysozyme-Moth Cytochrome C (B16.HELMCC), together with MCC-specific TCR transgenic CD4 T cells and HEL-specific BCR transgenic B cells. Growth of subcutaneous B16.HELMCC tumours in RAG-/- mice was controlled by adoptive transfer of T and B cells, T cells alone but not B cells alone. Anti-HEL serum IgM, IgG1, IgG2a, IgG2b and IgE were detected in the T+B group, IgM only in the B group and no antibodies in the T group. Anti-HEL IgG1 delivered intraperitoneally protected both RAG-/- and WT mice against tumour growth. Thus in our system, CD4 T cell help allows for the production of protective antibodies with anti-tumour reactivity.
We next assessed the role of naive tumour-specific B cells in T cell priming in RAG-/- hosts in which only transferred B cells expressed cognate MHCII. B cells induced only weak T cell proliferation, whereas strong proliferation was seen when RAG-/- host DCs could present antigen. Thus although B cells with anti-tumour reactivity cannot prime naive CD4 T cells, CD4-B cell interactions during an ongoing anti-tumour response may result in the production of protective antibodies.

P6.06.072
Comparison of monocyte derived dendritic cells versus leukemic blasts derived dendritic cells for immunotherapy of AML

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Background: Despite consolidation chemotherapy, relapse occurs in majority of patients with acute myeloid leukemia (AML) due to the presence of minimal residual disease. Dendritic cell (DC) based immunotherapy can help to maintain patients in remission. DCs can be generated from peripheral leukemic blasts (AML-DC) as well as peripheral blood monocytes(mo-DC). The aim of this study was to compare mo-DCs with AML-DCs in terms of maturation and functional properties.
Methods: Isolated blasts from 15 AML patients and monocytes from 20 healthy donors were differentiated into DCs in the presence of rhGM-CSF and rhIL-4. Different TLR agonists were used for DC maturation. Generated DCs were compared for efficiency of production, percent of recovery, maturation and functional properties.
Results: Results demonstrated that AML-DCs could be generated from 73% of patients versus 100% of that mo-DCs. Median recovery of mo-DCs was higher compared to AML-DCs. Also Mo-DCs showed higher expressions of surface molecules (HLA-DR, CD40, CD11c, CD83, CCR7 or CD86). However AML-DCs showed lower but reasonable capacity to stimulate allogeneic T cells in vitro as well as higher production of IL-12p70.
Conclusion: Although production of AML-DC is not feasible in all patients, once generated can be used as a proper alternative in immunotherapy of patients in which production of mo-DCs is not possible.

P6.06.073
IMCgp100: a novel bi-specific biologic for the treatment of malignant melanoma

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Despite significant advances in the treatment of metastatic melanoma, long-term remission for the majority of patients remains elusive. Kinase inhibitors provide potent but short-term responses for a significant proportion of patients and immunotherapy elicits long-term responses with the prospect of cure, but only in a minority.
IMCgp100 is a novel bi-specific immunotherapy comprising a soluble, affinity enhanced, T cell receptor (TCR) specific for the melanoma-associated antigen gp100, fused to an anti-CD3 specific antibody fragment (scFv). The engineered TCR portion of the drug targets and binds the gp100 peptide 280-288 antigen, which is over-expressed and presented by HLA-A2 on the surface of melanoma cells. The anti-CD3 scFv portion captures and redirects T cells to kill the melanoma cells; normal antigen negative tissues are spared destruction. *In vitro*, IMCgp100 potently redirects T cells from late stage cancer patients to target melanoma tumours exhibiting HLA-down regulation even in the presence of high numbers of regulatory T cells. Target cell killing is observed within hours, and, is associated with the release of pro-inflammatory cytokines and dendritic cell cross-presentation of gp100 and other melanoma-associated antigens. Thus, IMCgp100 demonstrates the potential to elicit potent short term responses and trigger longer-term anti melanoma activity *in vivo*.
IMCgp100 is undergoing Phase I clinical testing in advanced melanoma patients; 22 patients have received single IV infusions so far and 7 patients have subsequently received weekly infusions for 6 weeks. The drug is well tolerated with evidence of T cell mobilisation, specific cytokine release and tumour shrinkage after dosing.

P6.06.074
RNActive - A novel mRNA-based vaccination technology induces strong T- and B-cell responses in phase I/IIa trials in non-small-cell lung cancer and prostate carcinoma patients

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RNActive® technology is a novel vaccination approach using self-adjuvanted mRNA molecules. Pre-clinical experiments showed that intradermal-injection of RNActive® vaccines induces boostable and balanced effector and memory immune responses and provide a strong anti-tumor effect. We already tested CV9201 and CV9103, therapeutic RNActive cancer vaccines for non-small cell lung cancer (NSCLC) and castrate-resistant prostate cancer (PCa) in clinical phase I/IIa trials.
CV9201 is composed of mRNAs coding for NY-ESO-1, MAGE-C1, MAGE-C2, Survivin, 5T4; CV9103 of mRNAs coding for PSA, PSCA, PSMA, STEAP-1. For phase I/IIa trials, blood samples were taken before and after vaccination. Humoral and cellular responses were assessed *ex vivo* by IgG and IgM ELISA, ELISPOT , ICS (IFN-gamma, TNF-alpha,IL-2), tetramer analysis. Phenotyping was performed to identify various lymphocyte subsets.
Immune monitoring of CV9103 demonstrated antigen-specific T- and/or B-cell responses against at least one antigen in 79% of the patients. Importantly, 58% of responders reacted against more than one antigen. In the CV9201 trial, antigen-specific immune responses were detected in 65% of patients, 65% thereof were multiple responders. A significant 2-13 fold shift from naïve B cells to pre-germinal center B cells was detected in 61% of patients. Overall 84% of the treated NSCLC patients had a detectable antigen-specific immune response and/or an increase in germinal center B cells. The

presence of antigen-specific memory B cells was demonstrated with an antigen-specific B cell proliferation assay.
These results suggest that intradermal immunization with self-adjuvanted mRNA vaccines constitutes a highly immunogenic, novel vaccination approach able to induce antigen-specific B-and T-cell responses.

P6.06.075
Limitation of systemic exposure to transgenic interleukin-12 upon local treatment with armed oncolytic adenoviruses

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Oncolytic viruses armed with immunostimulatory transgenes are attractive therapeutic agents against cancer. We have developed oncolytic adenoviruses (OAVs) adapted as vectors for interleukin-12 (IL-12). However, high expression of IL-12 mediated by the replicative vector causes severe toxicity in permissive hosts such as Syrian hamsters. With the aim of increasing the therapeutic index of these agents, we have performed modifications in the expression cassette to reduce systemic exposure to the cytokine. Target sequences for microRNA 122 were introduced in the 3'UTR of IL-12 to prevent expression in hepatocytes. In addition, membrane anchoring of IL-12 was attempted by incorporation of the glycosylphosphatidylinositol (GPI)-anchor signal from the folate receptor or a transmembrane (TM) domain from the CD4 protein.
These modifications achieved membrane display of IL-12 in the surface of pancreatic cancer cells and specific inhibition of transgene expression in hepatocytes in vitro. However, only the TM-dependent anchorage was able to reduce the release of IL-12 in the supernatant of cells.
OAVs carrying these constructs were administered locally in different pancreatic cancer models. In hamsters bearing intra-hepatic tumors, the virus carrying the IL12-TM-miR122 cassette were well tolerated and caused lower seric concentrations of IL-12, compared with non-modified IL-12. However, these differences were less pronounced when the viruses were inoculated in orthotopic (intra-pancreatic) tumors. These results suggest that the main mechanism for reducing IL-12 in serum is the miR122-dependent inhibition of transgene expression in hepatocytes.
Therefore, membrane anchoring of IL-12 may be limited by local factors in the tumor microenvironment.

P6.06.076
Isolation of T cell receptors specific for validated cancer epitopes for cancer immunotherapy.

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The human immune system can theoretically identify malignant cells by inspecting cell surface Class I HLA -peptide complexes for the presence of disease-associated epitopes. Indeed, many cancer patients generate CD8 cytotoxic T cell responses to tumour-associated antigens; the majority of patients, however, fail to clear tumours since T cell avidity for self-antigens tends to be weak, and cancer cells employ escape mechanisms for avoiding destruction by T cells. To overcome these issues, we have engineered novel, bi-functional protein therapeutics termed ImmTACs (Immune Mobilising mTCR Against Cancer) which re-direct the immune system to target and destroy tumour cells with a high degree of potency and specificity. An ImmTAC comprises a high affinity 'monoclonal' T cell Receptor (mTCR) targeting a cancer-associated HLA-peptide complex, fused to an anti-CD3 scFv domain which activates an anti-tumour T cell response.
We have developed an integrated in-house process leading to the isolation of TCRs specific for validated cancer epitopes forming the starting material for ImmTAC production. The critical steps in this process are: antigen selection, epitope identification, T cell cloning, TCR isolation and binding to soluble peptide:MHC on the BIAcore. We describe each of these areas in more detail and show data to illustrate the successful isolation of a number of TCRs specific for various tumour associated antigens such as prostate and cancer testis antigens leading from this procedure.

P6.06.077
Dichloromethane fractions of Scrophularia oxysepala induce apoptosis in MCF-7 human breast cancer cells

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Background: Breast cancer is the most common malignancy in women, especially in developing countries. It has been observed that some of the plants contain a rich source of anti-cancer drugs ingredients. This paper tends to evaluate the effect of cytotoxic activity of Scrophularia oxysepala fractions, which is located at induction of apoptosis in MCF-7 breast cancer cell line on the breast cancer. Hence, a herbal based treatment for breast cancer is mostly stipulated in this work.
Method: Dichloromethane fractions were examined based on MTT (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Trypan-blue assays were performed in MCF-7 breast cancer cell line to analyze the cytotoxic activity of the extract of Scrophularia oxysepala. In addition, the apoptosis inducing action of the extract was determined by TUNEL (terminal deoxy transferase (TdT)-mediated dUTP nick- end labeling) test and DNA fragmentation.
Results: The results revealed that the dichloromethane fractions effectively inhibited cell growth and viability in dose (30, 50, 100 , 200, 300 µg/µl) and time (12, 24 and 36 hours) dependent manner, regardless of the inducing damage to non-cancerous cell lines. Furthermore, cell death assay and DNA fragmentation analysis using TUNEL indicated induction of apoptosis by dichloromethane fractions of Scrophularia oxysepala in MCF-7 cell.
Conclusions: The results obtained illustrate for the first time that Scrophularia oxysepala fractions may cause apoptosis in breast cancer. The findings indicate that Scrophularia oxysepala fractions contain potential anti-cancer components inhibiting breast cancer cell proliferation through DNA damage.

P6.06.078
Targeted multivalent scTRAIL fusion proteins for tumor therapy

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In contrast to other TNF superfamily members, TNF-related apoptosis inducing ligand (TRAIL) shows selective cytotoxicity on tumor cells sparing normal cells and has therefore been extensively investigated for the treatment of cancer. Clinical trials, however, revealed only limited therapeutic efficacy of recombinant soluble TRAIL. Thus, there is need for new formats of TRAIL-based therapeutics with increased activity. Functionality can be improved by targeted delivery to the tumor via fusion to a recombinant antibody. Simultaneously, these fusion proteins mimic, with respect to its activity, the membrane-bound form of TRAIL and thus efficiently activate both death receptors 4 and 5. Especially, formats displaying single-chain versions of TRAIL in a dimeric assembly show increased cytotoxicity. We created two new targeted formats of scTRAIL that force dimerization by either using a bivalent diabody (Db) or via fusion of a single-chain variable fragment (scFv) to a homodimerization domain derived from IgE (IgE heavy chain domain 2, EHD2). We could show that these targeted and dimeric versions of scTRAIL exert improved selectivity towards antigen-expressing tumor cells and enhanced apoptosis induction in vitro and in vivo. These results indicate that Db-scTRAIL and scFv-EHD2-scTRAIL fusion proteins are promising candidates for next generation TRAIL therapeutics showing increased efficacy.

P6.06.079
Long-term CTL induction and therapeutic tumor suppression by antigen- and adjuvant-carrying polymer nanoparticles

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Encapsulation of a model antigen and a TLR agonist (adjuvant) within a polymer-based nanoparticle (NP) leads to strong and rapid induction of adaptive immune responses. Efficient CTL response is induced after a single inoculation, with an NP-encapsulated antigen being a peptide, several peptides or a protein. Prime immunization followed by a tumor injection and repeated therapeutic immunizations at a tumor-distant site resulted in 100% survival in a mouse model. At the same time, a strictly therapeutic regimen was only weakly protective when NPs were administered at a tumor-distant site with peritumoral delivery being more efficient. An improved manner of antigen formulation within the NP led to augmented survival upon tumor-distant subcutaneous therapeutic immunization with 25-70% animals staying tumor-free for >6 weeks after inoculation with 0.25x106 EG.7-OVA cells. This enhanced survival corresponded to a preferred NP uptake by several classes of APC and to prolonged kinetics of CTL induction with a strong systemic in vivo CTL activity (75-90% specific cytotoxicity) seen up to 21 days after a single NP administration and being still detectable at 31 days. This superior long-term CTL induction was demonstrated by several approaches to persist both locally (draining lymph nodes) and centrally (spleen) and to parallel the induction of cells with T cell effector memory phenotype at the same locations. Similar approach using NPs carrying dominant immunogenic peptides and TLR agonist has shown a protective potential against intravenously delivered B16-F10 melanoma cells.

P6.06.080
Antitumor activity of Her2 liposomes

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The purpose of this study is to determine the potential benefit of liposomes as a delivery vehicle for HER2/neu-derived peptide and it' s adjuvant activity to induce CD_8^+ T cell response. P5 peptide encapsulated in nanoliposome consisting DSPC/DSPG/Chol were prepared as a cancer vaccine. Mice were immunized subcutaneously with peptide encapsulated in nanoliposome. 4 mice per group were sacrificed for immunological assays and 6 mice per group were transplanted subcutaneously with TUBO cell line. ELISPOT and flow cytometric analysis were used for the detection of intracellular cytokines (IFN-γ and IL-4) both in CD_4^+ and CD_8^+ T cells as a means to determine the antigen-specific response. An *in vitro* CTL activity assay was also performed to determine the effectiveness of peptide-liposomal vaccine in stimulating CD_8^+ T cells. ELISPOT results showed that immunization with P5 encapsulated in nanoliposome enhanced the antigen-specific IFN-γ response more than mice immunized with P5 alone. Also using CD_8 as a second marker and intracellular flow cytometric analysis, it was found that the IFN-γ response was contributed by CD8 T cells, confirming the induction of cytotoxic T lymphocytes by this vaccination method. Also results from *in vitro* CTL assay shown that encapsulated form of peptide caused more CTL stimulation compares to other groups. The group immunized with encapsulated P5 has a lower tumor size and longer survival time. Our results demonstrated that liposomal construct with Tc peptide antigens can induce efficient antigen-specific antitumor immunity and represent promising synthetic delivery systems for the design of specific antitumor vaccines.

P6.06.081
Antitumor activity of HER2-MPL Liposomes

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The induction of potent antigen-specific $CD8^+$ T lymphocyte (CTL) response is a major requirement in the development of efficient anti tumor peptide-based vaccines . To this aim in the present study we coupled P5 , the new designed peptide containing CTL multi epitope derived from the rat HER2/neu oncogene to phosphatidylethanolamine-based pH-sensitive nanoliposomes by using DSPE-mPEG-maleimide. Monophosphoryl lipid A (MPL) was incorporated to liposome formulation (DMPC,DMPG,Chol,DOPE) as an adjuvant. The immunization of BALB/c mice with MPL adjuvanted peptide-liposome conjugate resulted in significantly higher IFN-gamma production and CTL responses in comparison with control groups .In this work, intracellular flow cytometric analysis, ELISpot assay and in vitro CTL activity test with using TUBO tumor cells were performed to evaluate antigen -specific CTL responses. CTL responses induced by effective formulation also led to the lowest tumor size and the longest survival time in mice with TUBO tumor model. These results suggest that P5 peptide coupled to adjuvanted pH-sensitive liposome might be promising approach as an anti tumor vaccine.

P6.06.082
Antitumor activity of Her2/CpG liposomes

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The rat HER2/neu peptide (P5) is a synthetic, hydrophobic short peptide which can induce tumor -specific immunity against HER2-over expressing breast tumor in mice. Typical methods used for encapsulating peptides in lipid vesicles result in very low encapsulation efficiencies. In this study a highly efficient method for encapsulating peptide (P5) into liposomes composed of different molar ratios of DOTAP / DOPE /Cholesterol with or without CpG-ODN as an adjuvant was developed and their efficiencies in CD8+ T-cell response induction were evaluated in-vivo. Dried lipid film were hydrated with HEPES-Dextrose-Urea buffer containing P5 and sonicated for 10 minutes at 45° and further down sized by extrusion through 200 and 100nm polycarbonate membrane. In order to remove urea, DMSO and un-entrapped peptide, large unilamellar vesicles (LUVs) were dialyzed against HEPES-Dextrose buffer. All the preparation was characterized for their size, zeta potential and percent P5 encapsulation. Female BALB/c mice were immunized with three times SC injection with two weeks interval. Two weeks after the last booster CD8+ T-Cell responses were evaluated by Flow cytometeric and Enzyme-linked immunospot assay (ELISPOT). The results indicate that P5 encapsulated in Colestrol:DOTAP:DOPE liposomes with CpG enhances the CTL epitope delivery and induces CD8+ mediated immune response.

P6.06.083
Apoptosis induction in head and neck squamous cell line with combined drug therapy

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Introduction: Apoptosis or programmed cell death plays an essential role in the development and homeostasis of multicellular organisms. Because many anticancer drugs kill tumor cells by inducing apoptosis, mutations or deregulation of pro- and anti-apoptotic

proteins can contribute to the acquisition of chemoresistance. Head and neck cancer is a relatively well characterized human tumor, more than 90% of this cancer type has squamous origin and common sites include hypopharynx, larynx, oral cavity, nasopharynx, oropharynx, paranasal sinus, nasal cavity, parathyroid and salivary glands. The cause of head and neck squamous cell carcinoma (HNSCC) is multifactorial and, despite recent advances in treatment, the long-term survival rate has remained at 50% with high rates of associated mortality. Late presentation of lesions, lack of suitable markers for early detection and failure of available chemotherapy response in advanced lesions contribute to a poor outcome of HNSCC. In addition, little is known about the molecular mechanisms underlying this type of cancer. The aim of this study was to investigate the anticancer efficacy of drugs in HNSCC cells treatment.
Methods and Results: HNSCC cells (FaDu, SCC-25 and HaCat) was maintained in DMEM containing 10% fetal bovine serum and were treated with actinomicin-D, ara-C, etoposide and cycloheximide (CHX), for 18, 24 and 48 hours and the apoptotic cells were detected by DNA fragmentation analysis. We observed that tumor cells treated with apoptogenic stimulus in vitro showed decreased in the resistance of death.
Conclusion: HNSCC cells are sensitive when treated with actinomicina-D and CHX.
Supported by: CNPq

P6.06.084
Murine CTL response using rHER2/neu-petide

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One way to improve the immunogenicity of peptide vaccines is employment of multiepitope long peptide (MLP). MLPs are knows as exogenous peptides so taking up, processing and presenting by professional APCs, therefore, able to generate immune responses with longer survival. In this study BALB/c female mice were vaccinated with effective rHER2/neu-specific CTLs epitopes (p5 and p435) that was designed in previous study and conjugated together by double-Arg (RR) sequence as multiepitope long peptide by or without CpG-ODN adjuvant and PBS as control. 14 days after last vaccination 4 mice per group were euthanized and Immune responses were studied in their spleens for CTL cytotoxicity and IFN- by Elispot. Six mice per group challenged by live TUBO cell line and were followed for tumor size and survival. We observed that mice vaccinated with p5/p435 multiepitope long peptide in combination with CpG as adjuvant induced higher antigen-specific T-cell responses compared with group without CpG or groups vaccinated with P5 or P435 alone and PBS control group. Also tumor in the mice that received the long peptide vaccine grew slowly compared to those received the p5 or p435 or PBS and survival rate was significantly improved. Results of this study revealed that vaccination with p5/p435 multiepitope long peptide in combination with CpG is capable of generating potent antigen-specific CTL immune responses and antitumor effects in vaccinated mice.

P6.06.085
Synergistic effect of combined local irradiation and dendritic cell vaccine in murine fibrosarcoma tumor

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Objective: Neither radiotherapy alone nor dendritic cell (DCs) vaccination can successfully eradicate the tumor. Radiation induces tumor cells apoptosis, resulting in tumor antigens and danger signals releasing which are favorable for DC capturing antigens. Hence,combined irradiation and activated DC vaccine may be a novel strategy for treating solid tumors.
Methods: WEHI-164 a Balb/c derived fibrosarcoma cells were injected subcutaneously (SC) to female Balb/c mice. Bone marrow cells were cultured with GM-CSF and IL-4 for 5 days, Followed by adding Listeria monocytogenes antigens and tumor cell lysate for another 2 days. 7 days after tumor challenge, localized single dose (10 Gy) irradiation was applied. Activated DCs were injected SC around the tumor site, the day after irradiation. Tumor size was monitored every other day. Two weeks after immunization cytotoxic activity of splenocytes was measured with a LDH cytotoxicity detection kit. Intercellular INF_γ assessment was performed using a FACScalibur cytometer with WinMDI2.9 software.
Results: Immunotherapy with activated DCs after irradiation has a potent cellular response. The cytotoxicity of splenocytes in combined therapy specially which received complete cocktail was more than other groups (P< 0.016). Evaluation of intra-cellular INF-γ staining showed significant increase in animal which had received combined therapy (P<0.001).
Tumor size monitoring and mice survival showed reduced tumor size and increased survival in group which received both treatment (P=0.011, P <0.001).
Conclusion: Combination therapy using activated Listeria monocytogenes DCs and local irradiation induced noticeable efficient cellular antitumor response against poorly immunogenic tumor as well as tumor regression.

P6.06.086
Molecular diagnosis of human papilloma virus (HPV) infection in Tunisian women with various types of cervical lesions

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The human papilloma viruses (HPVs) are recognized as the main etiologic agents of cervical cancer.
In this work, we assessed the prevalence of high and low risk HPV types in cervical samples of two groups of Tunisian women: a first group of 50 legal sex workers at high risk of HPV infection and a second group of 50 women from the general population presenting with various types of genital lesions. A group of 11 women without genital lesions was taken as control. We used simple and nested conventional PCR in parallel, to detect HPV DNA in cervical samples. We used the MY09/MY11 and GP5 +/ GP6 + primers for PCR.
HPV typing was performed by sequencing of the amplified DNA. Our results show that none of the women in the control group were positive for HPV DNA by both PCR techniques whereas 60% of the sex workers and 30% of the second group were positive for HPV DNA by nested PCR. On the other hand, only 50% of sex workers and 8% of the second group appeared positive for viral DNA by simple PCR. This discrepancy between the simple and nested PCR data may be explained by a relative lack of sensitivity of the simple PCR or a difference in detectability and distribution, in the two populations studied, of the various HPV types. This is in line with the preliminary sequencing data which showed a predominance of high-risk HPV types in the sex workers group.

P6.06.087
Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells

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Genetic modification of T cells with chimeric antigen receptors (CAR's) has emerged as a powerful approach for enhancing cancer immunotherapy. However, the existence of different immunosuppressive mechanisms restricts the full potential of adoptive T cell therapy. Given that engagement of the PD (Programmed Death)-1 receptor with its ligands PD-L1/PD-L2 can dramatically reduce T cell immunity we examined whether administration of a PD-1 blocking antibody could increase the therapeutic activity of CAR T cells. In this study we first demonstrated

a significant increase in the level of PD-1 expressed on transduced anti-Her-2 $CD8^+$ T cells following antigen-specific stimulation with PD-$L1^+$ tumor cells. Furthermore, enhanced proliferation and function of CAR T cells cocultured with Her-2^+ PD-$L1^+$ tumor in combination with anti-PD-1 antibody was observed *in vitro*. In adoptive transfer studies in Her-2 transgenic recipient mice, we demonstrated a significant improvement in growth inhibition of two different Her-2^+ tumors treated with anti-Her-2 T cells in combination with PD-1 blockade. Strikingly a significant decrease in the percentage of $Gr1^+$ $CD11b^+$ MDSC cells was observed in the tumor microenvironment of mice treated with the combination therapy. Importantly, anti-tumor effects were not associated with any autoimmune pathology in normal tissue expressing Her-2 antigen. This study demonstrates that specifically blocking PD-1 immunosuppression can potently enhance CAR T cell therapy which has significant implications for potentially improving therapeutic outcomes of this approach in cancer patients.

P6.06.088
Immunostimulatory properties and antitumor activity of short double-stranded RNA against hepatocarcinoma G29 and melanoma B16

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Short double-stranded RNAs containing specific sequences are able to activate the mammalian innate immune system. Here we identified a set of 19-bp RNA duplexes with 3-nucleotid overhangs in the 3'-ends with original sequence (here and after immunostimulating RNA, or isRNA), which has no significant homology with human mRNAs.
The results in vitro experiments demonstrate that isRNAs under study efficiently inhibit growth of human cancer cells (KB-3-1, SK-N-MC), and induce synthesis of IFN-α and pro-inflammatory cytokines IL-6 and TNF-α in the human PBMCs. The examination of isRNAs sequence/activity relationships revealed that the introduction of substitutions in the middle part of the isRNA sequence does not alter the antiproliferative and immunostimulating activities, while substitutions in the 3'-end region of isRNA substantially reduce the activities.
The results of in vivo experiments show that single intravenous injection of isRNA complexed with transfection reagent efficiently increases the level of IFN-α, and to a lesser degree the level of pro-inflammatory cytokine IL-6 in mice blood serum. We found that isRNA reduces the metastases area in the liver, kidneys and heart of CBA/LacSto mice with hepatocarcinoma G-29 and cause a slight, but reliable inhibition of the tumor growth. The results of experiments with C57BL mice with implanted melanoma B16 show that isRNA efficiently inhibits tumor growth (3.5-5-fold) and metastasis spreading in the lungs (5-fold).
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P6.06.089
EXOLIGOS: a CpG oligonucleotide loaded exosome nanovesicle delivery platform suitable for effective immune modulation

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Exosomes are naturally occurring, membranous nanovesicles of 40-100 nm in diameter. To explore the therapeutic potential of exosomes, we investigated whether cell line-derived exosomes could be used to deliver, protect and enhance activity of CpG ODNs.
Exosomes were isolated supernatants RAW264.7 cell lines and loaded with D-CpG ODNs. FACS and confocal microscopy studies established the binding and uptake kinetics of loaded vesicles into RAW264.7 cells. In vitro, ex-vivo, and in vivo performances of CpG-loaded vesicles, compared to free ODN were analyzed by Th1 cytokine production profiles. ODN encapsulation led to resistance from nuclease digestion (>90% activity retention). Internalization mechanism was found to be dependent on the scavenger receptors.
Immunization experiments against OVA in C57BL/6 mice revealed that anti-OVA IgG production was Th1-biased and this trend was persisting even after 4 months post-booster injection. OVA immunized mice were challenged with OVA expressing EG-7 cells (5 million cells/animal, s.c.) and D-ODN loaded exosomes treated mice demonstrated >85% tumor clearance.
As a result, exosome-mediated vaccine delivery is a promising source of individualised and biocompatible therapeutic vaccine vehicle suitable for immunotherapy of aggressive tumors.

P6.06.090
In vitro treatment of colon cancer cells with non-toxic concentration of 5-fluorouracil enhances the effectiveness of tumor RNA-transfected DC vaccine

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We have recently observed that human DCs treated with low doses of antitumor chemotherapeutic agents became them more efficient to stimulate T lymphocytes, whereas treatment of tumor cells with low concentrations of drugs made them more immunogenic than wild type cells. In this study we aimed to evaluate whether transfection of DC with drug-treated tumor cells RNA, enhances the effectiveness of DC-based vaccine. Human colon cancer cells HCT-116 were *in vitro* treated with non-toxic concentration of 5-fluorouracil (%-FU) and their total RNA was transfected to human monocyte-derived DC obtained from health donors. Phenotyping showed that DC transfected with tumor RNA had an increased HLA-DR expression. Analysis of their ability to stimulate the alloreactivity showed that RNA from drug-treated cells are more effective for sensitizing DC than untransfected control. Transfection of RNA obtained in such conditions was also effective to induce the *in vitro* generation of tumor-specific cytotoxic T cells in a 14 days DC:T autologous co-culture. Analysis of cytotoxic activity showed significant improvement compared with untransfected control DC. Specific antitumor activity of these T cells also resulted in higher levels of IFN-y production but not on IL-10. In conclusion, our results showed that a) 5-FU is able to increased the immunogenicity of tumor cells; b) immunogenicity of tumor cells can be transferred to normal DC by transfection of tumor RNA, and c) RNA of drug-treated tumor cells is more effective for sensitizing DC.

P6.06.091
Clever-1/Stabilin-1 controls cancer progression by regulating migration of tumor-infiltrating leukocytes and tumor cells

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Common Lymphatic Endothelial and Vascular Endothelial Receptor-1 (Clever-1/Stabilin-1) is a scavenger receptor present on type II macrophages and lymphatic endothelium. The aim was to study whether Clever-1/Stabilin-1 is involved in tumor immunity and tumor cell trafficking, and ultimately if Clever-1/Stabilin-1 can be used as a target for anti-cancer therapy.
Blocking the function of Clever-1/Stabilin-1 inhibited the migration of tumor cells via lymphatics to the draining lymph nodes. Clever-1/Stabilin-1 expression was induced in the intratumoral vessels, where it mediated the binding of blood leukocytes. Antibody therapy targeting Clever-1/Stabilin-1 selectively decreased the number of type

2 macrophages and FoxP3 positive lymphocytes within the tumors. This enhanced the activation status of tumor-infiltrating leukocytes and led to apoptosis of tumor cells. As a result, both the primary tumors and metastases remained small. Same results were also observed in Clever-1 knockout mice.
These results indicate that anti-Clever-1/Stabilin-1 treatment targets two different mechanisms controlling cancer behavior: the entrance of immunity-regulating leukocytes via the blood vessels into the tumors, and tumor cell traffic within the lymphatics. In conclusion Clever-1/Stabilin-1 is a potential target to suppress tumor growth and metastatic spread.

P6.06.092
Harnessing the IgA FcαRI receptor for antibody therapy

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Humans express large quantities of monomeric serum IgA in contrast to most other mammalian species where dimeric IgA predominates. Interestingly, in monomeric form these antibodies can engage FcαRI receptors bound on the surfaces of numerous innate immune cells to potentiate both activating and inhibitory function depending on the multimericity of the interaction. Here we investigate the potential to harness FcαRI function in the context of an engineered IgG/IgA chimera which retains FcγR affinity. Using this new antibody we aim to both open new avenues of therapeutic treatment by recruiting neutrophils for the killing of cancer cells and investigate further the role FcαRI plays with FcγRs in innate immunity.
Our engineered antibody variant shows near wildtype binding to FcαRI, FcγRI and FcγRIIa and significantly reduced binding to FcγRIIb by SPR. We used alanine scanning to determine the most critical residues at the IgA:FcαRI interface to guide our design. Antibody performance is to be evaluated in ADCC assays to determine the efficacy of neutrophil activation. The biochemical nature of the mutations that enable binding to the receptors will be discussed and the nature of ADCC elicited by these antibodies in the killing of Her2+ tumor cells will be described.

P6.06.093
A new method for *in vitro* expansion of cytotoxic Natural Killer cells in gastro intestinal cancer

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Background: Gastrointestinal (GI) cancer is one of the most common cancers in worldwide. Cell and gene therapy of cancer has received much attention in past decade. One of the recent applications in immunotherapy of tumors is human natural killer (LAK) cells.
Objective: In this study we consider the effects of PC_1 and G_2 adjuvants on NK cells, inducing them to become LAK cells, and the effect of LAK cell cytotoxicity.
Methods: Patients with GI cancer, including stomach cancer or colorectal cancer, were qualified to this study. PBMCs from each patient (10^5 cell/ml) were seeded in triplicate in 96-well plates. In each well either IL-2, G_2 adjuvant or PC adjuvant were added separately and cytotoxicity was evaluated by LDH assay.
Results: Results of this study indicate that G_2 and PC_1 stimulate NK cell and increase their cytotoxicity.This study indicates that PC and G_2 induce a powerful NK cell stimulation in gastro intestinal cancer.
Conclusion: This finding suggests that G_2 and PC_1 adjuvants may be potentially useful for cancer immunotherapy. Further in vivo studies to assess their potential use in clinical settings need to be carried out prudently.

P6.06.094
KLF6-SV1 is overexpressed in T-cells from patients with chronic lymphocytic leukemia

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Communication between the tumor cells and the surrounding microenvironment are of importance for the survival of Chronic Lymphocytic Leukemia (CLL) cells. T-cells seem to be a key player in the pathogenesis of CLL contributing to the development of a microenvironment in which the leukemic clones evade apoptosis. In this microenvironment the leukemic cells exhibit an increased proliferative activity that sustains the growth of the malignant B-cells. The Krüppel-like transcription factor (KLF) family has been identified as important regulators of proliferation, differentiation, tumorgenesis and cell death.
In the present study we examined the expression of KLF6 and the KLF6-splice variant 1 at the mRNA and protein level in CD4+ (purity≥96%) and CD8+ (purity≥94%) T-cells from 39 patients with CLL and 10 patients with multiple myeloma as well as 10 normal donors. siRNA technology was used to down-regulate KLF6-SV1 mRNA. Sequence specific siRNA treated purified T cells and non-treated leukemic B cells were cultured together or alone for 5 days. Apoptosis of tumor cells was analysed using flow cytometry.
Western blot showed the expression of KLF6 wild-type in purified CD4+ and CD8+ T-cells. Real time PCR revealed a significant overexpression of KLF 6-SV1 in CD8+ T- cells of CLL patients compared to normal donors (p=0.002). siRNA KLF6-SV1 transfection of T cells induced a significant downregulation of KLF6-SV1 and in co-culture experiments a significant increase of apoptosis of B cells was noted. The results may indicate that the KLF6-SV1 might be involved in a dysregulated microenvironment supporting the growth of CLL cells.

P6.06.095
ImMucin, anti-MUC1 therapeutic Vaccine: Interim analysis from a phase I/II study in myeloma patients

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Ideal cancer vaccines should induce robust, diverse and long lasting B and T-cell responses in the majority of the patient population. Signal peptide (SP) domains were found to have exceptionally high number of MHC class I and II epitopes per sequence length which relies on their hydrophobic nature but also bare antigen specificity. Namely, SPs exhibit antigen specificity while maintaining their consensus motif as chaperones.
ImMucin, is a 21-mer peptide, encoding the entire SP domain of MUC1, a tumor-associated antigen expressed by most solid and hematological tumors including multiple myeloma (MM).
A phase I/II study was initiated to assess the safety and efficacy of ImMucin, in 15 MUC1-positive patients with a biochemical relapse MM following ASCT. Patients received 12 bi-weekly vaccination of 100µg ImMucin plus 250µg hGM-CSF. Interim analysis conducted on 7 patients completed the treatment suggests a high safety profile for the vaccine with temporary mostly local mild adverse events which resolved spontaneously. Immunomonitoring analysis by ICS-IFN-gamma, and anti-SP antibodies demonstrated a unique combination of robust ImMucin-specific CD8+ and CD4+ T-cell response with pan HLA in all patients after 2-4 vaccinations, along with increase in anti-ImMucin sera concentration in 5/7 patients. Moreover, a sharp decrease in soluble MUC1's levels was observed in all patients who had abnormal levels at screening. Lastly, stabilization or reduction in

disease markers and tumor cells in patient bone marrow was detected in 4/7 patients.
Our results, confirm a safety profile, robust and diverse immunity and hints of efficacy for ImMucin in MM patients.

P6.06.096
Enhanced tumor antigen specific T cells in prostate cancer patients received in situ gene therapy of repeated HSV-tk injection and ganciclovir administration

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(OBJECTIVE) The high relapse rate of the prostate cancer has been a problem after the radical prostatectomy. For the reduction of relapse rate, various neoadjuvant therapies are examined. Previous report has showed that immune responses were increased in patients treated with adenoviral vector-mediated herpes simplex virus-thymidine kinase (HSV-tk) gene delivery followed by ganciclovir (GCV) injection. However, the details of immune responses after gene therapy were not clear from that study. This study is designed to evaluate it.
(METHODS) This study was approved by the ethical committee of Kitasato University, and by the Ministry of Health, Labour and Welfare. Five patients who agreed this Phase I/II trial were repeatedly given 2 weeks of intravenous GCV administration following the intraprostatic HSV-tk injection. Peripheral blood samples were periodically collected after treatments. The lymphocyte subsets were analyzed by flow cytometry. We also measured intracellular IFN-γ produced by T cells to responding prostatic acid phosphatase and NY-ESO-1 overlapping peptides. Local immune responses were evaluated by Immunohistochemistory.
(RESULTS) The activated and central memory CD8+T cells of three patients markedly increased during second treatment. In three patients, tumor antigen-specific T cells were clearly increased after treatments. Macrophages and CD8+ T cells invasion were highly observed in apex and mid of all prostates.
(CONCLUSION) Immune cells were effectively infiltrated to prostate gland by repeated HSV-tk + GCV treatments. Prostate cancer antigen-specific T cells and increased memory CD8+ T cells may cotribute to reduce relapse rates. This gene therapy could be expected as one of effective neoadjuvant therapies.

P6.06.097
Development of the approach based on dendritic cells transfected with polyepitope DNA-construction for stimulation of antitumor cytotoxic response in colorectal cancer.

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Currently the scientists are searching and developing effective cancer vaccines based on dendritic cells (DC) loaded with various tumor antigens, including DNA-constructions that carry certain sequences of tumor antigen epitopes. These vaccines can efficiently and selectively activate the T-cell immune response. The aim of the study was to evaluate the effect of DCs transfected polyepitope DNA-construction pCI-UB-POLYEPI on the immune response in the culture of mononuclear cells (MNC) in patients with colorectal cancer. In this study we used venous peripheral blood of patients and a sample of the tumor. Dendritic cells were obtained from mononuclear adherent cells of peripheral blood by culturing in the presence rhGM-CSF, rhIL-4. Further we added rhTNF-α to the culture. Thereafter mature DCs were exposed to magnetic transfection with DNA-construction pCI-UB-POLYEPI. Then we cultured non-adherent MNCs with transfected DCs and performed the test for cytotoxicity of MNCs to autologous tumor cells and assessed the relative content of perforin-positive lymphocytes. By assessing the cytotoxic test we found a significant increase in tumor cell death in culture of MNCs cultured with DC transfected with pCI-UB-POLYEPI in comparison with control group by 1.9 times. By evaluating the content of perforin-positive cells we showed the greatest increase in their number in MNCs cultured with DC transfected with pCI-UB-POLYEPI, indicating that one of their mechanisms for realization the cytotoxic effect of the MNCs. Thus, mature DCs transfected with DNA-construction pCI-UB-POLYEPI stimulate cytotoxic response and increase content of perforin-positive cells in the culture of MNCs in patients with colorectal cancer.

P6.06.098
Receiving mature dendritic cells in vitro in patients with tumors of different localization

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In this study we used peripheral venous blood of patients with epithelial tumors of different localization (gaster, lung, colon, ovary). MNCs were isolated from peripheral blood on gradient. Then isolated monocytes by adhesion on plastic, followed by culturing for 48h in the presence GM-CSF (50 ng/ml), IL-4 (100ng/ml) and for 24h in presence TNF-α (25ng/ml). Maturation of DCs was assessed by flow cytometry on the expression of maturation markers (CD83, CD86, CD11c, HLA-DR) on cells and capture FITC-dextran. The protocol used cultivation DC with mature phenotype (high expression of CD83, CD86, CD11c, HLA-DR, low endocytic activity), which was shown in experiments in vitro on cells conditionally healthy donors. This protocol is used to assess the ability of cancer patients to MNCs maturation and differentiation into mature DCs. It was shown that in the culture of patients with epithelial ovarian cancer and colorectal cancer is significantly increased expression of CD83, HLA-DR, CD86, and decreased endocytic activity, which may indicate the ability of intact cell precursors of dendritic cells (monocytes) to differentiate into mature forms of DC. In patients with gastric cancer or lung when cultured DCs was no significant increase of maturation markers and changes in endocytic activity, which points to a violation of differentiation and maturation of dendritic cells in cancer of the stomach and lung.
Thus, in the used cellular protocol has been shown that the various types of cancer can vary the capacity for maturation of dendritic cells in vitro conditions in the presence of IL-4, GM-CSF, TNFa.

P6.06.099
Recombinant lipidated HPV E7 induces a Th-1-biased immune response and protective immunity against cervical cancer in a mouse model

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The E7 oncoprotein of human papillomavirus (HPV) is an ideal target for developing immunotherapeutic strategies against HPV-associated tumors. However, because protein-based immunogens alone are poor elicitors of the cytotoxic T-lymphocyte (CTL) responses, they have been difficult to exploit for therapeutic purposes. In this study, we report that a recombinant lipoprotein consisting of inactive E7 (E7m) biologically linked to a bacterial lipid moiety (rlipo-E7m) induces the maturation of mouse bone marrow-derived dendritic cells through toll-like receptor 2 (TLR2), skews the immune responses toward the Th1 responses and induces E7-specific CTL responses. We further studied the ability of rlipo-E7m to provide protection against a TC-1 tumor cell challenge in an animal model. Mice prophylactically immunized with two 10-μg doses of rlipo-E7m were

found to be free of TC-1 tumor growth. Experiments in a therapeutic immunization model showed that the tumor volume in mice receiving a single dose of rlipo-E7m was less than 0.01 cm^3 on day 40, whereas the tumor volume in mice treated with rE7m was 2.28 ± 1.21 cm^3. The tumor volume of the entire control group was over 3 cm^3. In addition, we demonstrated that the CD8+ T cells play a major role in anti-tumor immunity when administration of rlipo-E7m. These results demonstrate that rlipo-E7m could be a promising candidate for treating HPV-associated tumors.

P6.06.100
In-vivo testing of PSMA-targeted T-cell immunotherapy for prostate cancer

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The aim of this project is to develop prostate cancer immunotherapy using T-cells that express the P28z chimeric antigen receptor, targeted against prostate-specific membrane antigen (PSMA). The first step in this process is to develop an animal model of metastatic PSMA+ prostate cancer. The PC3LN3 prostate cancer cell line undergoes metastatic spread to draining lymph. When engineered to express PSMA, the resultant PC3LN3-PSMA cells are highly metastatic, but do not spread predominantly to bone. Increasing evidence suggests that bone metastasis requires the interaction between E-selectin on bone marrow endothelium with sialyl Lewis antigen-containing E-selectin ligands, expressed on cancer cells. However, neither cell line expresses E-selectin binding ligands or sialyl Lewis antigens. To investigate the mechanism underlying this, we quantified expression of glycosyltransferases required for sialyl Lewis antigen expression. Using real-time PCR, low fucosyltransferase (FT) 3 expression was consistently found. In preliminary studies, we have observed that delivery of a FT3-encoding retroviral vector to PC3LN3 and PC3LN3-PSMA enables them to express sialyl Lewis X and to acquire E-selectin binding activity. We also showed that FT3 promotes increased PC3LN3-PSMA motility and invasiveness in vitro. Bioluminescence imaging result showed their pattern of metastatic spread in SCID Beige mice. This was served as a platform to test immunotherapy using P28z+ T-cells. Next, SPECT imaging will be used to track tumour status (Tc-99m MDP) and T-cell migration (Tc-99m pertechnetate) in the same mouse. Using this approach, we hope to test and optimize the efficacy of P28+ T-cell immunotherapy against PSMA-expressing prostate cancer.

P6.06.101
Effects on apoptosis and cell cycle arrest contribute to the antitumor responses of interleukin-27

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Objective: Interleukin (IL)-27, composed of p28 and Epstein-Barr virus-induced gene 3 (EBI3) subunits, has diverse functions in regulating immune systems. To observe the effect and mechanisms on cell cycle and apoptosis of IL-27 through IL-27 treated on human esophageal carcinoma cells.
Methods: The expression of IL-27 in different esophagel carcinoma cells was detected by RT-PCR and Western-blot, rIL-27 was added to the high expression of IL-27R of esophagel carcinoma cells. Flow cytometry was used to analyze cell cycle and apoptosis, the relating gene and protein was detected by RT-PCR and Western-blot.
Results: The high (TE-1 and Yes-6) and low expression (TE-2) of IL-27R of esophagel carcinoma cells line were obtained. After the the high expression of IL-27R stimulated by IL-27, the apoptosis was increased. The cell cycle was arrested in G0 and G1($P < 0.05$), the expression of cell cycle relating protein p21 was increased, but the expression of survivin was reduced ($P < 0.05$), the apoptosis relating protien Bcl-2 was up-regulated, while Bax was down-regulated ($P < 0.05$).
Conclusion: IL-27 could have a direct role on high expression IL-27R of esophagel carcinoma cells, and induction of cell cycle arrest and apoptosis could be the mechanism of IL-27 in tumor regression.

P6.06.102
Anti-tumor effect of folate-conjugated chitosan nanoparticles containing the IP-10 gene in mice with hepatocellular carcinoma

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The relative insensitivity of hepatocellular carcinoma (HCC) to chemotherapy and radiotherapy has initiated great efforts to make proper immunotherapeutic strategies. Interferon-inducible protein-10 (IP-10) is a chemotactic factor which exerts its anti-tumor activity via recruitment of T lymphocytes and inhibition of angiogenesis. IP-10 is known to be associated with strongly liver fibrosis in hepatocellular carcinoma resected patients with chronic hepatitis C. It is also known to have prognostic utility as a marker of treatment outcome in chronic hepatitis C patients. In this study, chitosan, an economic and degradable natural nano-biomaterial, was applied as a vector for immunotherapy of HCC. A novel ion template method was used to prepare chitosan nanoparticles which were then modified with folic acid (FA) to confer tumor specificity. With conjugation of FA-chitosan with mouse IP-10 cDNA (FA-CS-IP-10), it generated nanoparticles which could target cancer cells and protected the IP-10 gene from endonuclease degradation. The underlying mechanisms of the antitumor effect of intravenous FA-CS-IP-10 in HCC-bearing BALB/c mice were investigated. FA-CS-IP-10 had shown significantly inhibited cancer growth, suppression of angiogenesis, increased apoptosis of cancer cells, and prolonged the survival time of HCC mice. FA-CS-IP-10 also reduced the proportion of regulatory T cells and induced cytotoxic T lymphocytes (CTL) to upregulate IFN-γ secretion. FA-CS-IP-10 also showed a good safety profile. We demonstrated the feasibility of using FA-modified chitosan nanoparticles to target IP-10 DNA to HCC tumors and suggested that FA-CS-IP-10 is a promising candidate for further evaluation as an immunotherapeutic agent to treat HCC patients.

P6.06.103
Rapid and massive localization of multiple lymphocyte subsets to specific tissues following administration of Interleukin-15

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The cytokine Interleukin (IL)-15 has potential applications in cancer immunotherapy and in vaccination as it expands anti-tumor T and Natural Killer (NK) cells and promotes long-lived T cell memory, respectively. While studying the immunological impact of intravenous IL-15 administration in a Phase I clinical trial in patients with cancer, we observed that lymphopenia preceded target cell expansion. Polychromatic flow cytometry of lymphocyte subpopulations in the peripheral blood of humans and, subsequently, rhesus macaques revealed that NK and memory T cells completely disappeared from the blood within minutes upon administration, thus suggesting localization to tissues. Among memory T cells, effector memory/terminal effector cells responded the fastest, followed by central memory, stem cell memory and naïve cells. Overall, the cytokine affected CD8+ more than CD4+ T cells. Animals sacrificed at 6 hours post administration indicated a massive increase in the proportion of memory CD8+ T cells in the proximal (jejunum) and distal (rectum) intestine. Conversely, naïve T cell proportion preferentially increased in the lymphnodes. The absence of proliferation (Ki-67) and activation (CD38, HLA-DR) markers indicates redistribution rather than expansion of resident cells. NK and CD8+ T cells returned in the circulation ~24 hours post treatment and were highly proliferating and activated. These data indicate the potential of IL-15 not only to expand discrete immune subsets but also to induce their localization in specific sites of the body, including mucosal

tissues. Vaccination strategies aimed at inducing mucosal immunity should exploit this novel function of IL-15.

P6.06.104
Enhancing the effector functions of tumor-infiltrating lymphocytes with a combination of new genetic adjuvants

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Adoptive cell therapy (ACT) using ex-vivo expanded and activated tumor-infiltrating lymphocytes (TILs) is currently the most effective treatment of metastatic melanoma and is extensively explored in other cancers. Yet, severe hurdles still limit its clinical outcome and broader application. These include full T cell differentiation and T cell exhaustion following lengthy ex-vivo propagation, the presence of inhibitory cells in heterogeneous TIL populations, the need to overcome immunosuppression at the tumor microenvironment and the dependence of T cell survivability on the systemic administration to patients of high-dose IL-2, which is often intolerably toxic.
For solving at least some these problems we have recently generated a set of new genetic adjuvants comprising constitutively active (ca) TLR4, caCD40 and a membrane-attached derivative of IL-2. The electroporation of polyclonal human CD4 and CD8 T cells and anti-melanoma TILs with in-vitro-transcribed mRNA encoding these adjuvants greatly enhanced their effector functions, while combinations of these adjuvants exerted a synergistic effect. Anti-melanoma TILs transfected with adjuvant mRNA, but not with irrelevant mRNA, exhibited markedly enhanced reactivity against autologous melanoma but not against HLA-mismatched tumor. Moreover, the combination of these mRNAs further improved the magnitude of this enhancement.
Our findings suggest that this new set of genetic adjuvants can improve the anti-tumor reactivity of TILs and offer a new tool for cancer ACT.

P6.06.105
Transgenic expression of soluble human CD5 enhances experimentally-induced autoimmune and anti-tumoral immune responses

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CD5 is a type I glycoprotein constitutively expressed in thymocytes, T lymphocytes and a subtype of B cells, called B1a; current data support the view that CD5 is a negative regulator of antigen-specific receptor-mediated signalling in T and B1a lymphocytes, and that this would be achieved through interaction with CD5 ligand/s (CD5L) of still undefined nature expressed on antigen-presenting cells. To explore the functional relevance of the CD5/CD5L interactions *in vivo* we report here the generation and characterization of transgenic mice expressing elevated circulating levels of a human soluble form of CD5 (shCD5) in sera. We hypothesized that shCD5 would be able to bind its natural ligand/s in the mouse and, as a result, impair intracellular signalling mediated by CD5. Interestingly, analysis of the shCD5 transgenic mice showed a significantly reduced proportion of Treg cells (CD4+CD25+FoxP3+), as well as decreased numbers of peritoneal IL-10-producing B1a (CD5+) lymphocytes. Moreover, NKT cells were increased in the spleen and peritoneum of transgenic mice. The observed reduction in these regulatory cell subpopulations, coupled with the increase in effector subpopulations, resulted in an enhancement of the immune reponse, as shown by the more severe forms of experimental autoimmune disease observed in these mice, as well as an increased anti-tumoral response in *in vivo* cancer models. These data reveal the relevant role played by CD5/CD5L interactions on the homeostasis of some functionally relevant lymphocyte subpopulations resulting in the enhancement of immune responses to autologous antigens.

P6.06.106
T cell costimulation in cancer immunotherapy with anti-CD137 monoclonal antibodies is mediated by K63-polyubiquitin-dependent signals from endosomes

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Agonist anti-CD137(4-1BB) mAbs enhance CD8-mediated antitumor immunity. Agonist anti-human CD137 mAbs covering four distinct epitopes on the CD137 glycoprotein co-stimulated T cell activation irrespective of the engaged epitope or its interference with CD137L binding. CD137 perturbation with all these agonist mAbs resulted in antigen and antibody internalization towards an endosomal vesicular compartment. Internalization was observed in activated T lymphocytes from humans and mice, not only in culture but also in antibody-injected living animals. These in vivo experiments were carried out upon systemic intravenous injections with anti-CD137 mAbs and showed internalization in tumor-infiltrating lymphocytes and in activated human T cells transferred to immunodefficient mice. Efficient CD137-internalization required K63-polyubiquitination and endocytosed CD137-containing vesicles were decorated with K63-polyubiquitins. CD137 stimulation activates NF-κB through a K63-linked polyubiquitination-dependent route and CD137-associated TRAF2 becomes K63-polyubiquitinated. Consistent with a role for TRAF2 in CD137 signalling, transgenic mice functionally deficient in TRAF2 showed a delayed immunotherapeutic activity of anti-CD137 mAbs. As a whole, these findings advance our knowledge of the mechanisms of action of anti-CD137 immunostimulatory mAbs such as those currently undergoing clinical trials in cancer patients.

P6.06.107
Chemokine-receptor modified Adoptive T cell Therapy (ACT)

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Adoptive Cell Therapy (ACT), using expanded ex vivo cancer patient T cells, has developed into a promising strategy for tumor therapy. The local immunosuppression of the tumor and the insufficient homing of transferred lymphocytes to the tumor site limit the efficacy of this technique.
Taking advantage of tumor chemokine production, we hypothesized that adoptively transferred CD8+ T cells, transduced with chemokine receptors matching the chemokines expressed at the primary tumor or the metastatic sites, would preferentially be attracted to their required sites of action. Performing chemokine expression analysis at the primary tumor and metastatic sites, we found that CCL2 in the primary tumor and CXCL12 in the lymph node metastasis are the predominant chemokines. To formally demonstrate that we can induce preferential homing of modified CD8+ T cells to the metastasis, we are using as the TRAMP tumor system that has similarities with the human disease. Our preliminary results show that CD8+ T cells transduced with the matching chemokine receptor preferentially reach the draining lymph nodes of the tumor, where the metastasis occurs. To investigate the benefit in survival following ACT, we are now performing experiments using an induced tumor model, based on injection of the TRAMP-C2 cell line.

P6.06.108
Competent dendrite cell maturation by cocktails containing TLR7/8 agonist for cancer immunotherapy

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Background: In spite of large advances in using dendritic cell (DC) vaccine for treatment of malignancies, the optimal maturation cocktail for producing efficient mature DCs have not yet been established. The aim of this study was to determine the best combination of TLR agonists with the synergistic effect on maturation of human monocyte-derived DCs for immunotherapy of malignancies especially acute myeloid leukemia.
Methods: DCs were generated in vitro from CD14+ monocytes from healthy donors. Different TLR ligand cocktails were used for DCs maturation. Subsequently, matured DCs were assessed phenotypically. IL-12p70 production and allostimulatory capacity of DCs also were analyzed. Ultimately, the ability of DCs in CTL induction against Leukemia blasts was determined.
Results: Results demonstrated that combination of particular agonists resulted in generation of mature DCs with high expression of CD83 and IL-12p70. Among different agonists, combination of TLR7/8, 4 and TLR3 or 2/6 showed prominent effect on DCs. Such potent DCs showed higher capacity to stimulate allogeneic T cells and autologous cytotoxic T cells than immature counterparts. Moreover increased killing capacity of induced CTLs against leukemic targets was shown.
Conclusion: Synergistic TLR stimulation could be efficient approach in generation of ex vivo produced DCs for immunotherapy of cancer in clinical setting.

P6.06.109
Therapeutic effect of anti- pan HLA-class II mAb 4713 on human lymphoma cells, including Hodgkin lymphoma and Adult T cell leukemia cell lines

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To develop a new therapeutic device for Hodgkin lymphoma, we immunized BALB/c mouse with living cells of 2 Hodgkin lymphoma cell lines alternately. We hybridized spleen cells from this immunized mouse with myeloma cells, P3U1. And we screened hybridoma clones by the direct cytotoxicity for the Hodgkin lymphoma cell line not used for immunization. Newly established mouse anti-human monoclonal antibody, raised by this strategy, rapidly induced serum complement independent cell death on not only Hodgikin cell lines but also Burkit lymphoma cell lines. Intriguingly IL-2 dependent not advanced Adult T cell Leukemia cell lines were not sensitive but aggressive IL-2-independent cell lines were sensitive for 4713 induced cytotoxicity. Consequently, this mAb named 4713 revealed to be mouse anti-human pan HLA class II specific monoclonal antibody, for recognizing HLA-DP, DQ, DR transfected cells but not non-transfected cells. Based on their cytolytic activity, it appears that anti-pan HLA class II monoclonal antibody may be most likely to contribute to the therapy of some types of lymphoma/leukemia including Hodgkin Disease, Burkit lymphoma and advanced ATL.
Treatment of this mAb induced large pore formation on target lymphoma cells. This fact suggests that cell death induced by anti-pan HLA class II mAb may share same death signals induced by cytolytic anti-pan MHC class I mAb, which we reported previously to induce large pore formation on target cell surface.

P6.06.110
Identification of unique colorectal cancer T-cell antigens by next generation sequencing of somatically mutated genes

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Immunotherapy holds promise for cancer treatment. Unique TAAs, encoded by somatically mutated cancer genes, may generate strongly immunogenic epitopes that induce tumor control by the host's immune system. We are using massive DNA sequencing of somatic mutations in colorectal cancer cells (CRC), and possibly also in their Cancer Stem Cells (CSCs), to identify patient-specific unique TAAs. We established 8 tumor cells lines from CRC patients, two of which also cultured as CSCs. Pools of mRNAs encoding 20 CAN-genes were subjected to high throughput sequencing, confirming the presence of somatic mutations in some of these genes. Synthetic peptides spanning the mutated proteins expressed by each CRC cell line are tested for their ability to elicit an in vitro immune response. Results obtained in one CRC sample show that the frameshift mutation occurring in the APC gene generates a neo-epitope that is presented by HLA-DR molecules in tumor cells and it is recognized by specific CD4+ T cells. Peptides corresponding to the point mutations in TP53 and SMAD4 gene products elicit CD8+ and CD4+ T cells that specifically react against the same CRC cells. Conversely, an autologous T cell line induced by this CRC cell line recognize the mutated SMAD4 peptide. These preliminary results would support the feasibility of the approach to identify unique TAAs in CRC by massive sequencing and reverse immunology.

P6.06.111
Examination of an immunoregulator in a syngeneic animal model

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Dialyzable leukocyte extracts (DLE), described by Lawrence in the mid-20th century, have been used widely as an adjuvant therapy for various diseases due to their immunoregulatory activity. Clinical studies on DLE in combination with conventional therapy against cancer have demonstrated that they are beneficial, reducing metastases and the side effects of chemotherapy and increasing life span. Nevertheless, their mechanism of action in cancer remains poorly understood.
Diffuse large B-cell lymphoma is a highly aggressive neoplasm that is resistant to many therapies, prompting the development of new therapies, particularly those that increase the immune response. This study examined the effects of DLE in A20 cell lymphoma in a syngeneic murine model. In vitro, the proliferation and cell cycle progression of A20 cells were unaltered on incubation with DLE of human origin. In vivo, treatment with various doses of DLE in mice that were subcutaneously isotransplanted with B cell lymphoma effected less tumor growth than in untreated mice but was insufficient to control the tumor. Mice that received combination therapy of cyclophosphamide and DLE experienced less tumor growth than those that were treated with either agent alone. Further, combination therapy delayed tumor recurrence resulted in fewer cases of recurrence than cyclophosphamide. In conclusion, DLE enhance the response to conventional treatment in a syngeneic murine tumor model.

P6.06.112
Evaluation of anti-proliferative activity of ruthenium (III) compounds on human cancer cell lines using RTCA - xCELLigence System

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The clinical use of Cisplatin and its analogues might be limited by tumour drug resistance and adverse effects, therefore other transition-metal agents that possess anti-tumour and anti-metastatic activities such as ruthenium (III) compounds could be considered as alternative drugs. In order to evaluate the anti-proliferative activity of several ruthenium compounds with anti-tumour activity and find the optimal time points and concentrations for high throughput predictive toxicological assessment, we have used the xCELLigence system comparing to other cytotoxicity assays (MTT, XTT). The xCELLigence system is a real-time cell analyzer based on label-free platform technology that continuously monitor cellular responses to a given treatment using the inherent morphological and adhesive characteristics of cells, and combine them with cellular endpoint assays such as flow-cytometry. Our complex interdisciplinary approach aims to develop pharmaceutical product models with significant anti-tumour action and minimal side effects. The study focused of evaluation of anti-proliferative activity of ruthenium (III) compounds compared to Cisplatin on various cancer cell lines (breast MDA-MB-231; colon LoVo; cervix HeLa; ovarian Sk-Ov-3; tongue PE/CA-PJ49 cells) using different co-solvents systems. In addition, percentages of apoptotic cells were evaluated using Annexin V/FITC and propidium iodide (PI) double staining (BD Annexin V-FITC Apoptosis Detection Kit), while DNA progression through cell cycle phases was estimated using PI staining (BD Cycletest Plus/DNA Reagent kit), followed by flow-cytometry. Extension of the research toward the solubilization of ruthenium compounds in order to improve their pharmacokinetics and testing their anticancer activity in soluble forms might improve their potential therapeutic use as anticancer agents.

P6.06.113
Competent maturation of human dendritic cells by protein fractions of listeria monocytogenes

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Objective: Dendritic cells (DCs) are sentinels of the immune system that instruct the activation of T and B lymphocytes. DCs are responsible for initiating immune responses against tumor antigens. DCs Maturation is essential for induction of appropriate anti-tumor responses. Until know, a potent activator for DCs maturation has not been introduced. Therefore, in this study, the ability of protein fractions of listeria monocytogenes in human monocyte-derived DCs maturation was analyzed.
Material and Methods: The listeria monocytogenes suspension was prepared. Subsequently, Bacteria were spun down by centrifugation, and were sonicated in the presence of protease inhibitors cocktail. Protein extract was fractionated firstly by stepwise precipitation with increasing amounts of ammonium sulfate and secondly by Ion-Exchange chromatography. The ability of achieved fractions in inducing DCs maturation was determined by different immunological assessments. Ultimately, the proteome of matured DCs was compared between different matured DCs.
Results: The result showed that one protein fraction of listeria monocytogenes is more potent in inducing DCs maturation. The protein components of selected fraction were characterized by SDS-PAGE and Mass-spectrometry. Proteomics analysis clarified that there are significant differences between DCs cytoplasmic proteome.
Conclusions: We conclude that some proteins of intracellular bacteria such as listeria monocytogenes can be considered as potent activators of human DCs in order to instruct immune responses against tumor antigens.

P6.06.114
Cloning and expression of human L- Asparaginase and study of its anti growth effect on leukemia cell lines

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Objectives: L-Asparaginase catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonia. L-Asparaginase has an antineoplastic activity selectively reduces the level of L-asparagine in blood and decreases the proliferation of cancerous cells. Consequently, it have been widely used as a therapeutic agent in the treatment of acute lymphoblastic leukemia. In the present study, we aim to produce of human L-Asparaginase enzyme and investigate of its antineoplastic effect on leukemia cell line.
Method: Total RNA was extracted from PC3 cell line and converted to cDNA. L-Asparaginase gene was amplified by RT-PCR and cloned into the PGEM- T vector. The positive clones were digested and finally confirmed by sequencing. The product was expressed in E. coli using pET-22b expression vector. Recombinant protein contained hexahistidine tag was purified using nickel-nitrilotriacetic acid chromatography. C105 cell line were treated by different concentration of purified enzyme. The cytotoxic effect of enzyme were measured by MTT assay.
Result: The sequence of cloned L-Asparaginase gene consisted of 937 bp showed 100% identity with previously reported sequence of this gene. Expression of L-Asparaginase gene in E. coli resulted in high levels of protein with molecular weight of 32 kDa in SDS-PAGE. The result of MTT assay showed that the C105 cell line is considerably sensitive to deduced protein.
Conclusion: In this study the recombinant L-Asparaginase was expressed and the anti proliferative effect of this product on a leukemia cell line was confirmed. Hence, it would be of interest to employ this recombinant protein as anti leucemic drug.

P6.06.115
Exosomes displaying high expression of CD24 and HSP90 induced cellular and humoral immune response "in vivo"

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Exosomes have emerged as a new approach for the diagnosis and therapy of cancer. The use of these nano-vesicles as modulators of the immune system is being studied presenting them as readily available and stable tumor antigens. The objective of this work was to characterize exosomes derived from the murine T-cell lymphoma LBC and their immunogenic properties. The expression of proteins of immunological relevance, such as MHC I, CD8, CD24 and Hsp90 on exosomes membrane was demonstrated. We demonstrated surface expression of Hsp90 and intralumen Hsp70, indicative of a different protein expression pattern with the cells from which they derive. An over-expression of tumor associated antigens on LBC exosomes when compared to LBC cell lysates or even to intact LBC cells, corresponding to proteins of 51 kDa on LBC-derived exosomes that could not be detected in LBC cells, was found. These tumor antigens were recognized not only by memory lymphocytes but also induced a primary immune response in vitro and a humoral and cellular immune response in vivo, as INF gamma secreted LBC-sensitized splenocytes and specific antibodies were identified in the sera of mice immunized with LBC exosomes. In conclusion, the association of tumor-associated antigens and other molecules of immunologic interest such as MHC, Hsp or costimulatory molecules on tumor exosomes might determine the their immune properties. All these findings confirm exosomes as promising defined acellular tumor

antigens for the development of an antitumor vaccine for the immunotherapy of cancer.

P6.06.116
Imiquimod enhances anti-lymphoma response in a tumor cell lysate vaccine and inhibits tumor cells growth

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Our aim was to study the immunomodulating and proapoptotic properties of the TLR7 agonist Imiquimod to be used as adjuvant in a tumor vaccine. Imiquimod showed an antiproliferative effect on LBC T-cell lymphoma in vitro at 48 and 72 h with doses between 0.5 and 200 µg/ml, inducing apoptosis in a dose dependent manner. BALB/c mice inoculated intraperitoneally twice with LBC cells lysates with or without 100 µg/mouse of Imiquimod and challenged one week later with LBC cells intraperitoneally, showed a significant increase in survival compared to un-immunized controls (LBC-lysate<0.05, LBC-lysate + IMQ<0.0005) and to mice receiving Imiquimod (p<0.0002; Logrank).Tumor incidence was only 5±5% in LBC-lysate+IMQ group whereas in LBC-lysate group was 52.5±2.5%. Although the percentages of specific cytotoxicity were above normal in both immunized groups (p<0.05, ANOVA), LBC-lysate+IMQ immunization induced the highest levels. A significant enhance in peritoneal cavity CD8+, CD4+ T and B cells (p<0.0001, ANOVA) with an increase IFNγ in supernatant from peritoneal cavity cells and splenocytes was demonstrated in all vaccinated groups compared to normal (p<0.001, ANOVA). Our vaccine also generated circulating antibodies in all immunized mice that rejected the tumor up to 75 days after challenge. Finally, mice that rejected the first tumor challenge were re-challenged with LBC tumor with an increased in survival in all immunized groups over the control (p<0.01, Logrank) with rejection rates close to 100%. We demonstrated that Imiquimod not only modulates immune response efficiently to reject tumor generating a long-lasting memory, but also is capable of inducing tumor apoptosis.

P6.06.117
Therapeutic activity of a combination of immunostimulatory monoclonal antibodies (anti-B7-H1, CD137 and OX40) on a c-myc-driven spontaneous transgenic model of hepatocellular carcinoma

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Several immunostimulatory monoclonal antibodies (ISmAbs) that derepress and unleash antitumor immune responses are showing efficacy in cancer clinical trials. MAbs anti-B7-H1 (PD-L1) block a critical inhibitory pathway for T cells, while antibodies anti-CD137 and OX40 provide intense T cell costimulation. Documenting efficacy of immunotherapies on spontaneous tumors arising in oncogene transgenic mice is considered more predictive than experiments on transplanted tumors. A combination of these ISmAbs has been tested on a transgenic mouse model of spontaneous primary liver cancer in which c-myc drives transformation and the tumor cells express ovalbumin as a surrogate transgenic antigen. The induced tumor lymphocyte infiltrates and immune mechanisms of action were studied. The triple combination of mAbs clearly extended survival of mice bearing hepatocellular carcinomas (HCC) and synergized with adoptive T cell therapy with activated TCR-transgenic T cells that recognize OVA. Mice undergoing therapy showed a clear increase in the tumor tissue infiltration by activated and blastic CD8 T lymphocytes expressing the ISmAb-targeted receptors. The triple combination of ISmAbs did not result in enhanced OVA-specific CTL activity but other antigens in cell lines derived from such HCC were recognized by the elicited tumor infiltrating T lymphocytes. Indeed adoptive transfer of OT-1 cells to tumor bearing mice resulted in their tolerization, unless the triple mAb therapy was instigated. Our results emphasize the role of combinational immunotherapy approaches including ISmAbs to impact on agressive and highly T cell-tolerizing hepatocellular carcinomas.

P6.06.118
Establishment of human cancer stem-like cell targeting immunotherapy

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Cancer stem-like cells (CSCs) have been reported to be resistant to chemotherapy and radiotherapy. However, it is still not clear whether if CSCs are sensitive to immune cells. In the present study, we analyzed gene expression profiles of human colon CSCs and evaluated the sensitivities to immunotherapy. RT-PCR and microarray analysis revealed that tumor-associated antigens can be classified into three groups according to expression in CSCs and non-CSCs. 'Shared antigens' are expressed in both CSCs and non-CSCs, 'CSC antigens' are preferentially expressed in CSCs and 'non-CSC antigens' are preferentially expressed in non-CSCs. The potencies of CSC antigens and shared antigens were compared by using cytotoxic T lymphocyte (CTL) clones specific for a CSC antigen (OR7C1) and a shared antigen (CEP55). The CEP55-specific CTL clone and OR7C1-specific CTL clone showed almost the same level of cytotoxicity *in vitro.* On the other hand, the OR7C1-specific CTL clone showed a greater anti-tumor effect than that of the CEP55-specific CTL clone *in vivo*. These results raised the possibility that treatment-resistant CSCs are sensitive to CTLs and that CSC-targeting immunotherapy using CSC antigens is feasible.

P6.06.119
Schedule-dependent anticancer therapy by targeting TNF-alpha to tumor vessels in combination with melphalan and gemcitabine

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L19-tumor necrosis factor-alpha (L19mTNF-a, L), a fusion protein consisting of mouse TNF-a and the human antibody fragment L19 directed to the ED-B of fibronectin, is able to selectively target tumor vasculature, and act synergistically with melphalan (M) in syngeneic mouse tumor models. We have studied the antitumor activity of single L treatment in combination with M and gemcitabine (G) using different administration protocols in two different murine tumor models: WEHI-164 fibrosarcoma and K7M2 osteosarcoma. Single L/M therapy and two injections of G alone exerted similar therapeutic effect curing 80-90% of WEHI-164 tumor, respectively, and 20% of K7M2 tumor-bearing mice. Combination studies of the three drugs showed that when 2 injections of G were administered after L/M the percentage of cured mice was unchanged in WEHI-164 tumor treated mice (80%), whereas in K7M2 tumor mice greatly improved (from 20% up to 80%). On the contrary, when G was administered before L/M, a strong antagonist effect was induced with reduction of tumor cure rate. All responding mice showed significant reduction of CD11b+Gr-1+ MDSC and an increase of CD4+ and CD8+ T cells in tumor infiltrates, as well as significant reduction of CD4+CD25+ Treg cells at the level of draining lymph nodes. What is important, is that all cured mice rejected tumor challenge up to one year after therapy. Targeted delivery of L19mTNF-a synergistically increases the antitumor activity of melphalan and gemcitabine, but optimal administration schedules are required. This investigation provides information for designing clinical studies using L19mTNF-a in combination with chemotherapeutic drugs.

P6.06.120
High-level expression of IGFBP-3 by recombinant vaccinia virus leads to its incorporation into IMV and affects virus replication and immunogenicity

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The aim of this study was to investigate, whether the tumor-suppressive properties of Insulin-like growth factor-binding protein-3 (IGFBP-3) can be used to enhance the efficiency of therapeutic immunization against the HPV16 E7 oncogene associated with cervical cancer. We constructed recombinant vaccinia viruses (rVACV) co-expressing the immunogen SigE7Lamp and IGFBP-3. The expression of IGFBP-3 was controlled either by the early promoter H5 or by the synthetic early-late (E/L) promoter. Our results show that IGFBP-3 expression under the H5 promoter (P13-SigE7Lamp-H5-IGFBP-3) is significantly higher than under E/L promoter(P13-SigE7Lamp-E/L-IGFBP-3) (***p<0.001). The mice bearing TC-1 tumors treated with P13-SigE7Lamp-H5-IGFBP-3 had significantly smaller tumors than PBS-treated mice by day 11 (*p<0.05), whereas mice treated with P13-SigE7Lamp-E/L-IGFBP-3 or the control virus P13-SigE7Lamp-TK$^-$ had significantly smaller tumors by day 18 (***p<0.001). Co-expression of IGFBP-3 increased T cel l response against VACV antigen but not against HPV16 E7 antigen as determined by ELISPOT IFN-γ. *In vitro* analysis of virus multiplication has shown that P13-SigE7Lamp-H5-IGFBP-3 has significantly higher replication rate than P13-SigE7Lamp-TK$^-$ or P13-SigE7Lamp-E/L-IGFBP-3 (***p<0.001) and that adding an IGFBP-3 neutralizing antibody significantly decreased yields of P13-SigE7Lamp-H5-IGFBP-3 (**p<0.01). Furthermore, using Western-blot analysis we detected insertion of IGFBP-3 into virions of P13-SigE7Lamp-H5-IGFBP-3. We therefore hypothesize that incorporation of IGFBP-3 into intracellular mature virus helps virus replication. Indeed, our results show that cell absorption rate of P13-SigE7Lamp-H5-IGFBP-3 is significantly higher than P13-SigE7Lamp-TK$^-$ (*p<0.05). Summarized, higher cell absorption leads to overall better replication and thus resulting in prolonged and enhanced antigen stimulation during immunization.

P6.06.121
Syntesis of matrix and matrix metalloproteases in 2D and 3D cultures of pancreatic cells

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Degradation of tissue matrix and vessel formation is essential for tumor growth. Matrix metalloproteases (MMPs) constantly degrade surrounding matrix and form new niches for growth. Models possessing ability to study cell interactions with matrix are essential. The aim of this work was to study synthesis of matrix and MMPs in 3D cultures of pancreatic cell lines. Supernatants of 3D cultures of AsPC-2, Colo 357, BxPC-3, and Su86.86 pancreatic cell lines, obtained on poly-HEMA films, were collected at 24, 48 and 72 hrs and subjected to casein and gelatin zymography to identify enzymatic activity. Matrix formation was studied by confocal microscopy in 3D cultures stained with cumarin. We demonstrated that different cells secrete different sets of MMPs however bands with MW 20; 40; 88; 95; 118 kDa were present in all gelatin zymorgams; while 40 kDa band varied in casein gels. Maximum production for all type of cells was observed at 24 hrs. Both active and latent forms of MMP 7 and 9 were identified. Higher secretion was found in 2D cultures, possibly due to the easier MMPs secretion. Secretion of MMPs decreased in a row: BxPC-3>AsPC-1>Colo-375>Su86.86=PANC-1. MCTS formed acellular matrix around spheroids which also differed for different cells. The depth of matrix decreased in a row: BxPC-3>AsPC-1>Colo 357. We were unable to register matrix shell around Su86.86 and PANC-1. There results show a good correlation between MMP and matrix synthesis.
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P6.06.122
Selective killing of activated B-cells and inhibition of antibody production with bispecific CD20 x CD95 antibodies

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Multimeric monoclonal antibodies directed to the death receptor (CD95/APO-1/Fas) are capable of inducing apoptosis in CD95 expressing and -sensitive cells. However, clinical application of such agonistic antibodies, e.g. to achieve killing of CD95 expressing human cells is precarious, due to expression of CD95 on normal tissue, such as liver.
In 2001 we reported that CD95 antibodies, hybridized to a second antibody directed against a different target antigen on the same cell, such as CD20 and CD40, induce apoptosis of the cells if the expression of the target antigen exceeds a certain threshold level. This concept seeks to refine CD95 targeting by developing bispecific reagents which restricts CD95 agonistic activity to cells carrying a second target antigen.
While this principle was originally applied to malignant cells the data we present here demonstrate that CD20 X CD95 bispecific antibodies kill activated CD95 expressing human B-cells and that antibody production by pokeweed mitogen (PWM) stimulated B cells is markedly reduced. The bispecific antibodies are significantly more effective in mediating these effects than monospecific CD20 antibodies. Our results confirm that normal B-cells acquire sensitivity to CD95 mediated apoptosis and that bispecific CD20 X CD95 antibodies may be used to effectively suppress antibody production not only in vitro but also in patients with antibody mediated autoimmune diseases. Compared to monospecific CD20 antibodies those reagents offer a new effector principle and specificity for activated rather than resting B cells.

P6.06.123
Modulating effects of different doses of 5-flurouracil (5-FU) on the maturation and function of dendritic cells (DC) in vitro

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Backgound: Cancer chemotherapy drugs are detrimental to immune cells due to their generalized cytotoxic effects. However, accumulating evidences demonstrate that suboptimal doses of some of these drugs, are not only cytotoxic, but also can augment tumor immunity by various mechanisms, especially by affecting tumor suppressive cells such as myeloid derived suppressor cells (MDSC). Moreover, in recent years, combination of chemotherapeutic agents and dendritic cell therapy has shown promising results in cancer immunotherapy. In this research, we evaluated effects of different non-cytotoxic doses of 5-Fluorouracil (5-FU) on the maturation and functional characteristics of bone marrow derived dendritic cells (BM-DC) of mouse.
Methods: DCs were generated from C57BL/6 bone marrow cells by standard protocol in the presence of different concentrations of 5-FU. Production of IL-10 and IL-12 by DCs were evaluated using ELISA and maturation markers for DCs by flowcytometry.
Results: The results showed that different doses of 5-FU have modulating effects on the maturation and functional characteristics of DCs.The effect of 5-FU on DCs was dose dependent.
Conclusion: According to the findings of the current study suboptimal doses of 5-FU have different effects on DCs function, which should be considered in combination therapy of cancer in clinical setting.

P6.06.124
Outer membrane proteins and vesicle proteins targeted as new vaccine strategies for pathogenic gram negative bacteria through particulate delivery system

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Virulence-related outer membrane proteins (OMPs) are expressed in pathogenic Gram-negative bacteria such as *Aeromonas hydrophila and Vibrio cholerae* and are essential to bacterial survival within macrophages and for eukaryotic cell invasion. The outer membrane proteins of enteric pathogens are one of several factors involved in the interaction between the bacterium and the epithelial cell surface, and confer bacterial resistance to bile salts and to host defense factors such as lysozyme and leukocyte proteins. OMVs (Outer membrane vesicles) also function in transport of virulence factors, adherence to and entry into host cells, or modulation of the host response. So the undertaken work is important in predicting PLA/Chitosan conjugated Omp/Omv microparticles (MPs) as a better delivery system and may offer an enhanced optimal immune response against Omp, so that Omp and Omv can be established as successful vaccine candidates both in lower and higher vertebrates against respective gram negative bacteria. Outer membrane proteins (Omps) from *A. hydrophila*, one of the bacterial pathogens causing aquatic diseases has been isolated and characterized by SDS PAGE. PLA based microparticles encapsulating Omps/Omvs antigens from *A. hydrophila* have been formulated using a double emulsion-solvent (W/O/W) evaporation technique. Preparation of Chitosan based antigen loaded microparticles and Chitosan coated PLA microparticles encapsulating antigens are under progress. Various encapsulation studies, interaction studies with the microparticles have been performed. We are continuing with stabilization experiments of Omp and Omv proteins from *A. hydrophila*.

P6.06.125
Down-regulation of pro-angiogenic and pro-metastatic genes in scFvs treated breast cancer cells

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Introduction: Intra-tumoral angiogenic and metastatic genes expression seem to be closely linked to HER2 expression level on breast cancer cell. Tumor cells secrete chemokines and matrix metalloproteinases (MMPs) in the tumor microenvironment. The expression levels of CXCL10, CXCL10L (CXCR3) and MMP-2 are closely related to each other and play critical roles in tumor cell proliferation, angiogenesis, and metastasis. In this study the effects of single chain antibodies (scFvs) against HER2 on the expression of pro-angiogenic and pro-metastatic genes were investigated.
Methodology: Following 48 hours treatment of scFvs (three human anti-HER2 scFvs had been previously isolated against HER2 epitopes in our laboratory) with SKBR3 cell line, total RNA was extracted and used for cDNA synthesis. Quantitative real-time PCR was done in triplicate using specific primers to measure CXCL10, CXCL10L (CXCR3) and MMP-2 transcripts.
Results: After 48 h treatments of SKBR3 cells with scFvs, down-regulation of CXCR3, CXCL10 and MMP2 gene transcripts were observed.
Conclusions: Studies have revealed a role for endogenous CXCL10/CXCR3 axis in the cell proliferation and invasion of breast cancer. MMPs are associated with tumor cell metastasis and angiogenesis, by clearing a path for migrating tumor cells and activating pro-angiogenic molecules. In the present study, gene expression of pro-angiogenic and pro-metastatic factors were investigated in scFvs treated breast tumor cells. Anti-HER2 scFvs, down-regulated the CXCR3 , CXCL10, and MMP2 transcripts in breast cancer cells. The results suggest that anti-HER2 scFvs are able to inhibit breast cancer cell line invasion via signaling pathways involving MMP-2 and CXCR3/CXCL10 axis.

P6.06.126
Down-regulation of Epidermal Growth Factor in a breast cancer cell line treated with anti-HER2 single chain antibodies

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Introduction: Epidermal growth factor receptor 2 (HER2) is over-expressed in certain different carcinomas such as breast cancer. Homo- and hetero-dimerization of HER2 with other members of HER family induce phosphorylation of tyrosine residues within the intracellular domain of receptor. These residues provide docking sites for adaptor proteins and link the HER2 to downstream survival, growth and angiogenic networks. In the current study the effect of specific scFvs against HER2 epitopes on the Epidermal Growth Factor (EGF) expression in cancer cells were investigated
Methodology: Three human anti-HER2 scFvs previously isolated against HER2 extracellular domains in our laboratory were used to treat SKBR3 breast cancer cell line individually and in combination. After 48 hours the expression of Epidermal Growth Factor was measured using real time PCR.
Results: Significant downregulation of EGF was observed when a cocktail containing the three scFvs were used.
Conclusions: Expression profile of HER family members on tumor cells, growth and survival of breast tumor cells may be affected by an autocrine loop between the HER family members and their ligands; like Epidermal growth factor (EGF). This loop could be disrupted by down-regulation of EGF expression in breast cancer cell line following treatment by anti-HER2 scFvs cocktail. However, individual scFvs were not able to inhibit EGF gene expression.This offers that the scFvs cocktail containing three specific scFvs against HER2, could be more useful for inhibition of tumor growth and proliferation.

P6.06.127
IL-12 immunotherapy: characterization of a CD4+ effector population

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The ability of interleukin-12 (IL-12) to initiate anti-cancer immune responses is well established, and this cytokine was recently ranked 3rd on a list of agents rated for their potential to treat cancer during the NCI Immunotherapy Agent Workshop. We have therefore used a murine leukaemia model to characterize the immune response initiated by a cell-based immunotherapy approach employing IL-12. This approach leads to protective immunity, but initial rejection is surprisingly dependent primarily on a CD4+ cellular subset. In contrast, injection of recombinant IL-12 leads to CD8+ T cell-mediated rejection. This highlights that the mode of IL-12 delivery has a distinct impact on the immune response initiated.
To further characterize this CD4+ effector population, as well as derive mechanistic information, in vitro assays have been established. We have demonstrated that NKT cells play a critical role in activating a DC population that subsequently drives the maturation of these cytotoxic CD4+ T cells, eventually leading to clearance of the leukaemia cells. Though CD4+ T cells are known to possess cytotoxic capacity, little is understood about the physiological role that a CD4+ CTL might play. Description of such a population that arises naturally, without targeted manipulation of the experimental system, is of great interest. Our work begins to address what stimuli are responsible for activation of this CD4+ effector population and the mechanisms they use to mediate cancer eradication.

P6.06.128
EBV-transformed Lymphoblastoid Cell Lines as Vaccines against Cancer Testis Antigen-positive Tumors

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EBV-transformed lymphoblastoid cell lines (LCL) are potent antigen-presenting cells. To investigate their potential use as cancer testis antigen (CTA) vaccines, we studied the expression of 12 CT genes in 20 LCL by RT-PCR. The most frequently expressed CT genes were SSX4 (50 %), followed by GAGE (45 %), SSX1 (40 %), MAGE-A3 and SSX2 (25 %), SCP1, HOM-TES-85, MAGE-C1, and MAGE-C2 (15 %). NY-ESO-1 and MAGE-A4 were found in 1/20 LCL and BORIS was not detected at all. Fifteen of 20 LCL expressed at least one antigen; 9 LCL expressed ≥2 CT genes, and 7 of the 20 LCL expressed ≥4 CT genes. The expression of CT genes did not correlate with the length of *in vitro* culture, telomerase activity, aneuploidy or proliferation state. While spontaneous expression of CT genes determined by real time PCR and Western Blot was rather weak in most LCL, treatment with DNA methyltransferase 1 inhibitor alone or in combination with histone deacetylase inhibitors increased CTA expression considerably thus enabling LCL to induce CTA-specific T-cell responses. The stability of the CT gene expression over prolonged culture periods makes LCL attractive candidates for CT vaccines both in hematological neoplasias and solid tumors.

P6.06.129
ENO1-specific Tregs frustrate Th1/Th17 effector cells isolated from pancreatic cancer patients

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Pancreatic cancer (PC) is an aggressive disease with dismal prognosis. Surgical resection is the recommended treatment for long-term survival, but patients with resectable PC are the minority (with a 5-year survival rate of 20%). Therefore, development of novel therapeutic strategies, such as anti-PC immunotherapy, is crucial. α-Enolase (ENO1) is an enzyme expressed on the surface of pancreatic cancer cells, and it is able to promote cell migration and cancer metastasis. The capacity of ENO1 to induce an immune response in PC patients renders it a true tumor-associated antigen. In this study we characterized the effector functions of ENO1-specific T-cells, isolated from PC patients and we specifically evaluated the successful role of intra-tumoral Th17 (T helper 17 cells) and the inhibitory role of Tregs (regulatory T cells) in promoting or reducing respectively the cancer-specific immune response. In this ex-vivo study, we have demonstrated, for the first time, that in PC patients, ENO1-specific Th17 cells have a specific anti-cancer effector function, and that there are decreased levels of these cells in cancer compared to healthy mucosa. In PC patients, there are elevated levels of ENO1-specific Tregs which leads to inhibition of the antigen-specific effector T cells, thus highlighting a possible role in promoting PC progression. These results might be relevant for the design of novel immunotherapeutic strategies in pancreatic cancer.

P6.06.130
IgE as a new player in tumor surveillance

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IgE is a key player in anti-parasitic immunity and allergies. Several research groups found that IgE may exert powerful anti-tumor potency *in vivo*, opening a new field of investigation named AllergoOncology. In our approach, mouse and human IgE have been employed as vaccine adjuvants, highlighting the importance of the IgE-FcepsilonRI interaction in the establishment of a strong allergy-like inflammatory response at the tumor site, with the induction of an adaptive anti-tumor immune response and memory. These results and epidemiological studies suggesting an inverse association between allergy and malignancies, directed our attention towards a possible role played by endogenous IgE in tumor surveillance. Strikingly, transgenic (high IgE-producing) mice immunized with irradiated TS/A-LACK mammary tumor cells are completely resistant to tumor growth after challenge with living TS/A-LACK cells. Using N2C cells, a HER2/*neu*-expressing tumor model, absence of tumor development occurs even without immunization. In order to definitely prove the involvement of endogenous IgE in the anti-tumor protection observed in this *in vivo* system, we generated a double mutant (high IgE-producing/FcepsilonRIalpha knock out) mouse. As expected, this new transgenic mouse develops the N2C tumor due to the inability of IgE to exert its effect in the absence of FcepsilonRI. Furthermore, sera from high IgE-producing mice challenged with N2C cells contain tumor-specific IgE, as evinced from a cell-based mediator release assay.
Among the future directions, a relevant effort is being spent in the detection and isolation of tumor-specific IgE mAbs from challenged high IgE-producing mice, opening new perspectives in cancer treatment.

P6.06.131
First exploration of clinical value of telomerase expression in triple-negative breast cancer tissues

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The human telomerase is an enzyme which is involved in cell senescence and division, providing DNA-protective functions during replication. It is suppressed in most of normal somatic cells but becomes active in malignant tumors. The goal of this project was to evaluate expression of human telomerase reverse transcriptase (hTERT), which is a catalytic subunit of telomerase, in triple-negative breast cancer tissue samples and the breast cancer cell line MDA-MB-435.
For evaluation of hTERT protein expression, immunohistochemistry (IHC) with a polyclonal antibody (600-401-252S, Rockland) against hTERT was performed; for gene expression qRT-PCR was employed. For IHC, 178 triple-negative breast cancer cases were assessed on tissue microarrays (TMA), for qRT-PCR the cell line MDA-MB-435. Since active telomerase associates with the nucleus, evaluation of hTERT by IHC was judged on the absence or presence of nuclear staining. Cases with nuclear staining were considered as positive. Staining results were statistically compared with clinicopathological data of the patients and their 5-year overall and disease-free survival (n=146).
Nuclear staining was observed in 29% (43 out of 146 patients) of the cases. Presence of nuclear staining was statistically significantly correlated with low-grade (G1) tumors (p=0.008). Patients with nuclear expression of hTERT had a tendency for better 5-year overall survival (p=0.068). In the MDA-MB-435 cell line, expression of hTERT gene was very low in comparison with the expression of the house-keeping gene HMBS.
Expression analyses of hTERT provided interesting results, but larger collectives of triple-negative breast cancer are needed for further investigation.

P6.06.132

Tumor-associated antigen-specific CD4$^+$ T-cell immunity augmented by CTL-epitopes vaccination in patients with malignant tumor

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We identified novel tumor associated antigens (TAAs) including CDCA1, LY6K and KIF20A that are overexpressed in lung cancer, head and neck malignant tumor (HNMT), pancreatic cancer, and other various malignancies, but not expressed in many adult normal tissues except for testis and fetal organs by using cDNA microarray analyses. Recently,
we identified TAAs-derived CTL-epitopes that can activate tumor-reactive and HLA-A2 or -A24-restricted CTLs in both *in vitro* preclinical and *in vivo* peptide vaccine clinical studies. In this study, we identified these TAAs-derived promiscuous 20~26-mer long peptides (LPs) bearing CTL-epitopes that can induce Th1 cells restricted by several frequent HLA class II and tumor-specific CTLs restricted by HLA-A2 or -A24. The LPs were naturally processed and presented by dendritic cells. In addition, these LPs induced propagation of TAA-specific CTLs by cross-presentation in both human *in vitro* and HLA-class I transgenic mice *in vivo*. Furthermore, these LPs and Th1 cell clone enhanced induction of TAA-specific CTLs of HNMT patients. Significant frequencies of TAA-specific Th1 cells were detected after short-term *in vitro* stimulation with LPs in HNMT patients vaccinated with TAA-derived CTL-epitope peptides and patients before vaccination, but not in healthy donors; responses were augmented by repeated vaccinations. These results show for the first time the presence of novel TAA-specific Th1 cell responses in HNMT patients and underline the potential utility of these LPs for propagation of both antigen-specific Th1 cells and CTLs.

P6.06.133

Effect of mature dendritic cells primed with autologous tumor antigens, patients with epithelial ovarian cancer to stimulate the cytotoxic activity of mononuclear cells in vitro

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Along with conservative treatment of epithelial ovarian carcinoma, which has the highest frequency of occurrence of gynecological cancers, specific immunotherapy is a modern and advanced way of treating the disease. Special role in the immunotherapy vaccine therapy is based on dendritic cells (DC). Therefore, the purpose of this study was to assess the effectiveness of the modulation of cytotoxic activity in vitro (in a culture of mononuclear cells) using autologous dendritic cells and tumor antigens patients with epithelial ovarian cancer.
We used peripheral venous blood and tumor biopsy samples obtained during surgery for removal in 23 patients with ovarian epithelial carcinoma at the age of 41-80 years. Dendritic cells were obtained by adhesion on plastic. To obtain antigen-loaded DCs after 48 hours of culture was added to immature DCs tumor antigen (autologous tumor lysate). As the factor of maturation after 24 hours was introduced rhTNF-α. A day later, received dendritic cells were cultured for five days with a non-adherent fraction of MNCs in the ratio of 1:10 with the addition rhIL-18 and rhIL-12. Modulation efficiency was assessed by cytotoxic activity MNCs against autologous tumor cells, production of IFN-γ and the number of perforin-containing lymphocytes. Demonstrated the ability of DCs primed lysate antigens of tumor cells to stimulate the cytotoxic activity of MNCs in more than 2-fold compared with the DC, untreated lysate, increase the number of perforin-containing lymphocytes and increased production of IFN-γ).Addition of IL-12 and IL-18 contributed to the strengthening of the effects obtained in co-culture with DCs.

P6.06.134

Immunotherapeutic and toxic effects of a triple fusion protein encompassing apolipoprotein A-I, interleukin-15 and the interleukin-15 receptor α sushi domain

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Interleukin 15 (IL-15) effects on CD8 T and NK lymphocytes hold promise to treat cancer. Fusion proteins have been engineered to provide IL-15 receptor alpha (IL-15Rα) mediated trans-presentation to lymphocytes and extend the plasma half-life of the cytokine. In this study, we report on a triple fusion protein combining apolipoprotein A-I, IL-15 and IL-15Rα's sushi domain. Apolipoprotein A-I conveys IL-15 to high-density lipoproteins (HDL), from which the cytokine is trans-presented by the IL-15Rα´s sushi domain. Such a construction was tested by hydrodynamic gene transfer to the liver of mice. Lethal toxicity was observed upon injection of 10µg of the expression plasmid. Mice died from an acute lymphocytic pneumonitis in which T and NK cells dominate a severe inflammatory infiltrate. Importantly, mice devoid of NK cells were not susceptible to such toxicity and mice lacking granzymes A and B also survived the otherwise lethal gene transfer. Lower plasmid doses (<2.5µg) were tolerated and dramatically increased the numbers of NK and memory CD8 T lymphocytes in the liver, spleen and lungs, to the point of rescuing the deficiency of such lymphocyte subsets in IL-15Rα$^{-/-}$ mice. Doses of plasmid within the therapeutic window successfully treated metastatic tumor models, including B16OVA lung metastasis of melanoma and MC38 colon cancer liver metastasis. Sushi-IL15-Apo as a recombinant protein was also bioactive *in vivo*, became conjugated to HDL and displayed immuno-therapeutic effects against metastatic disease.

P6.06.135

Cytotoxic effect of methanolic fractions of Scrophularia oxysepala on MCF-7 human breast cancer cell line

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Background: Cancer is a major public health problem in both developed and developing countries. It is the second largest common disease spread world-wide. Drug toxicity and resistance to chemotherapeutic agents make a struggle to treat cancer. For this reason, initial research focuses on traditional medicines or herbal formulations. As we know, breast cancer is the most commonly occurring cancer among women (about 25%). So, the cytotoxic effect of methanolic fractions of Scrophularia oxysepala was investigated in human breast cancer MCF-7. Scrophularia oxysepala plant was collected from Eastern Azarbaijan province, Iran. This is the first time that cytotoxic effect of Scrophularia oxysepala fractions and cell death mechanism of it, is studied.
Methods: The MCF-7 and L929 cells were seeded in 96-well culture plates in the presence of different concentrations (30, 50, 100, 200, 300 µg/µl) at 12, 24, 36 hours and then results were followed to determine cytotoxic effects on viability and apoptosis by MTT, TUNEL and DNA fragmentation.
Result: The obtained results declared that certain concentrations have cytotoxic effect on MCF-7 cell line and induce apoptosis while L929 cells, as normal cell line, remained intact.
Conclusion: Increased concentration of the fractions and treating time reduced cell viability. Our data showed that methanolic fractions of Scrophularia oxysepala have an apoptotic effect on MCF-7 cells and it might be an effective agent in cancer treatment.

P6.06.136
Discovery of chemotherapy induced ovarian cancer antigens by interrogating memory T cells

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Immunogenic cell death has been defined in mice by the ability of inducing a protective CD8 mediated immune response in tumor challenge experiments. To prove this hypothesis in human we took advantage of a reverse immunology approach by which apoptotic immunogenic antigens have been identified in ovarian cancer patients. Proteins induced ex-vivo in primary tumors by chemotherapy treatment were isolated and ranked according to immunological properties as indicated by IFNγ ELISPOT. A small number of proteins turned out to be immunogenic and were further analyzed by FACS analysis. Both memory CD4 and CD8 responses were observed upon overnight stimulation with selected proteins showing either a Th1 or a Th17 phenotype. Interestingly immune responses showed a statistical significant increase in patients who respond to chemotherapy than in non-responders. Immunogenic proteins isolated from 2D gel were therefore identified by mass spectrometry. Analysis of protein annotations showed an enrichment of anti apoptotic pathways suggesting a potential biological role of the immune correlates. The immunogenic properties of a subset of proteins were further confirmed using recombinant proteins opening the possibility of developing a predictive assay based on the immune responses. The T cell interrogation system represents a new strategy of reverse tumor immunology that proposes to identify chemotherapy induced tumor-associated antigens, which may then be validated as possible diagnostic tumor biomarkers or included in the design of neoadjuvant tumor vaccines.

P6.06.137
G1-4A, a polysaccharide from T. Cordifolia induces killer phenotype and increases dendritic cell immunogenicity in murine lymphoma model

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Dendritic cells (DC) play a central role in the development of adaptive immune response against tumor. DC are the only professional antigen presenting cells which generate a specific T cell response against tumor cell by the process of cross-presentation. In addition to activating cytotoxic T cells, DC also possess direct killer activity against tumor cells. In this study, we have investigated the effect of TLR4 agonist G1-4A, an arabinogalactan derived from *T. Cordifolia* to induce killer activity as well as maturation of DC. Immature bone marrow derived dendritic cells (BMDC) were treated with G1-4A for 48 hr. We observed increased expression of co-stimulatory molecules CD40/80/86 and MHC II which also serve as DC maturation markers. Augmentation of IL-12 and TNF-α production was also seen. Functional maturation of G1-4A treated DC was assessed by mixed leucocyte reaction. In addition to activating cytotoxic T cells, G1-4A treated DC co-cultured with tumor cells (E:T- 25:1) showed 35% increase in direct cytotoxicity as assessed by flow cytometry. This increased killer activity was associated with an increase in iNOS expression and nitric oxide production. DC can acquire tumor antigens through uptake of soluble antigens released from dying tumor cells or phagocytosis of apoptotic tumor bodies and this process could be expedited by direct killing of tumor cells. Tumor cell lysate pulsed mature BMDC (G1-4A) significantly decreased the tumor burden in a murine lymphoma model. The implication of differential efficacy of lysate pulsing and direct tumor cell killing to DC based tumor immunotherapy will be discussed.

P6.06.138
Bivalent CD28 agonistic aptamer: a novel adjuvant-vaccine for lymphoma

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Tumor vaccination is one of the main cancer immunotherapy strategies. It aims at stimulating a systemic immune response targeted to antigens expressed in the tumor lesions. One of the main challenges in developing effective vaccination protocols are the identification of expressed tumor-specific antigens and effective adjuvants which can stimulate a robust and durable immune response. Among the very few tumor-specific antigens described so far, probably the only one that can be identified in all patients with B-cell lymphoma is the idiotype, which is contained in the variable region of the tumor immunoglobulin. In this work, we have selected by SELEX a CD28 aptamer. As previously described, the agonistic capacity of an aptamer is achieved by dimerization. Two different dimeric forms were tested. Even though both dimeric forms preserved similar affinity for CD28, the costimulatory capacity was considerably improved, with a shorter and more flexible linker, surpassing the costimulatory capacity of agonistic anti-CD28 antibodies. In order to study the adjuvant effects of the CD28 dimeric agonistic RNA aptamer, A20 lymphoma-bearing mice were vaccinated with the lymphoma-idiotype complex with the CD28 agonistic aptamer. Mice treated with this vaccine formulation showed a significant increase in the overall survival compared to control groups. Mice vaccinated with A20 idiotype complex with the CD28 agonistic aptamer showed a more potent immune response, measured by IL-2 and INF-g production, and higher titers of anti-idiotype antibodies.

P6.06.139
Abrogation of tumor-associated immunosuppression by targeting tumor-infiltrating regulatory T-cell function can restore impaired T cell responses in patients with liver cancer

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Growing evidence shows that regulatory T cells (Treg) hamper the development of effective anti-tumor immunity in patients with cancer, and present a major hurdle for tumor immunotherapy. We recently described accumulation of activated CD4+FoxP3+ Treg at the tumor site in patients with liver cancer (hepatocellular carcinoma or metastasized colorectal cancer). These tumor-infiltrating Treg are potent suppressors of the local anti-tumor T cell responses, and they are characterized by the expression of higher levels of CTLA-4 and GITR than Treg in normal liver tissue or blood from the same patients. Now we show that treatment with a soluble form of the natural ligand of GITR (GITRL), or with blocking antibodies to CTLA-4, reduces the suppression mediated by tumor-derived Treg in *ex vivo* assays, restoring proliferation and cytokine production by effector T cells. These results suggest that modulation of intra-tumoral Treg function by either GITR-ligation or blocking CTLA-4 may be a promising strategy for alleviation of intra-tumoral immunosuppression, thereby contributing to immunotherapy induced effective immune responses in liver cancer patients.

P6.06.140
Cancer from hell: Devil Facial Tumour Disease

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Devil Facial Tumour Disease (DFTD) is a rare form of cancer that spreads like a contagious disease. It is a parasitic clonal cell line transmitted between hosts through biting. The disease is limited to Tasmanian devils, which are carnivorous marsupials endemic to Tasmania, an island state of Australia. It has a 100% mortality rate,

has reduced the population by 80% and may cause extinction of the Tasmanian devil in the wild within the next 30 years.
The lack of immunogenicity may be one possible mechanism used by DFTD cells to escape the host's immune system. Because of the lack of molecular tools and limited access to Tasmanian devils we developed a mouse model of DFTD. The tumour cells are immunogenic as immunocompetent BALB/c and C57BL/6 mice rejected xenograft implantation of 10^6 DFTD cells while immunocompromised NOD/SCID mice accepted the xenograft and developed DFTD tumours. Evidence for the immunological basis of the rejection was demonstrated with adoptive protection by splenocytes transferred from BALB/c to NOD/SCID mice.
In vitro stimulation of devil lymphocytes induced cytotoxic anti-DFTD activity. To determine if this could be effective in vivo, two NOD/SCID mice were co-injected with 5 x 10^5 DFTD cells and 5 x 10^6 stimulated devil lymphocytes. Seven weeks later the tumour growth was less than 10% compared to the control. Replication of this work with 10^4 DFTD cells plus lymphocytes failed to engraft the tumour providing insight into possible immunotherapy approaches for the disease.

P6.06.141
Expression patterns of cancer/testis antigens constitute a stable patient-specific fingerprint in multiple recurrent lesions of evolving metastatic melanoma

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Rare cases of metastatic melanoma with multiple, timely well-separated recurrences provide a unique opportunity to directly analyze the sequence of events during late stage disease progression, and identify conserved aspects of each patient's cancer that remain stable throughout multiple recurrences.
Here we present results of a study following 8 metastatic melanoma patients with an unusually protracted disease course, who developed a total of 26 recurrences over several years. Melanoma cells isolated from subsequent metastatic lesions were investigated by comparative genomic hybridization and global transcript analysis. We observed that subsequent recurrences did not display orderly trends toward a more aggressive phenotype in individual patients. Case-specific characteristics dominate late-stage cancer evolution, as differences among patients clearly exceed within-patient lesion variability, both at DNA copy number ($p<0.001$) and RNA gene expression level ($p<0.001$). Interestingly, tumor immunity-related melanoma genes were enriched among stable patient-specific traits, exemplified by several cancer-testis antigens (MAGE-A4, -B2, -C2, and BAGE-2) and antigen presentation-related transcripts (MHC I and II cluster, CIITA).
These results suggest that the late evolution of metastatic melanoma, which dramatically turns an indolent disease into a lethal phase, occurs through a series of random events that do not follow a consistent step-wise process. Within this random drift, however, key aspects of the immunologic profile of metastatic melanoma remain conserved even after years of progression, making advanced melanoma ideally targetable by personalized cancer immunotherapies. This study was supported by the Associazione Italiana per la Ricerca sul Cancro, IG 11746 and the Hungarian Academy of Sciences "Lendület" grant LP2012-49/2012.

P6.06.142
Targeting Th17-associated cytokines with a bi-specific antibody improves anti-myeloma responses

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Elevated levels of cytokines (interleukin-6, transforming growth factor-beta, and IL-1) in bone marrow (BM) micro-environment play an important role in both sustaining multiple myeloma (MM) cell growth and immune dysfunction in MM. We have previously demonstrated that interleukin-17 (IL-17) producing Th17 cells and associated cytokines, particularly, IL-23, are significantly elevated in blood and BM in MM and IL-17 promotes MM cell growth in vitro and in vivo (*Blood*. 2010; 115:5385-5392) making it an important therapeutic target for MM. Therefore, we pre-clinically evaluated a bi-specific antibody (AZ17) that targets both IL-23 and IL-6, the 2 cytokines with both direct and indirect effects on the initial differentiation of Th17 cells and the activation of effector Th17 cells in MM. We observe significant inhibition of MM cell growth (N=9) by AZ17 in the presence of BM stromal cells (BMSC). In addition AZ17 bi-specific antibody significantly reduced MM cell-colony size and number in MethoCult agar media. AZ17 significantly down-regulated IL-6 production in MM-BMSC co-culture (N=3). Moreover, we evaluated impact of AZ17 on CD8+ cytotoxic T lymphocytes (CTLs), generated from HLA-A2+ MM patient PBMCs using MM-specific peptides (derived from XBP1, CD138 and CS1), and cultured with or without HLA-A2+ MM cells and intra-cellular granzyme B was analyzed as an indicator of cytotoxicity. AZ17 bi-specific antibody significantly enhanced expression of granzyme B in MM-specific CTLs (N=5). These in vitro observations suggest efficacy of AZ17 in myeloma and provide the rationale for its in vivo and subsequently clinical evaluation both for anti-myeloma effects.

P6.06.143
Anti-tumor activities of human Vγ9Vδ2 T cells in combination with zoledronic acid in a pre-clinical model of neuroblastoma

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Neuroblastoma (NB) is the most common extracranial solid tumor in children. High-risk NB patients still have poor clinical outcome despite aggressive chemotherapy. Low to absent expression of surface major histocompatibility complex (MHC) class I molecules and defects in antigen processing machinery make human NB cells appropriate targets for MHC unrestricted immunotherapeutic approaches. Human Vγ9Vδ2 lymphocytes exert MHC-unrestricted anti-tumor activity and are activated by phosphoantigens whose expression in cancer cells is increased by aminobisphosphonates. These compounds are well established in the clinic as inhibitors of osteoclastic activity and possess antiangiogenic and proapoptotic properties for different human tumors. We have investigated the in vivo anti-NB activity of human Vγ9Vδ2 T cells and zoledronic acid (ZOL).
SH-SY-5Y human NB cells were injected in the adrenal gland of immunodeficient mice. After three days, mice received ZOL or human Vγ9Vδ2 T cells or both agents by intravenous administration once a week for 4 weeks.
A significantly improved survival was observed in mice receiving the combined treatment. Inhibition of tumor cell proliferation, angiogenesis and lymphangiogenesis, and increased tumor cell apoptosis were detected. Human Vγ9Vδ2 T lymphocytes were attracted to NB-tumor masses of mice receiving ZOL where they interfered with tumor microenvironment by expressing the cytotoxic molecule TIA-1 and by secreting IFN-γ that in turn induced CXCL10 expression in NB cells.
This study shows that human Vγ9Vδ2 T cells and ZOL in combination can inhibit NB growth in vivo and may provide the rationale for a phase I clinical trial in high-risk NB patients.

P6.06.144
Analysis of IL-10 serum levels in epithelial ovarian carcinoma in comparison to Ovarian benign Cyst

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Background: Tumor cells are influenced by the components of tumor milieu for instance; cytokine expression in tumor microenvironment can affect both anti-tumor immune response and tumor cell survival. Interleukin-10 (IL-10) is an immunosuppressive cytokine and several studies suggest that this cytokine could suppress anti-tumor immune response and might have key role in tumor progression.
Method: We aim to evaluate IL-10 content in serum of malignant ovarian cancer, benign cyst patients and normal individuals by ELISA.
Result: Serum level of IL-10 in ovarian cancer patients was higher than in benign cyst patients and normal individuals (P >0.05). In addition, IL-10 was significantly elevated in serum of normal individuals compared to benign cyst patients (P = 0.02).
Conclusion: These data support that Th2 cytokines such as IL-10 may be important in the tumor microenvironment and might play pivotal role in tumor progression by suppressing anti-tumor immune response. So, this cytokine can be used in the molecular targeting of ovarian cancer.

P6.06.145
Equipotent antagonism towards both ligands and excellent anti-tumor efficacy with a peptide inhibitor of PD-1 immune check point pathway

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Pioneering success of anti-CTLA4 antibody and the impressive clinical data on agents that target PD-1 or its ligands have opened novel avenues in the area for caner immunotherapy. However, along with impressive clinical activity, immune-related toxicities have also been observed in significant (25-30% with anti-CTLA4 and up to 15% with anti-PD1) patient population. Sustained inhibition of PD-1 signaling as a result of a long half-life (>15-20 days) is likely contributing to severe adverse effects observed in clinical trials with antibodies targeting PD-1 signaling. In the present study, we sought to characterize in detail a peptide antagonist of the PD1 signaling providing desired anti-tumor efficacy with shorter exposure for effective management of severe adverse effects.
A novel peptide AUR-012 (29-mer) constructed with elements from PD-1 displayed equipotent antagonism towards PD-L1 and PD-L2, and potent activity in rescue of proliferation and effector functions of lymphocytes. Sustained changes in circulatory immune cells and ability to secrete IFN-γ indicated that pharmacodynamic effects persist even after the clearance of AUNP-12 in animal models. In models of melanoma, breast, kidney and colon cancers, AUR-012 showed excellent efficacy in inhibition of primary tumour growth and metastasis. AUR-012 showed additive anti-tumor activity when combined with vaccination or a chemotherapeutic agent known to induce “immunological cell death”. In 14 days repeated dose toxicity studies, AUR -012 was well tolerated at 100x efficacious doses.
The findings demonstrating equipotent antagonism of both PD-L1 and PD-L2 signaling along with impressive efficacy in preclinical models support further development of AUR-012 for clinical use.

P6.06.146
Development of a monoclonal antibody against the canine CCR2 as a new targeted anticancer therapy in the scope of comparative oncology

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Introduction: In this project, it is proposed that the clinical application of a monoclonal antibody against the receptor of the Monocyte Chemotactic Protein-1 (MAb anti-CCR2), would hinder cancer progression, inhibiting macrophages recruitment. Canine mammary tumours (CMT) will be used as a model of human breast cancer, in face of molecular, histologic and clinical similarities of the disease in both species. Herein, the initial stages of the production of a MAb anti-CCR2 are described, aiming the future clinical application.
Material and Methods: RNA was extracted from canine bone marrow, CCR2 cDNA was synthesized by RT-PCR, cloned into a plasmid vector, and then confirmed through DNA sequencing. Transformed BL21 DE3 *E.coli* were used as a system of bacterial expression. The bacterial lysates were purified through an affinity chromatography column, to isolate the CCR2 protein which was subsequently verified through mass spectrometry.
Results: The purity of the isolated protein was confirmed by mass spectrometry analysis and it is now suitable for the mice immunisation. Throughout the purification process, the inclusion of urea in the lysis, wash and elution buffers was crucial to withdraw the most of the protein from the bacterial lysates.
Conclusion: Hitherto, the antigen production stage was concluded, obtaining a pure form of CCR2, ready for the immunisation of mice. Hence, it is now possible to progress to the following phases in the development of a Mab anti-CCR2 using hybridoma technology. Afterwards, an experimental therapy delivering anti-CCR2 monoclonal antibody to the tumour site will be applied to dogs with advanced CMT.

P6.06.147
Anti EGFR monoclonal antibody inhibits prostate cancer PC3 cells growth

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Epidermal growth factor receptor (EGFR) network appears to be a rich target for prostate cancer. Thus, in this current investigation, to pursue our newly developed anti EGFR monoclonal antibody (mAb) in prostate cancer, its inhibitory effect was investigated in PC3 cells. The binding specificity of antibody was examined by flow cytometry and immunofluorescence staining. Cultured cells were treated with various doses of the anti-EGFR mAb at different time points and the cellular and/or molecular impacts were assessed. MTT assay was utilized to examine the cytotoxic effects. Semi-quantitative RT PCR was used to evaluate the expression of EGFR and some important apoptosis signaling molecules (e.g., MAPK1, STAT5, Akt1 kinase). Flow cytometric and immunofluorescence staining analyses showed that the anti-EGFR mAb can bind to EGFR with high specificity. The results revealed that the anti-EGFR mAb can inhibit cell growth in a dose and time dependent manner. RT PCR analysis revealed that the binding of anti-EGFR mAb to its receptors can eventually result in down regulation of Akt1 kinase gene, but not MAPK1, STAT5 and EGFR genes. Based on our findings, it can be concluded that Akt1 is the most important downstream signaling molecules affected by anti-EGFR mAbs.

P6.06.148
Characterization of a genetic mouse model of lung cancer: a promise to identify oncoantigens associated with different stages of NSCLC progression

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Non-small cell lung cancer (NSCLC) accounts for 81% of all cases of lung cancer and they are often fatal because 60% of the patients are diagnosed at an advanced stage. Besides the need for earlier diagnosis, there is a great need for additional effective therapies. Lung cancer progression in $p53^{R172H\Delta g}$/$Kras^{G12D}$ mice that develop aggressive NSCLC, mimicking features of human lung cancer, was characterized by using non-invasive magnetic resonance imaging (MRI) and immunohistochemical analysis. To identify new NSCLC associated oncoantigens against which addressing the immune system, we performed gene expression and RNA seq profiling from total RNA extracted from lungs of 10, 20 and 30 week-old $p53^{R172H\Delta g}$/$Kras^{G12D}$ and wild type mice. Transcription profiling was performed taking in account the relationship between murine and human lung tumor progression. 282 genes were found significantly differentially expressed during the increment of tumor mass. Interestingly, 10 transcripts related to inflammatory response and 44 transcripts associated to inflammation disorder class were identified as up-regulated and down-regulated genes respectively, agreeing with the link existing between chronic immune activation and tumorigenesis in human NSCLC. RNA-seq library constructions was performed in order to identify novel oncoantigens and fusion transcripts associated with NSCLC, potentially useful as therapeutic targets. The receptor tyrosine kinase ROS1 was identified as the most promising candidate for vaccination against NSCLC since its overexpression correlates with poor prognosis for overall survival in human stage I NSCLC and increases in recurred NSCLC. A DNA vaccine against ROS1 has been generated and is now under investigation in $p53^{R172H\Delta g}$/$Kras^{G12D}$ mice.

P6.06.149
Tumour vaccine for oral cancer: prospects and challenges

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Oral cancer is one of the major reasons for morbidity and mortality worldwide. Despite significant advances in radiation, surgery and chemotherapy, substantial gap remains in its effective control. Even with all recent advances, the overall five-year survival rate for oral cancer has remained status quo at around 55% and hence, a need for effective vaccine has been felt for long that could confer long-term immunity and have a huge impact in control of this deadly disease. We did a retrospective literature search with key terms “Tumors Vaccines” AND “Oral Cancers” AND “Dentistry” through Pub Med, MEDSCAPE, MICROMEDEX, relevant immunology, dental and medicine journals and this was done independently by the authors. Majority of tumor vaccine research in dentistry is limited to Human Papilloma Virus (HPV) vaccines. However these are more focused on cervical cancer, with oral malignancy as only one of the secondary outcomes. It has been observed that HPV has a definitive role in oral cancers and these vaccines could have a positive impact in prevention of its ever-increasing incidence, though gray areas in research like optimal dosing schedule, efficacy in men, duration of protection and oral cancer specific studies remain unanswered. Emergence of second generation tumor vaccines against oral cancer does seem promising. There is a need to sensitize dental professionals with the latest advancement in tumor vaccines. A subtle shift from bench side research to bed side availability of an effective tumor vaccine in dentistry armamentarium does not seem to be a distant dream.

P6.06.150
Multifaceted immunotherapeutic effects of vitamin D-binding protein-derived macrophage activating factor (GcMAF) on human breast cancer and neuroblastoma cells

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The vitamin D axis includes vitamin D, its receptor (VDR) and vitamin D-binding protein (or Gc-globulin) that is the precursor of the Gc-globulin-derived Macrophage Activating Factor (GcMAF), a protein that has been proposed as an immunotherapeutic agent in the treatment a variety of conditions ranging from cancer to neurological disorders. Here we demonstrate that GcMAF (Immuno Biotech Ltd) added to human breast cancer cells (MCF-7, HPA Culture Collection) inhibited their proliferation, induced morphological changes, reduced vimentin expression and inhibited cancer cell-induced angiogenesis. We also describe the effects of GcMAF on the activation of macrophages added to MCF-7 cells. Macrophages (Raw 264.7, HPA Culture Collection) were activated by culturing them in the presence of 100 ng/ml GcMAF for 72 h prior to addition to the MCF-7 cells. The macrophages were added at a ratio of 1:1 to the MCF-7 cells. Photography was taken over a 60 h period using an Olympus CK2 microscope and a GXCAM-3 with NCH Debut capture software. After about 60 h, the irregular growth of the breast carcinoma cells was arrested and carcinoma cells were phagocytised by the activated macrophages. Finally, we demonstrate the GcMAF inhibited proliferation of human brain neuroblastoma cells (SH-SY5Y, ATCC) and induced morphological changes consistent with induction of apoptosis. Taken together, these results support the hypothesis that GcMAF has multiple biological effects: it inhibits cancer cell proliferation and metastatic potential; it reverts the neoplastic phenotype; it inhibits cancer cell-induced angiogenesis; and it stimulates tumoricidal macrophages that phagocytise cancer cells.

P6.06.151
In vitro cytotoxic effects of dichloromethane extract of Scrophularia atropatana on WEHI-164 cells

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Background: Cancer is one of the most prevalent diseases worldwide which is a major public health issue in both developed and developing countries. Recent studies have shown that medicinal plants are potential agents for cancer therapy .The present study was designed to explore the cytotoxic effects and the cell death mechanism of dichloromethane extract of Scrophularia atropatana on fibrosarcoma cell line (WEHI -164) compared to non-malignant (L929) cells.
Materials and Methods: WEHI -164 and L929 cells cultured in RPMI 1640 were exposed to a range of concentrations (100, 150, 200, 300, 400, 500, 600 µg/ml) of the extract and then incubated for 24, 36 and 48 h under specific condition (37 °C, 5% CO2). Cytotoxic effects were measured using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To detect apoptosis, cell death assays, TUNEL test and DNA fragmentation assay were performed.
Results: In the present study, the dichloromethane extract of Scrophularia atropatana exhibited considerable cytotoxic effects in WEHI-164 cells in a dose and time-dependent manner without cytotoxic effect on L929 cells. TUNEL, cell death and DNA fragmentation assays showed that the dichloromethane extract of Scrophularia atropatana induces apoptosis in WEHI-164 cells.
Conclusion: The data obtained demonstrate that dichloromethane extract of Scrophularia atropatana has a proapoptotic effects on WEHI-164 cell line and it may be effective agent in treatment of cancer.

P6.06.152
Tumour cell lysate loaded-Dendritic Cell vaccine induces biochemical and memory immune response in castration-resistant prostate cancer patients

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Purpose: Previously, we described a method to produce ex vivo antigen presenting cells (APCs)loaded with a melanoma cell lysate named TRIMEL as antigen source and activation factor. Tumour-antigen presenting cells (TAPCells)-vaccine constituted a safe therapy for melanoma patients, inducing immunological responses and increasing survival. Herein, we investigate prostate cancer cell lysate (PCCL)/TRIMEL-loadedTAPCellseffect in castration-resistant prostate cancer (CRPC) patients, co-injected with the *Concholepas concholepas* Haemocyanin (CCH) as an adjuvant. **Experimental Design:**Cell lysate capacity for inducing APCs maturation and T cell activation was analysed by flowcytometry and Elispot. Delayed-type hypersensitivity (DTH) reaction against PCCL and frequency of blood circulating $CD8^+$ memory T cells (Tm) were measured after treatment of twenty CRPC patients. Biochemical responses were analysed by measuring the prostate specific antigen (PSA) in serum. **Results:** The lysate mix induced phenotypic and functional mature DCs capable to activate PCCL-specific T cells. No relevant adverse reactions were observed. Six out of 14 patients showed a significantly decrease of PSA levels in serum. DTH+ patients showed a prolonged PSA doubling-time after treatment. An expansion of both central and effector $CD8^+$ Tm capable to produce IFN-γ, Granzyme B and Perforin was detected. All biochemical-responders showed a DTH reaction against prostate tumour lysate. **Conclusions:** Treatment of CRPC patients with PCCL/TRIMEL-loaded TAPCells,co-injected with CCH as adjuvant, is a safe procedure. Biochemical and memory immune responses were detected, although the limited number of cases makes necessary the confirmation of results in a phase II clinical trial.

P6.06.153
Evaluation of anti-FoxP3 vaccine efficacy following DNA-protein prime-boost immunization in mice

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FoxP3+Regulatory T cells (Tregs) are main obstacle against anti tumor immune responses and depletion of these cells is purpose of many studies in the field of cancer research. In this study we evaluated the breakdown of tolerance against FoxP3 by targeting FoxP3 as most specific marker for Tregs in comparison to CD25 using DNA-protein prime-boost vaccination.
C57/BL16 mice were primed against FoxP3 by one subcutaneously injection of DNA vaccine coding FoxP3 and boosted by two subcutaneously injections of FoxP3 recombinant protein. Three weeks after last injection, splenocytes separated, IFN-γ ELISPOT assay and proliferation assay were done to compare FoxP3 specific T cells in vaccinated and control groups. Serum of mice was evaluated for detecting anti-FoxP3 total IgG and IgG subclasses including IgG1, IgG2a, IgG2b and IgG3 by ELISA.
Our results indicated that IFN-γ producing cells and FoxP3 specific T cells significantly increased in vaccinated group. Serum levels of anti -FoxP3 total IgG in vaccinated group was significantly higher than control group. The analysis of anti-FoxP3 IgG subclasses showed a significant increase in IgG2a and IgG2b levels.
In conclusion, this study demonstrates that anti-FoxP3 DNA-protein prime-boost vaccination is possible to breakdown of T-cell and B-cell tolerance against FoxP3. Increased number of anti -FoxP3 IFN-γ producing cells and elevated levels of IgG2a and IgG2b can be considered as a consequence of a type 1- immune response that is proper for the purpose of this vaccination.

P6.06.154
The relevance of heat shock protein 90 and its role in cancer treatment

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Heat Shock Proteins (HSP) constitute a family of chaperones. These proteins are very important for cytoprotection and tolerance build-up against ischemia, hypoxia, high temperatures, infection, among others. They can be classified by their molecular masses. In this study, we deal with HSP90. Hematological malignancies and solid tumors have increased levels of HSP90. This overexpression accounts for the ability of cancer cells to maintain homeostasis in a hostile environment. HSP90 also allows the cancer cells to tolerate genetic alterations that would be fatal otherwise. The purpose of this study is to review the literature concerning HSP90, its functions, its role in immunology and the advances in the clinical development of HSP90 inhibitors on different kinds of cancer.

P6.06.155
Pseudo-exhaustion of CD8+ T cells in acute myeloid leukemia (AML)

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The prognosis of acute myeloid leukemia (AML) is poor due to high relapse rates after induction chemotherapy. Dendritic cell (DC)-based immunotherapy is a promising strategy to stimulate AML-specific immunity, particularly T cells. However, the functionality of T cells in AML patients is not well described. T cell exhaustion, characterized by an increased expression of inhibitory molecules and funtional deficits, has been suggested to contribute to immune evasion in various solid and hematological malignancies.
To characterize T cell exhaustion in AML, CD8+ T cell surface expression of CD244, CD160, PD-1 and TIM-3 as well as proliferation and cytokine production were measured. Results were compared to healthy controls (HCs) and untreated HIV-infected patients. To specify the stimulatory effect of DCs on AML patient-derived T cells, we cocultured DCs with autologous T cells.
We detected increased frequencies of CD244+, CD160+ and PD-1+ CD8+ T cells in HIV patients, while AML patients at time of diagnosis and relapse or during refractory disease had higher frequencies of CD244+ and TIM-3+ or PD-1+ CD8+ T cells, respectively. However, no functional impairment of AML patient-derived T cells was observed. DC stimulation resulted in upregulation of PD-1 and TIM-3, accompanied by high IFNγ secretion, that could be enhanced by PD-1 blockade.
Thus, we found elevated levels of exhaustion-associated inhibitory molecules on CD8+ T cells of AML patients, which were further upregulated upon DC stimulation. Our data suggest that immunotherapeutic strategies combining DC vaccination and immunomodulatory antibodies directed against T cell inhibitory molecules are particularly suited for AML treatment.

P6.06.156

Functionalized polyethyleneimine-based nanoparticles improve antigen cross-presentation of murine bone marrow derived macrophages and dendritic cells depending on size and zeta potential

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Nanoparticles (NP) have recently been used in several different applications in the field of nanomedicine. In most cases they are used as carriers for drug delivery, to increase solubility or to reduce drug toxicity, especially in cancer therapy. The activation of $CD8^+$ cytotoxic T cells, which thus are able to specifically attack and kill cancer cells is another promising application for NPs. Antigen presenting cells (APCs), like dendritic cells (DCs) and macrophages, play a key role in effectively inducing such a $CD8^+$ T cell immune response. They have the capability to take up antigens and present them on MHCII or cross-present them on MHCI, thereby inducing subsequent immunological processes.
We used NPs based on linear or branched poly(ethylene imine) of differently molar masses and varying concentrations of the murine model antigen ovalbumin (Ova). After incubation of bone-marrow-derived macrophages or dendritic cells with different NP concentrations, we coincubated $CD8^+$ OTI T cells and measured the expression of activation markers (CD69, CD80, MHCI, MHCII) as well as inflammation-related cytokines (IL-2, IFNgamma, IL-12, TNFalpha) with FACS and ELISA respectively.
For several NP combinations, the $CD8^+$ T cell and APC activation was significantly increased by more than tenfold compared to free Ova. In addition, this increase was most prominent in positively charged NP with a PEI:Ova relation of 0.04, independent of the polymerization architecture (linear or branched).
These PEI-Ova-NP are a promising tool as further conjugation with TLR-Ligands or other functionalizations might increase their immunogenicity.

P6.06.157

Immunopharmacodynamic response to blinatumomab in patients with relapsed/refractory B-precursor acute lymphoblastic leukaemia (ALL)

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Background: Blinatumomab is an anti-CD19/anti-CD3 bispecific T cell engager (BiTE®) that induces target cell-dependent, polyclonal T cell activation and proliferation, resulting in redirected lysis of $CD19^+$ target cells.
Methods: In a phase 2 study, adult patients (N=36) with relapsed/refractory B-precursor ALL received continuous blinatumomab IV infusion for 28 days ≤5 treatment/consolidation cycles.
Results: Lymphocytes in all patients responded in a similar fashion. After infusion start, peripheral B cell counts dropped to ≤1 B cell/µL in <1 week and remained undetectable throughout treatment. Peripheral T cell redistribution was characterized by swift disappearance within the first 2-6 hrs and subsequent recovery to baseline within several days. Otherwise, T cell counts remained at least stable in most patients. In some patients expansion of the T cell compartments was observed, likely due to specific proliferation of activated T cells. During the first infusion days, a significant proportion of T cells newly expressed the activation marker CD69, and granzyme B was detectable in serum. Additionally, a transient cytokine release dominated by IL-10, IL-6 and IFN-γ was observed in most patients shortly after infusion start, which was alleviated or absent in subsequent cycles. Consistent with previous studies, blinatumomab serum steady state concentrations (mean±SD) were 198±61 pg/mL and 694±236 pg/mL at doses of 5 and 15 µg/m²/d, respectively.
Conclusions: Immunopharmacodynamic response to blinatumomab was characterized by B cell depletion, T cell activation and redistribution, and release of granzyme B and cytokines, suggesting T cell engagement according to the expected BiTE® mode of action.

P6.06.158

Role of the transcription factor forkhead box P3 in breast cancer and metastasis

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Background: The transcription factor forkhead box P3 (FOXP3), known for its critical role in regulating the development and function of regulatory T cells, can be also expressed in cancer cells. We have previously shown that FOXP3 expression in breast cancer represents a strong prognostic negative factor for distant metastases-free survival. Thus, we investigated the role and molecular mechanism of action of FOXP3 in the metastatic process using breast cancer models. **Methods and results:** MDA-MB-231 human breast cancer cells, expressing low levels of FOXP3, was used to establish a tet-off inducible expression system, in which full-length (WT) FOXP3 or splice variant form (Δ2) were induced upon doxycycline removal. Real Time PCR and Western Blot analysis confirmed that in this condition FOXP3 mRNA and protein levels were significantly increased in both WT and Δ2 FOXP3-tet-off MDA-MB-231 clones. *In vitro*, an up-regulation of WT and Δ2 FOXP3 inhibited cell proliferation, but resulted in an increased migration and invasion. *In vivo* experiments, initially performed using Δ2 FOXP3-tet-off clones, revealed a significant increase in the number of spontaneous and experimental lung metastases, obtained by mammary fatpad and intravenous injections, respectively, when Δ2 FOX3 expression was induced *in vivo* by doxycycline removal. Accordingly, a significant modulation of the expression of genes relevant for dissemination and/or control of the immune response has been observed in Δ2 FOXP3-induced when compared to not-induced ones by gene microarrays analysis. **Conclusions**: These findings indicate that in breast tumor FOXP3 expression inhibits tumor growth while promotes metastatic capability.

P6.06.159

Production of lacZ inducible T cell hybridoma specific for human and mouse gp10025-33 peptides

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The key event in T cell activation is the recognition of a peptide bound to major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells (APCs). The enormous pool of peptides displayed on MHC makes it almost impossible to detect a given peptide-MHC complex on the surface of APCs by using conventional indirect methods. On the other hand, direct recognition of a selected peptide by the TCR results in generation of intracellular signals leading to initiation of the primary stages of T cell activation. To facilitate measurement of T cell activation and to enable identification of individual clones, β-galactosidase (*lacZ*) inducible $CD4^+$ and $CD8^+$ T cell hybrids were developed. Previous studies showed that heterologous *Escherichia coli* β-galactosidase (*lacZ*) gene, under control of the IL-2 entire enhancer region or the nuclear factor in activated T cells (NFAT) element alone, is specifically induced in transfected and activated T cells. Thus, activation of transfected T cells, results in synthesis of both IL-2 and *lacZ* gene products. Moreover, since the *lacZ* remains sequestered within the activated cells, chromogenic or fluorogenic substrate enables measurement of an activating event in a single T cell. Generation of the hybrids is relatively easy and allows maintenance in culture and the *lacZ* assay provides a rapid, sensitive and non-radioactive method for measuring T cell activation. In this study we isolated T cells from Pmel-1 mice and generated a *lacZ* inducible $CD8^+$ T cell hybridoma.

P6.06.160
Dendritic cells transfected with allel-specific polyepitope DNA-constructs stimulate cytotoxic response mononuclear cells of breast cancer patients in vitro

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Breast cancer (BC) has the highest frequency of women's cancers. We investigated the efficacy of DC transfected polyepitope constructs containing HLA-A0201-associated peptide CTL epitopes of different tumor-associated antigens for breast cancer to stimulate the cytotoxic response in culture mononuclear cells of patients with BC.
Monocytes from mononuclear cells (MNCs) of peripheral blood from BC-patients were cultured in the presence of rhIL-4, rhGM-CSF and rhTNF-a for generation of mature DC. Delivery of DNA-construct containing HLA-A0201-associated peptide CTL epitopes, in mature DCs was performed using magnetic transfection. To stimulate the cytotoxic activity was carried co-culture of DC and MNCs in several parallel cultures at meeting the same culture conditions - the ratio of DC: MNC 1:10 and stimulate Th1-polarized by rhIL-12 and rhIL-18. Autologous tumor cells and cell lines MCF-7 we used as target cells. DNA-transfected mature DC stimulate the cytotoxic potential of MNCs against autologous tumor cells, and against MCF-7. Addition of IL-12 and IL-18 at cocultivation MNCs and transfected DC is an effective method of stimulation the specific cytotoxic response against tumor cells. The use of autologous tumor cells as a target cell results in a more pronounced cytotoxic response, compared with the cell line MCF-7.
Thus, the use polyepitope DNA constructs for antigenic load of DC and stimulation of MNCs culture with transfected DC leads to the stimulation of cytotoxic response directed at tumor cell lysis.

P6.06.161
Supportive involvement of tumor-associated myeloid cells in RNA adjuvant therapy for cancer

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Tumor-associated myeloid cells including tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) contribute to tumor growth by inducing smoldering inflammation and immune suppression. On the other hand, acute phase inflammation elicited by the activation of pattern recognition receptors leads to tumor regression. PolyI:C, a synthetic analog of double-stranded (ds) RNA,induces retardation of tumor growth in mice by inducing Th1-type immune response. PolyI:C stimulates dendritic cells and macrophages to mature and produce proinflammatory cytokines through TLR3 and MDA5 receptors, which activates the adaptor molecules TICAM-1 (TRIF) and MAVS, respectively. The effect of polyI:C on tumor-associated myeloid cells has not been fully understood. Here, we show that polyI:C treatment induces phenotype switch of TAMs and MDSCs from tumor-supportive to tumor-suppressive in tumor-bearing mice. PolyI:C administration provoked TNF-α-dependent hemorrhagic necrosis and retardation of 3LL tumor growth. TAMs were the main producers of TNF-α and exerted cytotoxic activity. Furthermore, MDSCs as well as TAMs in polyI:C-treated mice exhibited the increase in the expression of Th1-inducing genes. PolyI:C-stimulated TAMs and MDSCs induced NK cell activation, which required the type-I IFN signaling. Both TICAM-1 and MAVS were involved in the activation of TAMs and MDSCs in vivo. These data suggest that tumor-associated myeloid cells contribute to tumor regression by directly inhibiting tumor growth and enhancing antitumor immune response by turning an NK activator in dsRNA treatment. Adjuvant immunotherapy using dsRNA will be a promising treatment for not only activating the innate array of immunity but also converting tumor-associated myeloid cells to antitumor effectors.

P6.06.162
The cross-talk between plasmacytoid DC and conventional DCs shapes the anti-tumor memory CD8$^+$T cells

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A key goal of vaccine immunotherapy is the generation of long-term memory CD8$^+$ T cells capable of mediating immune surveillance. The path from naïve precursor to effector and memory CD8+T cell development begins with interactions between mature dendritic cells and naïve T cells. However, the developmental program of generating memory T cells at DC level has not been established in detail.
We previously demonstrated that an iNKT cell-triggered DCs in vivo, linked innate and adaptive immunity by an administration of iNKT ligand-loaded tumor cells. In the current study, we have further elucidated a novel intercellular pathway governing the development of potent memory CD8$^+$ T cell responses against tumor antigens that is mediated through cross-presentation by CD8$^+$ DCs. Generation of CD8$^+$ memory T cells against tumor cells loaded with iNKT ligand depended on cross-talk between CD8$^+$ DCs and pDCs that was regulated by IFNalpha/IFNalphaR signals. The IFN-alpha production by pDCs was stimulated by OX40 signal from the iNKT cells, and HMGB1 signal from the dying tumor cells. Thus, integrating some key parameters on DCs can program the ensuing CD8$^+$ memory T cell response. These findings reveal an integrative pathway of intercellular collaboration on DCs for the generation of tumor-specific CD8$^+$ memory T cells that can be exploited for strategic vaccination in the setting of tumor immunotherapy.

P6.06.163
Immunization against tumor-associated macrophages - enhancement of DNA vaccine potency against legumain

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Tumor-associated macrophages (TAMs) are one of tumor-stroma components that are most important for tumor progression. Asparaginyl endopeptidase legumain that is overexpressed in M2-polarized TAMs have been identified as a suitable target for elimination of these cells. To enhance the efficacy of DNA immunization against legumain, we performed several modifications in legumain that could improve induction of immune responses. First, we mutated the RGD motif into GGD or RGG sequences. This alteration resulted in diminished maturation of legumain and impaired cellular localization into the Golgi apparatus. Then, as tolerance to self-antigens can be broken by activation of CD4$^+$ T cell help, we tried to enhance the immunogenicity of legumain by insertion of a foreign helper epitope, namely the p30 epitope from the tetanus toxin (aa 947-967). Finally, both modifications of legumain were combined. After DNA immunization of C57BL/6 mice with these constructs via a gene gun, we identified the CD8$^+$ T cell epitope $Lgmn_{111-119}$ that binds to H-2D^b molecules. Furthermore, we showed that mutagenesis in the RGD motif significantly enhanced the immune response against legumain. The addition of the p30 helper epitope induced the specific production of IFN-γ by T cells, but did not further increase legumain-specific immunity which might be caused by simultaneous activation of a Th2 response demonstrated by the production of IL-4. However, the beneficial effect of the helper epitope was proved after the depletion of regulatory T cells with antibody against CD25.
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P6.06.164
New role for Toll-like receptor 9 agonists, CpG-ODN as cancer cell "chemosensitizer"

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Synthetic oligodeoxynucleotides expressing CpG motifs (CpG-ODN) are TLR9 agonists that have shown antitumor activity in preclinical models and in humans based on their unique ability to stimulate both the innate and adaptive arm of the immune system. Here, we investigated the efficacy of CpG-ODN alone or in combination with different therapeutic agents (bevacizumab, Poly(I):Poly(C), gefitinib, cetuximab and cisplatin) in human ovarian carcinoma xenografts. We found that only the combinations CpG-ODN/cetuximab and CpG-ODN/Cisplatin increased the Median Survival Time (MST) vs. CpG-ODN alone. For a more advanced stage of ovarian cancer, the triple combination, CpG-ODN/cetuximab plus cisplatin strongly increased survival (MST=105.5; P=0.001 vs CpG-ODN/cetuximab group) as compared with other treatment groups. We demonstrated that the synergism between peritumorally administered CpG-ODN and cisplatin, a DNA-damaging chemotherapy agent, is due to the ability of CpG-ODN to induce down-modulation of the expression of DNA repair genes directly and/or by modulation of microRNAs expression (i.e. hsa-miR-302b). In contrast to tumor cells, analyses conducted on immune cells revealed an upmodulation of DNA repair genes. This suggests that, upon detection of an infectious agent via TLR9, immune cells might regulate DNA repair genes to decrease their susceptibility to possible pro-apoptotic signals during infections and induce down-modulation of DNA repair gene expression in infected (or transformed) cells to facilitate their elimination. Since experiments were performed using athymic mice which lack T lymphocytes, our findings highlight the importance of innate immune cells present in the tumor microenvironment in sensitizing cancer cells to DNA-damaging chemotherapy and, in general, during TLRs immunotherapy.

P6.06.165
Study of the anti-tumor effect of X- radiation in combination with DC vaccine

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Radiotherapy is one of the most important methods against cancer. By means of modifying the tissue structure and activity of the tumor, X-ray can enhance the immunogenicity of tumor cells, which release "danger signals", such as HMGB-1and hsp70, and up-regulates the expression of adhesion molecules, such as VCAM-1, MHC-I and ICAM-1. In our study, the aim is to observe the synergy effect between X-ray and DC vaccine against tumor. X-ray at a dose of 10Gy for sequential 3 times interval of 24 hours could induce 4T1 cells apoptosis as well as necrosis. The expression level of HMGB-1 indicated a increasing trend, while TGF-β with a decreasing trend. Compared with the control group, DCs loaded with protein from X-irradiated 4T1 could obviously induce the expression of ICAM-1,MHC II and CD86, and could powerfully stimulate the proliferation of allogeneic T lymphocytes.X-ray combined with DC vaccine significantly inhibit tumor cells growth *in vivo,* the production of IFN-γ of spleen lymphocytes and CTL activity enhanced. The combination of X-ray and DC vaccine up-regulated the number of $CD8^+$T cells, and had no obvious regulatory role for the T- Reg cells, but raising the ratio of $CD8^+$T/T-Reg. Moreover, the expression of IL -6 and IL -10 significantly decreased whereas the CXCL16/CXCR6 markedly up-regulated. Conclusion, X-ray could work in coordination with DC vaccine on anti-tumor by significantly improving the tumor microenvironmen.

P6.06.166
Dual targeting by TCR-redirected T cells enables remission from autochthonous mouse prostate cancer and long-term disease-free survival

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Hematopoietic cell transplantation and adoptive cell therapy can overcome mechanisms of central and peripheral tolerance, which limit protective immunity in tumor-bearing patients. In clinical trials, the infusion of tumor-derived lymphocytes, or of autologous PBMCs genetically engineered to express a tumor-specific TCR, has demonstrated some therapeutic efficacy in several solid tumors. We now report that the infusion of TRAMP (TRansgenic Adenocarcinoma of the Mouse Prostate)-derived autologous TCR transduced mouse T cells with specificity for an epitope from a tumor-associated antigen (the large T antigen) is per se insufficient for tumor remission. When tumor redirected T cells are however co-infused with autologous T cells engineered to recognize a minor histocompatibility antigen (HY-encoded Uty), therapeutic Graft versus Tumor Responses are observed and mice survival is significantly prolonged. Of note, Graft Versus Tumor Responses are not accompanied by Graft versus Host Disease in spite of ubiquitous HY expression, which may best be explained by alloreactive T cells remaining responsive only at the tumor-site. We hypothesize that the synergy of tumor and minor H antigen redirected T cells results from dual targeting of the transformed epithelium and/or the tumor associated stroma, among which HY-expressing fibroblast-like cells and endothelial cells can readily be identified. Current studies are aimed at characterizing the tumor-associated stroma, at visualizing the dynamics of T cell infiltration into the tumor by non-invasive in vivo imaging, and at testing various combinations of TCR engineered T cells.

P6.06.167
Targeting the self-antigen nucleolin for antibody immunotherapy of breast and other cancers

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Not all autoreactive antibodies are harmful, despite their role in autoimmune disease. Autoantibodies targeting tumor antigens, growth factors and their receptors are currently used therapeutically for treatment of cancer and autoimmunity. Ordinarily, healthy subjects do not exhibit strong secondary antibody responses to self-proteins, because most autoreactive B cells do not receive sufficient T cell help required for the germinal center reaction and for differentiation into IgG secreting cells. Our data demonstrate that rare, potentially therapeutic autoreactive B cells can be efficiently rescued from tonsil with EBV immortalization, and manipulated *in vitro* to secrete IgG, by supplying factors that mimic antigen stimulation and T cell help. Because this process is performed wholly *in vitro*, it constitutes a rapid and cost effective technology, resulting in the isolation of fully human antibodies. In addition, antibodies can be generated against highly toxic antigens, since immunization is unnecessary. As proof of concept, we have developed a panel of human monoclonal antibodies targeting the self-tumor antigen nucleolin for use in cancer immunotherapy. Nucleolin is normally expressed in the nucleus of all cells, but it is over-expressed in both the cytoplasm and on the cell surface of tumor cells in the most common forms of breast cancer, making nucleolin a new type of tumor antigen. The nucleolin specific antibodies elicit complement and ADCC-independent cytotoxicity of breast tumors, without affecting normal breast tissue. This is an important attribute for treatment of immunosuppressed cancer patients. The antibodies are currently undergoing pre-clinical testing against breast cancer.

P6.06.168
Low dose radiation-mediated enhanced effector function regulated by the ERK1/2 pathway in human T cells

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Radiation therapy (RT) is a primary treatment in many haematological and solid cancers, causing irreparable damage to tumour cell DNA. However, immune cells are also exposed to radiation during RT. We previously reported that T cell responses to recall antigens were enhanced by low dose radiation in vitro. Here we study the characteristics and mechanism of low dose radiation-induced enhancement of T cell function. We used PBMC from healthy donors or prostate cancer patients, T cells purified from PBMC and peptide-specific CD8+ T cell lines. Irradiation was carried out using a 137Cs-source (0.637Gy/min). Low dose radiation (0.6-2.4Gy) enhanced both peptide- and TCR cross linking-induced T cell proliferation and IFNg production. Irradiated T cells responded to 10-fold lower concentration of peptide with a broader Th1 cytokine profile. Cytotoxic activity of tumour antigen-specific T cells was also elevated in a dose-dependent and antigen-specific manner. T cells remained “primed” to give enhanced responses to stimulation even 24h after radiation. Similar results were observed with T cells from prostate cancer patients who received 2.0-2.4Gy localised (pelvic) radiation, as TCR cross-linking resulted in significantly elevated IFNg and TNFa production 24h after RT. Significantly increased ERK1/2 phosphorylation was observed in the irradiated plus stimulated T cells. The role of this pathway in enhancing T cell responses was subsequently confirmed in blocking experiments. We report here a direct effect of low dose radiation on T cells resulting in a significant enhancement of T cell function. This effect maybe exploited to boost T cell responses in immunotherapeutic settings.

P6.06.169
Enhancement of antigen-specific CTL inducing ability in the leukemic plasmacytoid dendritic cell line (PMDC11) by lentiviral vector-mediated transduction of caTLR4 gene

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A leukemic plasmacytoid dendritic cell (pDC) line with HLA-A*02:01/24:02, PMDC05, which was established in our laboratory, acquired a higher expression of CD80 after transduction with lentiviruses encoding the CD80 gene. CD80-expressing PMDC05 was named PMDC11. In order to establish a more potent antigen presenting cells for cellular immunotherapy in tumors or severe infections, PMDC11 cells were transduced with a constitutively active (ca) TLR4 gene by using the Tet-On system (caTLR4-PMDC11). CD8+ T cells from healthy donors with HLA-A*02:01 were co-cultured with mutant WT1 peptide-pulsed PMDC11 or caTLR4-PMDC11 cells. IL-2 (50 IU/ml) and IL-7 (10 ng/ml) were added on day 3 of culture. Priming with mutant WT1 peptide-pulsed PMDC11 or caTLR4-PMDC11 was performed every week and two thirds of the IL-2/IL-7 containing medium was replenished every 3-4 days. Immediately before the priming with PMDC11 or caTLR4-PMDC11 cells, cultured cells were analyzed for the percentage and number of CD8+/WT1 tetramer+ T cells. CD8 T cells positive for both wild and mutant WT1 tetramer were generated after 2-3 weeks of culture and CD8+/WT1 tetramer+ T cells were much increased in caTLR4-PMDC11-primed CD8+ T cell culture compared with PMDC11-primed CD8+ T cell culture. These CD8+ T cells co-cultured with caTLR4-PMDC11 cells were demonstrated to be cytotoxic to WT1-expressing target cells. These data revealed that the antigen-specific CTL inducing ability of PMDC11 is enhanced by transducing caTLR4 gene, and that caTLR4-PMDC11 cells could be applied as potent antigen presenting cells for generating antigen-specific CTLs in adoptive cellular immunotherapy against tumors and severe viral infections.

P6.06.170
TAPCells based vaccination induce specific anti NY-ESO1 immune response in melanoma patients

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Introduction: Malignant melanoma is a highly aggressive skin cancer with increasing incidence worldwide. Though its epidemiological importance, few effective therapies have been developed. Our dendritic cells based immunization (TAPCells) is a interesting approach that, in our hands, improved median survival rate over three times on patients that developed a DTH reaction to a melanoma lysate (TRIMEL). NY-ESO1 is a highly immunogenic tumor associated antigen (TAA) that seems relevant in melanoma and has been used in other protocols of immunotherapies.
Materials and Methods: Sera from 20 healthy donors and 40 TAPCells immunizated advanced stage melanoma. Anti NY-ESO1 specific antibodies were tested by ELISA. All experiments were approved by the Bioethical Committee for Human Research of University of Chile, Faculty of Medicine.
Results: Melanoma patients have higher anti NY-ESO1 titers than controls. Even more, TAPCells immunization induce an increase in these titers in both responding and non responding melanoma patients. Survival rates according to the anti NY-ESO1 levels are currently under analysis.
Conclusion: Beside inducing cellular immunity, TAPCells based immunotherapy induce humoral response anti tumoral associated antigens in melanoma patients.

P6.06.171
HSV-1 activated plasmacytoid dendritic cells inhibit melanoma cell growth

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Malignant melanoma is an aggressive tumor with increasing incidence, frequent metastasis, and poor prognosis. On the other hand, melanoma is a very immunogenic type of cancer with repeatedly reported spontaneous regression. To date, the success of immunotherapies using tumor antigen-pulsed monocyte-derived dendritic cells for advanced stages of melanoma has been limited, although significantly correlated with the induction of tumor-specific cytotoxic T lymphocytes.
Our aim is to improve these tumor-vaccination approaches using virus-activated plasmacytoid dendritic cells (PDC). PDC can engulf, process, and present tumor antigens; have cytotoxic activity; are recruited into the skin to melanoma lesions; and are the main producers of type I interferons, which contribute to activation of the adaptive immune system. INF alpha is currently used as adjuvant therapy in melanoma patients. Our hypothesis is that upon appropriate vaccine stimulation, PDC are able to induce immune responses against melanoma cells. Therefore, we activated PDC with a replication-deficient herpes simplex virus type 1 and cocultured them with five melanoma cell lines and six patients’ primary isolates. Cell-free PDC supernatants reduced the melanoma cell growth only to a minor extent, whereas cocultures with virus-activated PDC effectively killed nearly all melanoma cell lines. To better define the underlying mechanism, we used a neutralizing antibody against the IFN-a receptor (IFNaR Ab). The effects of soluble components were mainly due to IFN-a, whereas effects of activated PDC could not be neutralized by the IFNaR Ab. Taken together, our results suggest that virus-stimulated PDC inhibit melanoma cell growth via a cell-contact dependent mechanism.

P6.06.172
Polysaccharide nanocomplexes harboring split vaccine and TLR ligands are potent vaccine carriers against influenza

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Nucleic acid based TLR ligands are widely used in the vaccine formulations as potent adjuvants against pathogens. Yet, elimination of these labile ligands either by nucleases or by adsorption onto serum proteins restricts their *in vivo* therapeutic applications. Delivering these labile agents within proper depot carrier systems can provide increased *in vivo* stability and efficient targeting/internalization to relevant immune cells. We recently demonstrated that β-glucan polysaccharide (PS) is recognized by TLR2 and initiates proinflammatory signaling cascade. Moreover, nanocomplexes of PS with pIC (TLR3L), R848 (TLR7L) and CpG ODN (TLR9L) induce synergistic and more pronounced immune activation *in vivo*. Here, nanocomplexes with SV (split vaccine of influenza virus) along with different TLRL were used as a candidate vaccine against flu. C57BL/6 mice either i.n. or i.d. immunized with the mixtures of TLRL+SV, and their PS nanocomplexes. Sera of nanocomplex treated animals` induced robust IgG and Th1-dependent antibody production after booster i.n. injection. Additionally, significantly higher IgA titers were detected from both nasal wash and sera of PS(pIC+SV) treated mice. Spleen T-cells from i.n. immunized mice with PS(TLRL+SV) nanocomplexes strongly responded against both MHC-I and MHC-II influenza-specific peptides as-evidenced by IL-2 and IFN-γ productions. However, after i.d. immunization of PS/TLRL+SV vaccine formulations, IgG2c levels at all investigated titers after booster injection were lower than the levels of IgG1 suggesting a Th2-biased response. Taken together, our results implicated that i.n. injection of PS(TLRL) nanocomplexes led to a more pronounced mucosal as well as humoral responses against H1N1 Ag along with efficient CTL response.

P6.06.173
Protein chimera of IL-2-S4B6 mAb exerts similar or even higher biological activity as IL-2/S4B6 mAb immunocomplexes in vivo

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In vivo biological activity of IL-2 can be dramatically increased by complexing IL-2 with certain anti-IL-2 mAbs. The immunocomplexes of IL-2 and anti-IL-2 S4B6 mAb (henceforth IL-2/S4B6) are highly stimulatory for recently activated naïve $CD8^+$ T cells, memory $CD8^+$ T and NK cells ($CD122^{high}$ populations), and moderately stimulatory for T_{reg} cells. Moreover, they were reported to have potent anti-tumor effects.
We designed and produced protein chimera consisting of IL-2 linked to light chain of anti-IL-2 mAb S4B6 through flexible oligopeptide spacer $(Gly_4Ser)_3$ and characterized it in terms of structure that proved to be similar to IL-2/S4B6 immunocomplexes. Moreover, *in vitro* and *in vivo* experiments showed that the protein chimera mimics IL-2/S4B6 immunocomplexes not only structurally, but also functionally as it was able to stimulate proliferation of purified and activated OT-I $CD8^+$ T cells *in vitro* and induce vigorous expansion of purified CFSE-labeled OT-I $CD8^+$ T cells activated by injection of low dose of SIINFEKL peptide *in vivo*. This *in vivo* expansion was even higher than that seen after administration of IL-2/S4B6 immunocomplexes.
In conclusion, IL-2-S4B6 mAb protein chimera mimics IL-2/S4B6 immunocomplexes structurally as well as functionally, and it is probably even more potent than its immunocomplex counterpart.
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P6.06.174
Search for an association between JC virus (JCV) and colorectal cancer in Tunisian patients

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Colorectal cancer (CRC) is one of the most common malignancies throughout the world. Several risk factors, both genetic and environmental, have been linked with colorectal carcinogenesis. Recent reports suggested an association of some viruses, especially JCV and human papilloma virus (HPV) with CRC. In the present study, we looked for the presence of JCV DNA and T-Ag expression in fresh colorectal tumor biopsies and in paired adjacent normal mucosa collected from forty seven Tunisian patients. DNA was extracted from fresh tissues using the Invitrogen Purelink Genomic DNA mini Kit. Two PCR methods were used to amplify a region of the T gene: a simple PCR with the JCTs/JCTas primer pair specifiying a 154 bp sequence, and a nested PCR using JCTs/JCTas as external and the pair 4129/4209 specifiying a 98 bp sequence, as internal primers. T-Ag expression was determined by immunohistochemical staining using a mouse monoclonal antibody (clone PAb416) directed against SV40 T-Ag that cross reacts with JCV T-Ag. PCR data indicated that 10 of 47 (22%) CRC tumors were positive for JCV DNA whereas all paired adjacent normal mucosa biopsies were negative. On the other hand, immunohistochemical staining showed nuclear T-Ag expression in all ten JCV DNA- positive samples and in 3 additional tumor samples which appeared DNA-negative by PCR. In conclusion, our data are in line with an association of JCV with at least a subpopulation of colorectal tumors.

P6.06.175
Chemotherapy can enhance trastuzumab-mediated ADCC

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Recent clinical data indicated a synergistic therapeutic effect between trastuzumab and taxanes in neoadjuvant setting in HER2-positive breast cancer patients. We hypothesized that the mechanism by which trastuzumab synergizes with taxanes might be mainly through ADCC and that it might depend on the ability of drug-induced stress to improve NK cell effectiveness.
A rapid and dynamic surface expression upmodulation of NK activator ligands was observed in HER2-positive BT474 and MDAMB361 breast carcinoma cell lines treated with taxotere both *in vitro* and *in vivo*, accompanied by about a 15-40% increase in *in vitro* trastuzumab-mediated ADCC. Consistently, antibodies blocking NK receptors (NKG2D and CD226) specific for these ligands significantly reduced this enhancement. We obtained PBMC from 8 HER2-positive breast carcinoma patients isolated at different time points during the neoadjuvant treatment protocol with taxanes followed by trastuzumab. Analysis of NK cells showed that even if their amount among PBMC decreased, their overall activation status increased after chemotherapy, resulting in *in vitro* ADCC levels on BT474 similar to those obtained with PBMC isolated before treatment. Notably NKG2D receptor on NK cells was significantly enhanced by chemotherapy treatment.
Altogether, our results indicate that taxanes can increase tumor susceptibility to ADCC acting on both tumor and NK cells, raising the possibility of identifying optimal chemotherapy administration schedules and new potential strategy to maximize trastuzumab-mediated ADCC.
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P6.06.176
Osteosarcoma antigen PBF-derived peptides could elicit immunological response in patients with refractory osteosarcoma

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Osteosarcoma is the high-grade malignant tumor of bone and occur mainly in the 1st and 2nd decades. Although the introduction of neo-adjuvant chemotherapy increased 5-year survival rate to 70%, the prognosis of non-responders to chemotherapy is still poor. Towards the development of peptide vaccination therapy for osteosarcoma, we previously identified autologous CTL-defined osteosarcoma antigen PBF and CTL epitope in the context of HLA-B55 using cDNA library expression cloning. Next, we identified and characterized HLA-A24 and HLA-A2-restricted PBF-derived peptides (PBF A24.2 and PBF A2.2) using limiting dilution/MLPC/tetramer procedure. On the basis of these results, we started clinical phase I trial of vaccination of PBF-derived peptides in HLA-A24+ or HLA-A2+ patients with refractory PBF+ osteosarcoma. Each PBF A24.2 (1mg or 10mg) and PBF A2.2 (1mg) was vaccinated with IFA in five and three cases, respectively. Six-time vaccination schedule was completed in two. After vaccinations, peptide-specific immunological response was observed in four of five HLA-A24+ and two of three HLA-A2 patients using tetramer or ELISPOT. All patients were evaluated as PD under RECIST criteria. In one HLA-A2+ patient, however, necrotic change and infiltration of CD8+ cells in metastatic cutaneous lesion was observed and suggested the effect of peptide vaccination. CPA was occurred in one patient after 5th vaccination of PBF A24.2. In this case, the cause was conceived as adriamycin cardiomyopathy. No other severe adverse events were observed. For further characterization of the effect of vaccination, the clinical trial is still continued.

P6.06.177
Tattoo delivery of Semliki Forest virus-based immunization strategies against cervical cancer

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Cervical cancer is the second most prevalent cancer among women worldwide. The disease develops as a result of infection with high-risk human papillomavirus (HPV) through persistent expression of early proteins E6 and E7 with transforming capacities in cervical epithelial cells. Numerous immunotherapeutic strategies have been described to induce both cellular and humoral immune responses against the early proteins. Our group pioneered the application of a replication-defective recombinant viral vector system based on Semliki Forest virus (SFV) for vaccination against cervical cancer. In preclinical studies, we demonstrated that a replicon vaccine, based on recombinant SFV encoding HPV E6 and E7 (rSFVeE6,7), induces robust HPV-specific cellular immune and memory responses resulting in excellent therapeutic anti-tumor efficacy. DNA vaccine administration by tattooing can induce potent immune responses. Yet little is known about the efficacy of viral vectors delivered by tattooing.
In this project, the efficacy of tattoo injection with rSFVeE6,7 and SFV-based DNA vaccines was explored.
Our results showed that intramuscular (i.m.) injection of a SFV-based DNA vaccine and rSFVeE6,7 resulted in lower antigen expression in contrast to tattoo injection. Despite the lower antigen expression, tattoo injection was associated with a higher immune response with both vaccine strategies. rSFVeE6,7 also elicited a more potent immune response in contrast to our SFV-based DNA vaccine.
This preliminary study suggests that tattoo immunization has a higher intrinsic immune potentiating activity compared to i.m. immunization. The explanation for this enhanced efficacy will be further studied to unravel the immune mechanisms behind this effect.

P6.06.178
A new identified CD20-spliced protein as potential target for T cell-based immunotherapy in B-cell lymphoma/leukemia patients

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CD20 is an antigen expressed on the cell surface of B lymphocytes. It is targeted by therapeutic monoclonal antibodies such as Rituximab in various B cell lymphomas and leukemias. We have identified an alternative transcript variant (ΔCD20) of the human MS4A1 lacking most of the transmembrane segments (Henry et al., Blood 2010). Whereas it was not detected in quiescent B lymphocytes from healthy donors, its expression is detectable in activated EBV infected B cells and in various B cell pathologies such as follicular lymphoma, chronic lymphocytic leukemia, diffuse large B-cell lymphoma and mantle cell lymphoma.
In the present study we show that the Rituximab treatment of normal B lymphocytes or B-cell lymphomas induces the expression or overexpresses the ΔCD20 protein respectively. Thereby ΔCD20 could be an attractive target for T cell immunotherapy in association with Rituximab treatment. Thus, with a reverse immunology approach we sought to identify MHC-I and -II restricted epitopes derived from the ΔCD20 specific sequence. The HLA-A2/DRB1*0101 mouse model was used to identified a ΔCD20 specific epitope presented in the HLA-A*0201 context, and HLA-DR epitopes. ΔCD20 specific CD4+ T cell producing IFN-γ were also observed in lymphoma patients with various HLA class II contexts. Finally, isolated ΔCD20 specific CD4+ T cells clones are capable to induce autologous lymphoma B cell apoptosis after in vitro co-culture.
These results identify ΔCD20 as an attractive target for T cell-based immunotherapy and Rituximab combination treatment for B cell lymphomas and leukemias.

P6.06.179
Immunotherapy for the liver metastases prevention with a vaccine strain of an attenuated *Salmonella* Typhi

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In previous studies we demonstrated that an attenuated *Salmonella* Typhi vaccine strain (CVD 915) induces tumor-specific immune and therapeutic responses, following intratumoral and subcutaneous inoculations, in mice bearing a subcutaneous mammary adenocarcinoma (LM3). The aim of this work was to study whether orogastric (og) administration of CVD 915 can prevent tumor cell implantation in liver, in a mouse model of liver metastases. Each BALB/c mouse received og $5x10^9$UFC of CVD 915 or PBS (control group). After 24 hours, mice were subjected to surgery and 50,000 LM3 cells were injected into their spleens. Then, a splenectomy was performed. After 24 h of bacterial treatment, CVD 915-treated mice exhibited elevated IFN-γ serum levels. Seven days later, *Salmonella* treatment stimulated the proinflammatory Th1 cytokines production (enhanced IFN-γ level in serum, $p<0.001$, and detectable serum TNF-α and IL-12 levels). The prophylactic treatment with *Salmonella* significantly decreased the occurrence of liver metastases as well as their average volume (1.9 vs. 6.2 mm^3) 21 days after surgery, as compared with control mice ($p<0.05$). Moreover, at that time, an increased frequency of $CD4^+$ lymphocytes but a decreased proportion of B cells ($B220^+$) in both liver and peripheral blood were observed in CVD 915-treated animals ($p<0.05$). These results indicate that a CVD 915-based immunotherapy used preventively via og route induces an antitumor immunity and prevents the implantation of metastasis in a vital organ. The use of this vaccine as an adjunct therapy to conventional cancer treatments, such as surgery, should be assessed in the future.

P6.06.180
Antitumor activity of a *Salmonella* Typhi vaccine strain in a mouse model of T-cell lymphoma

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Previously we demonstrated the therapeutic efficacy of an attenuated vaccine strain of *Salmonella* Typhi as a cancer immunotherapy in a mouse model of subcutaneous T cell lymphoma. EL4 tumor-bearing mice treated with *Salmonella* Typhi CVD 915, by injection into the tumor and in the area of the draining lymph nodes, showed reduced tumor growth and increased survival. In this work, we further studied the therapeutic efficacy and the antitumor effector mechanisms promoted by this bacterium. Our results indicate that the treatment with *Salmonella* promoted a reduction in the mitotic index of the tumors (p <0.05) and delayed the development of palpable metastases in the lymph nodes (p <0.001). Furthermore, the administration of *Salmonella* in tumor-bearing mice promoted leukocyte infiltration, mainly neutrophils (p <0.01) and decreased IL-10 levels in the tumor microenvironment (p <0.05), and also reduced LTreg frequency in tumor draining lymph nodes. Under these conditions, *Salmonella* remained viable within the tumor, and tumor-infiltrating neutrophils released TNF-α and expressed bacterial antigens. Finally, *Salmonella* was found to bind to tumor cells and exert oncolytic activity upon direct contact with the tumor cell. These results demonstrate the efficacy of a vaccine strain of *Salmonella* Typhi as an oncolytic and immunotherapeutic agent against a highly malignant tumor.

P6.06.181
Lcn2 secreted from DCs induces CD8+ T-cell apoptosis, it is involved in T-cell priming, and upon DCs vaccination trigger a Th1 phenotype

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Lipocalin 2 is expressed from dendritic cells during the late phases of Dex and LPS maturation. DCs are professional antigen presenting cells capable of regulating immunity. Besides the classical understanding concerning mouse DC subsets, new studies are suggesting that DCs follow a multi-stage differentiation programme after encountering danger signals, which facilitates the fine-tuning of the immune responses. Tolerance maintenance, immune-stimulation and immune-suppression are time dependent functions of the DCs. Lcn2 is active in defence against bacteria via capturing of iron, and it is involved in autocrine apoptosis of T-cells. In order to understand the function of Lcn2 in the DC/T-cell interaction, we stimulated mouse bone marrow Lcn2-/- or wild type DCs with Dex and LPS and co-cultured with T-cells from OT-I or OT-II transgenic mice. The induction of apoptosis in T-cells was highly reduced using the Lcn2-/- compared to WT DCs. An in vivo Cytotoxic T-Lymphocyte killing assay using LPS treated Lcn2-/-- or WT-DCs as vaccine showed that the specific killing ability was diminished in mice that had received Lcn2-/--DCs (in WT-DCs 96,18 ± 0,5057, in Lcn2-/--DCs 87,12 ± 2,034, p<0.01). We conclude that Lcn2 is involved in the priming of CD8+ and CD4+ T-cells in a dose dependant manner, and induces a TH1 microenvironment. In DCs treated with Dex and LPS high expression of Lcn2 is involved in CD8+ T-cell apoptosis. This study aims to contribute to better understand the DCs' biology and to improve their use in cancer vaccination.

P6.06.182
Allogeneic dendritic cells induce potent antitumor immunity by activating KLRG1$^+$CD8 T cells

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There is increasing evidence that strong antitumor effects can be elicited by allogeneic vaccines, although the underlying mechanisms remain undefined. In this context, first, we observed that allogeneic DC (alloDC) vaccination could significantly inhibit tumor progression in C57BL/6 mice, as well as in immunodeficient $\beta_2m^{-/-}$ mice. Then, we found that alloDC immunization could result in a predominant increase in a subpopulation of CD8 T cells co-expressing T cell–activated markers and NK receptors, among which the most representative is KLRG1. Subsequently, we revealed the positive correlation between the amount of KLRG1$^+$CD8 T cells and antitumor efficacy in alloDC-vaccinated mice, followed by a further exploration demonstrating the involvement of KLRG1$^+$CD8 T cells in tumor rejection, as they showed more efficient migration to tumor sites and more potent antitumor effects when adoptively transferred into naïve mice. Finally, alloDC-activated KLRG1$^+$CD8 T cells were shown to exert direct cytotoxicity on tumor cells by the granule exocytosis pathway. The data we present in this study suggest that the small KLRG1$^+$CD8 T cell subset, which was thoroughly ignored in normal mice, represents an important allo-stimulated antitumor effector subset. Based on the high antitumor efficiency of these KLRG1$^+$CD8 T cells, a therapeutic vaccination using allogenic APCs might be a potential anti-tumor approach.

P6.06.183
Expression of cancer testis antigens (CTAs) and its correlation with clinical parameters in Chinese hepatocellular carcinoma

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Background: Many therapies such as surgery, chemical or physical approaches have been used for treatment of HCC; however, the outcome is still poor. Cancer immunotherapy is considered to be one of the promising strategies in recent years. We aim to evaluate the expression of cancer testis antigens (CTAs) in Chinese hepatocellular carcinoma and its correlation with clinical parameters. Methods: Expression levels of CTAs including MAGE-A3, MAGE-A4, MAGE-C2 and NY-ESO-1 mRNA were detected with reverse transcription polymerase chain reaction (RT-PCR) in HCC tissues and corresponding adjacent non-cancerous tissues from 54 HCC patients. Genes of five samples with positive PCR results were sequenced. Results: 87.0% of HCC tumor tissue samples expressed at least 1 CTA. HCC adjacent non-cancerous tissues did not express CTA. 77.8% tumor tissue samples expressed MAGE-A3 mRNA, 35.2% samples expressed MAGE-A4 mRNA, 68.5% samples expressed MAGE-C2 mRNA and 13.0% samples expressed NY-ESO-1 mRNA. The expression of CTA showed correlation with Ki67(r=0.35, P0.05). The expression of MAGE-A3 showed correlation with the high expressions of AFP in serum (r=0.42, P<0.01) and lymph node metastases (r=0.31, P=0.02). The expressions of NY-ESO-1 showed no correlation with the expressions of AFP and lymph node metastases (P>0.05). Conclusion: Our findings demonstrate the tumor-specific expression of CTAs genes show correlations with

tumor stages and proliferation and may represent useful targets for tumor specific immunotherapy in HCC patients.

P6.06.184
Cell surface delivery of TRAIL strongly augments the tumoricidal activity of T-cells

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Adoptive T-cell therapy has the potential to enhance antitumor immunity, although it is generally ineffective in patients with solid tumors. This is mainly caused by resistance of malignant cells to cytotoxic defense mechanisms of the immune system. During normal tumor immune surveillance, natural killer (NK)-cells use surface expressed TNF-related apoptosis-inducing ligand (TRAIL) to kill transformed cells. In contrast, the expression of TRAIL on T-cells is typically low. In order to enhance the tumoricidal activity of T-cells, we designed a strategy to specifically deliver TRAIL to the T-cell surface. Hereto, we constructed two TRAIL-fusion-proteins directed to T-cell antigens CD3 and CD7, designated anti-CD3:TRAIL and K12:TRAIL, respectively. Both anti-CD3:TRAIL and K12:TRAIL selectively bound to T-cells and accreted TRAIL at the T-cell surface. This potently enhanced the tumoricidal activity of T-cells toward cancer cell lines and primary patient-derived malignant ovarian carcinoma. Furthermore, T-cell surface delivery of TRAIL inhibited tumor growth and increased survival time of xenografted mice more than 6-fold. Interestingly, both fusion proteins had a distinct mode of action, as anti-CD3:TRAIL mainly induced granzyme/perforin-mediated cell lysis, whereas K12:TRAIL activated initiator caspase-8/-9 and effector caspases. In addition, anti-CD3:TRAIL also activated resting T-cells by triggering CD3 signaling. In conclusion, cell surface delivery of TRAIL to T-cells potently augments the tumoricidal activity of T-cells *in vitro* and *in vivo*, and could be easily integrated in current T-cell-based anti-cancer therapies.

P6.06.185
NK depletion enhances the rejection of established melanoma by tumor-specific CD4+ T cells

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Five-year survival rates for patients diagnosed with metastatic melanoma are less than 5%. Adoptive cell transfer (ACT) has achieved an objective response of 50% by Response Evaluation Criteria in Solid Tumors (RECIST) in this patient population. For ACT to be maximally effective, the host must first be lymphodepleted. The lymphodepletion procedure is often accompanied by myelotoxic, nephrotoxic, and pulmono-toxic adverse effects. It is hypothesized that lymphodepletion may remove regulatory elements and cytokine sinks, or increase that activation and availability of antigen presenting cells (APCs). We are using an *in vivo* model of ACT of tumor-specific CD4+ T cells (TRP1 cells) to study the interaction between natural killer (NK) cells and CD4+ T cells during malignancy. We found that depletion of natural killer (NK) cells enhances therapy. NK depletion increases the number of tumor-specific $CD4^+$ T cells and APCs, and the serum concentration of pro-inflammatory cytokines. Additionally, we observe increased autoimmune vitiligo and fewer tumor relapses after ACT when combined with NK cell depletion. Our data suggests that NK depletion removes a cytokine sink. Understanding this mechanism may help develop new therapies that would obviate the need for toxic lymphodepletion prior to ACT.

P6.06.186
IL-17A promotes immune cell recruitment in human esophageal cancers

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We previously reported that tumor-infiltrating IL-17A-producing cells play a protective role in human esophageal squamous cell carcinoma (ESCC). However, the potential mechanisms involved remain unclear. In the present study, we investigated the effects of IL-17A on immune cell recruitment and function in ESCC. In vitro chemotaxis assays using the ESCC cell lines EC109 and KYSE30 demonstrated that while IL-17A showed no significant direct effects on the migration of T cells, NK cells as well as DCs, it could induce ESCC tumor cells to produce inflammatory chemokines, e.g. CXCL9, CXCL10 and CCL2, CCL20, which are associated with the migration of T cells, NK cells and DCs respectively. In addition, IL-17A enhanced the cytotoxic effects of NK cells against tumor cells by augmenting the expression of cytotoxic molecules, e.g. TNF-α, IFN-γ, perforin and granzyme B and activation receptors, e.g. NKp46, NKp44, NTB-A and NKG2D on NK cells. Furthermore, immunohistochemical analysis revealed that the density of IL-17A-producing cells was positively and significantly associated with the density of CD1a+ dendritic cells (DCs) in tumor tissues. With the analyses of 181 ESCC patients, we found a correlation of higher number of tumor infiltrating CD1a+ DCs with significantly improved overall survival of patients with ESCC. This study provides further understanding of the roles of Th17 cells in ESCC, which may contribute to the development of novel cancer immunotherapy strategies.

P6.06.187
The effects of esomeprazole combined with classical cancer drugs on lung cancer cells and peripheral blood mononuclear cells

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Ascidity of tumour microenviroment is one of the important factors in resistance to chemotherapeutics. Proton pumps play a critical role by transfering H^+ ion from cell cytoplasm into extracellular compartment. In this study, to examine if esomeprazole, a proton pump inhibitor, increases the sensitivity of A-549 cells to classical drugs used for lung cancer treatment. For this aim, the combination of classical drugs used for lung cancer treatment with esomeprazole were applied onto A-549 cells, and then, their cytotoxic effects were evaluated with Methyl Thiazol Tetrazolium (MTT) Assay, ATP-TCA and M30 ELISA. It was demonstrated that combinations of esomeprazole only with cisplatin and carboplatin lead to significant increase in cytotoxicity. Additionally, M30 levels were measured in order to determine the cell death mechanism. The increase of M30 levels in carboplatin alone and in combination with esomeprazole exhibit that carboplatin causes cell death by apoptosis. The fact that no increase was observed with ciplatin alone and in combination with esomeprazole suggested that cisplatin, unlike carboplatin, leads to cell death by necrosis. Furthermore, the cytotoxic effect of esomeprazole alone and in combination with cancer drugs on lymphocytes were investigated with MTT and flow cytmetric cell cycle analysis. When compared with control group apoptotic cell ratio was dose-dependently increased with both esomeprazole alone and in combination with cisplatin or carboplatin. Our study suggests that espomeprazole augments the sensitivity of tumour cells to chemotherapeutics, and also, may protect the host from side effects caused by high doses of cancer drugs.

P6.06.188
Establishment of a lymphoblastoid cell line with B1 cell characteristics derived from a chronic lymphocytic leukemia patient by in vitro EBV transformation

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Infection of normal B cells by Epstein Barr Virus (EBV) induces activation and subsequent transformation of B cells to lymphoblastoid cell lines (LCLs). Chronic lymphocytic leukemia (CLL) cells can be infected by EBV in vitro, however, they are refractory to transformation. We describe establishment of a LCL line with B1 characteristics following in vitro EBV infection and costimulation with CpG and CD40 ligand from a mutated CLL patient. Infection with EBV alone did not result in establishment of growing LCLs. The growing LCL was found to co-express CD5 and CD19 implying its possible leukemic origin, however, immunoglobulin heavy chain variable gene (IGHV) analysis revealed disparity in VH rearrangement pattern of the LCL (VH3) and the corresponding CLL B cells (VH6). Further flow cytometry revealed expression of CD5, 43, 27, 20 and CD23 and dim expression of activation markers CD38 and CD25, suggesting derivation from the normal B1 circulating pool. Sequencing of IGVH gene demonstrated expression of IGHV3-23*01 and IGHJ4*02 gene which belong to the skewed human B1 BCR repertoire. The LCL cells secrete secreted IgM, as determined by ELISA.
Our results indicate that despite employment of an improved EBV transformation method which enhanced the B cells transformation efficiency, the leukemic CLL B cells are refractory to EBV transformation.

P6.06.189
Identification of surface molecules with enzymatic and signaling proprieties as targets for mAb-immunotherapy of human prostate cancers

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We developed a panel of murine monoclonal antibodies (mAb) for diagnosis and therapy of human prostate cancers by selecting targets with enzymatic and/or signaling proprieties. mAb selection was made by exploiting different approaches on fresh and prostate cells). The selection grid lead to the selection of mAbs tested for their ability to modulate the transcriptome of the LNCaP line. Out of these, one was characterized as an anti-PSMA, a glycoprotein provided with enzymatic activities of N-acetylated α-linked acidic dipeptidase (NAALADase) and also of pteroyl poly-γ-glutamyl carboxypeptitdase (i.e., folate hydrolase). The molecule is homologous with the transferrin 1 and 2 receptors. The anti-PSMA mAb was efficiently used for radio-immumoscintigraphy in mice bearing a human prostate tumor. *In vitro* mAb ligation did not induce significant variations in the transcriptome on LNCaP, as tested by microarray.
Other reagents selected were agonistic mAb regulating gene activation. GF/3E8 modulates the expression of genes involved in carcinogenesis, metastatic spreading and transcription of cancer biomarkers.
GF/3D3 and C5 mAbs bind a 60 kDa heat-shock protein.
This study has enriched the armamentarium of mAbs specific for human prostate. The ongoing efforts are to use the regents non giving activatory signals as carriers of radioactive tracers (or toxins), ideal tools to trace primary and secondary lesions. Next step of the project is to use of the enzymatic characteristic of PSMA to convert pro-drug in to activate *in situ* anti-cancer drugs. Ambitious goal will be to use agonistic mAb for differentiation therapies or to induce cell death.

P6.06.190
Modulation of tumor microenvironment associated with tumor regression in melanoma patients receiving adoptive cell transfer therapy

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Mechanisms of tumor regression in melanoma patients after adoptive T cell transfer have not been fully understood. In this report, the interaction between the tumor microenvironment and adoptively transferred T cells in melanoma patients was examined. The gene expression of gp100 and MART-1 decreased dramatically in the fine needle aspiration biopsies from patients with objective responses after adoptive immunotherapy, suggesting that analysis of gp100 and MART-1 expression represents a surrogate marker for clinical responses in melanoma patients. Following T cell transfer, significant increases in the expression of CD8, IFNγ, TGFβ1, TNFα and ARG1 were observed in tumor deposits. Responders demonstrated significantly increased ratio of CD8 to CD4 and higher level of TNFα expression in tumors than non-responders, suggesting that expression of this inflammatory cytokine may be associated with tumor regression. In contrast, non-responders demonstrated higher levels of CTLA-4, IDO and IL-10 expression in tumors than responders, implying that they may impede tumor regression. Moreover, the expression levels of CD8, CD25, IL-2 and GM-CSF in tumors one week after adoptive cell transfer significantly correlated with clinical response duration. Specific clonotypes derived from the transferred T cells selectively accumulated in tumors one month after adoptive cell transfer, indicating that they may play a role in tumor microenvironment modulation and tumor regression. The results indicate that clinical response may be associated with relatively high levels of expression of T cell activation markers and stimulatory cytokines and relatively low levels of expression of inhibitory molecules modulated by adoptive cell transfer immunotherapy.

P6.06.191
A tumor vaccine against chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is incurable with conventional therapies. Malignant B cells in CLL are able to be antigen-presenting cells (APC) themselves and are ideal targets of immunotherapy. However, various immune-based therapeutic approaches that were tried haven't improved the survival of patients significantly. In this study, CLL cells were treated with PMA, IL-2, Imiquinod (TLR-7 agonist) which are all B cell activators for 48 hours. The treated cells developed branched projections and had an elevated expression of CD54, CD83, CD80 and CD86 on cell membrane, which are all characteristics of mature dendritic cells (DC). Moreover, these treated cells are more immunogenic in cytotoxic T lymphocyte assay (CTL) with peripheral blood mononuclear cells (PBMC) from healthy donors than original CLL cells. CLL animal models (NOD.SCIDIL-$2^{\gamma-/-}$ mice), which had CLL cells engrafted in peritoneal cavity (PC) and spleen (SP), were infused with treated autologous CLL cells twice as tumor vaccine. Then CFSE-labeled autologous CLL cells were given to the mice to test if engrafted T cells were activated by the vaccination in vivo. Cells in PC and SP were collected for counting and CTL assay *ex vivo*. The vaccinated mice had less CLL cells engrafted both in PC and SP, especially those mice that are treated with anti-CD200 antibody and anti-CD20 antibody in advance. The CTL assay confirmed that cells from vaccinated mice were able to kill more CLL cells and anti-CD200/anti-CD20 treatment augmented the ability. Our

data suggest an effective immunotherapeutic strategy for CLL which may be used clinically.

P6.06.192
Construction and functional activity of shRNA targeting Wnt-1 gene hyperexpressed by Wnt-1 breast tumors in mice

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RNA interference is used to down regulate key genes involved in tumor progression. For biomedical applications, expression of short hair-pin interfering RNA (shRNA) in viruses possesses the advantage of safe gene delivery to tumors by intravenous injections. The aim of this work was to construct shRNA interfering Wnt-1 gene involved in breast tumor formation in mice. Five Wnt-1 shRNA, coding different regions of Wnt-1 gene, were obtained in HEK-293T cells in the form of non-replicating lentivirus particles (LVP) using pLV-neo system. LVP were concentrated and purified using PEG and the titers were estimated on 3T3 fibroblast monolayers. Functional activity was verified in vitro using primary Wnt-1 cells obtained from tumors. Incubation with LVP2, 5 and their mixtures suppressed in a dose-dependent manner Wnt-1 expression as was determined by flow cytometry using antibodies to Wnt-1. LVP1, 3 and 4 did not affect Wnt-1 expression. LVP2 and LVP5 were injected intravenously into mice with Wnt-1 tumors three times a week during 3 weeks. Control mice were treated with chitosan nanoparticles loaded with doxorubicin (Chi-DOX) or doxorubicin alone. Both DOX and Chi-DOX effectively inhibited while LVPs significantly stimulated the growth of tumors. These results are rather unexpected and require further study.
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P6.07 Cell-based therapy

P6.07.01
Repeated intra-muscular injections of allogeneic mesenchymal stem cells (MSC) promote donor-specific T-cell hypo-responsiveness but also induce allo-antibody

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MSC from allogeneic donors (allo-MSC) have therapeutic potential for immune-mediated diseases and may be immune-privileged. For clinical translational, however, clearer understanding of allo-MSC immunogenicity is needed. In this study, anti-donor T-cell and B-cell responses following single or repeated intra-muscular injections of allo-MSC were examined in mice.
BALB/c mice received 10^6 allo(B6)-MSC, allo(B6)-splenocytes, syngeneic(BALB/c)-MSC or vehicle once or on two occasions 2 weeks apart. One week later, anti-donor T-cell and antibody responses were assessed. In T-cell “re-call” assays stimulated by donor(B6) or third-party(FvB) DCs, increased donor-specific $CD4^+$ and $CD8^+$ T-cell proliferative responses occurred following single or repeated allo-splenocyte injections and single allo-MSC injections compared to vehicle and syngeneic MSC. In contrast, repeated allo-MSC injection was associated with donor-specific T-cell hypo-responsiveness. Key results: B6-DC-induced $CD4^+$ T-cell proliferation (%-divided, mean±SD): Vehicle injections: 67.8±7.9%; Repeated allo-splenocyte injections: 85.1±4.1%; Repeated allo-MSC injections: 37.4±12.8%. $CD8^+$ T-cell proliferation: Vehicle: 68.2±9.6%; Repeated allo-splenocyte: 88.6±0.5%; Repeated allo-MSC: 47.3±12.4%. FvB-DC-induced T-cell proliferation did not differ between the groups.
Flow cytometry-based assays indicated similar titres of donor-specific (anti-B6) IgG among recipients of single and repeated allo-splenocytes as well as single and repeated allo-MSC. However, IgG isotype-specific assays indicated that IgG1:IgG2a ratio was higher in serum of repeated allo-MSC recipients compared to repeated allosplenocyte recipients (2.6±0.25 vs 0.6±0.22; p=0.000004). Nonetheless, sera from recipients of multiple allo-MSC injections were associated with complement-mediated lysis of B6 target cells *in vitro*.
Thus, repeated intra-muscular inoculations of allo-MSC induce distinctive donor-specific immune responses that include T-cell hypo-responsiveness and IgG1-dominant allo-antibody production.

P6.07.02
Generation of tolerogenic monocyte-derived dendritic cells from patients with primary Sjögren’s syndrome that suppress Ro/La specific T cell responses

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Primary Sjögren’s syndrome is a chronic autoimmune disease, characterized by mononuclear cell infiltrations preferentially in salivary and lacrimal glands that lead to dryness of mouth and eyes. In a majority of the patients, autoantibodies against SSA (Ro52 and Ro60) or SSB (La) are present. There exists no cure, and current therapies rely mostly on alleviating the symptoms. Therapeutic vaccination with antigen specific tolerogenic dendritic cells (DC) might become a future option of individualized therapy for patients with autoimme diseases like Sjögren’s syndrome. Here we generated monocyte-derived tolerogenic DC from patients with primary Sjögren’s syndrome using dexamethasone, vitamin D3 and LPS (DexVD3 DC). The phenotype was analyzed by flow cytometry and cytokine profile was investigated using 25-plex Luminex assay and ELISA. The capacity to induce Ro/La specific T cells was evaluated by an autologous MLR. Furthermore, the suppressive capacity of the induced T cells was analyzed in a suppression assay. DexVD3 DC generated from patients with primary Sjögren’s syndrome had an increased expression of CD83 compared to DexVD3 DC from healthy controls. They induced little antigen specific T cell proliferation, but the induced T cells successfully suppressed Ro/La specific T cell proliferation. Therefore, DexVD3 DC presenting Ro/La antigens might be a promising new therapeutic option for patients with primary Sjögren’s syndrome.

P6.07.03
Mesenchymal stem cells (MSC) modulate the expression of adhesion molecules involved in activated T-lymphocyte extravasation into the central nervous system (CNS): contribution to the therapeutic effect of MSC in inflammatory CNS diseases?

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Multiple Sclerosis (MS) is an autoimmune disorder associated with an abnormal leucocyte migration into the CNS through a multistep process characterized by the interaction between activated leucocytes and endothelial cells of the brain blood barrier (BBB) and choroid plexus. This interaction is mediated through several molecules expressed on activated leukocytes like α4β1 integrin and LFA-1, which bind to VCAM1 and ICAM-1, respectively, therefore blocking of receptors and/or ligands implicated in this interaction results in amelioration of the disease. Furthermore the majority of T cells in the cerebrospinal fluid (CSF) express CXCR3, suggesting its fundamental role in T cell extravasation into the CNS. Mesenchymal stem cells (MSC), potent immunoregulators that modulate effector functions of cells of innate and adaptive immune system through juxtacrine and paracrine mechanisms, are being investigated for therapy to MS. Accordingly, we have studied the effect of MSC in vitro on the expression of the receptors and their ligands on T cells and endothelial cells. We show that MSC decrease the expression of α4β1 integrin and ICAM-1 both at $_m$RNA and plasma membrane levels. The expression of CXCR3 on T cells was also decreased by

MSC together with the CXCR3-mediated migratory activity. MSC decreased the expression of ALCAM and ICAM-1 which become up-regulated on the plasma membrane of endothelial cells and are crucial for extravasation of T lymphocytes into the CNS. Our transwell experiments indicate that the dual mode of action of MSC on T lymphocytes and on endothelial cells is likely to be through paracrine mechanisms.

P6.07.04
Umbilical cord blood T cells cultured with IL-7 in addition to IL-2 exhibit a higher degree of polyfunctionality and superior proliferation potential

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BACKGROUND: Umbilical cord blood transplantation (UCBT) is an increasingly used stem cell source for allogeneic stem cell transplantation. One disadvantage to UCBT is that treatment with donor lymphocyte infusion (DLI) is not possible. We wanted to explore if cultured T cells from the UCB graft could offer an alternative to DLI in this setting. METHODS: T cells were positively selected with CD3+/CD28+ magnetic beads and cultured with IL-2 at escalating concentrations. In addition, IL-7 (20 ng/ml) was added to half of the cultures. After 7 days of expansion the T cells were analyzed by flow cytometry to determine phenotype, and cytokine production.
RESULTS: There was significantly greater proliferation in cultures to which IL-7 had been added. Phenotypical differeces such as a higher CD4+/CD8+, a lower percentage of central memory T cells (CD45RO+ CCR7+) and a higher percentage of effector memory T cells (CD45RO+ CCR7-) was seen with IL-7. We assessed the intracellular production of IL-2, TNF-alpha, IFN-gamma and CD107a, and found that a higher percentage of polyfunctional T cells (positive for three or four factors) was seen in cells cultured with IL-7 in CD3+ and CD3+CD8+ cells, with a trend towards the same association also in CD3+CD4+ cells.
CONCLUSION: There seem to be a proliferation advantage in adding IL-7 to a lower dose of IL-2. Addition of IL-7 also seems to have an impact on the differentiation status of the cells. T cells cultured in IL-7 had more polyfunctional traits, possibly increasing the activity of a putative future DLI.

P6.07.05
A simple and quick method for isolation of mononuclear cells from peripheral blood and buffy coat

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Isolated mononuclear cells (MNC) are fundamental requirement for a number of immunological experiments. At present, density gradient centrifugation is the method of choice to isolate MNC from human peripheral blood and buffy coat, but it is cumbersome, unsuitable for small volumes e.g. 50 ul, needs 90 minutes to be performed and there is a loss of a number of cells during this process.
We developed a new method based on solution called Genekam MNC Isolater containing different inorganic chemicals. This solution can be mixed with peripheral blood or buffy coat at ration of 1:3 or 1:10 and kept at room temperature for 10 minutes. Subsequently it is washed two times with phosphate buffer to get a clean pellet containing MNC. We used it successfully on 50 ul to 1 ml samples to get the sufficient number of MNC within 25 minutes for culturing. Cultured cells remain vital upto 30 days. Number of cells produced in our method are much higher than density gradient centrifugation, hence it makes it more stable for applications, where large number of MNC from small volume of blood and huge number of MNC from large volume of blood or buffy coat are needed. The cells are examined microscopically for their morphology and through flowcytometry for different biomarkers of lymphocytes e.g.CD3, CD4, CD19, C20 etc.
We developed simple, quick and economical method to isolate MNC, which may accelerate the research work in immunology. Now we are applying it to perform different other immunological experiments in our laboratory.

P6.07.06
Altered B cell development and fuctions in Adenosine Deaminase deficient patients

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Adenosine deaminase (ADA) deficiency causes severe cellular and humoral immune deficiency and dysregulation due to metabolic toxicity. Alterations in B cell development and function have been poorly studied in these patients. Enzyme replacement therapy (ERT) and hematopoietic stem cell gene therapy (GT) are therapeutic options in patients lacking a suitable bone marrow transplant donor. We studied bone marrow (BM) of ADA-deficient patients and investigate the ability of ERT and GT to restore normal B cell differentiation and functions in BM and PB. We found that BM B cells from untreated ADA-deficient patients show an increased proportion of pre-B1 B-cells and a progressive decrease in later stage of maturation. This is in agreement with observation that the strongest selective advantage for ADA-transduced cells is observed at the transition from immature to naïve cells. BM alterations were overcome in patients treated with GT or ERT, but in the latter group immature B cells were expanded. In the periphery, transitional B cells accumulate under ERT and persist long-term, while after GT, transitional B cell frequency progressively normalized. Down regulation of BAFF-R and higher BAFF plasma levels were observed both in ERT and GT patients. B cell proliferative responses after BCR/TLR triggering were severely impaired in ERT patients but improved after GT. Our findings confirm that transfer of ADA gene into autologous hematopoietic stem cells restores B-cell development and functions.

P6.07.07
The effector function of antiviral T cells in stem cell donors is strongly impaired by G-CSF mobilization

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Cellular therapies have become a powerful tool to complement impaired immune reconstitution in patients after hematopoietic stem cell transplantation (HSCT). Recent publications suggest that G-CSF-mobilized donors might be a source for antiviral T cells for adoptive therapy. As information on number and functionality of antiviral T cells is scarce, we aimed to (1) identify the immunomodulatory effects of G-CSF on antiviral T-cells, (2) detect influence of the apheresis procedure and (3) discuss optimal time points to collect antiviral T cells for transfer.
We assessed (1) numbers of CMV-, EBV- and ADV-specific T cells using 14 HLA-matched multimers, (2) functionality by IFN-γ ELISpot in response to overlapping peptide pools, (3) immunophenotyping using bio-plex, Granzyme B ELISA and multicolour flow cytometry for T-effector and -memory cell subsets in order to characterize the influence of G-CSF and enrichment procedure.
Absolute numbers of antiviral T cells detected after G-CSF stimulation and compared to G-CSF-untreated samples and *in vitro* expansion rate upon antigenic stimulation was not influenced. However, functionality as expressed by mean reduction in IFN-γ (75% *in vivo*, 40% *in vitro*) and Granzyme B secretion (32% *in vitro*) was significantly impaired in response to all tested antigens tested.
Our results suggest that antiviral T cells from mobilized stem cell grafts might not be the optimal source for adoptive therapies due to their functional impairment. For patients with early viral complications whose donors is still under the influence of G-CSF or those with seronegative donors, third party T cells might be an attractive alternative.

P6.07.08
Tie2 monocyte-mediated delivery of interferon-alpha to the liver as a novel treatment for colorectal carcinoma liver metastases

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Colorectal carcinoma (CRC) is the third most frequent and the fourth leading cause of cancer-related mortality worldwide. The liver is the most common site of CRC metastases, which constitute the major cause of CRC-related deaths also due to the partial efficacy of the available treatments.
Tie2-expressing monocytes (TEMs) are a subset of circulating monocytes recruited at the tumor site in response to pro-angiogenic stimuli.
In this study we investigate the possibility to utilize engineered TEMs to treat CRC liver metastases by delivering low amounts of interferon-alpha (IFN-α) specifically at the tumor site *in vivo*.
Briefly, we established a model of CRC liver metastases by injecting CT26 mouse colon cancer cells in the spleen of immunocompetent inbred CB6 mice (Balb/c x C57B6 F1). 7 weeks prior to injection, mice were transplanted with syngenic bone marrow where hematopoietic stem/progenitor cells were transduced *in vitro* in order to express IFN-α under the control of the Tie2 regulatory elements. After bone marrow reconstitution we assessed the ability of TEMs-IFN-α to repress tumor growth. Notably, our results indicate that when compared to controls, mice bearing TEMs-IFN-α display reduced tumor burden, delay in tumor appearance and tumor regression. Initial evidence suggests that IFN-α can act directly by reducing tumor cells proliferation and indirectly by enhancing both innate and adaptive immune responses. Moreover, no side effects related to the administration of type I interferon molecules were observed. Taken together our results suggest that TEMs-IFN-α might represent a valuable tool for the treatment of CRC liver metastases.

P6.07.09
Overexpression of galectin-9 in islets prolongs grafts survival via downregulation of Th1 responses

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The differential activation of T helper (Th) cells and production of cytokines contribute to graft rejection or tolerance. In general, the Th1-type cytokines and cytotoxic T cells are detected consistently in host undergoing rejection, whereas Th2 responses are linked to a tolerance condition. Galectin-9 modulates Th1 cell immunity by binding to the T-cell immunoglobulin mucin-3 (Tim-3) molecule expressed on the Th1 cells. We investigate whether overexpression of galectin-9 in islets prolongs grafts survival in diabetic recipients. Islets were transduced with lentiviruses carrying galectin-9 and were then transplanted to streptozotocin-induced diabetic NOD/SCID recipients. The normoglycemic recipients then received splenocytes from diabetic NOD mice. Blood glucose concentration was monitored daily after adoptive transfer. The histology of the islet grafts and flow cytometric analyses were assessed at the end of the study. Overexpression of galectin-9 in islets prolonged grafts survival in NOD/SCID mice after challenge with diabetogenic splenocytes (mean graft survival, 38.5 vs 26.0 days, n = 10, respectively; P = 0.0096). The galectin-9-overexpressed grafts showed decreased infiltration of IFN-γ-producing $CD4^+$ and $CD8^+$ T cells, but not of IL-17-producing $CD4^+$ T cells. Strikingly, this islet-specific genetic manipulation did not affect the systemic lymphocyte composition, indicating that galectin-9 may regulate T cell-mediated inflammation *in situ*. We demonstrate that galectin-9 protects grafts from Th1- and Tc1-cell-mediated rejections, suggesting that galectin-9 has preventive and/or therapeutic benefit in transplant therapy for autoimmune diabetes and may be applied further to the transplantation of other organs or tissues.

P6.07.10
T and B cell restricted MOG expression through retroviral transduction of bone marrow stem cells mediates protection against EAE induction

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Irradiated mice transplanted with bone marrow stem cells (BMSC) transduced with the autoantigen myelin oligodendrocyte glycoprotein (MOG) are tolerant upon MOG35-55 peptide immunisation and do not develop the clinical symptoms associated with experimental autoimmune encephalomyelitis (EAE). However due to the large number of cell lineages that derive from bone marrow stem cells it is difficult to attribute the induced tolerance to a specific cell lineage. To address this, I have established a novel method of generating cell lineage specific gene expression following retroviral transduction of bone marrow stem cells. This strategy utilises the Cre lox system to mediate a single DNA inversion event within the encoded provirus DNA that aligns the encoded gene of interest with the retroviral promoter, thus allowing gene expression only in Cre expressing cell lineages. Utilising this novel strategy we have restricted MOG expression to T or B cells utilising lck-cre and CD19-cre transgenic BMSCs. Upon challenge with MOG35-55 peptide immunisation, we have found a 50% reduction in the incidence of EAE in both lck-creMOG and CD19-creMOG BM recipient mice compared to non-manipulated lck-cre or CD19-cre BM recipient or normal C57BL/6 mice. Furthermore, of the CD19-creMOG mice that did develop EAE, we found EAE onset was significantly delayed. This work provides evidence that following transfer of transduce BM, MOG autoantigen delivery through T or B cells can play a part in mediating protection against EAE induction. Further studies are currently underway to determine the mechanism by which immune tolerance to MOG is mediated in both settings.

P6.07.11
Intrathecal transplantation of neural stem/precursor cells hampers the CNS-confined immune response of experimental autoimmune encephalomyelitis

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Transplantation of neural stem/progenitor cells (NPCs) in experimental autoimmune encephalomyelitis (EAE) has consistently shown that NPCs promote neuroprotection through pleiotropic mechanisms, including a strong, but only partially characterized immunomodulatory effect. We investigated if and how the intrathecal transplantation of NPCs could modulate the CNS-restricted effector phase of EAE, the key event in disease initiation and maintenance.
We found that intrathecal transplantation of NPCs in MOG-immunized C57BL/6 EAE mice induced a significant and persistent amelioration of clinical disability when compared to sham treatment. At the end of follow-up, 80 days post immunization (dpi), neuropathology of NPC-transplanted mice showed lower demyelination and axonal loss. Flow cytometry analysis of CNS inflammatory infiltrate at 40 dpi revealed that these findings were preceded by a significant reduction of infiltrating myeloid cells as well as of encephalitogenic T helper cells.
Transplanted NPCs localized strategically in the meningeal perivascular spaces, in close contact with local antigen presenting cells, such as myeloid dendritic cells (DCs) and perivascular macrophages, suggesting that NPCs might interfere with the antigen-recall of autoreactive T cells within the CNS.
Indeed, in vitro co-culture of NPCs with bone marrow-derived DCs showed that NPCs inhibit, trough secreted factors, the maturation and the capability of reactivating myelin-specific T cells of DCs.
Our work confirms the efficacy of intrathecally transplanted NPCs in ameliorating EAE and suggests that NPCs can impair the antigen recall and terminal polarization of encephalitogenic T cells within the subarachnoid perivascular spaces, thus preventing CNS accumulation of infiltrating inflammatory cells responsible for disease progression.

P6.07.12
Exploring the immunogenicity and efficacy of bone marrow-derived allogeneic cell therapy in vivo using a humanised mouse model

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Allogeneic stem cell therapies offer great potential as off-the-shelf therapeutic agents to a wide range of degenerative and autoimmune diseases. The immunogenicity of stem cell products may have an impact on the safety and efficacy of allogeneic cell therapies. There is currently no reliable experimental model to monitor T cell responses against stem cell products in vivo. Using a fluorescent immunosorbent spot (FluoroSpot) assay, we found mice that received human bone marrow-derived mesenchymal stem cell (MSC) infusions have increased frequency of IFN-gamma producing cells against human antigen. This effect is mediated through both the direct and indirect pathway of antigen recognition. In the same xenogeneic setting, MultiStem®, another human bone marrow-derived stem cell product (Multipotent Adult Progenitor Cell) shows very limited long term engraftment after infusion. To investigate human T cell responses against stem cells in an allogeneic setting, a humanised mouse model was established using NOD-scid gamma (NSG) mice, reconstituted with peripheral blood mononuclear cells (PBMCs) from prescreened naïve human donors. We demonstrated that MultiStem did not sensitise naïve human responders through the direct pathway of alloantigen recognition. In addition, MultiStem treatment maintained body weight in a graft versus host disease (GVHD) model in NSG mice humanised with HLA mismatched allogeneic immune cells. Taken together, MultiStem demonstrates low immunogenicity and anti-inflammatory efficacy, therefore showing potential for treating human autoimmune diseases.

P6.07.13
Performance characterization of a novel cell processing sorter: microchip sorting without aerosols in a closed system

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Owl biomedical has developed a cell sorting platform with a closed, single -use fluidics path capable of safe, high-specificity, high-purity, and high-volume sorting. Cell samples are driven with low, positive pressure through a sorting microchip where cells are laser interrogated and sorted by a high-speed mechanical valve based on a user defined fluorescent phenotype. We developed a cell-based longitudinal stability assay (a "gage") using 1% CFSE+ labeled K562 cells spiked in a 2M/mL background of K562s to quantify the Nanosorter® sort performance and reliability. The data show the Nanosorter achieves highly repeatable sort results using a traditional purity metric and two metrics for yield; depletion yield measures the ability of the valve to sort target cells and absolute yield characterizes the target cell recovery from the entire closed fluidic system. In addition to sort performance data, the gage quantifies the Nanosorter mechanical and optical stability over time by trending metrics such as sample flow rate, detected events, dead volume, and missing counts. Over nine gage runs sorting at 2,200 cells per second, the Nanosorter enriched from 1% input to 91% ±3% pure target cells, with 88% ±6% depletion yield, and 55% ±10% absolute yield. A richer biological gage is in development using a 1:4 dilute whole blood (1B cells/mL) labeled with CD4-PE and CD45-APC.Cy7 with a no lyse no wash protocol. Preliminary data from this gage also shows high CD4+ cell enrichment and yield. The closed, single-use nature of the fluidics will be uniquely valuable for cell therapy manufacturing.

P6.07.14
Purifying Tregs with a new closed sorter

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Therapeutic use of regulatory T cells is of broad interest, and current purification strategies have limitations. We are developing a new sorting platform which achieves the multidimensional selection capabilities of droplet sorters and the single-use, closed fluid path typical of magnetic enrichment systems. We have characterized a first generation commercial device by sorting CD4+CD25hiCD127dim cells from human PBMCs. Performance metrics include assessments of the purity and recovery of the selected cells, and were found to vary as expected according to instrument settings, sample cell density and input Treg starting frequency. We find consistent enrichment between 10 and 20 fold, with 70-80% of the Tregs selected appropriately when sorting in the range of 2e7 PBMCs over 2.5 hours, without the need for operator intervention using a standard sort protocol. Design features of the device facilitate workflows typical in a cell therapy manufacturing environment and routine research applications. A second generation device is currently in development.

P6.07.15
Quality controls of Cell-Based therapies: Assessment of conditioned Dendritic Cells phenotypes by Global Gene Expression Profiles

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Whole-cell immunotherapies have shown promising results in clinical trials. Nevertheless, the complex nature of the cellular product and the individual variability associated to their preparation makes cellular immunotherapies less well characterized. Therefore, one major challenge in the development of whole cell therapies is the ability to demonstrate a clear comparability of the cellular product obtained after the application of different differentiation settings and conditioning protocols in the manufacturing process.
Research in our lab is focused towards the development of novel molecular assays that can be used as quality controls in cell-based therapies. Using a functional genomics approach we have been able to select a genetic signature of activation of DCs *in vitro*. We selected lists composed of 44 genes able to discriminate different DCs phenotypes. We validated the signature *in vivo* in DCs derived from a mouse model of inflammation. The selected signature has been further validated in human derived DCs in order to develop a quality control molecular assay for DCs-based therapies. Therefore, the basic methods to select a genetic signature of DCs that may predict *in vivo* functional activity and its applications in cellular immunotherapies are presented.

P6.07.16
Improved monitoring and generation of HAdV-specific T cells by a newly identified A*02 MHC class I multimer and short-term *in vitro* expansion

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Adenoviral infections are a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT) in pediatric patients. Adoptive transfer of donor-derived human adenovirus (HAdV)-specific T-cells represents a promising treatment option. However, the difficulty in identifying and selecting rare HAdV-specific T-cells, and the short time span between patients at high risk for invasive infection and viremia are major limitations. We therefore developed an IL-15-driven 6 to 12 day short-term protocol for *in vitro* detection of HAdV-specific T cells and identified a new adenoviral CD8 T-cell epitope derived from the E1A protein for the frequent HLA-type A*02:01. Using this novel and improved diagnostic approach we observed a correlation between adenoviral load and reconstitution of $CD8^+$ and $CD4^+$ HAdV-specific T-cells including central memory cells

in HSCT-patients. Adaption of the 12-day protocol to good manufacturing practice conditions resulted in a 2.6-log (mean) expansion of HAdV-specific T-cells displaying high cytolytic activity (4-fold) compared to controls and low or absent alloreactivity. Similar protocols successfully identified and rapidly expanded CMV-, EBV-, and BKV-specific T-cells. Our approach provides a powerful clinical-grade convertible tool for rapid and cost-effective detection and enrichment of multiple virus-specific T-cells that may facilitate broad clinical application.

P6.07.17
Effect of myeloid-derived suppressor cells on dendritic cells maturation and function

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Background: Myeloid-derived suppressor cells (MDSCs) are major regulators of the immune system that accumulate in tumors and are an important hindrance to cancer immunotherapy. Dendritic cells (DCs) are professional antigen-presenting cells in immune system. These cells have critical role in anti-tumor immunity. As it has been well documented that MDSCs interface with DCs maturation and function, therefore in this study the effect of MDSCs on DCs maturation and function was investigated.
Methods: Bone marrow derived DCs were generated in the RPMI1640 with 5% fetal serum supplemented with recombinant mouse GM-CSF and IL-4. Simultaneously, MDSCs were isolated from spleen of tumor bearing mice, and were cocultured with DCs in the presence of 5-fluorouracil and/or LPS for 48 and 36 hours respectively. Afterwards DCs were harvested and their activity to produce IL-12 was measured by standard ELISA. Besides, expression of DCs surface markers (CD40 and CD11c) was evaluated by flow cytometery.
Results: Our results showed that IL-12 production was significantly reduced by DCs co-cultivated with MDSCs. DCs also expressed lower surface costimulatory molecules.
Conclusion: Based on our experiments, MDSCs may interfere with anti tumor immunity through inhibition of DC's function. This finding should be considered in designing DC based vaccines for treatment of cancer.

P6.07.18
Cloning of human antigen-specific TCRs can confer the candidates for cancer gene therapy

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[Introduction] Antigen (Ag)-specific T-cell therapy or T-cell receptor (TCR) gene therapy is a promising immunotherapy for infectious diseases as well as cancers. Either TCR beta chain or alpha chain repertoire is currently analyzed; however, the availability of a suitable screening system for analyzing both Ag-specific TCR alpha/beta pairs from single T cell is limited. Here, we report an efficient cloning and functional evaluation system of TCR cDNA derived from a single Ag-specific human T cell by which we can obtain TCR cDNAs and determine their antigen specificity within 10 days. We designated this system the hTEC10 system (human TCR efficient cloning within 10 days)
[Method] In hTEC10 system, human antigen-specific T cells are detected by staining with antigen-specific MHC tetramers and single cells are obtained by FACS. TCR cDNA is amplified from single cells, cloned into an expression vector, and transduced into the TCR-negative T cell line TG40. The antigen specificity of the TCR is then assessed by staining the transduced TG40s with MHC tetramers. This entire process can be performed within 10 days.
[Results and Discussion] To evaluate this system, we first cloned and analyzed 379 Epstein-Barr virus-specific TCRs from 10 latent healthy donors. We then applied this system to clone tumor antigen-specific TCRs from cancer patients who had been treated with peptide vaccination. We obtained 199 α-fetoprotein (AFP)-specific TCRs and showed their cytotoxic activities toward AFP peptide-bearing cells. This system may provide a faster and powerful approach for TCR gene therapy for infectious diseases and cancers.

P6.07.19
A novel methodology of immune regulation using pluripotent stem cells

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In several decades in the last century, tissue or organ transplantation therapy has been developed for curing patients with end-stage organ dysfunction. However, shortage of donated tissue or organ has been one of the biggest problems to perform the therapy. Recently, a promise of regenerative medicine using pluripotent stem cells such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) as donor source has been increased. However, particularly when ES cells were used, immune-suppressive therapy should be required because the donor-recipient combination would be allogeneic. We successfully induced immunosuppressive cells in a differentiation process with granulocyte-macrophage colony-stimulating factor (GM-CSF) from mouse ESCs. The immunosuppressive cells were similar to so-called regulatory macrophages in the meaning of cell surface molecule and gene expressions. They efficiently suppressed allogeneic T cell proliferative responses, at least in part, in nitric oxide dependent manner. We applied these cells to in vivo allogeneic transplantation and found that they substantially prolonged ESCs-derived graft survival. These results open a new insight for development of a novel immune-regulatory strategy in the age of regenerative medicine based on the use of pluripotent stem cells.

P6.07.20
The application of in vitro expanded CD4 Th1 cells in adoptive cell therapies

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Adoptive cell therapies (ACT) have been used as an immunotherapy for cancer treatment; with most ACT focusing on tumor-reactive CD8 T cells. However, both clinical and animal studies suggest that therapeutic strategies that only focus on the use of CD8 T cells are not effective in eliminating tumor cells. In this study, we sought to investigate the therapeutic efficacy of mono-cellular therapy and combination therapies of CD4 and CD8 T cells. Methods for CD8 T cell expansion have been well established, while ex vivo/in vitro expansion of CD4 T cells has not yet been optimized. By using OT-II cells as a model, we have achieved more than 5,000 CD4 T cell expansions in 24 days. The majority of these in vitro expanded cells express early effector cell phenotypes as CD27+/CD28+/CD127+/PD-1-/CTLA-4-; and they were able to express multiple cytokines after short duration of antigen re-stimulation. These cytokines were predominantly IL-2, IFN-g, and TNF-a, and low level of IL-4, IL-17 and granzyme B, suggesting a Th1 cell phenotype. Preliminary in vitro cytotoxic assays showed both direct killing of target cells by these CD4 T cells; and also enhanced CD8 T cells cytotoxicity. The in vivo efficacy of ACT with CD4 T cells and/or CD8 T cells is currently under investigation.

P6.07.21
Chondrogenic differentiation of mesenchymal stem cells alters cellular immunogenicity in vitro and in vivo

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Osteoarthritis (OA) is characterized by articular cartilage degradation and joint inflammation. Allogeneic mesenchymal stem cells (MSCs) have potent regenerative and immunosuppressive potential and are being investigated as a therapy for OA, however little is known about the immunological changes that occur in allogeneic MSCs after induced or trans-differentiation. A fully allogeneic rat model was used for this study. Chondrogenic differentiation was induced in alginate layers. After 18 days, rat MSCs up-regulated Collagen 2 (>50 fold), Aggrecan and Sox-9 (> 4 fold). Allogeneic differentiated MSCs lose the ability to inhibit T-cell proliferation in co-cultures *in vitro*, which was associated with significantly reduced nitric oxide & prostaglandin E_2 ($p<0.001$) in co-culture supernatants. Differentiation altered immunogenicity as evidenced by up-regulation of MHC-I, MHC-II, CD86, induced proliferation of allogeneic $CD4^+$and $CD8^+$ cells ($p<0.01$), and increased susceptibility to cytotoxic lysis by allo-specific T cells. 10^6 undifferentiated or differentiated allogeneic MSCs were injected or implanted in alginate subcutaneously. *Ex vivo* recall analysis 6 weeks later, confirmed increased memory T-cell response in draining lymph nodes of animals injected with undifferentiated (>4-fold) and differentiated MSCs (>5-fold), however only differentiated MSCs induced systemic memory T-cell responses ($p<0.05$). Implantation of undifferentiated MSCs in alginate reduced local T-cell responses by 75%; however alginate-encapsulated differentiated allogeneic T cells induced detectable memory T-cell responses both locally and systemically ($p< 0.05$). H & E analysis of mononuclear cells at the implantation site confirmed this finding. Systemic allo-immune responses to differentiated MSCs indicate that they become immunogenic and will require immunosuppressive therapy to be used therapeutically.

P6.07.22
Migration of human mesenchymal stem cells towards inflammatory lesions after transfection with CD29 specific siRNA

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Background and aims: Mesenchymal stem cells (MSCs) are a population of non-hematopoietic stem cells with differentiation potential, which have the capacity to migrate towards inflammatory lesions. Several adhesion molecules, chemokines, and integrins are involved in the MSC response to inflammation. The aim of this study was to investigate the specific role of integrin beta1 (CD29) in MSC migration towards inflammatory skin lesions, in order to increase the MSC efficiency in wound healing.
Method: A GFP-expressing human MSC line (hMSCs) was purchased. The cell expression of CD29 was inhibited specifically by transfection with siRNA duplexes. An inflammatory skin lesion was produced in CD1 Nu/Nu mice, and the transfected hMSCs were injected subcutaneously. hMSC migration in vivo was evaluated macroscopically by a fluorescence imaging system and microscopically by immunofluorescence for CD29, vimentin, cytokeratin and Ki67.
Results: The intracellular and surface expression of CD29 was abundant, as shown by immunohistochemistry and flow cytometry (74.22±2.3% positive cells), respectively. CD29 was successfully inhibited, its expression being reduced by 76% in transfected hMSCs (18.02% positive cells). Transfected hMSCs also exhibited decreased expression of CD90 and CD105. When compared with control hMSCs, transfected hMSCs migrated more slowly to the skin inflammatory lesion site, but their proliferation rate was higher, as shown by their expression of Ki67. However, there were no significant differences between overall healing times.
Conclusion: CD29 inhibition has downstream effects by affecting the expression of MSC specific markers and MSC proliferation. It may offer a novel approach to the treatment of inflammatory skin lesions.

P6.07.23
Treatment with EBV-Cytotoxic T-Lymphocytes of pediatric liver transplant recipients affected by EBV-related Post-transplantation lymphoproliferative disease in early phase

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The Epstein-Barr virus (EBV) associated post-transplantation lymphoproliferative disorder (PTLD) is a severe complication of transplantation due to immunosuppressive regimen. The disease results in immortalization and transformation of infected B-cells and it is highly frequent in pediatric transplanted patients since it is related to the virus primary infection in EBV-seronegative recipients.
Aim of our study is to control disease progression by infusion of autologous EBV-specific T lymphocytes (CTL), expanded and activated in vitro, in order to provide persistent cytotoxic activity against infected B-cells in vivo.
Seven liver transplanted children, with a diagnosis of polyclonal EBV-related PTLD (3 polymorphic, 4 “early lesions”, median age at diagnosis 4 years-old), have been enrolled in our Institution. All patients showed adenotonsillar and gastrointestinal PTLD with positive EBV-encoded small RNA (EBER) staining; the IFN-γ secreting EBV specific lymphocytes measurement at ELISPOT assay resulted low (median value: 0,49±0.5 lymphocytes/µl of blood). EBV-CTLs, before infusion, are examined for sterility, immunophenotype, potency and EBV specificity. Treatment protocol consisted in 2 blocks of 3 monthly infusions (average dose 1.5x106CTL/Kg) followed by complete histological reassessment.
Infusions have been well tolerated and no adverse reactions have been recorded. Results of histological assessment after 6 infusions document downgrading of polymorphic PTLD to “Early Lesions” or to normal tissue in 5/7 patients (two complete regression); overall decrease in EBER positive lymphocytes is found in histological sections. These preliminary results are encouraging and confirm that CTLs, generated in our facility, are a reliable tool for cell therapy to mediate regression of low grade disease

P6.07.24
Immune system modulation during cytokine induced killer cells adoptive therapy in hematologic patients

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Adoptive therapy of cytokine-induced killer cells (CIK) have shown anti-leukemic activity. In a phase II study of allogeneic CIK cells, in patients relapsing after allogeneic hematopoietic stem cell transplantation, we performed a longitudinal immune monitoring based on gene expression profiling in adittion to phenotypic and functional characterisation of PBMCs. We isolated PBMCs from 4 patients before and 5 hours after each CIK infusion. Gene expression profiling was executed with HG-U219 array strips (Affymetrix, USA). Time course analysis was performed applying a regression model to estimate the effect of the interaction between time and classes of complete responders (CR) versus partial responders (PR) patients. We identified 23 probes corresponding to 17 genes whose variation of expression over time was different between classes. Based on the kinetic of gene modulation we were able to discriminate two different subgroups: one enriched in genes associated to inflammation and blood cell motility with similar co-regulation and a second characterised by genes associated to developmental disorder, embryonic development, hereditary disorder. Almost all the genes selected were up-regulated only in the PR at time zero while they were up-regulated after the first CIK infusion and down-modulated in the late time points analysed in CR. According to our data the infusion of allogeneic CIK cells induces a transient up-regulation of specific inflammatory cytokines in CR but after the second infusion it leads to a generalized anergic status of PBMCs, probably caused by the parallel over-expression of the G0S2 gene, known to inhibit hematopoietic cells proliferation.

P6.07.25
Toxicity evaluation of CD44v6-specific T cells in a xenograft model that fully recapitulates human hematopoietic development

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Introduction. Antitumor efficacy of genetically modified T cells (GMTs) in humans often associates with toxicity. In particular, off-tumor expression of the targeted antigen raises concerns when using GMTs expressing natural or chimeric receptors. We have developed a strategy for multiple-tumor eradication with GMTs expressing a CD44v6-specific chimeric receptor. Since CD44v6 is expressed at certain stages of hematopoietic development, it is mandatory to preclinically demonstrate that our strategy will not cause unbearable hematopoietic toxicity.
Aim. To develop a xenograft mouse model for predicting the spectrum of hematopoietic toxicities by CD44v6-specifc GMTs
Results. The immundeficient mouse strain NOD.Cg-Prkdc-Scid IL2rg-tmWjl/Sz (NSG) completely lacks T, B and NK cells. Human cord-blood derived CD34+ hematopoietic stem cells (HSCs) readily engrafted in irradiated NSG mice, but mainly reconstituted CD19+ B cells. Conversely, NSG mice transgenic for human IL-3, SCF and GM-CSF (3GS-NSG) also reconstituted T cells and myeloid cells. Reconstituted T cells were single positive for CD4 or CD8 and had a CD45RA+/CD62L+/CD95- naïve phenotype. When stimulated ex vivo with a clinical-level protocol for GMT generation, reconstituted T cells expanded and differentiated into CD45RA-/CD62L+ central memory T cells. Reconstituted myeloid cells were enriched for CD14+ monocytes. Importantly, when analyzed on all populations, CD44v6 expression was absent on HSCs and progenitors cells, but present on CD14+ monocytes, which is in accordance with the human situation.
Conclusions. We have developed a xenograft mouse model that fully recapitulates human hematopoietic development and that is suited for preclinically evaluating the potential hematopoietic toxicities of CD44v6-specifc GMTs and rescue approaches.

P6.07.26
TLR7/8 Ligand Is Necessary to Generate Potent Blast-Derived Dendritic Cells in Acute Myeloid Leukemia

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Active dendritic cell (DC) immunization protocols are quickly obtaining interest as an alternative therapeutic approach in patients with acute myeloid leukemia (AML). Despite progress in DC-based immunotherapy, some discrepancies were reported in generating potent DCs. Regarding the differentiation ability of leukemic blasts to DCs, here we generated DCs from various subtypes of AML blasts. For DC maturation, a combination of TLR agonists was used. Leukemic Blasts from 15 patients were differentiated and 6-day immature AML-DCs were cultured in the presence of TLR agonists for 24 hrs. The morphology, expression of surface molecules, allostimulatory activity, cytokine production and phagocytic function of resultant DCs were compared with immature DCs and primary blasts. To induce cytotoxic T cells in vitro, auotologous T cells of patients after remission were co-cultured with matured DCs for 21 days. Cytotoxicity of CTLs was assessed by CFSE staining of targets. Our results showed that although Poly(I:C) has a synergistic effect on LPS, the addition of TLR7/8 agonist (R848) is necessary to reinforce the effect of LPS or LPS+Poly (I:C) to produce highly efficient DCs with increased IL-12 production and substantial capacity to activate allogeneic T cells. Compared to immature DCs or blasts, AML-DCs matured with TLR4 plus TLR7/8 agonists with or without TLR3 agonist were able to induce CTLs with greater cytotoxicity against relevant targets. Therefore, a combination of TLR4 and TLR7/8 agonists (with or without TLR3 agonist) could be regarded as an appropriate maturation cocktail for AML-DC production, and have potential use for immunotherapy of AML patients.

P6.07.27
Mannose-α(1→2)-Mannose derivative for dendritic cell targeted delivery

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Dendritic Cells (DCs) are professional antigen presenting cells (APC) since they have the unique ability to activate naïve T lymphocytes (TL), initiating the adaptative immune response. To modulate it, many options have been developed including vaccines, immune gene therapies and specific targeting to DCs. DC-targeted vaccination is a promising alternative against certain infectious diseases and cancer. The use of DCs for this purpose is attractive because of their ability to present epitopes through the MHC class I and activate cytotoxic CD8+ T responses.
Mannose is a glucid that belongs to many pathogen-associated molecular patterns (PAMPs) which can be used anchored to nanovehicles such as liposomes to target their cargo to DCs. This is possible since PAMPs will bind to the pattern-recognition receptors on the DCs surface. C-Type membrane lectins, such as DC-SIGN and the mannose receptor, present on the DCs surface, recognize oligosaccharides containing mannose and/or fucose and mediate their specific endocytosis.
To target DCs we have used rhodamine-labeled Mannose-α(1→2)-Mannose-liposomes (Manα1-2Man-L) and uptake was observed with bone marrow-derived mouse DCs and monocyte derived human DCs in a specific manner. We showed by fluorescent microscopy and flow cytometry that fluorescent-labeled liposomes containing Manα1-2Man were taken up by DC in a specific manner: more than 65% and 53% of targeting respectively.
These results show that the Manα1-2Man-L are a good tool to load DC in vitro. We also showed that he liposomes are stable and negatively charged suggesting that binding specificity is due to the decoration and not the net charge.

P6.07.28
Therapeutic effect of bone marrow-derived clonal mesenchymal stem cells in DSS-induced colitis model

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Bone marrow-derived clonal mesenchyaml stem cells (BM-cMSCs), isolated by a subfractionation culturing method, are derived from single cell, highly homogeneous and show multilineage differentiation potential. Although recent studies indicate that BM-cMSCs have been recognized as cell therapy sources against certain diseases, it still remains unknown whether BM-cMSCs are therapeutically effective in ulcerative colitis. In this study we provide further evidences that mouse BM-cMSCs are used as cell therapy sources by examining the effects of mouse BM-cMSCs injection on the dextran sulfate sodium (DSS)-induced colitis model. BM-cMSCs, which were injected intravenously injected, relieved the pathological phenotypes of DSS-induced colitis mice, regarding loss of body weight, stool scores, the reductions of colon length and hematochezia scores. The *iv* injected BM-cMSCs were considerably distributed on inflammatory regions of colons in DSS-induced colitis. Furthermore, H/E staining showed significantly restored intestinal tissue damages induced by DSS and reduced inflammations. These findings strongly suggests that BM-cMSCs are therapeutically applicable in inflammatory diseases such as colitis.

P6.07.29
Update on IPEX syndrome: new diagnostic biomarkers and therapeutic perspectives

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Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome is a life-threatening autoimmune disease due to mutations in *Forkhead box P3* (*FOXP3*), a transcription factor crucial for $CD4^+CD25^+$ regulatory T cell (Treg) function. In IPEX autoimmunity results from the dysfunction and instability of Tregs, devoted to control immune responses to foreign and auto-antigens. Patients with similar symptoms but wild-type *FOXP3* (IPEX-like), or patients with atypical milder IPEX forms are not easily diagnosed.
We previously reported that quantification of Tregs by analysis of the demethylation status of the *FOXP3* locus characterizes a subset of IPEX-like patients. We show here that anti-harmonin, the 75kDa USH1C protein and, to a minor extent, anti-villin-autoantibodies, measured by LIPS assays, are detectable in the sera of all IPEX patients but not in IPEX-like patients, thus providing specific markers of IPEX, that also prove to be reliable markers to monitor response to therapy.
At present, therapies for IPEX are limited to immunosuppression, with only partial efficacy, and haematopoietic stem cell transplantation, which is not always available. We investigated whether lentivirus-mediated gene transfer of wild-type FOXP3 in IPEX $CD4^+$ T cells could convert effector T cells into Tregs ($CD4^{FOXP3}$). Patients-derived $CD4^{FOXP3}$ cells expressed Treg markers, were anergic, and displayed potent suppressive activity, both in vitro and in vivo, in a model of xenogeneic graft-versus-host-disease. Furthermore, ectopic expression of FOXP3 stably suppressed cytokine production, also in inflammatory conditions.
Overall, these findings pave the way for adoptive therapy with $CD4^{FOXP3}$ T cells and for more expert diagnosis and follow-up of IPEX patients.

P6.07.30
Investigation of mouse bone marrow Lin^- cells regenerative properties in vitro and in vivo

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The objective of our study was to determine the influence of skin tissue-specific microenvironment on mouse bone marrow-derived Lin^- cell proliferation and migration *in vitro* and *in vivo*. Female BALB/c mice were used. Cells were analyzed for the expression of CD117, CD34, Sca-1, CD90 and CD133 by flow cytometry. Lin^- cells exhibited a 2- to 13-fold higher expression of stem/progenitor cell surface markers compared with unpurified bone marrow cells. Proliferation of MACS-purified cells in 3D cultures was investigated by WST-8 assay. Lin^- cell migration was evaluated by *in vitro* scratch assay. The results obtained show that basement membrane matrix is more effective for Lin^- cell proliferation *in vitro*. However, type I collagen matrix better enhances the re-epitelization process, that depends on the cell migratory properties. Lin^- cells were administered subcutaneously to mice, in which skin lesion were created with the use of an 6-mm punch biopsy instrument. The most significant effect in full-thickness skin wound model *in vivo* was detected after treatment with Lin^- cells, integrated in type I collagen matrix.

P6.07.31
Promotion of corneal graft survival by donor bone marrow derived dendritic cells is mediated by creating an intragraft immunoregulatory milieu

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The *ex-vivo* generation of donor bone marrow derived rat dendritic cells (BMDCs) and glucocorticoid treated BMDCs with potent immunomodulatory properties for application in cornea transplantation is described.
BMDCs were generated via culturing BM precursor cells with rat GMCSF and IL-4 containing medium supplemented with dexamethasone (Dexa) to induce an immature, maturation resistant phenotype. BMDC and Dexa BMDC phenotype, antigen presenting cell function and their immunomodulatory properties were examined and characterised in detail. Both BMDC and Dexa BMDCs displayed significant immunomodulatory properties relative to freshly isolated OX62+ DCs, including but not limited to, a significant increase in mRNA expression of programmed death-ligand 1 and indoleamine 2,3-dioxygenase. Also, both BMDCs and Dexa BMDCs displayed a profound impairment in their capacity to stimulate allogeneic lymphocytes. Moreover, in a fully MHC I/II mismatched rat corneal transplantation model (Dark Agouti (DA) to Lewis (LEW)), injection of donor (DA) derived BMDC or Dexa BMDCs ($1x10^6$cells/animal, day -7) significantly prolonged corneal allograft survival. With both cell therapies, we observed a significant reduction in the level of allograft cellular infiltration and a significant increase in the ratio of intragraft FoxP3 expressing regulatory cells.
Taken together, our results demonstrate a detailed analysis of the significant therapeutic effect of donor derived BMDCs with and without glucocorticoid treatment in corneal allograft survival by modulation of the allo-immune response at the level of both the allograft and the draining lymph nodes. Without the need of additional immunosuppression, our study represents a novel therapeutic approach for the prevention of corneal allograft rejection.

P6.07.32
Ex vivo generation and expansion of polyclonal human Treg

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Administration of regulatory T cells (Treg) appears as a suitable strategy to modulate bone marrow and organ transplantation. Previous work performed in mice has shown the feasibility of generating and expanding ex vivo Treg from naive T cells (Tn). In the present study we aimed to generate polyclonal human Treg ex vivo as a first step to modulate the immune response.
Peripheral blood mononuclear cells were obtained from healthy volunteers, Tn isolated by negative immune-selection were polyclonally stimulated and cultivated in media supplemented with IL-2, TGFβ, all-trans retinoic acid (ATRA) and rapamycin at different concentrations. The number and proportion of Treg generated was determined after 6 days by flow cytometry through the expression of CD4, CD25 and Foxp3.
Results show that TGFβ is a key cytokine to generate Treg in vitro, however a contaminating population of non Treg was also observed. ATRA in conjunction with IL-2 and TGFβ increased marginally the number of Treg, whereas Rapamycin showed no additional effect. We also show that the expression of CCR9 and β7 is augmented by TGFβ while the expression of β7 is further increased by the addition of ATRA. Addition of Rapamycin increased the expression of CXCR4.
Our results show the feasibility of generating human Treg from Tn in the setting of polyclonal activation in cultures supplemented with IL-2 and TGF-beta. Addition of ATRA and Rapamycin contribute to the differential expression of homing receptors.
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P6.07.33
Generation of highly effective and stable murine alloreactive Tregs by combined anti-CD4 mAb, TGF-β and RA treatment

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Transfer of alloreactive Tregs (aTregs) into transplant recipients represents an attractive treatment option to improve long-term graft acceptance. We recently described a protocol for the generation of aTregs using a non-depleting anti-CD4 antibody (anti-CD4 mAb). Here we investigated whether adding TGF-β and Retinoic Acid (RA) or Rapamycin (Rapa) can further enhance their stability and in vivo function.
Methods: CD4+ T cells were cultured with allogeneic B cells in the presence of anti-CD4 mAb alone, anti-CD4 mAb+TGF-β+RA or anti-CD4 mAb+Rapamycin. CD4+CD25+ T cells obtained were analysed for Foxp3, Helios, t-bet and RORγt expression and cytokine production. Cells were restimulated with allogeneic B cells and cytokine release and stability of Foxp3 expression determined. aTregs were tested for their in vivo function by studying their ability to inhibit the rejection potential of co-transferred effector T cells.
Results: Addition of TGF-β+RA or Rapamycin resulted in a relative and absolute increase of CD25+Foxp3+ expressing T cells. However, production of inflammatory cytokines (IFN-γ, IL-17) was completely abolished in aTregs obtained from anti-CD4 mAb+TGF-β+RA treated cultures. Although CD25+Foxp3+ cells from all culture conditions displayed complete demethylation of TSDR region, only anti-CD4 mAb+TGF-β+RA Tregs showed stable Foxp3 expression upon restimulation. Consequently, anti-CD4 mAb+TGF+RA aTregs could more effectively inhibit acute graft versus host disease (aGvHD) and skin transplant rejection mediated by effector T cells in comparison to aTregs harvested from anti-CD4 mAb only or anti-CD4 mAb+Rapamycin cultures.

P6.07.34
Generation and Characterization of WT1-specific T cells derived from healthy donors for adoptive tumor therapy

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The Wilms tumor antigen 1 (WT1) is highly expressed on leukemic cells, but not on healthy tissue. WT1, therefore, is a favorable target antigen for allogeneic T cell therapy to prevent or treat leukemic relapse after stem cell transplantation.
Although, WT1-specific T cells have been detected in healthy individuals in low frequencies, the efficient expansion of these T cells for clinical use has remained a major challenge.
To date, generation of WT1-specific T cells has mostly been restricted to priming and expansion of either CTL or T helper cell clones. Although, the generation of a $CD4^+/CD8^+$-mixed T cell line using a time-consuming priming approach was reported recently, the T cell cultures displayed low frequencies of WT1-specific T cells; moreover, broad characterization of the expanded T cells was omitted.
In this study we aim to develop an improved method for the generation of functionally potent, polyclonal WT1-specific T cells from peripheral blood of healthy donors.
WT1-specific, reactive T cells are enriched directly from peripheral blood mononuclear lymphocytes (PBMC) by magnetic separation of T cells that upregulate the activation marker CD137 on the cell surface after an antigen-specific stimulation. After a 9-day expansion phase those T cell cultures show specific reactivity against WT1-presenting autologous cells, as detected by cytokine production after antigen-specific restimulation. Comprehensive phenotypic and functional characterization of those polyclonal WT1-specific T cell cultures reveals a potent T cell product.
In the future, our approach should be implemented for GMP-conform, highly automated generation of WT1-specific T cells for potential clinical use.

P6.07.35
Constitutively CD40-activated B cells regulate CD8 T cell inflammatory response by IL-10 induction

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B cells are exposed to high levels of CD40 ligand (CD40L, CD154) in chronic inflammatory diseases. In addition, B cells expressing both CD40 and CD40L have been identified in human diseases such as autoimmune diseases and lymphoma. However, how such constitutively CD40-activated B cells under inflammation may impact on T cell response remains unknown. Using a mouse model in which B cells express a CD40 ligand transgene (CD40LTg) and receive autocrine CD40/CD40L signaling, we show that CD40LTg B cells not only over produced IL-10 but also stimulated memory-like CD4 and CD8 T cells to express IL-10. This IL-10 expression by CD8 T cells was dependent on IFN-I and Programmed cell death protein 1, and was critical for CD8 T cells to counter-regulate their over activation. Furthermore, adoptive transfer of naïve CD8 T cells in RAG-1-/- mice normally induces colitis in association with IL-17 and IFNγ cytokine production. Using this model, we show that adoptive co-transfer of CD40LTg B cells, but not wild type B cells, significantly reduced IL-17 response and regulated colitis in association with IL-10 induction in CD8 T cells. Thus, B cells expressing CD40L can be a therapeutic goal to regulate inflammatory CD8 T cell response by IL-10 induction

P6.07.36
Cell therapy of cancer with alloreactive intentionally mismatched IL-2 activated donor lymphocytes targeting anti-cancer killer NK & T cells using monoclonal and bispecific antibodies

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Graft-vs.-leukemia or solid tumors effects following allogeneic stem cell transplantation (SCT) are well established, however, use of SCT may be hazardous and associated with unavoidble graft-vs-host disease (GVHD). We investigated whether short-term immunotherapy could be induced by intentionally mismatched alloreactive killer NK and T cells (IMAK), maximally activated with interleukin 2 with no SCT. Monoclonal and trifunctional bispecific antibodies (BSA) were used for targeting IMAK against antigens over-expressed on malignant cells confirmed efficacy of eradication of otherwise lethal inoculum of B16 melanoma while avoiding GVHD in mice. Successfully treated mice could also resist fresh tumor challenge, suggesting development of anti-cancer immunity by Fc binding of malignant cells to host dendritic cells and presentation to T cells cells. Commercially available monoclonal and BSA against target antigens (EGFR, VEGF, Her-2/neu, CD20, EpCAM) using Erbitux, Avastin, Herceptin, MabThera & Catumaxomab, respectively were used in a pilot clinical trial. We have confirmed that treatment of patients with hematologic malignancies and solid tumors with IMAK with (n=16) or without (n=40) targetting antibody is safe and potentially effective with no GVHD in patients with hematological malignancies and metastatic solid tumors. We are now investigating whether targeting of IMAK may be further improved using low energy acoustic shockwave therapy (AST) that may increase the perfusion of malignant tissue as well as increase binding of killer lymphocytes to cancer cells. Based on our cumulative experience we conclude that treatment with short-lived targeted IMAK may represent an effective future approach for personalized targeted cancer immunotherapy.

P6.07.37
Distribution of the lymphoid cells of various functional activity after transplantation

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Collective cell migration is an important process that determines cell reorganization in a number of biological events such as development, regeneration and immune responses. Bone marrow - the most important organ of the hematopoietic system and is also a key component of the lymphatic system. The spleen plays important roles in regard to the immune system. Bone marrow contains undifferentiated lymphoid cells, whereas more mature cells are located of spleen. Cells migration is necessary step of the immune response. Therefore the study of the this lymphoid cells function of varying degrees of differentiation is an important problem. Distribution of intravenously injected or implanted male mise bone marrow-derived cells or splenocytes inside female sibling host animals was traced using Y-chromosome-sensitive PCR. When injected via the tail vein, Y-chromosome-positive cells were found in all studied organs: heart muscle, skin, muscles, lungs, liver, spleen, lymph nodes, brain and bone marrow. Transplanted cells derived bone marrow and spleen migrated in all organs at all times after transplantation. The dynamics of the distribution is similar, but more intense splenocytes migrated to the lymphoid organs, such as lymph nodes and spleen.

P6.07.38
Tumor associated fibroblasts are endowed with increased ability to support tumor development

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Tumor associated fibroblasts (TAFs) from solid human tumors provide structural and functional support to tumor development and progression. The main purpose of this study was to investigate TAF phenotype and functions comparative to bone marrow-derived mesencymal stem cells (MSCs) and to evaluate the role of these cells in tumorigenesis. TAFs were isolated using enzymatic digestion from surgical removed breast tumor pieces and were cultured for several passages in standard cultivation conditions, similar to MSCs. Cellular analysis included transmission electron microscopy (TEM), immunophenotyping (HLA-DR, CD29, CD44, CD73, CD90, CD106, and CD117), cytoskeleton and matrix proteins expression based on immunohistochemistry, and tri-lineage differentiation potential towards adipocytes, osteoblasts, and chondrocytes. ELISA assay was used for determining cells secretory profile, and we found that TAFs secrete significantly increased amounts of VEGF, TGF-β1, IL-4, IL-10, and TNF-α. Tumor cell lines SK-BR3, MDA-MB231 and MDA-MB468 were used for flowchamber adherence studies, under progressively increasing shear stress from 0.35 to 15 dyne/cm2, on MSCs or TAFs cellular substrate. The results showed that tumor cells have increased adherence to TAFs, for all values of shear stress, mainly because VEGF and IL-4 secretion, so that we may conclude that even though MSCs and TAFs share functional and structural characteristics, TAFs represent a more specialized cellular type, able to provide support for tumor growth and development, and can be a target for further anti-tumor therapies.

P6.07.39
Mesenchymal stromal cell therapy prolongs corneal allograft survival in rats

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Mesenchymal stromal cells (MSCs) have potent immunomodulatory potential and are being investigated clinically to prolong allograft survival. Here, we investigated the ability of MSCs to prolong allograft survival in a rat corneal transplant model.
Syngeneic (syn), allogeneic (allo) or 3rd party MSCs were isolated from bone marrow of Lewis (LEW), Dark Agouti (DA) or Wistar-Furth rats, respectively. A fully allogeneic rat cornea transplant model (DA to LEW) was used for *in vivo* studies. Recipient (LEW) rats received one dose of 1x10^{6} MSCs (syn, allo or 3rd party) i.v. on day -7 and a second dose on the day of transplantation (day 0). Graft survival was monitored. Histology and flow cytometry was used to analyze cellular infiltration.
Untreated allografts were uniformly rejected (MST 16.6±1.5d, n=9) and, while syn-MSC treatment showed some evidence of attenuation of rejection (MST 18.3±4d, n=9), no clear pattern emerged following this treatment. In contrast, corneal allograft rejection was significantly delayed in approximately 90% of allo-MSC treated and 80% of 3rd party MSC treated allograft recipients. Flow cytometric analysis showed lower percentages of infiltrating NKT cells in corneas of both allo- and 3rd party MSC treated animals, coupled with a significantly higher frequency of splenic CD4+Foxp3+ regulatory T cells (Tregs), compared to controls.
Systemic administration of both allo- and 3rd party MSCs prolongs allograft survival with between 80 and 90% of allografts surviving for ≥ 30 days. This can be in part explained by the presence of fewer intragraft NKT cells and a higher frequency of splenic Tregs.

P6.07.40
TLR5 signaling enhances the proliferation of human allogeneic CD40-activated-B-cell-induced regulatory CD4 T cells

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Although diverse functions of different toll-like receptors (TLR) on human natural regulatory T cells have been demonstrated recently, roles of TLR-related signals on human induced regulatory T cells remains exclusive. Previously our group developed an *ex vivo* high-efficient system in generating human alloantigen-specific CD4^{hi}CD25^{+} regulatory T cells from naïve CD4^{+}CD25^{-} T cells using allogeneic CD40-activated B cells as stimulators. In this study, we investigated the role of TLR5-related signals on the generation and function of these novel CD4^{hi}CD25^{+} regulatory T cells. It was found that induced CD4^{hi}CD25^{+} regulatory T cells expressed an up-regulated level of TLR5 compared to their precursors. The blockade of TLR5 using anti-TLR5 antibodies during the co-culture decreased CD4^{hi}CD25^{+} regulatory T cells proliferation by induction of S phase arrest. The S phase arrest was associated with reduced ERK1/2 phosphorylation. However, TLR5 blockade did not decrease CTLA-4, GITR, and FOXP3 expressions and suppressive function of CD4^{hi}CD25^{+} regulatory T cells. In conclusion, TLR5 signaling enhances the proliferation of CD4^{hi}CD25^{+} regulatory T cells by promoting S phase progress through ERK1/2 signaling but is not involved in the suppressive function of human CD40-activated B cell-induced CD4^{hi}CD25^{+} regulatory T cells.

P6.07.41
Adoptive T cell therapy in patients with prostate cancer

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Prostate cancer is one of the most common malignant diseases in men over 50 years of age. Current therapeutic approaches are highly effective in the early stages; however, treatment of the metastatic disease is more complicated and associated with poor prognosis. Epidemiological data encourage intense research of innovative experimental methods that would improve the quality of life and prolong the survival of cancer patients. Among the treatment strategies, immunotherapy seems to be the most promising method.
The Department of Immunology 2nd Medical School and University Hospital Motol has been focusing on tumor immunology and antitumor immunotherapy for over ten years. We have developed a DC-based vaccine that proved the ability to induce antigen-specific T cells that recognize and eliminate transformed tumor cells. Due to low frequencies of induced tumor-specific T cells we try to find approaches that might increase the numbers of specific effectors. Therefore the aim of the project is to develop and optimize a large scale *ex vivo* expansion protocol for adoptive T cell transfer, using autologous tumor-specific T-lymphocytes induced by the DC-based vaccine. For clinical use, the aim is to adjust protocol settings to Good Clinical Practice (GMP) conditions. The combination of therapeutic vaccine and adoptive transfer offers a promising approach for the treatment of tumor diseases.

P6.07.42
Changes in T lymphocyte subsets accompany the clinical response to Mesenchymal Stem Cell (MSC) therapy - first experience in irradiation-induced colitis

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Background: Therapy by Mesenchymal Stem Cells (MSC) facilitates functional recovery and dampens the systemic inflammatory response in radiation-induced colitis. Bone marrow-derived MSC from the patients´ children were injected to four patients.
Patients and Methods: MSC were obtained by culture from bone marrow aspirates. A quantity of $2x10^6$ - $6x10^6$ MSC /kg were infused intravenously to the patients. Pain, hemorrhage, frequency of diarrheas and fistulisation as well as the lymphocyte subsets in peripheral blood were evaluated before MSC therapy and during the follow-up.
Results: Two patients revealed a substantiated clinical response for pain and hemorrhage after MSC therapy. In one patient pain reappeared after 6 months and again substantially responded on a second MSC infusion. A beginning fistulisation process could be stopped in one patient resulting in a stable remission for more than 3 years of follow-up. The frequency of painful diarrhea diminished from an average of 6/d to 3/d after the first and 2/d after the 2nd MSC injection in one patient. A decline of CD4+ and CD8+ T lymphocytes and an increase of potentially regulatory CD25+ T cells accompanied the clinical response in this patient after the MSC injections. In all patients prostate cancer remained in stable complete remission. No toxicity occurred.
Conclusion: A modulation of the lymphocyte subsets towards a regulatory pattern and diminution of activated T cells accompanies the clinical response in refractory irradiation-induced colitis. For patients with refractory chronic inflammatory and fistulising bowel diseases, systemic MSC injections represent a safe and effective option for salvage therapy.

P6.07.43
Genetically modified T cells expressing an affinity enhanced NYESO-1 T Cell Receptor in cancer therapy

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Thymic selection of T cell receptors (TCRs) ensures the low affinity of natural TCRs targeted to self-antigens, including those associated with tumours, such that they cannot recognize the level of self-antigens presented on most cancer cells. We have developed an approach that uses adoptive transfer of T cells genetically engineered to express optimised affinity TCRs that specifically target an epitope shared by the Cancer Testes (CT) antigens NYESO-1 and LAGE-1 (SLLMWITQC) in the context of HLA-A201. This approach has been evaluated pre-clinically and is currently being evaluated in a Phase I/IIa clinical study in multiple myeloma. To date 13 patients have reached the day 100 assessment and a CR/nCR response rate of 76% has been observed. An analysis of patient samples to determine bioactivity, trafficking, durable persistence and anti-tumour activity of the enhanced affinity TCR modified T cells has provided an insight into the mechanism and efficacy of the therapy. Data indicate T cell persistence in the responding patients is directly correlated with decreased NY-ESO-1/LAGE-1 antigen levels and decreases in the myeloma marker CD138. These observations indicate that incorporation of the affinity optimised tumour antigen specific TCRs into autologous T cells can overcome the limitations of thymic restrictions to the repertoire of TCRs able to target low levels of antigen on cancer cells.

P6.07.44
Exploring leukemia- and virus-reactive CD8+ T lymphocytes with stem-cell-like and central memory properties for effective immunotherapy in humanized mice

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Adoptive transfer of antiviral and tumor-reactive T cells has become a powerful therapy to treat cancer and virus-associated malignancies such as EBV-mediated lymphoproliferative disorders. However, terminally differentiated, high avidity effector T cells exhibit limited homing and self-renewal capacity to establish sustained memory. In this study we thus explored the modulation of the canonical Wnt-signaling pathway shown to affect T cell differentiation to generate HLA-A*0201 restricted, EBV- and Wilms Tumor1 (WT1) antigen-specific cytolytic T lymphocytes (CTL) from naïve human CD8+ cells with stem-cell-like memory (TSCM) and central-memory (TCM) properties.
Following repetitive stimulation with autologous DCs or PBMCs loaded with EBV- or WT1-peptide mix or EBV-transformed B cells (B-LCL) in the presence of IL-12, -7, -15, -21 and the glycogen synthase kinase-3β inhibitor TWS119 for 28-33 days we obtained strong expansion of EBV- and WT1-specific CD45RA+CD62L+CCR7+ TSCM and CD45RO+CD62L+CCR7+ TCM as compared to unmodulated control CTL. Additional studies revealed elevated ß-catenin levels and higher expression of eomesdermin supporting our phenotypic T cell differentiation analyses. Whereas both TWS119 treated and control CTL populations elicited comparable reactivity in vitro, EBV- and WT1-reactive TSCM and TCM showed prolonged persistence following adoptive transfer into human IL-15 supplemented NSG mice. Moreover, so far we observed superior killing of B-LCL engrafted into NSG mice as compared to control CTL in both preemptive and therapeutic situations.

We conclude that stem-cell-like and central memory type EBV- and WT1-reactive CTLs with increased homing capability and sustained effector functions can be generated from naïve T cells by modulating Wnt-signaling for improved immunotherapy.

P6.07.45
Intrapancreatic transplantation of allogeneic mesenchymal stromal cells (MSCs) decreases blood glucose levels in experimental type 1 diabetes

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Introduction: Mesenchymal stromal cells (MSCs) transplantation has been proposed to be one possible therapeutic alternative in type 1 diabetes (T1D). However, one challenge associated with MSCs transplantation is the route of administration for efficient cell delivery. Aim: Evaluating the therapeutic potential of intrapancreatic transplantation of allogeneic MSCs in experimental T1D. Methods**:** Diabetes was induced in C57BL/6 mice by streptozotocin. MSCs were isolated from adipose tissue of Balb/c mice. $1x10^6$ MSCs were administrated by intrapancreatic injection in hyperglycemic mice twelve days after diabetes induction. The control group was injected with PBS. The glycemia was monitored periodically. The glucose tolerance test (GTT) was performed 57 days after MSCs/PBS injection. The frequency of $Foxp3^+$ cells in spleen and pancreatic lymph nodes was evaluated at day 70^{th} post-treatment. Results: We observed that the intrapancreatic administration of MSCs could recover the hyperglycemia in 60% of diabetic animals. During GTT, the treated group showed a significant improvement of the peripheral response to glucose in all time points evaluated. The area under the curve of MSCs-treated group was lower when compared with the control (45.560 *versus* 71.884; p=0.003). At day 70^{th}, the fasting /non fasting glycemia of responders treated-mice were lower compared with the control (166.42/183.6 mg/dL *versus* 302.2/382.9 mg/dL, p<0.001). We did not observe differences in the frequency of $Foxp3^+$ cells. Conclusion: The intrapancreatic transplantation of allogeneic MSCs was effective in improving glucose control in diabetic mice. The intrapancreatic route represents a new approach to avoid MSCs pulmonary entrapment with promising results in T1D. Acknowledgment: FAPESP/CNPq.

P6.07.46
Generation of gene engineered mesenchymal stem cells and its efficacy against human breast cancer

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Breast cancer has become an escalating disease that has highly significant ramifications for future global health. In Malaysia, breast cancer is the number one cancer killer and affects the health of Malaysian women from all walks of life. Novel approaches on treatment of breast cancer are urgently needed to improve the clinical outcome of these patients. Recent studies have suggested that mesenchymal stem cells (MSCs) can home to and incorporate within the tumor tissues. This finding offers a novel way to deliver therapeutic agents to the tumor sites. In the study, we propose to generate genetically engineered MSCs (GE-MSCs) that express IL-12 and IL-18. The efficacy of these GE-MSCs will then be tested on a panel of breast cancer cell lines (MCF-7, MDA-MB-231 and T-47D). Bone marrow derived MSCs were characterized by immunophenotyping and differentiation studies. MSCs were found to selectively migrate towards breast cancer cells in a dose dependent manner. GE-MSCs were successfully generated by transfection with recombinant plasmids encoding IL-12 and IL-18 and the cytokines secreted by GE-MSCs were able to induce IFN-γ production by peripheral blood mononuclear cells. A marked increase of IFN-γ was observed when both cytokines were used, demonstrating their synergistic effects. Apart from that, these GE-MSCs retained properties of MSCs such as differential plasticity and migration abilities. The GE-MSCs appear to possess potent anti-proliferative and cytotoxic effects against breast cancer cells. The GE-MSCs also capable of activating unprimed T lymphocytes by up-regulated CD69 which may in part enhance the treatment efficacy.

P6.07.47
Endothelial cells: new targets for MSCs-mediated immunomodulation in mouse

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Chronic inflammation is involved in the pathogenesis of several human diseases, such as atherosclerosis, allergy and autoimmune diseases. Several anti-inflammatory pharmacological drugs are currently available but with limited efficacy and deleterious long-term consequences.
Long lasting tolerance induction may represent an effective resolution for these diseases and scientists have become interested in therapies based on cells with immunomodulatory activity. It has been demonstrated that Mesenchymal Stem Cells (MSCs) exert potent immunosuppressive effect both *in vitro* and *in vivo*, although the results of clinical trials are still disappointing and the biological mechanisms responsible for MSC effect remain poorly defined.
The aim of our study is to obtain a deeper understanding on MSC immunomodulatory mechanisms *in vivo*.
By transplanting alginate-encapsulated MSCs, we have recently shown that their therapeutic effect is completely independent of migration capacity or cell to cell contacts. Using TCR transgenic mice, we have found that encapsulated MSCs hamper recruitment of leukocytes and dendritic cells into inflamed lymph nodes. We speculated that this could be due to a direct effect on endothelial activation. Here we show that, *in vivo*, MSC hamper the up-regulation of VCAM and the increase in the number of CD31 positive vessels in inflamed lymph nodes. Furthermore, MSC-conditioned medium is able to down regulate the expression of VCAM1 and ICAM1 on endothelial cell lines in vitro. Altogether, these data strongly suggest that soluble factors secreted by MSC can modulate immune responses by directly inhibiting endothelial cell activation. The precise molecules involved in this process are currently under investigation.

P6.07.48
Treatment of autoimmune hepatitis by inhibiting CD8+T cells using hepatic stroma-induced regulatory DCs

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Dendritic cells(DCs) play a key role in triggering protected immune response against microorganisms or suppressing autoimmune inflammatory responses. We thus presumed that regulatory DCs (DCreg) may protect liver from $CD8^+$T cell-mediated liver damage. Autoimmune hepatitis(AIH) were induced in mice by transferring HBV-specific CTL into HBV transgenic mice. Liver significantly displayed damage from day1 to day3 after transfer of specific HBV-CTL, and gradually restored at day 6. During AIH, injection of liver stroma-induced DCreg significantly reduced the level of aspartate aminotransferase (AST), indicating a protective effect on liver. Liver DCreg were characterized by a longer life span, reduced expression of CD11c, Ia, CD80, CD86, CD40 along with increased expression of CD11b. They stimulated OT-1 $CD8^+$T cell to express CD25 and CD69 but inhibited mature DC-triggered $CD8^+$T cell proliferation. Compared with mature DCs, DCreg produced much higher nitric oxide (NO). Addition of NO synthase inhibitor PBIT remarkably reversed the DCreg inhibition of the proliferation of $CD8^+$T cells. These data provides a new idea for the treatment of autoimmune hepatitis by using regulatory DC in the future.

P6.07.49
Spatial distribution of transplanted bone marrow-derived mesenchymal stem cells in recipient's organs in the B16 melanoma model

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Introdaction. It is believed that the intratumoral integration of exogenously delivered bone marrow-derived mesenchymal stem cells (MSCs) may contribute to the stroma of tumors. MSCs were suggested as delivery vehicles for anticancer agents into tumors. However, the spatial distribution of MSC in recipient organisms during tumorogenesis has not yet been fully investigated.
Methods. We isolated MSCs from bone marrow of mice, fluorescently labeled the cells, and injected them into tail vein of mice bearing B16 melanoma. The recipient's organs (tumor, skin, lymph nodes, lung, liver, spleen, brain, the bone marrow) were harvested at different times after MSC transplantation: one hour, 3, 7 and 14 days. We detected MSCs in organs by histological analysis and real time polymerase reaction.
Results. After 1 hour donors MSCs were identified in all organs, with maximal donor cell markers in the lung, liver and spleen. In histological sections, cells were mainly observed in the blood vessels of organs.
After 3 days, highest levels of donor cell markers were observed in lymph nodes, bone marrow and lung. Analysis of distribution transplanted MSCs on day 7 showed accumulation of cells into the tumor and organs that are metastasized: lung, liver and spleen. Migration activity of MSCs into tumor and organs that are metastasized increased from 1h to 7 day after cells transplantation, when the tumors were visualized macroscopically. Overall, we believe that the concept that MSCs can be used as delivery vehicles for anticancer agents into tumors, is valid and worthy of further investigation.

P6.08 Stem cells in immunity

P6.08.01
Comparison of immunoregulatory properties of stem cells from human exfoliated deciduous teeth and bone marrow-derived mesenchymal stem cells

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Mesenchymal stem cells (MSCs) have remarkable capacities (stemness properties, unique immunoregulatory features and etc.) that suggest the application of them in various new therapeutic disciplines. Stem cells from human exfoliated deciduous teeth (SHED) are introduced recently. Because of their convenient accessibility, safety of harvest(despite BMMSC),and being ethically uncontroversial**,** SHED can be a preferable source for the ever-increasing MSCs' applications. While they are new, compared to other MSC populations, their immunoproperties have not been studied as much as necessary. In this study, we explored the effect of SHED on proliferation of T lymphocytes and compared to BMMSCs**.** With this aim, at first the isolated T lymphocytes were activated both specifically (by allogenic PBMCs) and nonspecifically (by phytohemagglutinin, PHA) in vitro and cocultured with SHED or BMMSCs under same conditions, subsequently their proliferation and cytokine secretion were measured.
In our experiment, BMMSCs and also SHED could inhibit the proliferation and cytokine production of both PHA and alloantigen stimulated T lymphocytes in a dose-dependent manner. Although the inhibition decreased by the separation of lymphocytes and MSCs by a semipermeable membrane, but it was not abolished.
This study showed that SHED can suppress the activation of human T lymphocytes in vitro. However, compared to BMMSCs, this suppression was distinctly alleviated. Moreover, in the equal condition the pattern of immune-modulation of BMMSCs and SHED was different, suggesting that SHED do not exert the exact mechanisms of BMMSCs' immunosuppression.

P6.08.02
Production and characterization of murine monoclonal antibodies against human synthetic peptide of CD_{34}

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The treatment of hematologic malignancies, solid tumors, and some non-malignant diseases like immunodeficiency diseases is offered by hematopoietic stem cells (HSCs) as a unique self-renewal and differentiation source which most commonly is selected by CD_{34} surface marker for HSC. By the advent of hybridoma technology, monoclonal antibodies applications exert extensive changes in medical fields such as diagnosis, treatment and purification. Antibodies are highly specific and functional in applications such as ELISA, Flowcytometry, Immunocytochemistry, and Western blot assays. In this study, splenocytes from Balb/c mice immunized with two 14-mer synthetic peptide were fused with SP2/0 murine myeloma cells. Fused cells were grown in hypoxanthine, aminopterine and thymidine (HAT) selective medium. Supernatant of hybridoma cells were screened by enzyme-linked immunosorbent assay (ELISA) for antibody production and the desired clones were selected and cloned by limiting dilution (L.D) assay. Eventually, the specificity and cross reactivity of secreted mAbs were further studied by ELISA and western blot analysis (WB). Isotype of this mAb was identified as γ heavy chain and k light chain. Reactivity of these antibodies was then evaluated in different immunological assays including ELISA, Immunofluorescence (IF), Western blot (WB) and Flowcytometry. Monoclonal antibody was purified by affinity chromatography on protein A-Sepharose and then confirmed by SDS-PAGE. These results indicate that peptide-based antibody against CD_{34} surface marker of HSC can be used for research, diagnosis and purification.

P6.08.03
The effect of TNF-α in differential gene expression pattern of CXCR4 on human marrow-derived mesenchymal stem cells

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Introduction: Cell therapy and tissue repair are used in a variety of diseases including tissue and organ transplantation, autoimmune diseases and cancers. Now mesenchymal stem cells (MSCs) are an attractive and promising source for cell-based therapy according to their individual characteristics. Soluble factors which are able to induce MSCs migration have a vital role in cell engraftment and tissue regeneration. Tumor necrosis factor α (TNF-α) is a major cytokine present in damaged tissues. Methods: We have investigated the pattern of gene expression of chemokine receptor CXCR4 in nine groups of human bone marrow-derived MSCs stimulated with TNF-α in different dose and time manner. Results: Comparison of TNF-α treated with untreated MSCs revealed the highest expression level of CXCR4 after treatment with 1 ng/ml, and 10 ng/ml of TNF-α in 24 hours, and the production of CXCR4 mRNA was regulated up to 216 and 512 fold, respectively. Our results demonstrated the differential gene expression pattern of chemokine receptor CXCR4 in human marrow-derived MSCs stimulated with inflammatory cytokine TNF-α. Conclusion: These findings suggest that in vitro control of both dose and time factors may be important in stem cell migration capacity, and perhaps in future-stem cell transplantation therapies.

P6.08.04
Association between T Reg marker polymorphism and tuberculosis

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Introduction: The FOXP3 gene encodes a transcription factor thought to be essential for the development and function of regulatory T cells(Treg), which are play a key role in balancing immune responses to maintain peripheral tolerance against antigens .The Studies have found an association between common polymorphisms in FOXP3 and some of infection diseases. However, data about FOXP3 gene in TB is limited. The aim of present study was to analyze the association between FOXP3 gene polymorphism and susceptibility to TB in the North of IRAN.
Methods: In a case–control study, 183 TB patients and 183 controls were recruited according to age and gender. We investigated one FOXP3 promoter single nucleotide polymorphism (SNP), -3279 A>C (rs3761548) by using PCR sequence-specific primer (PCR-SSP) technique.
Results: We found that there was no evidence of an increased risk associated with the FOXP3 and rs3761548 (-3279 AC) genotype (P > 0.05).
Conclusion: In this study, we showed that there were no differences between TB patients in comparison with controls. It is concluded that relationship between TB and Foxp3 gene needs to more experiments in difference population.

P6.08.05
Developmental endothelial locus-1 (Del-1) is a negative regulator of GCSF-dependent hematopoietic stem cell mobilization

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Hematopoietic stem cell (HSC) mobilization from the bone marrow to the peripheral blood is important in the course of bone marrow transplantation. HSC mobilization can be stimulated by granulocyte colony-stimulating factor (G-CSF) and CXCR4 antagonism (by AMD3100), which are broadly used in the clinical setting. Adhesive interactions, like VLA-4/VCAM-1 and LFA-1/ICAM-1 between the HSCs and bone marrow stromal cells regulate HSC retention in the bone marrow and thus modulate HSC mobilization. Del-1 is an endothelial-derived endogenous inhibitor of inflammatory cell recruitment by antagonizing LFA-1-dependent leukocyte adhesion to endothelial ICAM-1 (Choi et al., Science, 2008; Eskan et al., Nat Immunol, 2012). Here we found that Del-1 is expressed in the bone marrow, in both mesenchymal stem cells and endothelial cells. Interestingly, Del-1 KO mice showed higher numbers of HSC in the peripheral blood after G-CSF but not after AMD3100 stimulation. Upon G-CSF administration, the level of Del-1 in the endosteal bone is decreased, which is reminiscent of the reduced GCSF-mediated expression of CXCL12, Kit ligand and osteopontin, which are important factors for HSC retention. Moreover, bone marrow mononuclear cells (BMMNC) from Del-1-proficient mice can bind to immobilized Del-1 through LFA-1 in vitro, indicating that Del-1 could contribute to HSC retention directly. Our results suggest that Del-1 in the bone marrow is a negative regulator of HSC mobilization, and further studies are required to reveal the underlying mechanism.

P6.08.06
Differential efficacy of human mesenchymal stem cells on disease in murine lupus based on source of origin

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Systemic lupus erythematosus (SLE) is a devastating autoimmune disease that targets multiple organ systems. A population of non-hematopoetic stem cells, mesenchymal stem cells (MSC), are of increasing interest as a therapeutic option for autoimmune diseases such as SLE. MSCs can be derived from various sources such as bone marrow or umbilical cords. In this study we examine the efficacy of human MSCs with varying origin on disease in murine based lupus. Human MSCs were harvested from umbilical cords, healthy donor bone marrow, and lupus patient bone marrow. We showed that in MRL/lpr mice, MSCs from all sources improved survival, increased body weight, and decreased proteinuria. Glomerular IgG, but not C3, deposition is significantly decreased in mice receiving umbilical cord and healthy donor MSCs. However, MSC from lupus patients caused a significant increase in glomerular C3 and IgG. Although no differences were seen in the percentage of CD4+ or CD8+ T, mice receiving lupus MSCs experienced a 2-fold increase in the percentage of Foxp3+ cells in the spleen. These mice also experienced this percentage increase in TCRβ+, B220+, and CD138+ cells in the bone marrow. Human MSC from various origins all made an impact on the disease severity of lupus prone mice. However, these results suggest that MSCs from healthy donors or umbilical cords appear to me more effective in murine lupus than the MSCs derived from lupus patients.

P6.08.07
LT-beta receptor signaling promotes progression of chronic myeloid leukemia in mice

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Despite promising progress in long-term treatment of Leukemia, the removal of leukemic stem cells (LSCs) and therefore cure of the disease remains an unmet need. Numerous studies indicate that LScs are resistant to conventional treatment such as radiotherapy or chemotherapy. Tumor necrosis factors (TNF) and their receptors (TNFR) regulate cell survival, growth, apoptosis and differentiation. TNFR signaling is of importance in anti-tumor immunity but also contributes to chronic inflammation. Lymphotoxin-beta receptor (LTbR) is shown to play a role in regulating developmental programs and formation of lymphoid organs, but is also critically involved in the regulation of immune responses. In this study, we now show that LTbR is expressed on normal hematopoietic stem cells and on LSCs. Hematopoiesis in naïve LTbR-KO mice is comparable to naïve BL/6 mice. To investigate the role of LTbR signaling in leukemia, we induced chronic myelogenous leukemia (CML) in mice by retroviral transduction of bone marrow (BM) progenitor cells with BCR/ABL. Surprisingly, LTbR signaling on leukemia stem and progenitor cells significantly promotes CML development compared to LTbR-KO CML. Analysis of homing capacity of LSCs to BM revealed impairment of LTbR-KO LSCs to home into BM compared to control. In conclusion, LTbR signaling seems to positively affect leukemic development. Therefore, blocking LTbR signaling may be a strategy to target leukemia stem and progenitor cells directly.

P6.08.08
In vivo imaging approach for continuous monitoring of transplanted cells

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With significant developments in tissue engineering, the concern of immunogenicity of transplanted cells or tissue constructs becomes critical. Yet, current methods for assessment of cell survival are often limited to a single time point at the end of the engraftment period. To circumvent this limitation we developed an imaging protocol that allows continues tracking of transplanted cells within the same animal as well as simultaneously assessment of general inflammatory reaction caused by the same cells. To illustrate this technique we employed mouse fibroblasts of the H-2b haplotype obtained from C57Bl/6 mice and immortalized by transformation with Simian Virus-40 T large antigen. Immunogenicity of aforementioned cells was diminished by stable transduction with lentivirus encoding anti-beta2-microgobulin shRNA. Parental and modified cells were loaded with cell tracker of different colors and injected in the pinna of either right or left ear of syngeneic (C57Bl/6) mice. Parallel experiments were conducted using allogeneic (Balb/C) mice. Grafted cells were visualized by confocal microscopy while degree of general inflammation was assessed by ear thickness measurements. In vivo visualization experiments were conducted on daily basis under general anesthesia and allowed live monitoring of the engraftment process. Evans Blue was used to simultaneously measure blood flow to the graft area. The developed approach offers a convenient and easily adaptable way to evaluate survivability of grafted cells or tissue engineered cell constructs in syngeneic or xenogeneic environments (Supported by AHA/SDG5680036, NSF/CBET1231549 & NIH/1S10OD010710 awards)

P6.08.09
Preparation of a high affinity monoclonal antibody against morphine showing no cross reactivity with heroin

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Morphine (7, 8-didehydro-4, 5-epoxy-17-methylmorphinan-3, 6-diol) is obtained from opium, derived from the milky exudate of the incised unripe seed capsules of the poppy plant, Papaver somniferum. This compound is a potent narcotic analgesic with substantial potential for abuse and constitutes a significant health problem for human society. Drug testing is a rising demand to restrict the spread of substances of abuse and to provide more protection to the members of society.
Monoclonal antibody (MAb) with a high affinity towards morphine can be considered as a great tool for its detection. In this study we prepared a MAb specific for morphine with no cross reactivity with heroin.
To prepare the immunogen, a morphine derivative (6-hemisuccinate) was conjugated to cationized BSA. Mice were immunized for 4 months. ELISA technique was used to screen the presence of antibody in the serum and culture media. Beatty method was used to calculate antibody affinity.
Results indicate that prepared antibody has a high affinity (5.9×108 M-1) towards morphine and less than 0.001% cross reactivity with heroin.

P6.08.10
Recombinant human Cyclophilin A supports differentiation of granulocytes

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Cyclophpilin A (CypA) is an18 kDa protein is found in all mammalian tissues and have a wide spectrum of functions. As a secreted form, CypA is present in the plasma of healthy individuals and in human breast milk. CypA is a chemokine for stem cells and various types of bone marrow precursors (dendritic cells, granulocytes, T- and B-lymphocytes). Although CypA was shown to promote differentiating of stem cells into dendritic cells, whether CypA can support development of other hematopoietic cells has not been investigated.
The goal of our study was to evaluate the effects of a recombinant human cyclophilin A (rhCypA) on differentiation of hematopoietic stem cells in vivo in mice and in vitro in human cells. Administration of rhCypA to sub-lethally irradiated mice increased the numbers of stem cell colonies in the spleen. In cyclophosphamide (CTX) treated mice, administration of rhCypA induced a significant increase in leukocytes and differentiated granulocytes in the blood. RhCypA restored $CD31^+GR1^+$ granulocytes and $CD11b^+CD11c^+$ dendritic cells in the bone marrow of CXT-treated mice. The restoration of granulocytes by rhCypA was due to its effect on $SCA\text{-}1^+$ stem cells in the mouse bone marrow. Studies using human leukapheretic product and bone marrow showed that rhCypA supported differentiation of $CD33^+CD14^+$, $CD33^+CD64^+$, and $CD13^+CD14^+$ myelomonocytes in vitro. Morphological analysis of these cells revealed a significant accumulation of young granulocytes. Thus our data demonstrated the activity of rhCypA as a differentiating factor for granulocytes.

P6.08.11
Susceptibility of human cardiac progenitor cells to natural killer cell lysis

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Stem cells therapies as alternative cure for heart failure has been strengthened by findings showing the existence of human cardiac stem/progenitor cells (hCPC). The use of autologous hCPC is hindered by the state of patients and calls upon the use of allogeneic hCPC. However, their immunological risk should be fully understood for their clinical application. Natural killer (NK) cells could eliminate allogeneic cells expressing low levels of MHC class I molecules or high levels of ligands for NK activating receptors. The cytolytic activity of NK cells is triggered after integration of signals transmitted from their activating and inhibitory receptors. In this work, hCPC were characterized for the expression of ligands for activating and inhibitory NK cells receptors under inflammatory conditions or not. Then their susceptibility to NK cell-mediated lysis as well as their capacity to modulate NK cell activities in allogeneic settings was evaluated. hCPC express similar levels of ligands for activating NK receptors under inflammatory and non-inflammatory conditions and were lyzed by activated NK cells. However, the expression of ligands for inhibitory receptors was increased/induced under inflammatory conditions, which led to a drastic decrease in their susceptibility to NK killing. hCPC were also able to modulate the NK cytolytic activity towards target cells, and this was accompanied by a decrease in the expression of NK activating receptors. Collectively, these data indicate that allogeneic hCPC display low immunological risk, in particular in an inflammatory context, supporting thus, their applicability after myocardial infarction.

P6.08.12
Properties of TiCaPCON-based nanostructured coating as bioimplant constituent

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Biocompatibility of TiCaPCON-based nanostructured coating with multi-potent mesenchymal stromal cells (MMSC) isolated from mice red bone marrow that possessed high level of CD271 expression and with human dendritic cells (DC) generated from peripheral blood monocytes was analyzed.

The aim of work was to explore the possibilities of this material application as a bioimplant constituent to replace bone tissue defects. MMSC and DC were introduced in suspension to TiCaPCON-coated slide surface (NRTU «MISIS» films, Moscow). Coating-free slides served as control. Microphotography was performed using inverted microscope (Leica, Germany) daily for 7 days of incubation at 37°C. Studies conducted on 200 slides demonstrated that TiCaPCON appeared to be both biocompatible as did not render cytopathogenic action on mice MMSC and human DC and bioactive material as it favored adhesion, proliferation and colonization of cells under study.
Thus, TiCaPCON-coated and MMSC-colonized implant possessed the properties of accelerated fixation and biointegration in damaged tissue/organ site. Therefore, TiCaPCON appears to be promising as a composite bioimplant (material of natural or synthetic origin) constituent being able to provide reliable supporting and/or structure-forming function.

P6.08.13
Mass-production and characterization of anti-human CD_{34} monoclonal antibody in peritoneum of BALB/c mice

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Monoclonal antibodies or specific antibodies are now an essential tool of biomedical research and are of great commercial and medical value. The purpose of this study was to produce large scale of monoclonal antibody against CD_{34} in order to diagnostic application in leukemia and purification of human hematopoietic stem/progenitor cells. For large scale production of monoclonal antibody, hybridoma cells that produce monoclonal antibody against human CD_{34} were injected into the peritoneum of the Balb/c mice which have previously been primed with 0.5 ml pristane. Approximately, 5 ml ascitic fluid was harvested from each mouse. Evaluation of mAb titration was assessed by ELISA method. MAb was purified from ascitic fluid by affinity chromatography on protein A-Sepharose and then, the purity of mAb was assessed by SDS-PAGE and conjugated with FITC. Specific binding of conjugated mAb with CD_{34} marker of human hematopoietic stem/progenitor cells was evaluated using flowcytometry and immunofloresance techniques. This mAb showed appropriate reactivity in the following assays as if ELISA, Flowcytometry, Immunofluorescence staining and Western blot (WB). The results of immunological assays clearly showed that our antibody strongly reacts with CD_{34} antigen. The conjugated monoclonal antibody could be a useful tool for isolation, purification of human hematopoietic stem cells.

P6.08.14
Cell Cycle Analysis of Human Colorectal Cancer Stem Cells Reveals their Quiescent Nature

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Aim: The CD133 antigen has been identified as a putative stem cell marker in colorectal cancer tissues. The aim of this study was to investigate the cell cycle state of CD133 + and CD133 - cells, isolated from primary and metastatic human colorectal tumors. Materials and Methods: After mechanical and enzymatic dissociation of the tumor samples, CD133 + and CD133 - subsets were identified and separated by magnetic cell sorting. Flow cytometric analysis was performed to compare the cell cycle of both CD133 + and CD133 - cells isolated from primary and liver metastatic cancer cells. Results: The results indicated that CD133 + cells isolated from both primary and liver metastatic colorectal cancers were found in higher percentage in the G0/G1 phases. However, the CD133 - cells isolated from primary colorectal cancers were predominantly found in the S and G2/M phases. Surprisingly, the CD133 - cells isolated from liver metastatic colorectal cancers were mostly found in the G0/G1 phase. Conclusion: The present study provides evidence that CD133 + cells are in a quiescent state in colorectal cancer, representing a mechanism that would at least partially explain chemotherapy resistance and tumor recurrence in post-therapy patients.

P6.08.15
NF-kB *rela*-deficient macrophages fail to support bone formation and maintain the microenvironment for hematopoiesis after X-ray irradiation and HSC transplanatation

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In bone, cells derived from HSCs and MSCs are interacted each other. Bone is remodeled by osteoblasts derived from MSCs and osteoclasts from HSCs. The maintenance and differentiation of HSCs are supported by niche cells derived from MSCs. NF-κB is an important transcription factor for hematopoiesis, bone remodeling, and inflammation. *rela-/-* fetal liver chimeric mice, which are prepared by transferring fetal liver cells into lethally irradiated host mice, are known to have reduced lymphopoiesis, although their hematopoietic activity of HSCs is intact. We found that they also developed osteoporosis. In *rela-/-* chimeric mice, bone formation was declined, but differentiation of osteoclasts was not enhanced. The most severe osteoporotic *rela-/-* chimeric mice possessed enhanced mobilization of HPCs in the peripheral, implying an impairment of microenvironment for hematopoiesis in *rela-/-* chimeric mice. Recently, macrophages in bone marrow are known to be important to maintain the microenvironment and to support bone formation. In order to assess the ability of F4/80+ macrophages to support bone formation, we co-transferred wild type F4/80+ macrophages with fetal liver cells into the host mice. As a result, the bone formation was rescued, indicating that a defect of *rela-/-* macrophages caused reduced bone formation. *rela-/-* macrophages more express NOS2, a typical M1 type inflammatory gene, but less express PGE2, important for wound healing and bone formation. The failure of wound healing after X-ray irradiation caused by a defect of macrophage function may occur, resulting in reduced bone formation and impairment of microenvironment for hematopoiesis in *rela-/-* chimeric mice.

P6.08.16
The effect of the Immunomodulatory functions of adipose derived mesenchymal stem cells on the expression of major transcription factors in CD4 T cell subsets

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The potential and promising role of cultured adipose-derived mesenchymal stem cells (AD-MSCs) in regenerative medicine and new cell therapeutic concepts has been shown recently by many investigations. On the basis of the immunomodulatory functions of mesenchymal stem cells which have been indicated, It has been proposed that this function of MSCs play a pivotal role in establishment and leading of T lymphocytes, especially CD4 T cells, toward different functional subsets. In this context,
To detect the impact of AD-MSCs immunomodulatory and regulatory functions on the immune system in in vitro co-culture conditions at the gene expression level, we used Real Time-PCR as a sensitive method to investigate this effect on C57BL/6 spleen cells. Then, we analyzed the effects of AD-MSCs on master regulators and related major cytokine genes and on cell differentiation potential. Expression of c well-known CD4 effector T cell-related transcription factors, i.e. t-bet, GATA-3, Ror-gt and Foxp3 and cytokines, i.e. IFN-g, IFN-b and IL-b for Th1 cells, IL-4 for Th2 cells, IL-17 and STAT-3 for Th17 cells and IL-10 and TGF-b for regulatory T cells respectively, were evaluated by real time - polymerase chain reaction (RT-PCR).
In conclusion, the findings of this study confirm previous studies on MSCs and provide important implications for the role of immunomodulatory MSC effects in the differentiation fate of CD4-Tcell subsets.

P6.08.17
The effect of DiD on the immunosuppressive function and differentiation potency of Mesenchymal stem cells

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MSCs with immunomodulatory function and differentiation into various cell types can be used for treatment of autoimmune diseases. The fate of MSCs after injection helps the researchers to improve this therapy. In this regard, lipophilic carbocyanin fluorescent dyes with high emission spectra, such as DiD, avoid interference with background fluorescence and are suitable for in vivo imaging studies. In this study, in vitro and in vivo function of DiD labeled MSCs were examined. More than 99% of the MSCs were tagged with high intensity DiD. No significant change was observed in the Mitochondria Membrane Potential (MMP), Reactive Oxygen Species (ROS) production, proliferative capacity of the MSCs, and the morphology of adipocytes, osteoblasts, and Neural Progenitor Cells (NPCs), under proper differentiation media after labeling. DiD did not affect the expression of Nestin mRNA in NPCs derived from labeled and intact MSCs (p=0.7). No significant change was observed in production of PGE2 and IL-10 from the MSCs and NPCs after labeling. DiD labeled as well as intact MSCs significantly suppressed the proliferation of activated T cell in MLR by PGE2, reduced IFN-g and IL-17. Moreover, allogeneic MSCs with or without DiD similarly (p=0.63) improved the clinical score of the EAE mice. In comparison to the untreated EAE mice, a significant decrease in the levels of circulating IFN-γ and interleukin-17 and an increase in the IL-10 levels were observed in the EAE mice treated with labeled MSCs. This results revealed DiD is suitable for labeling of MSCs in neuronal autoimmune diseases.

P6.08.18
Human mesenchymal stem cells modulation of alveolar macrophage polarization in vivo and in vitro

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Rationale: We have previously reported that bone marrow-derived mesenchymal stem cell (MSC) treatment is protective in several models of ALI and sepsis. To effectively translate MSC into the clinical practice better understanding of the mechanisms mediating their effect is needed. ALI is a serious clinical syndrome characterized by dysregulated and excessive pulmonary inflammation. Key cellular mediators of the lung innate immunity are macrophages and neutrophils. It is recognized that macrophages can be polarized towards M1 (implicated in the development of ALI) or M2 (responsible for the resolution of inflammation). The current studies were undertaken to test 1) relative importance of macrophages vs. neutrophils to the MSC protective effect in vivo and 2) if human MSCs have a role in polarizing alveolar macrophages towards M2 phenotype in vivo and in vitro.
Results: In the in vivo model of E.coli pneumonia, when given to the neutrophil depleted mice, hMSC treatment demonstrated strong trend toward reduction of the severity of lung injury, improved bacterial clearance and reduced TNF-α levels in the BAL 24 h after infection, compare to PBS-treated animals, suggesting the central role of lung macrophages as cellular mediators of MSC effect. Both in vivo and in vitro hMSC administration was associated with significant up-regulation of CD206 (mannose receptor, widely accepted to be a marker for M2 activation) on AM and reduced TNF-alpha secretion.
Conclusions: Bone marrow-derived hMSCs have the capacity to change the phenotype and functional properties of alveolar macrophages, resulting in driving their polarization towards less inflammatory state.
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P6.08.19
The characteristic of minor cell populations in multiple sclerosis patients after cell therapy

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Introduction. Minor cell populations demonstrate functional diversity, from effector action to immunoregulation, depending on pathological conditions. Our previous results showed significant differences in numbers and functions of minor cell populations including γδT-cells, $CD8_{low}$T-cells, NK-cells and TNK-cells in multiple sclerosis (MS) patients compared with donors.
Objective. To estimate the influence of cell therapy upon minor cell populations' quantity in peripheral blood of MS patients.
Materials and methods. Peripheral blood mononuclear cells (PBMC) were obtained from relapse-remitting MS patients (n=15) with expanded disability status scale (EDSS) score median of 2,5 before and after intravenous infusion of autologous bone marrow-derived mesenchymal stem cells. PBMC were harvested at baseline and during 1 year after cell therapy and monitored with flow cytometer FC500 using appropriate antibodies.
Results. γδT-cells, which are able to regulate myelin-specific T-cell response, increased in peripheral blood of MS patients on day 10 after cell therapy ($p<0,01$) and continued elevation during 1 year of treatment ($p<0,05$) without any significant changes in $CD8^+$γδT-cells population with potential cytotoxic activity. The percent of $CD8_{low}$T-cells reduced after 1 month of cell infusion ($p<0,01$) and the decrease of $CD8_{low}CD45RO^+$T-cells and $CD8_{low}CD119^+$T-cells populations was observed during 9 months after treatment ($p<0,01$). The last one correlated with activated $CD3^+CD25^+$T-cells' percent ($R=0,59$, $p<0,05$). The number of NK-cells correlated with EDSS before treatment ($R=0,54$, $p<0,01$) and decreased after 1 month of cell therapy ($p<0,05$) without significant differences in TNK-cells percentage.
Conclusion. Cell therapy established immunoregulatory profile of minor cell populations in peripheral blood of MS patients following 1 year after treatment.

P6.08.20
Immunoregulatory activity of Decidual/Endometrial Stromal Cells in humans

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Decidua is the maternal tissue that is in close contact with the fetal trophoblast cells. During normal pregnancy tolerance mechanisms develop at the level of the decidua. In the case of abortion or pre-eclampsia caused by antiphospholipid antibodies or autoimmune diseases, an immunologic activation develops against trophoblast. Human decidual cells (DCS) from the uterus and parent cells, the endometrial cells (ESC), are closely related to the mesenchymal stem cells (MSC), and like the latter, can survive during xenotransplants, and probably during semiallogeneic transplants as well. DCS/ESC show advantages over bone marrow MSC in terms of collection. Additionally, they have surface antigen markers in common like HLA-G, due to their immunomodulatory activity. In our present work it has been proved that the expression of HLA-G from the DSC/ESC might be the responsible for the immunomodulatory effects of the DSC as well as for the effects o their equivalents, the ESC.
Methodology. DSC were collected from termed placenta and ESC were isolated from menstruation. Samples were purified and cultured. To study T cells proliferation and diferentation, T cells were isolated from peripheral blood from healthy donors and co-cultured with DSC/ESC. Immunomodulatory effects of the DSC/ESC were determined through monoclonal antibodies by flow cytometry, RT-PCR and Western Blotting.

Results. HLA-G expressed by the DSC/ESC was proved to contribute to T cells modulation.
Conclusion. HLA-G expressed by the DSC/ESC might be responsible for the immunomodulatory effects of DSC/ESC, proving its importance as immunosuppressive, useful for clinical therapies or gynecological-obstetrician immunological problems.

P6.08.21
Adipose Derived Stem Cells Isolated from Omentum: a Novel Source of Chemokines for Ovarian Cancer Growth

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The main site of ovarian cancer metastasis is the omentum. Omental adipose tissue is known for contribution to the tumor growth and metastasis through different mechanisms.
Objective: In the present study, adipose derived stem cells (ASCs) were isolated from the omentum of patients with ovarian cancer and those with ovarian cysts and the expression of SDF-1/CXCR4, IP-10/CXCR3, RANTES/CCR5, IL-4 and IL-10 were analyzed.
Methods: ASCs were isolated from 10 ovarian cancer and 25 ovarian benign cyst patients. Our investigations were done by quantitative Real Time PCR (qRT-PCR), flowcytometry, Western blot and also ELISA.
Result: Expression of IP-10 and CCR5 showed statistically significant difference between omentum derived ASCs of ovarian cancer patients compared to those with benign cysts ($p<0.05$). Expression of IL-10 also detected in the supernatant of cultured malignant ASCs.
Conclusion: Omental adipose tissue may play crucial roles for tumor promotion through the expression of tumor promoting chemokines. Accordingly, tumor-surrounding adipose tissue may be a novel target for immunotherapy of cancer.

P6.08.22
Isolation and Characterization of Mesanchaymal Stem Cells from Mouse Decidua

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Background: Mesanchymal stem cells (MSCs) have been isolated from different sources. These cells expand in vitro rapidly and under defined condition differentiate to other cells and tissues. Due to their immunomodulatory and tissue repairing potential MSCs are under intensive studies as therapeutic cells.
In this investigation we tried to purify cells with MSCs-like potency from pregnant mouse deciduas for the first time.
Methods: After scarifying femal Balb/c mouse in 13.5 days of pregnancy, the uterus was opened from antimesometrial side, all embryos and placenta were separated and the deciduas were chopped and digested with collagenase D and collagenase type I. The cell suspension was passed through the cell mesh and after washing the pellet was re-suspended and cultured in DMEM medium. After 4 days of culturing, the adherent cells were isolated and examined for morphological properties, surface markers, and differentiation markers.
Results: The isolated cells expressed several stem cell markers and showed the potency to differentiate to bone and cartilage tissues. In addition the isolated cells proliferate significantly faster and easier than BM-MSC and adipose derived MSC.
Conclusions: We conclude that deciduas are a useful source of MSCs and our protocol provides an efficient isolation method for MSCs separation from mouse decidua.

P6.08.23
The role of p15Paf oncogene in hematopoiesis

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The mechanisms that regulate hematopoietic stem cell (HSC) self-renewal and differentiation into multipotent progenitor populations remain unclear despite being an active and clinically relevant area of research. We have recently showed that the highly conserved proliferating cell nuclear antigen (PCNA) associated factor (Paf), an oncogene over-expressed in most malignancies, is one of the molecular switches that controls hematopoiesis. Mice carrying a targeted deletion of the Paf gene are leukopenic due to a disruption of the regulated proliferation of HSCs that resulted in their functional impairment. Both ubiqitylation and interaction with PCNA are important for Paf to promote cell survival upon the introduction of replication blocks. Data support that Paf association with PCNA regulates DNA polymerase switching during translesion synthesis (TLS). Whether Paf function in HSCs, potentially by regulating DNA replication and repair, is entirely PCNA dependent is still unknown. Therefore, to analyze the molecular mechanisms by which Paf mediates HSCs self-renewal and differentiation, transgenic mice were created that expressed a mutant PAF that does not interact with PCNA. The ability of the PAF PCNA binding mutant to reconstitute hematopoiesis when expressed in Paf-/- mice was determined using a series of phenotypic and functional assays to measure HSCs function and development. In the light of recent findings that Paf likely mediates TLS DNA polymerase switching, the results of our studies will be relevant not only for understanding the function of Paf in hematopoiesis, but will also provide information relevant to Paf function in cancer development.

P6.08.24
Cytotoxic CD8+ T cells regulate myelopoiesis by cytokine signals to bone marrow stromal cells

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Cytotoxic CD8+ T cells (CTLs) play a major role in host defense against intracellular pathogens. However, complete clearance of pathogens and return to homeostasis requires a regulated interplay of innate and acquired immunity. In contrast to T cells that have high proliferative potential, innate immune cells have to be continuously replenished from bone marrow (BM) hematopoietic stem- (HSCs) and progenitor cells. Inflammation-induced hematopoiesis, also known as emergency hematopoiesis, is critical for the control of systemic bacterial infections and may also be important in viral infections. Here, we show that interferon-gamma (IFNg) secreted by effector CTLs regulates hematopoiesis and induces myeloid differentiation resulting in an accumulation of myeloid cells. IFNg did not primarily affect hematopoietic stem or progenitor cells directly, but stimulated BM mesenchymal stromal cells (BMSCs) of the HSC niche to produce hematopoietic cytokines including interleukin-6. This reduced the expression of the transcription factors Runx-1 and Cebpa in hematopoietic progenitor cells resulting in increased myeloid differentiation. Our study indicates that during an acute viral infection, CTLs exert an indirect positive feedback on myelopoiesis via BMSCs.

P6.08.25
Large zinc finger transcrition factor Schnurri-3 functions in the osteochondroprogenitor cell to regulate bone mass

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The large zinc finger transcription factor Schnurri-3 (Shn3) was originally identified as a DNA binding protein of the heptameric recombination signal sequence for V(D)J recombination of immunoglobulin genes. Previous studies from our group have

revealed an unexpected role for Shn3 in the skeletal system and have identified Shn3 as a central regulator of postnatal bone mass. Mice lacking Shn3 display severe osteosclerosis primarily due to enhanced osteoblast activity. In this study we have characterized a specific cell population in which Shn3 functions to regulate bone mass. Based on analysis of mice lacking Shn3 in osterix or collagen II expressing cells, we hypothesized that Shn3 must be functioning in the common progenitor cell for osteoblasts and chondrocytes-the osteochondroprogenitor cell. However, characterization of this cell population has been limited due to lack of definitive cell surface markers to phenotype these cells. We established a flow cytometry based high throughput screen to identify a panel of unique cell surface molecules that can be used to identify and isolate osteochondroprogenitor cells. These osteochondroprogenitor cells not only formed bone and cartilage in vitro but also in vivo when transplanted under the kidney capsule of recipient donors. Lastly, we were able to demonstrate that Shn3 functions intrinsically in the osteochondroprogenitor cells to control bone mass.

P6.08.26
The advent of mucosal-associated invariant T (MAIT) cells from iPSCs opens a novel avenue for immunology

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Mucosal-associated invariant T (MAIT) cells belong to the innate type immune cells, play an important physiological role in host pathogen defense and may also be involved in inflammatory disorders and multiple sclerosis. The rarity and inefficient expansion of these cells have hampered detailed analysis and application. Here we report an iPSC-based reprogramming approach for the expansion of functional MAIT cells. We found that human MAIT cells can be reprogrammed into iPSCs using a Sendai virus harboring standard reprogramming factors without cell cycle stimulation. Under T-cell permissive conditions, these iPSCs efficiently redifferentiate into MAIT-like lymphocytes expressing the T cell receptor (TCR) Vα7.2, CD161, and interleukin-18Rα. Upon incubation with bacteria-fed monocytes, the derived MAIT cells show enhanced production of a broad range of cytokines including interferon gamma and tumor necrosis factor alpha. Following adoptive transfer into immunocompromised mice, these cells migrate to the bone marrow, liver, spleen, and intestine and protect against Mycobacteria abscessus, most likely via granulysin release. Our findings pave the way for further functional analysis of MAIT cells and determination of therapeutic potential. The work has been published in Cell Stem Cell (May 2013).

P6.09 Vaccine adjuvants

P6.09.01
Commensal bacteria-derived membrane vesicles supress Th-1 dominated immune responses in vaccinated and tumor-bearing mice

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Constitutive secretion of extracellular membrane vesicles is a common feature of cells from all domains of life including Archaea, Bacteria, and Eukarya. Although the contribution of gram negative bacterial outer membrane vesicles in disease pathogenesis has been extensively studied, whether commensal bacteria constitutively secrete such vesicles is still unknown. Given the importance of microbiota as regulators of immune homeostasis, we aimed to assess the immunomodulatory properties of extracellular vesicles secreted by 3 different human commensal lactobacilli isolates in comparison to E.coli derived outer membrane vesicles. AFM microscopy, dynamic light scattering and zeta potential measurements revealed that commensal-derived membrane vesicles (MVs) were nearly 250 nm in diameter and had high negative charge densities (-40 mV). Mice immunized with an inactivated viral vaccine against the foot an mouth disease virus showed suppressed FMD-specific IgG2a response when the vaccine contained MVs derived from commensals but not from E.coli. Similarly, commensal MVs suppressed anti-OVA antibody responses in OVA immunized mice and exacerbated tumor progression following challenge with EG.7 tumor cells, suggesting that commensal-derived MVs ameliorate Th-1 dominated inflammatory responses. In contrast, encapsulation of cyclic-di-GMP in commensal-derived MVs enhanced the immunostimulatory properties of this cyclic di-nucleotide and reversed the tolerogenic potential of the MVs. These results indicate that human commensal bacteria-derived membrane vesicles can have powerful immunomodulatory effects and can have potential therapeutic applications as novel anti-inflammatory agents.

P6.09.02
Immunomodulatory cytokine gene expression profiles and lymphoprolifration response in hamsters vaccinated by leptospiral vaccine with several adjuvants

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Introduction: Leptospirosis is an acute infectious disease caused by *Leptospira spp*. that produces a broad range of health problems mainly in the developing world. The present work aimed to quantify the mRNAs from cytokines ex vivo and its relationship with the lymphoproliferative response of vaccine-sensitized splenocytes as major elements in the protection against leptospirosis.
Materials and Methods: 64 four-week-old male hamsters separated in eight equal groups were subcutaneously inoculated with four-leptospiral vaccine and four-control adjuvant through a triple-injection regiment at fortnightly intervals. Animals in group one only received the plain vaccine preparation with no adjuvant while other groups were immunized with the same vaccine preparation but different adjuvants. Two weeks after the last injections, all animals were euthanized and subjected to splenectomy. After preparation of splenocytes, cytokine profiles and lymphoproliferative response has been evaluated against the recall antigen.
Results: Vaccination of hamsters led to significant lymphoproliferative response in all of the animals although differences were noticed in reaction levels between groups. The induction of Th1 ($IL12p_{40}$, IFNγ and TNFα), Th2 (IL4, IL10) and Treg (TGFβ) type cytokine were evaluated from tested animals lymphocyte stimulated by ex vivo antigen utilizing real time PCR
Conclusion: We have demonstrated that adjuvant plays a fundamental role in the type and extent of the immune responses raised by leptospiral vaccination. In our hands, alum triggered both type 1 and type 2 immune reactions. Giving the fact that both Th1 and Th2 responses were stimulated, we strongly recommend selection of alum in any formulation of leptospiral vaccine.

P6.09.03
Intradermal and subcutaneous adjuvant effects of type I enterotoxigenic *Escherichia coli* (ETEC) heat-labile toxin derivatives

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Type I heat-labile toxins (LT), produced by enterotoxigenic *Escherichia coli* (ETEC) strains have remarkable immunological properties but clinical trials raised concerns on the use of these toxins as vaccine adjuvants regarding both safety (when administered thorough the intra-nasal route) and effectiveness (after transcutaneous immunization). Nonetheless, the recent discovery of natural LT variants with altered biological features and the use alternative parenteral routes may unveil more rational approaches for the use of LT-based vaccine formulations. In the present study we determined the intradermal and subcutaneous adjuvant effects of three LT derivatives including the reference type I LT (LT1), a non-

toxic derivative (LTK63), and a natural polymorphic LT variant (LT2), frequently encoded by wild type LT^+ ETEC strains, using as a model antigen a recombinant HIV-1 p24 protein. In particular, we evaluated the differential activation of antigen-specific cytotoxic $CD8^+$ T lymphocytes (CTL), required for the control of intracellular pathogens such as HIV. Similar antigen-specific humoral (serum IgG) and T helper adjuvant effects were observed in BALB/c mice immunized with the tested LT derivatives. However, LTK63 showed reduced in vivo activation and induction of migratory behavior of dendritic cells when compared to the natural LT forms. In addition, mice immunized with LT1 or LT2 showed significantly higher in vivo activation of $CD8^+$ T cell-dependent cytotoxic responses when compared to mice immunized with the non-toxic LT derivative. Collectively, these results support the use of natural LT derivatives as parenterally delivered vaccine adjuvants particularly for the induction of strong CTL responses by protein-based vaccine formulations.

P6.09.04
Investigating Layered Double Hydroxides (LDHs) as Designer Inorganic Vaccine Adjuvants

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Despite its extensive use for decades, the vaccine adjuvant 'alum' consists of a heterogeneous mixture of poorly characterised compounds and has a limited ability to promote cellular (Th1-type) immune responses. The application of solid-state inorganic chemistry could lead to many potential new metal-based particulate adjuvants; layered double hydroxides (LDHs) are chemically homogeneous, crystalline materials that can be synthesised in a variety of chemical compositions and particle sizes. Previously, we found that LDHs of different chemical compositions can stimulate human dendritic cell (DC) responses *in vitro* and mouse antigen-specific antibody responses *in vivo*, and that some responses were significantly different from those induced by alum. Statistical analysis revealed direct correlations between the chemical and physical properties of the LDH and the immune response elicited, allowing accurate prediction of the immune response based on the material's properties. We have now synthesised LDHs of different chemical compositions with controlled particle sizes in the range 10 nm to 10 μm and tested their capacity to stimulate DCs. For a given chemical composition, we find that the particle size markedly alters the ability of the LDH to stimulate DC maturation, assessed using a variety of markers including co-stimulatory molecule expression and cytokine secretion. Our findings demonstrate that immunity can be driven purely by chemistry, and open the possibility of rational manipulation of immunity for therapeutic purposes.

P6.09.05
A novel allergen-adjuvant conjugate suitable for allergen-specific Th2 redirection

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Background: As the new TLR7-ligand 8-hydroxy substituted adenine SA-2 was found by our group to revert allergen-specific T_H2 responses both in vitro and in vivo, we studied a new compound (SA-26E) which was chemically conjugated to the purified natural allergen Derp2 (nDer p2-Conj) as a potential new adjuvant for possible application in allergen-specific immunotherapy (SIT).
Method: nDer p2-Conj was characterized by MALDI-TOF analysis and evaluated for induction of NF-κB in CD14+ cells, TLR-triggering in transfected HEK293 cell lines, cytokine production in BDCA4+ and CD14+ cells, Th-skewing effects in allergen-specific human T cell lines and T cell clones and allergenicity in BASOTEST assays.
Results: nDer p2-Conj induced innate cells to produce IFN-α and IL-12 via TLR7 triggering. As a consequence, the conjugate reverted T_H2-prone allergen T cell lines into IFN-γ-producing cells (T_H1/T_H0 phenotype) as assessed by cytokine production in the supernatants, intracellular cytokine expression and T_H-related transcription factor expression. nDer p2-Conj was also able to revert the phenotype of T_H2 established cells. The T_H1 differentiation induced by allergen conjugate could be reverted by the addition of neutralizing antibodies against T_H1 citokines. In addition, Derp2 specific T cell clones derived from the T cell lines cultured in the presence of Derp2 conjugate showed a T_H1/T_H0 profile. Finally, the nDer p2-Conj reduced basophils activation in comparison with uncoupled allergen protein.
Conclusion: A system of chemical conjugation to relevant proteins as allergens has been set up with maintenance of modulatory effects of these complexes, thus allowing the development of new vaccine strategies.

P6.09.06
Helix pomatia Hc (HpH) hemocyanin as protein-carrier and bio-adjuvant of bacterial and viral proteins

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Objectives. Killed viral vaccines and bacterial toxoids are weakly immunogenic. The hemocyanins are widely used as immune modulators. In the present study we promote the hemocyanin, isolated from the terrestrial gastropod Helix pomatia (HpH) as protein-carrier as well as bio-adjuvant. The purified HpH was combined with standard antigens and a construct of HpH with influenza virus hemagglutinin intersubunit peptide (IP) or HpH - tetanus toxoid were used for immunization.
Methods. HpH was isolated and purified from hemolymph by ultracentrifugation and chromatography.
Immunization: Balb/c mice were immunized i.p. with HpH-IP, or HpH combined with tetanus toxoid (TT). As controls commercial anti-flu and tetanus vaccines were used. The mice were bled and collected sera were kept frozen. ELISA: determination of anti-TT and anti-IP antibodies. Cytokine detection: IL4 and IFN-γ levels were measured by ELISA. Cytotoxic assay: We examined the CTL activity of isolated spleen cells to influenza virus-infected cells.
Results. An immunization of mice with Influenza peptide conjugated to Helix pomatia hemocyanin induced a strong anti-influenza cytotoxic response. The IgG antibody response to the TT combined with HpH was comparable to the response of the toxoid in CFA. Immunization of experimental animals with HpH combined with IP or TT led to generation of enhanced levels of key cytokines for development of Th1 or Th2 - related immune response.
Conclusions. The vaccination of mice demonstrates that the HpH is acceptable as a potential bio-adjuvant for subunit vaccines and it could be used as natural adjuvant or protein-carrier.

P6.09.07
Novel vaccine adjuvant for induction of cellular and humoral immunity

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Wittycell S.A.S. is a privately owned company pioneering a new approach in the development of vaccine adjuvants based on the stimulation of innate immune responses through iNKT cells and the amplification of adaptive immune responses through iNKT-dendritic cell interactions.
The most unique feature of WTCc is its ability to induce strong antigen-specific cytotoxic T-cell responses in classical preclinical models also used for benchmark adjuvant study, e.g. CpG. Immunization with WTCc/antigen induces the activation of iNKT as well as the generation of specific cytotoxic T cells. We demonstrate that engendered immune response can be protective in case of

infectious disease but also in tumor model. A statistic time to relaps is observed during tumor therapeutic vaccination with an endogenous antigen after one injection. This delay is associated with activated NK and tumor specific T cells detected not only in spleen but also into the tumor. His distinct characteristic, combined with a good toxicity profile and clear industrial manufacturing feasibility makes WTCc an attractive candidate for many applications, including mass vaccination.
After successful demonstration of the NTK activity and adjuvant effect of WTCc in mice and monkey using different indications and further confirmation of the NTK activity induced by WTCc in humans, Wittycell nominated WTCc as the first clinical candidate. Wittycell had finished a full GMP and GLP preclinical package and started end of 2011 an HBV clinical Phase I/IIa in normal volunteers to evaluate clinical and laboratory safety, tolerability profiles and determine efficacy based on Ab and T cell induction.

P6.09.08
Designing small molecules as optimal vaccine adjuvants

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Adjuvants increase vaccine potency largely by activating innate immunity and promoting inflammation. These processes may result in side effects, which constitute a major hurdle for adjuvant use in humans. Traditional adjuvants are complex poorly characterized molecules or mixtures that provide little opportunity for rational improvements in terms of potency and safety. They are also difficult to manufacture, purify and formulate. Here we describe new principles for engineering small molecule immune potentiators (SMIPs) as adjuvants with increased therapeutic indices.
First, we used medicinal chemistry to identify and optimize a novel class of TLR7 SMIPs, benzonaphthryridines (BZN). We then postulated that limiting the biodistribution of the SMIP adjuvant could increase vaccine efficacy and minimize side effects associated with systemic and generalized inflammation. To test this hypothesis, BZN analogs were designed to be less soluble than traditional LMW drugs to facilitate retention at the injection site. These lipophilic compounds were more potent in vivo than soluble compounds as adjuvants although they were less potent TLR7 agonists and exhibited minimal systemic exposure and no systemic immune activation. However, low solubility renders industrial develop of these first generation SMIPs difficult. This drawback was overcome by designing soluble SMIPs with linkered phosphonates that could be adsorbed to aluminum hydroxide (AlOH) via ligand exchange. Through novel medicinal and formulation chemistry and extensive immuno-pharmacology we show that remarkable potency can be achieved with little to no systemic exposure, localized innate immune activation and short in vivo residence times of SMIP-based adjuvants.

P6.09.09
Long-term antigen-specific antibody responses against RSV glycoprotein by an osmotic active nanocarrier via enhanced phagocytosis

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Recently, subunit vaccines are becoming favorable choice for effective immunization. However, systemic degradation often hampers their immunogenicity. To achieve the best efficacy without drawback, adequate delivery of subunit vaccine is a critical issue. Recently, we developed a polysorbitol-based osmotically active transporter showing high efficacy as a nanocarrier for gene delivery. Here, we report this polysorbitol-mediated transporter (PSMT) for effective delivery of respiratory syncytial virus glycoprotein (R-Gp) as a subunit vaccine candidate. The physicochemical properties of PSMT and PSMT/R-Gp nanoparticles were found suitable; the structural integrity and biological effectiveness (in vitro indexes of immune stimulation) of R-Gp was well protected when complexed by PSMT. Osmotic PSMT was found entirely non-toxic both in vitro and in vivo. A routine intranasal administration of PSMT/R-Gp nanoparticles elicited strong antigen-specific antibody (IgG1, IgG2c, IgA and IgM) responses which was significantly higher compared to that of naked R-Gp and well comparable to cholera toxin (CT)/R-Gp used as controls. The enhanced antibody response was likely due to the osmotic property of PSMT and effective transportation of R-Gp. A long-term antibody persistency was found up to six months after primary immunization. The mechanism study elucidated that macrophage exhibited finger-like projections as a sign of activation upon PSMT/R-Gp treatment and showed internalization through phagocytosis by the osmotic transporter followed by intracellular release from phagosome via proton sponge effect. Furthermore, the precise mechanism of PSMT/R-Gp nanoparticles during the phagocytosis was confirmed by inhibition assay. This study suggests that PSMT could be a safe and potent adjuvant system for subunit vaccines.

P6.09.10
Autologous hsp70 enhances the expression of adhesion, fusion receptors, HLA and costimulatory molecules on murine peritoneal macrophages in a T cell lymphoma

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Tumor microenvironment has been known to suppress the function of macrophages and induce the polarization into tumor-associated macrophages that ultimately support tumor growth and progression. However, various biological response modifiers such as LPS, Interferon, various types of colony-stimulating factors are able to activate macrophage and potentiate the immune system against various immunopathologies. Heat shock protein 70 is one of the natural substances that have been reported to activate macrophages and therefore by virtue, it has been suggested as an effective immunotherapeutic approach against a variety of tumors. However, effect of hsp70 on the expression of adhesion molecules, fusion receptors, costimulatory molecules and HLA molecule have not yet been done. Therefore, in this study, an attempt has been made understand the exogenous application of hsp70 on the expression CD54, Mac-1, CD172a, CD47, CD80, CD86 and HLA-H2d(b) on the macrophages of tumor-bearing host. Macrophages were harvested from normal healthy and Dalton's lymphoma-bearing mice, adherent purified, characterized and single cell monolayer was prepared. Peritoneal macrophages were treated with 10µg/ml of autologous hsp70 purified from DL cells and further experiments were performed. It has been observed that tumor progression suppresses the expression of receptor molecules that are CD54, MCP-1, CD172a (SIRP-α), CD47, CD80, CD86 and HLA-H2d(b) while treatment of autologous hsp70 resulted in significant increase. Therefore, it can be concluded that exogenous application of tumor-derived autologous hsp70 enhances the expression of those molecules that are important for adhesion, fusion, antigen presentation and costimulation on normal as well as tumor-associated macrophages.

P6.09.11
Mast cells redirect the differentiation of dendritic cells away from tolerance and towards immunogenicity

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Administration of mast cell (MC) activators with vaccine antigens induces protective antigen-specific immune responses. The mediators secreted by activated-MCs may act by modulating the balance between effector (LTeffec) and regulatory (LTreg) T lymphocytes. Yet, we demonstrated that the tolerant- or immunogenic-priming of T cells by dendritic cells (DCs) partly depends on GILZ (Glucocorticoid-induced leucine zipper) protein. DCs expressing high GILZ levels acquire a tolerant phenotype and induce LTreg, while they must downregulate GILZ expression to induce LTeffec. We thus hypothesized that MC mediators decrease GILZ expression in resting DCs and thereby direct their phenotype away from tolerance and towards immunogenicity.
We first demonstrated that circulating resting pDCs and mDCs freshly isolated from healthy donors express high GILZ levels. GILZ expression in DCs is under the control of endogenous steroids, IL-10 and TGFβ, and therefore dramatically drops after blood DCs isolation. Thus, GILZ is never detected in IL-4-Monocyte-derived DC (IL4Mo-DCs), but its expression is restored to similar levels as in freshly isolated DCs by treatment with exogenous steroids. Using such GILZ-expressing "resting-IL4Mo-DCs", we showed that activated-MC culture supernatants and histamine significantly reduced GILZ expression in resting-IL-4Mo-DCs through an ERK signaling pathway. This decline of GILZ results in a loss of ability to activate antigen-specific LTreg.
Collectively, these data reveal that MC mediators act as adjuvants by decreasing GILZ expression in DCs and thus their regulatory potential. These novel insights on MC activity highlight new potential pathways in adjuvant therapy. Moreover, they highlight GILZ as a new target to modulate DC functions.

P6.09.12
Adjuvant potential of QS-21 on protective efficacy of DPT vaccine

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Background: Combined use of vaccines and adjuvants is emerging as one of the innovative approaches in adjuvant development. Various immunomodulators such as QS-21, MDP and MF-59 are currently under consideration for possible inclusion in alum-containing vaccines.
Methods: Guinea pig model was used for the study. Host challenge procedure was used wherein diluted doses of DPT vaccine (1:80 & 1:160) and diphtheria toxin ($10LD_{50}$) were used as antigen and challenge respectively. Antitoxin levels, IFN-γ/IL-4 level ratios, sera cortisol and survival rate were used as markers of immune efficacy. Vero cell assay and ELISA techniques were used to determine antitoxin and cytokine levels respectively.
Results: Co-administration of QS-21 (250 µg/Kg) with DPT vaccine resulted in significant increase ($p<0.05$) of after-challenge diphtheria antitoxin levels (0.219 ± 0.060 IU/ml) as compared to same and double dose of control (0.076±0.045 & 0.160±0.064 IU/ml respectively). Moreover, IFN-γ/IL-4 ratios were significantly ($p<0.05$) raised (0.80 vs 0.51), suggesting a mixed Th1/Th2 response of QS-21 as compared to more limited responses of alum alone. Serological findings were supported by decreased morbidity and mortality (100% survival in QS-21 treated group vs 57% & 80% in control groups.
Conclusion: Study establishes adjuvant potential of QS-21 with DPT vaccine as indicated by significantly enhanced protective efficacy (Kaplan-Meier survival analysis), significantly higher antitoxin and cytokine levels (Mann-Whitney test) even at higher dilution (1:160) of vaccine, suggesting antigen sparing effect. Such approaches will be helpful in further reduction of antigenic requirements for obtaining protective immunity, thus increasing production capacity and decreasing cost.

P6.09.13
An adjuvant, fungal immunomodulatory protein (FIP)-*fve*, potentiates the immune response by engaging the co-stimulatory molecules, CD27 and CD134 (OX-40)

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Fungal immunomodulatory protein (FIP)-*fve* has been shown to be an immunomodulator in tumor immunology and allergy. However, the molecular mechanism by which FIP-*fve* modulates the immune response to exert its adjuvant effect is yet to be elucidated. Our *in vitro* results demonstrated that FIP-*fve* required the interaction between mouse T cells and bone marrow-derived dendritic cells (BM-DCs) to induce the optimal T cell proliferation and secretion of several pro-inflammatory cytokines and chemokines, such as IFN-γ, MIG, MIP-1α, MIP-1β, and IP-10. In addition, we found that FIP-*fve* could directly activate T cells in a dose- and time- dependent manner, shown by the up-regulation of CD69, CD25, CD134 (OX-40), CD137 (4-1BB) on both gated $CD4^+$T and $CD8^+$T cells as well as CD27, CD28 on gated $CD8^+$T cells mostly. Interestingly, blocking of the interaction between CD134 (OX-40), CD27 and their corresponding ligands significantly reduced the FIP-*fve*-induced T cell proliferation and cytokine/chemokine secretion. Furthermore, when FIP-*fve* was injected into the mouse footpad, we observed that the absolute cell number of $CD4^+$T cells, $CD8^+$T cells, and B cells were increased tremendously in the draining lymph node. However, our *in vivo* bromodeoxyuridine (BrdU)-incorporation assay suggested that the proliferating cells upon FIP-*fve* injection were mostly $CD8^+$T cells and $CD4^+$ T cells. To confirm the T cell activation in draining lymph node, FIP-*fve* was also shown to up-regulate the expression of CD69 and the co-stimulatory molecules, CD27 and CD28, on the gated T cells. Thus, FIP-*fve* may function as an adjuvant via activation and modulation of immune system.

P6.09.14
SCE 350ET: A plant derived adjuvant inducing Th1 and Th2 type immune response

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Adjuvants are the vaccine additives that efficiently induce antigen specific immune response. In the present study, we have evaluated the adjuvanticity of the supercritical CO2 extract of Seabuckthorn leaves (SCE350ET) in Balb/C mice. The efficacy of SCE350ET as adjuvant was determined using diphtheria toxoid (DT) antigen. The dynamic changes in the immune response were measured in terms of humoral and cell-mediated immune response (CMI). The results indicated that the levels of DT specific immunoglobulins IgG and its isotypes IgG1, IgG2a and IgG2b were significantly enhanced by SCE350ET. In addition, mitogenic stimulation of splenocytes isolated from SCE350ET and DT immunized group of mice showed significant lymphocyte proliferation and production of Th1 and Th2 cytokines interferon (IFN)-γ and interleukin-4 (IL-4) respectively. Furthermore, increased expression of CD25, MHCII and effector memory markers (CD44hi CD62Llow) by SCE350ET, confirmed its potential to generate strong CMI. The increased antibody response may be due to the increased expression of Oct-2, which skewed B cells to antibody producing plasma cells. In conclusion, this study suggests that SCE350ET has potent immunostimulatory activity and hence, seems to be a promising balanced Th1 and Th2 directing immunological adjuvant.

P6.09.15
Oligomannose-coated liposome as a novel antigen-delivery vehicle and an adjuvant for induction of cellular immune responses

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Antigen-delivery systems are important for inducing and modifying immune responses. A key to development of vaccines is the ability to deliver antigens to antigen-presenting cells (APCs) and to activate APCs more efficiently for induction of adoptive immunity. Thus, strategies that target APCs and modulate APC functions in vivo have significant implications for vaccine design.
We have demonstrated that oligomannose-coated liposomes (OMLs), which consist of phosphatidylcholine, cholesterol, a mannotriose-containing neoglycolipid, can induce strong cellular immunity. In this study, we summarize OMLs as novel antigen-delivery vehicles that have a strong adjuvant effect on induction of Th1 immune responses and CTLs specific for the encased antigen. This property of OMLs is due to their ability to assist selective cellular uptake by APCs in vivo and to promote subsequent APC maturation, effective presentation of the encased antigens on both MHC class I and class II molecules, preferential secretion of IL-12 from APCs, and migration of APCs into lymphoid tissues from peripheral tissues, where both naïve CD4+ and CD8+ T cells are activated.
Administration of OML-based vaccines can eliminate an established tumor, inhibit elevation of the serum level of IgE against an allergen, and prevent progression of some protozoan infections in mouse models. OMLs with an encased antigen are also able to induce antigen-specific CTLs in PBMCs obtained from patients. These feasibility studies have revealed potential of OML-based vaccines for clinical use in vaccination for diseases in which CTLs and/or Th1 cells act as effector cells.

P6.09.16
Fucoidan effect on mononuclear leukocyte immunophenotype and lymphoid organ morphology in mice

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The aim of investigation was to study the fucoidan effect on immunophenotype characteristics of spleen mononuclear leukocytes (ML) and on structure and cell composition of mice lymphoid organs. Fucoidan was administrated to CBA mice in a dose of 200 mg/kg of body weight. Subset structure of lymphocytes isolated from spleen was examined with flow cytometry. ML immunophenotype assay was carried out under the administration of two types of preparation - fucoidan japonica and cidcorideus to splenocyte cultures in a dose of 10 μg/ml. Paraffin sections of lymphoid organs were stained by histological and histochemical methods. Intraperitoneal fucoidan injection evoked 1,5-2-fold increment in natural killers-T cells, by 2-6-fold - the amount of T- and B-lymphocytes, and by 1,5-fold - the number of cytotoxic lymphocytes. Morphological analysis revealed the expansion of thymus lobe cortex, enlargement of periarterial muffs area, lymphoid nodes frequently merging in conglomerates in spleen. Following the introduction each of two fucoidan preparations to ML mice spleen cultures there were observed similar changes in splenocyte: statistically significant increase in amount of NK, NKT-cells, activated T-helpers, B-lymphocytes, and decrease in $CD3^+$ and $CD8^+$ cells. Pronounced TLR expression by cell surface was noted. It was demonstrated that fucoidan modulated the effector activity of innate and adaptive immunity and resulted in lymphoid cell proliferation in primary and secondary lymphoid organs. Its ability to promote Toll-like receptor expression by ML surface seems to be promising for its use in immunotherapy. This work was supported by RFBR grant №11-04-96037r_ural_a and administrative body of Perm Region.

P6.09.17
Chitosan effect on the inactivated influenza vaccine immunogenicity

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Inactivated influenza vaccines are one of the most effective means of preventing influenza. This type of vaccine reduces the protective properties in the elderly and poorly protected against drift variants of influenza virus.
Given the lack of immunogenicity of inactivated influenza vaccine, we investigated the effect of chitosan on the activation of immune effector mechanisms under the combined administration with inactivated influenza vaccine.
As adjuvant for influenza vaccines the chitosan glutamate solution (MW 200 kD) was taken, deacylated by 85%, to a final concentration of 0.5%. Mice were vaccinated intramuscularly with 0.2 ml of a formulation containing the vaccine and 0.5% chitosan glutamate.Mice were vaccinated intramuscularly injecting 0.2 ml of a formulation containing 3 mg for each component of the vaccine, and 0.5% of the preparation of chitosan (final concentration) or buffer.
It is shown that parenterally administered chitosan derivatives in combination with inactivated influenza split vaccine strengthened the activation of innate and adaptive immunity in mice, which was manifested in the expression of Toll-like receptors on the spleen(-s) (TLR2, TLR9), induction of cytokine synthesis in blood (IL -12, INF-γ, IL-17, IL-1β and IL-5), increasing the number of T (CD3, CD4, CD8, γδT), B lymphocytes (CD19, CD5), NK (CD16/32), NKT (CD3 / NK), T-reg (CD4/CD25/Foxp3) cells.
The expediency of chitosan glutamate application to enhance the immunogenicity and protective efficacy of inactivated influenza vaccines that activate the mucosal and systemic immunity by parenteral immunization is demonstrated.
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P6.09.18
Functional and immunophenotype characteristics of peripheral blood mononuclear leukocytes from healthy donors under sodium nucleinate effect

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Aim: investigation of sodium nucleinate (SN) - sodium salt of low-molecular weight RNA produced from baker's yeast - against functional activity and immunophenotype of cells-effectors of immunity from healthy donor peripheral blood.
There were analyzed killer activity relatively to K-562 cell line, proliferative activity and immunophenotype of mononuclear leukocytes isolated from healthy donor blood and cultivated for 48 h with the addition of sodium nucleinate.
Investigation demonstrated that lymphocytes obtained from peripheral blood mononuclear leukocytes (PBML) as a result of extracorporal activation with sodium nucleinate were characterized by significantly greater functional activity as compared with the initial cells. Cytotoxic properties of activated cells increased from 49,5% (control) to 69,7-74,7% at SN concentration of 1-100 μg/ml. Proliferative activity increased from 16,1% (control) to 56,3-61,3% after 1-10 μg/ml of SN addition in cultures. Smears made from cultural cell suspension demonstrated large amount of pro-lymphocytes and immunoblasts, some cells in mitotic division that reflected the lymphoid cell blasttransformation as a result of their SN-induced activation. While studying the immunophenotype of SN-activated PBML from donors it was revealed that those expressed activation molecules CD38, CD25, molecules of II-type major histocompatibility complex (HLA-DR), adhesion molecules (CD57, CD58) on their membranes.
Thus sodium nucleinate renders promoting effect on natural killers of donors and results in formation of lymphocytes similar to lymphokine-activated killers by their immunophenotype. SN ability to activate PBML evidences for its promising use for immune disease prevention

and as nonspecific stimulator of immunity effectors in therapy of oncological patients.

P6.09.19
Studies with novel synthetic immunogens as a tool for efficient antibody generation

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With the aim to develop efficient and highly defined immunogens for a robust induction of anti-peptide antibody responses we investigated Three-Component Immunogens that only incorporate the minimally required components: a B cell epitope; a specific, highly effective promiscuous T_H cell epitope; and covalently attached synthetic immunostimulatory adjuvant such as muramyl dipeptide (MDP). In this study the Three-Component-Immunogen PTH-Polio-MDP was analyzed for its potential in the production of monoclonal antibodies compared to a conventional immunogen incorporating the carrier protein KLH as well as a Three-Component-Immunogen containing a cationic peptide. Balb/c mice were immunized with the different immunogens - with or without additional adjuvant (such as CFA/IFA) - and subsequently hybridoma cells were prepared and analyzed regarding the produced antibodies. Furthermore the potential of MDP to activate bone marrow-derived dendritic cells was examined. We found that the Three-Component-Immunogen with MDP (PTH-Polio-MDP) exhibits a clear immunostimulatory effect even without the use of an additional adjuvant. In contrast, the standard immunogen with a carrier protein required the use of adjuvant to induce a sufficient humoral immune response.

P6.09.20
EDA-streptavidin fusion protein conjugated to biotinylated HCV-NS3 protein for the induction of T cell immune responses against NS3

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Aims: Recombinant proteins are poor immunogens and do not induce strong T cell responses if they are not combined with appropriate adjuvants, or modified to improve their capture by dendritic cells (DCs). In previous works we have shown that fusion of an antigen to the extra domain A from fibronectin (EDA) targets the antigen to dendritic cells via TLR4 and improves its immunogenicity. In this work we have prepared a fusion protein between EDA and streptavidin (EDAvidin) to allow its interaction with biotinylated antigens. We have tested the immunogenicity of biotinylated NS3 combined with EDAvidin to induce strong anti-NS3 immune responses as a potential vaccination strategy against HCV infection. Methods: Recombinant EDAvidin, EDA-NS3 fusion protein and NS3 were produced and purified by affinity chromatography. NS3 was biotinylated using Sulfo-NHS-biotin reagent. The capacity of EDAvidin to bind to biotinilated proteins was tested by surface Plasmon resonance, western blot and ELISA. Immunogenicity of the recombinant proteins was tested in HHD transgenic mice (expressing human HLA-A2) by ELISPOT and in vivo killing assays. Results: Recombinant EDAvidin tetramerizes and binds very efficiently to biotynilated proteins. EDAvidin retains the proinflammatory properties of EDA, activating TLR4 signaling pathway. Immunization of mice with EDAvidin combined with biotinylated NS3 induce a strong anti-NS3 immune responses similar to that induced by the fusion protein EDA-NS3. Conclusions: EDAvidin can bind biotinylated proteins and improve their immunogenicity in vivo. EDAvidin combined with biotinylated NS3 can be considered as a vaccination strategy against hepatitis C virus infection.

P6.09.21
Development of a new microparticle vaccine adjuvant with the ability to deliver peptides to dendritic cells

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MIS416 is a novel vaccine adjuvant generated from propionibacterium acnes cell wall skeletons comprising naturally occurring NOD-2 and TR9 ligands with the ability to induce effective adaptive Th1 immunity (1). In this research project we aim to enhance the therapeutic potential of MS416-adjuvanted vaccines by directly conjugating specific peptides derived from Tumors Associated antigens (TAA), achieving co-delivery of antigen and adjuvant. We hypothesized this will enhance the quality and quantity of ensuing adaptive immunity. Using the high-affintiy streptavidin-biotin complex as a conjugation strategy for peptide attachment, we were able to irreversibly couple different fluorescent molecules to the surface of MIS416 as proof of principle. The OVA-derived peptide SIINFEKL, was then coupled to MIS416 and this MIS416-peptide conjugate was pulsed onto BMDC (c57bl6 mice) showing that DC were activated by the conjugate. Flowcytometry, analysis showed that levels of MHCII,CD40,CD80 and CD86 were upregulated by our novel vaccine conjugate. Proliferations assays have been carried out to prove that DC pulsed with MIS416-SIINFEKL are able to activate OT1 T cell and upregulate activation markers such as CD69. Future work will test MIS416-TAA conjugates in therapeutic transplanted tumor models.
[1] Girvan et al, Vaccine. 2011 Jan 10;29(3):545-57

P6.09.22
PolyICLC increases the immunogenicity and efficacy of the filovirus virus-like particle (VLP) vaccine

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The filoviruses Marburg and Ebola have reported mortality rates of as high as 90% and occur naturally in Africa and the Philippines. We examined the efficacy of a promising dsRNA mimic, polyICLC (Hiltonol), in combination with the filovirus virus-like particle (VLP) vaccine. VLP vaccination of cynomolgous macaques resulted in 100% protection from Ebola Sudan challenge at low VLP dose levels, when vaccination was in combination with 100ug of polyICLC. To further understand the efficacy of the adjuvant, we used progressively decreasing doses of polyICLC with a non-protective dose of vaccine in mice. We show that at dose levels as low as 100ng, polyICLC increases survival after Ebola challenge from 10% to 100% and increases anti-glycoprotein IgG titers. To further elucidate the impact of the adjuvant on the immune response, we compared the anti-glycoprotein T cell response in mice vaccinated with a protective dose of VLP, with and without polyICLC. PolyICLC increased the antigen-specific T cell response, resulting in a detectable, polyfunctional, and predominantly CD4+T cell response. Finally, we explored the relationship between adjuvant efficacy and serum cytokine levels as a measure of potential toxicity. We collected serum 1 hour, 5 hours, and 24 hours after vaccination, and we examined serum levels of type I interferon as well as a panel of chemokines and cytokines. These data demonstrate that polyICLC is a highly efficacious adjuvant at dose levels far lower than those previously explored, and it increases the immunogenicity and efficacy of the VLP vaccine.

P6.09.23
Physicochemical and immunological parameters of the anti-allergic adjuvanted vaccine PROLINEM-DS

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Introduction: Th1 promoting adjuvants, as the Proteoliposome from Neisseria meningitides (PL) seemed to be helpful for designing new anti-allergic vaccines. The novel anti-allergic vaccine candidate comprising allergens from Dermatophagoides siboney mite and PL

adsorbed onto alum, showed a protective anti-allergic response in Balb/c mice. The objective of this work was to test the physics-chemical and immunological parameters for stability to real time of the new vaccine. Methods: Following the ICH methodology, samples of three pilot scale GMP batches were stored at 4°C and assayed at 0,3,6,9,12,18 and 24 months. Possible desorption from alum gel was monitored, testing the supernatant for allergenic activity (IgE-inhibition ELISA), Der s1 content (MAb-ELISA) and total protein content. Preservation of antigen's integrity was checked by SDS-PAGE and Western-Blotting after forced desorption. Other tests were applied for measuring preservative content, pH stability, and sterility. Since, a potency test is not yet established for this new vaccine, allergen-specific immunogenicity in Balb/C mice was determined at the beginning and end of the study. Results: After 24 months no deviations of quality specifications were detected in any parameter. Although a slight tendency toward increasing the allergen activity and Der s 1 content in the supernatant was noted, it was not statistically significant (regression analysis, $p<0.05$). The immunogenicity test showed the expected outcome regarding induction of allergen-specific IgG, IgG1 and IgG2a antibodies (is PL adjuvant effect dependent), similarly to initial results. Conclusion: This study proved the vaccine stability during 24 months as a basis for approval of a reliable expiration period.

P6.09.24
Immunogenic and adjuvant properties of OmpS1 and OmpS2 porins

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Salmonella enterica serovar Typhi (*S*. Typhi) is the causal agent of typhoid fever, a disease that primarily affects developing countries. Various antigens from this bacterium have been reported to be targets of the immune response, with the highly abundant OmpC and OmpF porins being of particular importance. Recently, the S. Typhi genome has been shown to encode two porins - OmpS1 and OmpS2 - that are expressed at low levels under in vitro culture conditions; however, the immunogenic properties of these proteins and their contributions to the bacterial immune response have not been investigated. In this study, we demonstrate that immunising mice with either OmpS1 or OmpS2 induced production of specific, long-term antibody titres and conferred protection against *S*. Typhi challenge. We also found that OmpS1 is a Toll-like receptor (TLR) 4 agonist, whereas OmpS2 is a TLR2 and TLR4 agonist. Both porins induced the production of TNF and IL-6, and OmpS2 was also able to induce IL-10 production. Co-immunisation of OmpS1 or OmpS2 with ovalbumin (OVA) increased anti-OVA antibody titres, the duration and isotype diversity of the OVA-specific antibody response, and the proliferation of T-lymphocytes. These porins also had adjuvant effects on the antibody response when co-immunised with either the Vi capsular antigen from *S.* Typhi or inactivated 2009 pandemic influenza A(H1N1) virus (A(H1N1)pdm09). Taken together, the data indicate that OmpS1 and OmpS2, despite being expressed at low levels under in vitro culture conditions, are potent protective immunogens with intrinsic adjuvant properties.

P6.09.25
The role of alum-induced host DNA release in antigen-presenting cell activation and T cell priming

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Aluminum salts (alum) are widely used as adjuvants in human vaccines even though their mechanism of action remains unclear. It is known that host DNA rapidly coats injected alum and may be responsible for immune cell stimulation. Intramuscular immunization with alum plus influenza A nucleoprotein stimulates robust CD4+ T cell responses. We have confirmed that this response is attenuated if DNAse is co-injected with alum and nucleoprotein. Our experiments suggest that host DNA, induced by alum, increases the interaction time between antigen specific T cells and antigen presenting cells (APCs). Following alum immunization, at least three distinct populations of antigen-loaded cells appear in the draining lymph nodes: monocytes, migratory dendritic cells, and B cells. We therefore hypothesize that host DNA, released upon immunization with alum, contributes to alum's adjuvanticity by activating one or more of these sets of APCs. DNAse treatment does not prevent any of these cell populations from taking up antigen or migrating to draining lymph nodes, but it may alter the stimulatory qualities of these cells. Therefore, we are currently assessing how DNAse co-injection alters the activation profiles of antigen-loaded APCs and their ability to prime CD4+ T cells. We expect that one or more subsets of APCs depend on host DNA stimulus to effectively prime T cells upon alum immunization. These studies contribute to our understanding of the role of host DNA release in alum's adjuvant effect.

P6.09.26
Salmonella typhi porins are adjuvants on the specific immune responses to co-inmunized antigens

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TLR agonists as CpG and flagellin have been used as adjuvants on a variety of antigens. We have previously shown that Salmonella typhi porins are highly immunogenic proteins which antibody responses are modulated by TLRs and that induce diverse and long lasting antibody responses against themselves. Here, we evaluated the adjuvant effect of S. typhi porins on antibody and cellular immune responses over a model antigen, as ovalbumin, and an inactivated pandemic influenza virus. We found that S. typhi porins exert adjuvant effect on the antibody responses elicited against ovalbumin, that were characterized by the diversification of isotypes and avidity maturation of antibodies. The adjuvant effect elicited by porins was also observed on T cell responses, as we found augmented proliferation of CD4 specific T cells and cytokine secretion, mainly IFN-γ and IL-2. Finally, we evaluated if S. typhi porins could also exert and adjuvant effect on immune responses against an immunogen, as an inactivated pandemic influenza virus. We found that porins augment the magnitude and persistence of antibody responses against influenza virus, and that this response was isotype diversified and displayed characteristics of avidity maturation. All together, these results suggest that Salmonella typhi porins could be used as adjuvants because of the immunostimulatory properties on the antibody and cellular immune responses that can elicit.

P6.09.27
Therapeutical effects of a novel nDer p2-conjugated TLR7 in a mouse model of lung inflammation

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There is a growing interest in the use of Toll-like receptor 7 ligands for the new therapeutical strategy of allergic diseases. Modified adenines (MA) which trigger TLR7 represent a new group of adjuvants. We investigated the ability of a synthetic MA, called SA26E, chemically conjugated to nDer p2 to trigger murine TLR7. We analyzed the therapeutical effect of SA26E conjugated in a murine model of lung inflammation.
C57Bl/6 mice were intraperitoneal immunized with nDer p2 at d0 and d7. At d14 mice were intratracheally challenged with nDer p2, while at d21 and d28 mice were intraperitoneal treated with an administration of nDer p2-conjugate or nDer p2 alone. At d49 and d53 mice were challenged with nDer p2 and sacrified at d56 for the analysis.
SA26E mantained the ability to trigger HEK293 cell line transfected with murine TLR7 after conjugation. Moreover MA-nDer p2 conjugate reduced eosinophils in bronchoalveolar lavage of mice treated but not of control mice. This effect was associated with a shift in the antibody profile from a type 2- (IgE and IgG1) to a type 1-associated (IgG2a). A reduction of IL-13 and an increase of IFN-γ was observed in the supernatants of mononuclear cells from lung in vitro stimulated. A concomitant significant increase of IL-10 levels was found in the supernatants of nDer p2-stimulated cells of spleen and draining lymph nodes.
Data suggest that the nDer p2-SA26E conjugate regulates and redirects the immune response in a therapeutical fashion and may constitute an approach for immunotherapy of allergic diseases.

P6.09.28
Inhibitory effects of silibinin on invasive properties of human breast cancer cell line, MDA-MB-231, through suppression of the proteolytic cascade of cathepsin B/ urokinase-type plasminogen activator

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Tumor cell dissemination to distant organs is thought to be the main reason for death in patients with advanced breast cancer. Although new treatments including multiple new cytotoxic drugs has been developed for metastatic breast cancer these therapeutics exert a minor impact on outcome of disease. So, establishment of more effective preventive and curative regimens with a low cytotoxicity profile against MBC seems crucial.
This study aimed to study the effects of silibinin, a natural non-toxic anti-cancer drug, on metastatic breast cancer cell line, MDA-MB-231. Herein, MTT metabolic activity assay, bromodeoxyuridine cell proliferation assay, cell-based ERK 1/2 activation assessment, cathepsin B activity assay and quantitative real-time PCR were performed to appraise the effects of silibinin on breast cells.
Silibinin inhibited metabolic activity, cell proliferation, ERK 1/2 activation, cathepsin B enzymatic levels in MDA-MB-231 cells. In addition, an expressive decrease in mRNA levels of cathepsin B, urokinase plasminogen activator receptor and hyaluronidase-2 were observed.
Altogether, silibinin inhibits proliferation of breast tumor cells. Moreover, our data revealed that silibinin could inhibit invasive features of a invasive breast cell line, through suppression of the proteolytic cascade of cathepsin B/urokinase-type plasminogen activator. Furthermore, silibinin might disrupt the activation of ERK 1/2 through suppression of uPAR. silibinin might control the transcriptional levels of uPAR through induction of programmed cell death 4 (PDCD4).

P6.09.29
Effective mucosal protection against sexually-transmitted viral infections is provided by MEC/CCL28

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Background: MEC/CCL28 binds to CCR3 and CCR10 and recruits IgA-secreting plasma cells (IgA-ASCs) in the mucosa. The suitability of CCL28 as an adjuvant for the elicitation of optimal innate and acquired immunity was assessed in mice using HIV-1, Influenza A and HPV-16 Virus-like Particle models.
Materials and methods: Balb/c mice were immunized intramuscularly with a prime-boost regime based on HIV1IIIB-, H7N1- or HPV16-VLPs in the presence/absence of CCL28 and of the control CCL19. Flow citometry evaluation of CCR3 and CCR10 was performed on splenocytes. Th1 and Th2 cytokine production was performed on splenocytes and mucosal biopsies, whereas IgG and IgA antibodies were evaluated in sera and mucosal secretions. Immune sera and mucosal secretions were tested for ex vivo neutralization of either HIV-1 subtype B and C strains, H7N1 strain or HPV-16. IgA-ASC recruitment at the mucosal level was verified with immune-histochemistry analyses.
Results: The following immune parameters were significantly augmented in VLP-CCL28 mice compared to all other groups in all three animal models: 1) the percentage and the surface density of CCR3 and CCR10 on CD19+ splenocytes; 2) antigen-specific IFN-γ, IL-4 and IL-5 production in splenocytes and mucosal specimens; 3) total and antigen-specific IgG and IgA titers in sera and mucosal secretions; 4) serum and mucosal secretion, mostly IgA-dependent, neutralizing activity; 5) IgA-ASCs in either rectum, lungs or uterine cervix.
Conclusions: CCL28 has a robust immunomodulatory effect on both systemic and mucosal immunity and could play a useful role in increasing the efficacy of preventive vaccines for mucosal viral infections.

P6.09.30
Adjuvants as possible solution to polysaccharide unconjugated vaccines

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Background. Plain polysaccharide (Ps) vaccines were the first meningococcal vaccines developed. Nevertheless, they neither function in new born / infant nor induce memory response and could induce antibody hyporesponsiveness. Therefore, covalent conjugated vaccines offer a particular type of adjuvation where the carrier gives the T cell help. However, adjuvants in the restricted sense never were considered as an alternative solution. Therefore, we developed a proprietary adjuvant Finlay platform derived from Neisseria meningitidis. Aims. To determine if adjuvants could be use as an alternative solution to covalent conjugated Ps vaccines. Results. The Ps used were: PsVi from Salmonella Typhi and PsA from N. meningitidis serogroup A respectively. The adjuvants used were: Outer membrane vesicles (Proteoliposome, AFPL™, Adjuvant Finlay PL) from N. meningitidis serogroups B, A, W135 and AFCo (Cochleate) 1 derived from PL. MenAfricVac™ as well as unconjugated plain Ps were use as controls. Anti Ps IgG class and IgG1, IgG2a, IgG2c and IgG3 subclasses were evaluated after two parenteral or three mucosal doses. The conventional memory response was evaluated after a booster dose. Non-conventional T and B memory response was also evaluated. AFPL™ overcomes the thymus-independence of Ps inducing a Th1 typical pattern. AFPL™ induces similar anti Ps responses than covalent conjugated vaccines. They induce memory response at B and T cell levels. Conclusion. Adjuvants could be an alternative to covalent conjugated vaccines. The mechanism of T cell memory induction needs to be fully explored.

P6.09.31
Influence of phosphate salts and alum content on to the immunogenicity and stability of a *Dermatophagoides siboney* adjuvanted vaccine

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Background: AFPL1 is a novel combination adjuvant containing alum-adsorbed Outer Membrane Proteoliposomes from Neisseria meningitidis. Phosphate ions interfere with adsorption of Dermatophagoides allergens to Al(OH)3. In addition, alum is associated to the development of granulomas in the injection site, so its content should be minimized.
Aim: To evaluate the immunogenicity and stability of formulation variants of a novel Dermatophagoidessiboney AFPL1-adjuvanted vaccine, using different content of phosphate salts and Al(OH)3.
Methods: A 23 experimental design was used for evaluating the effect of phosphate (0-8.5mmol/L) and alum content (0.5-2mg/mL) on to adsorption of Der s1. Stability during storage was assessed following standard ICH guidelines. Four formulation variants were selected for immunogenicity testing in Balb/C mice, using three weekly injections (2 µg Der s 1). Allergen-specific antibodies IgG, IgG1, IgG2a, and IgE were measured in serum by ELISA; IL-13, IL-5, IL-10 and INF-γ cytokines were measured in cell culture supernatants by ELISA.
Results: Best variants regarding Der s1 adsorption were in the range 0-4.26mmol/L of HPO4and0-2.5mmol/L of H2PO4, achieving values up to 99.9%. All variants were able to maintain Der s1 and protein adsorption levels during storage. Regarding immunogenicity, all tested variants induced a mixed response of IgG1 and IgG2a antibodies in a similar extent, as well as, a moderate increase of IFN-γ, and decrease of Th2 cytokines and lung tissue inflammation as compared to allergic control.
Conclusions: Reduction of alum and phosphate content could be considered as pharmaceutical improvements with no disadvantages regarding immunogenicity and safety of this experimental allergy vaccine.

P6.09.32
GM-CSF enhances the cellular immune response elicited by a DNA vaccine against HIV even in a low dose approach.

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The cytokine GM-CSF has been used in several clinical trials with vaccines and have shown great adjuvant efficacy when combined with vaccine formulations that aimed induction of cellular immune response. This property is mainly due to GM-CSF ability to attract and stimulate antigen-presenting cells to the immunization site. Here we demonstrate that GM-CSF co-administration can enhance the magnitude and quality of the immune response induced by a HIV-specific DNA vaccine. The DNA vaccine HIVBr18, which encodes 18 conserved, multiple HLA-DR-binding peptides from the HIV-1 subtype B consensus sequence, was mixed with a plasmid encoding murine GM-CSF. Balb/c mice were immunized with 100 µg of HIVBr18 plasmid alone, or in combination with 100 µg of the GM-CSF plasmid. After three doses we observed that the group of animals that received the combination of plasmids developed a cellular immune response with higher magnitude and better quality, as demonstrated by a higher number of cells producing simultaneously IFN-g, IL-2 and TNF-a. We also observed that reducing the amount of HIVBr18 to 25 µg, in association with GM-CSF, led to a cellular immune response with similar magnitude to that found with 100 µg of the HIVBr18 plasmid administered alone antigen administrated alone. Our data corroborate the adjuvant properties of GM-CSF on DNA vaccines observed in different models. DNA vaccines that are more immunogenic and generate higher quality immune responses could be instrumental in the development of a new formulation for a HIV vaccine.

P6.09.33
Proteus mirabilis flagellin, neither a good adjuvant nor a protective antigen against urinary tract infection

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P. mirabilis, a gram negative bacterium associated with complicated urinary tract infections (UTI), expresses several virulence factors including MR/P fimbriae and flagella. Flagellin from certain bacteria has shown interesting adjuvant and protective properties but *P. mirabilis* flagellin has not been analyzed so far.
Here, we evaluated the role of *P. mirabilis* flagellin as protective antigen in a mouse UTI model, and its adjuvant capacity for the protective antigen MrpA (MR/P fimbriae structural subunit).
Four groups of mice were intranasally immunised at days 0, 3, 10, 17 and 24 with either MrpA, flagellin, both proteins and PBS only. Before and after immunisation urine and blood samples were collected to evaluate antibodies induction. Cytokine production was analysed on day 30 in splenocytes. At day 31 mice were challenged using an ascending *P. mirabilis* UTI model, being sacrificed 7 days after for bacteria quantification in kidneys and bladders.
MrpA-immunised mice showed a significant increase in serum and urine specific IgG and IgA while mice immunised with MrpA-flagellin or flagellin did not. Animals did not show significant anti-flagellin antibodies induction. MrpA-immunised showed a significant decrease in bacteria recovered from bladders and kidneys compared with any other group of mice. Instead, infection levels in flagellin, MrpA-flagellin and PBS-immunised mice showed no significant differences between them. Remarkably, IL-10 was significantly induced in the mice receiving flagellin or MrpA-flagellin.
These results showed that *P. mirabilis* flagellin is not a good adjuvant and did not protect mice against UTI, and suggested that it may have an immunomodulatory role.

P6.09.34
Circulating influenza-specific $CD4^+$ $ICOS1^+$ $IL\text{-}21^+$ expand after vaccination, exert helper function and predict antibody responses in humans

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Protection against influenza is mediated by neutralizing antibodies, and their induction at high and sustained titers is key for successful vaccination. We searched for early immunological parameters that associates in a predictive manner with the rise in neutralizing antibody after influenza vaccination. T follicular helper cells (Tfh) have been identified, in lymph-nodes and tonsils, as the T cell subset specialized in helping B lymphocytes with IL-21 and ICOS1 playing a central role in this function. We searched for antigen specific $IL\text{-}21^+$ $CD4^+$ T cells in peripheral blood. We show that influenza-specific $ICOS1^+$ $IL\text{-}21^+$ $CD4^+$ T cells can be measured in human blood upon *in vitro* stimulation, expand after MF59-adjuvanted influenza vaccination and their expansion associates and predicts the rise of functional antibodies. We also show that blood derived $CXCR5^-$ $ICOS1^+$ $CD4^+$ T cells are enriched in T cells able to help influenza-specific B cell differentiation into antibody-secreting cells
in vitro in an IL-21- and ICOS1-dependent manner. We suggest that the expansion of an antigen-specific $IL\text{-}21^+$ $CD4^+$ T cell sub-population in peripheral blood is an early predictor of the ability of a vaccine to prime the immune system and could become a useful parameter to test vaccine immunogenicity in humans.

P6.09.35
Immunological characterization of M13 phage vaccine

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We have recently shown the immunological basis of M13 phage vaccine that is dependent on MyD88-signaling. Mice can be immunized by administrating phosphate-buffered solution containing M13 phage without any adjuvant materials. M13 phage evokes strong IgG response as early as 14 days after primary immunization and lasts its immunological memory for long term (BBRC 402: 19-22, 2010).
We have also demonstrated that M13 phage displaying less than a few copies of amyloid β 42 (Aβ42)-mimotope peptide at the N-terminus of g3p induces a significant level of Aβ42 fibril-specific IgG antibody in mice (J. Neuroimmunol. 236: 27-38, 2011). These results suggest promising features of M13 phage displaying a given epitope-inserted g8p in place of g3p for vaccine vehicle because a phage bears 2700 copies of g8p in contrast to 5 copies of g3p. We demonstrate the immunological features of motif-integrated g8p-phage in murine models of Alzheimer disease and cedar pollinosis, respectively.
Safety issues and the potential significance of bacteriophages for therapeutic agents have been demonstrated (EMBO reports 13: 20-21, 2012).
Thus, peptide-displaying filamentous bacteriophage induces strong immune responses to displayed peptides, suggesting a novel application of phage-display library as bacteriophage vaccine.

P6.09.36
New proteoliposomevaccineformulation from N. meningitidisserogroup B, without aluminum hydroxide, retains its antimeningococcalprotectogenic potential as well as Th-1 adjuvant capacity

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Proteoliposomes purified from the Outer Membrane of *Neisseria meningitidis* B, have been successfully used as core for adjuvants and vaccine formulations .We have tried to increase their structural definition and to conserve their efficacy and stability avoiding the addition of the aluminum hydroxide to the final formulation.Liposomal particle systems were prepared from components of defined molecular structure, such as a *Neisseria meningitidis* B protein complex, extracted and purified without forming vesicle structures.Liposomes were prepared from a mixture of dioleoylphosphatidyl serine and cholesterol, using the classical dehydration-rehydration method.Transmission Electron Microscopy (TEM) was used to characterize the liposomes.BALB/c mice were used for animal testing procedures.Analysis of specific IgG response, serum bactericidal activityas well as DTH reaction was carried out. Isolation and purification of mRNA and real-time PCR, was performed to determine the dominating Thlymphokine pattern.The new antimeningococcal formulation without aluminum hydroxide prepared with components of defined molecular structure assembled itself into Neoproteoliposomes(NPL) ranging from 50 to 70 nm in diameter. (PD-Tp),as well as the NPL-formulation favors a Th1 response pattern, suggested by the higher percentages of DTH, increased expression of proinflamatory lymphokine mRNAs when administered by intramuscular and intranasal routes.It stimulates systemic bactericidalantibody response against *Neisseria meningitidis* B and immunologic memory similar to VA-MENGOC-BC® vaccine, even at lower dosages and is less reactogenic in comparison with the formulation with aluminum hydroxide.This new adjuvant formulation could be applicable to the development of new and improved vaccines against meningococcal disease, and eventually as modulators of the immune response against other diseases.

P6.09.37
Evaluation of the Adjuvant properties for Chitosan-Polyvinylpyrrolidone-based Nanoparticles.

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Polymeric Nanoparticles (NPs) have recently been shown to possess significant potential as drug or antigen delivery systems. In addition to the carrier function, the adjuvant capacity is also an important and desirable property in the context of vaccination. Here, we evaluated the adjuvant properties for a new NPs formulation based on two-polymer combination: Chitosan and Polyvinylpyrrolidone (CS-PVP). The NPs were synthesized by ionic gelation and characterized by transmission electron microscopy (TEM), Dinamyc Light Scattering (DLS) and Fourier Transform Infrared Spectroscopy (FTIR). Moreover, biological properties of NPs were addressed in vitro by studying their effect on cell viability and Dendritic Cells (DCs) maturation. 500,000 bone marrow DCs were stimulated with CS-PVP NPs at different concentrations for 24 hours. At the end of the time, we analyzed the surface expression for costimulatory molecules by flow cytometry. Our results showed an enhanced surface expression of CD40, CD80, CD86 and MHC II on NPs stimulated-DCs at two different concentrations: 250 y 500 µg/mL, confirming the maturation phenotype. Using Human Monocyte-derived Dendritic Cells, we evaluated by ELISA the production of TNF-α and IL-6. 200000 Human DCs were stimulated for 24 hours with 300 µg/mL of CS-PVP NPs. Intermediate levels of both TNF-α (260 pg/ml) and IL-6 (75 pg/mL) were detected comparing against LPS. These results clearly indicate that CS-PVP NPs formulation active both murine and human DCs, opening the possibility for using such as a potential adjuvant in vaccination.

P6.09.38
A Non-lytic form of Listeriolysin O induces Human Dendritic Cells to produce IL-12 and IL-23

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Introduction. LLO, a pore-forming citolysin is a key virulence factor of Listeria monocytogenes (LM). LLO induces a wide range of biological effects during the infection by LM or by itself as recombinant antigen. Here we studied the effects of a non hemolytic form of LLO on the production of two key cytokines for coordinating innate and adaptive immune responses against intracellular pathogens such as IL-12 and IL-23. Objective. We aimed to test the innate immune response of Human Monocyte-derived Dendritic cells (hDCs) to a non-hemolytic form of LLO named as DetoxLLO (mutated at C484A, W491A, W492A positions). Materials and methods. Human Monocyte-derived DCs were stimulated with DetoxLLO (0.5 or 1µg/mL). DCs were stimulated with DetoxLLO in the presence or absence of 50 µg/mL of polymyxin B; 24 hours after stimulation, sups were tested to measure the concentration of TNF-α, IL-6, IL-12 and IL-23 by ELISA technique. Results: DetoxLLO induced the production of IL-6 (1,615.66 pg/mL), TNF α (2,739.15 pg/mL), IL-12 (2,471.53 pg/mL) and IL-23 (269.60 pg/mL). Cytotoxic effects were undetected after 24 hours stimulation with DetoxLLO. Conclusion: We have identified a very potent innate immune response when DetoxLLO is used as an exogenous antigen on human DCs. The production of IL-12 and IL-23 by human DCs after the recognition of LLO could be very important for the immune response during the human infection by Listeria. As a potential adjuvant for human vaccination purpose, DetoxLLO could be use to polarized the immune responses toward a Th1 or Th17 responses.

P6.09.39
Adjuvant-induced muscle ATP release potentiates response to vaccination

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Vaccines are the most effective agents to control infections. In addition to the pathogen antigens, vaccines contain adjuvants that are used to enhance protective immune responses. However, the molecular mechanism of action of most adjuvants is ill known, and a better understanding of adjuvanticity is needed to develop novel adjuvants based on molecular targets that further improve vaccine efficacy. This is particularly important for tuberculosis, malaria, AIDS and other diseases for which protective vaccines do not exist. Release of endogenous danger signals has been linked to adjuvanticity, however the role of extracellular ATP during vaccination has never been explored. Here, we tested whether ATP release is involved in the immune boosting effect of four common adjuvants: aluminium hydroxide, calcium phosphate, incomplete Freund's adjuvant and MF59. We found that only MF59 induced a rapid and transient release of ATP at the injection site. Immunization of mice with an MF59-adjuvanted influenza vaccine and apyrase, an ATP-hydrolyzing enzyme, strongly inhibited the recruitment of immune cells within the muscle and reduced antigen-specific T cell responses and antibody titers. This demonstrates that a transient ATP release is required for innate and adaptive immune responses induced by MF59 and link for the first time extracellular ATP to an enhanced response to vaccination.

P6.09.40
Effect of monogalactosyldiacylglycerol from different marine macrophytes on conformation and immunogenicity of hemagglutinin of Influenza A virus H1/N1 A/California/07/2009

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Tubular immunostimulating complexes (TI-complexes) consisting of cucumarioside A2-2, cholesterol and monogalactosyldiacylglycerol (MGDG) from marine macrophytes are a most promising antigen delivery system, providing much higher adjuvant effect than classic ISCOMs or Freund's complete adjuvant. Earlier, we have shown that conformation and immunogenicity of porin, incorporated in TI-complexes, depend on physicochemical properties of MGDG. In order to check the universality of this approach for optimization of TI-complex, we used recombinant monomeric hemagglutinin (HA) of Influenza A virus H1/N1 A/California/07/2009. MGDG samples with different physicochemical properties were isolated from Sargassum, Ulva and Zostera. In spite of different effect of MGDGs on conformation of HA the level of anti-HA antibodies, induced by respective TI-complexes, similarly increased by 2 times compared with HA alone. The absence of the expected effect is probably due to the absence of conformational antigenic determinants in the selected antigen.
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P6.09.41
Cyclic-di-GMP based immune stimulatory formulations as novel vaccine adjuvants and anti-cancer agents

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3´,5´-Cyclic diguanylic acid (c-di-GMP) is a bacterial derived small cyclic dinucleotide that functions as the universal bacterial secondary messenger. To date, studies concerning its immunostimulatory effects revealed that cytosolic sensing of c-di-GMP by the innate immune receptor STING, induces a robust type-I interferon production in antigen presenting cells, leading to their maturation. However, the chemical structure and the anionic nature of c-di-GMP limit its efficient entry through cellular membranes, requiring its transfection to cytosol. Here, we present a simple molecular complexation strategy that improves the intracellular delivery and boosts the immunostimulatory activity of c-di-GMP. Moreover, we also show that bacteria derived membrane vesicles (MV) can be utilized as vesicular carriers of this cyclic dinucleotide. Vaccination of mice using model antigen OVA adjuvanted with c-di-GMP/nonaarginine peptide complexes or c-di-GMP encapsulated in bacterial MVs, induced significantly higher levels of antigen-specific total IgG when compared to OVA or OVA+free c-di-GMP immunized groups. In contrast to OVA immunized mice, OVA+c-di-GMP/nonaarginine and OVA+c-di-GMP/bacterial MV adjuvanted groups showed complete protection from EG.7 tumor challenge. Consistent with the ability of these formulations to induce anti-tumorimmunity, ex-vivo re-stimulation of spleen cells harvested from immunized mice with the SIINFEKL peptide resulted in 8 to 10 fold increase in IFN-γ production. These findings suggest that c-di-GMP/nonarginine complexes or c-di-GMP/bacterial MVs are effective vaccine adjuvants and can generate tumor-specific CD8 T cell responses.

P6.10 Mucosal vaccines

P6.10.01
Determinants of immune responses to mucosal delivery of a malaria parasite protein on Lactococcus lactis cell walls and co-administration of an inducer strain

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Lactococcus lactis are potentially useful vectors for mucosal delivery of vaccines. We report on oronasal immunisation of different mouse strains with the *Plasmodium falciparum* merozoite surface antigen (PfMSA2), magnitude of immune response by co-administration of recombinant vector and nisin inducer *L. lactis* strain and related histopathological changes.
PfMSA2 was covalently attached to cell walls of live *Lactococcus lactis* (MSA2cP) and non-covalently attached to *L. lactis* cell wall ghosts (MSA2cA), and used for immunisation. Immune responses and effect of co-administration on immune responses in mice and histopathological changes in gut associated lymphoid tissue were investigated.
Serum IgG antibodies to MSA2 were elicited by both immunogens in strain-dependent manner. The IgG isotype antibodies of these mice reflected the influence of Th1 and Th2 cells. Serum and faecal IgA anti-MSA2 antibodies were also detected. Antigen specific IFN-γ producing T cells were detectable in spleens of all inbred mouse strains immunised with MSA2cA and in C57 mice immunised with MSA2cP. Co-inoculation of inducer *L. lactis* significantly improved antibody response to MSA2. Enlargement of mesenteric lymph nodes, increased lymphatic infiltration of the lamina propria as well as germinal centre formation in the spleen were noted in mice fed with *L. lactis*.
Results suggests that mucosal immunization of *Pf* MSA2 is able to generate protective levels of systemic antibodies and cellular immunity in mice dependent on strain and anchoring method and co-administration of an inducer strain elicited better immune responses. These findings are relevant for developing better vaccine delivery systems for human use.

P6.10.02
Mucosal delivery of ACNPV baculovirus driving expression of the Gal-lectin LC3 fragment confers protection against amoebic liver abscess in hamster

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Mucosal vaccination against amoebiasis using the Gal-lectin of E. histolytica has been proposed as one of the leading strategies for controlling this human disease. However, most mucosal adjuvants used are toxic and the identification of safe delivery systems is necessary. Here, we evaluate the potential of a recombinant Autographa californica baculovirus driving the ex-pression of the LC3 fragment of the Gal-lectin to confer protection against amoebic liver abscess (ALA) in hamsters following oral or nasal immunization. Hamsters immunized by oral route showed complete absence (57.9%) or partial development (21%) of ALA, resulting in some protection in 78.9% of animals when compared with the wild type baculovirus and sham control groups. In contrast, nasal immunization conferred only 21% of protection efficacy. Levels of ALA protection showed lineal correlation with the development of an anti-amoebic cellular immune response evaluated in spleens, but not with the induction of seric IgG an-ti-amoeba antibodies. These results suggest that baculovirus driving the expression of E. histolytica vaccine candidate antigens is useful for inducing protective cellular and humoral immune responses following oral immunization, and therefore it could be used as a system for mucosal delivery of an anti-amoebic vaccine.

P6.10.03
Mucosal influenza vaccine comprising of M2e-HSP70 formulated with N-Trimethyl chitosan(TMC) particles induces strong M2e specific immune responses

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Nasal vaccines can induce systemic IgG and mucosal IgA antibody responses, which establish two layers of immune defense against infectious pathogens like influenza.
Mucosal vaccines have to overcome several limitations, including mucociliary clearance and the inefficient uptake of soluble antigens. Therefore, nasal vaccines require potent adjuvants and delivery systems to enhance their immunogenicity and to protect their antigens.
In this study, ectodomain of the conserved influenza matrix protein 2 (M2e) which has been found to induce heterosubtypic immunity was fused to C-terminus of Mycobacterium tuberculosis (MBT) HSP70 and a derivative of chitosan, N-trimethyl chitosan (TMC), was used as a carrier for this fusion protein.
Ionically crosslinked nanoparticles were formulated with M2e-HSP70 protein using the ionic gelation technique with pentasodium tripolyphospate (TPP) as a crosslinking agent. Particles had a size in the range of 200-250nm and positive surface charge. Intranasal immunization with M2e-HSP70/TMC in BALB/c mice induced significantly higher M2e specific antibody, IgG in serum, than those induced in control groups (M2e-HSP70, TMC and HSP70). The anti-M2e IgG subtype induced was mainly IgG2a. Cellular immune response was evaluated by cytokine production assay of splenocytes after in vitro stimulation.
M2e-HSP70/TMC-immunized mice were fully protected against lethal challenge (MLD90) of influenza A infection (PR/8) compared to control groups of mice with survival rates of 20% to 30%.
This data confirmed that M2e-HSP70 fusion protein formulated with TMC nanoparticles could be an effective construct to induce strong immunogenicity and obtain full protection as a promising candidate mucosal influenza vaccine.

P6.10.04
Beta-glucan particles as novel antigen delivery systems: towards oral vaccination

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Gastro-intestinal infections are still a main cause of enteric diseases and mortality among humans and animals. Oral vaccination is crucial in generating an adequate local mucosal immune response, however the hostile environment of the intestinal tract and oral tolerance remain huge obstacles that inhibit the ability to successfully develop new mucosal vaccines. A promising strategy for vaccination with safe, biodegradable non-replicating antigen delivery systems has gained increased interest for eliciting cellular and humoral immune responses. The current study evaluates the potential of β-glucan (BGP) and calcium carbonate (CaCO3)- and mannitol-templated polyelectrolyte particles as mucosal antigen delivery systems and their adjuvant characteristics. All microparticle types are efficiently internalized by Caco-2 and HT-29 cell lines and in particular the BGP triggered the expression of pro-inflammatory cytokines IL-23p19, IL-8 and beta-glucan receptors in activated Caco-2 cells and CCL20 in HT-29 cells. In contrast, the expression level of TGF-b, an important mediator of the active component of oral tolerance, was significantly downregulated in HT-29 cells. Oral administration of BGP induced intestinal adaptive immune responses characterized by an increased sIgA and secretory component production. Interestingly, adoptive transfer experiments pointed out the proliferation of naïve OVA-specific CD4+ OT-II cells and increased IL-17 production in spleens of BGP-fed mice upon antigen restimulation. These results demonstrate that BGP enhances MHC-II-presentation and promotes mucosal immune responses preferably steered towards a Th17 response and represents a promising strategy for oral vaccination.

P6.10.05
Hsp65-producing Lactococcus lactis prevents experimental colitis by IL-10- and TLR2-dependent pathways

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Disruption of the delicate balance between tolerance and immunity in the gut mucosa can cause inflammatory bowel diseases (IBD) in genetically susceptible hosts. Heat shock proteins (Hsp) are conserved proteins that are highly expressed in inflammation. Thus, they are good therapeutic antigenic targets for oral tolerance in IBD. Herein, we investigated the effects of administration of Lactococcus lactis genetically engineered to secrete Hsp65 in murine colitis. Mice received Hsp65-L. lactis orally during four days. Ten days thereafter, colitis was induced by 1.5% dextran sodium sulfate (DSS). Hsp65-L.lactis administration completely prevented colitis with diminished levels of TNF-α, IL-6 and IL-4 and enhanced IL-10 levels in the colonic mucosa. In IL-10 deficient mice, Hsp65-L.lactis fail to prevent intestinal inflammation. The same was observed in TLR2-/- but not in TLR4-/- mice. Interestingly, frequency of lamina propria IL-10+ B cells enhanced in mice treated with Hsp65-L. lactis. In conclusion, Hsp65 delivered by Lactococcus lactis prevents intestinal inflammation via IL-10 and TLR2 pathways.
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P6.10.06
Prime-boost strategies in mucosal immunization affect local IgA production and the type of Th response

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Combination of different delivery routes for priming and boosting represents a vaccination strategy that can modulate magnitude,

quality, and localization of the immune response. A murine model was used to study T-cell clonal expansion following nasal and subcutaneous priming, and secondary immune responses after boosting by either homologous or heterologous route. Antigen-specific T-cell priming studies, performed by using the adoptive transfer model of OVA-specific transgenic T cells, showed that both nasal and subcutaneous immunizations efficiently elicited primary antigen-specific CD4+ T cells clonal expansion in the respective draining lymph nodes, that disseminated towards distal lymph nodes and spleen. After boosting, a very high serum IgG response was induced in all groups, while significant levels of local IgA were detected only in mice boosted by the nasal route. Mucosal priming drove a stronger Th1 polarization than the systemic route, as shown by serum IgG subclass analysis. IFN-γ production was observed in splenocytes of all groups, while prime-boost vaccine combinations including the mucosal route yielded higher levels of IL-17 production. Memory lymphocytes were identified in both spleen and draining lymph nodes in all immunized mice, with the highest number of IL-2 producing cells detected in mice primed and boosted by the nasal route. Studies of priming of pathogen-specific T cells are ongoing using multimeric MHC-peptide complex technology.
These data provide relevant insights for the design of optimal prime-boost strategy.

P6.10.07
Nanogel-based PspA nasal vaccine induces S. pneumoniae - specific neutralizing antibody immune responses in nonhuman primates

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We previously developed an affective vaccine delivery system with self-assembled nanosize hydrogel (nanogel), which composed of a cationic type of cholesteryl group-bearing pullulan (cCHP). In our recent study, we demonstrated that intranasally immunized pneumococcal surface protein A (PspA) with cCHP vaccine (PspA-nanogel) induced strong antigen-specific systemic and mucosal antibody (Ab) immune responses with protective immunity in mice. In this study, we examined whether PspA-nanogel nasal vaccine could induce PspA-specific Ab responses in nonhuman primates. The cynomolgus macaques nasally immunized with 25 μg of PspA-nanogel five times at two weeks intervals showed significantly increased levels of PspA-specific serum and broncho alveolar lavage fluid (BALF) IgG, and nasal wash (NW) S-IgA Ab responses when compared with control macaques nasally immunized with 25 μg of PspA alone or PBS. Further, increased levels of IFN-γ, IL-4 and IL-17 production were found in PspA-nanogel immunized macaques but not control macaques. When the Balb/c mice were challenged with the mixture of 7.5 x 103 CFUs of S. pneumoniae strain A66.1 (Xen10) and the serum from macaques given nasal PspA-nanogel vaccine, they showed a full protection. However, the other mice, which were challenged with the mixture of the same level of S. pneumoniae and the serum from 25 μg of PspA alone or PBS as control macaques, were died within 2 days after challenge. These results show that nasal PspA-nanogel vaccine can effectively induce PspA-specific serum IgG with neutralizing activity and S-IgA Ab immune responses in nonhuman primates.

P6.10.08
Immunisation using a sustained release vaccine generates functional CD8 T cell memory

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Generating an effective memory population of quantity, quality and in the correct biological location is key in having a good vaccination method. Although humoural immune responses conferred through current vaccination methods are effective, in many cases there is a need for vaccination that will confer a cytotoxic CD8 T cell response. We investigated the generation of murine memory CD8+ T cells using a sustained antigen release vaccine vehicle (chitosan gel) containing ovalbumin protein or conventional vaccination using dendritic cells loaded with protein. The aims of this work were to evaluate the efficacy of sustained release vaccines and to compare their ability to generate peripheral versus mucosal CD8 T cell memory.
Mice were euthanised at memory time points following subcutaneous vaccination and cell populations were assessed in peripheral and gut associated lymphoid tissues using flow cytometry and ex vivo cytotoxicity assays. In vivo cytotoxicity was also assessed. We could detect CD8 T cell memory populations in both peripheral and gut associated lymphoid organs specific for the ovalbumin protein vaccine and a cytotoxic response was seen in vivo up to 60 days following this. These results indicate that subcutaneous vaccination with a sustained release vaccine can generate a population of functional CD8 memory cells in gut associated lymphoid tissues. We have established an orthotopic mouse model of colorectal cancer and are testing the efficacy of this vaccine in protective tumour immunity. This form of vaccination could be used to induce immunity in sites that are not easily accessible such as the gut.

P6.10.09
In vitro Inhibition of Staphylococcus aureus Isolates by Anti-Anti-Idiotypic Antibodies to Staphylococcal Protein A (SpA)

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This study investigates the ability of antibodies to SpA (a protein produced by the bacterium *Staphylococcus aureus*) to inhibit the growth of the bacteria. Chickens immunised with SpA produced anti - SpA antibodies in their eggs (primarily the yolk). These anti-SpA antibodies were used to feed chicks, which produce antibodies that recognise the original antigen. When these antibodies were purified from their sera and included in the growth medium of the *Staphylococcus aureus*, there was inhibition of the bacterial growth. This indicates that these antibodies had specifically bound to the cell surface of the bacteria and prevented growth of the bacteria, i.e. the antibodies could protect against bacterial infection. The potential of such antibody in the hyper-immune egg to act as oral therapeutic agents is discussed.
The anti-SpA hyperimmune egg may be considered as an oral mucosal vaccine that estimulated the production of antibodies that recognised the original antigen on the surface of *Staphylococcus aureus.*

P6.10.10
Nanogel-based pneumococcal surface protein A (PspA) intranasal vaccine prevents invasive disease and nasal colonization by pneumococcus

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To establish a safer and more effective vaccine against pneumococcal respiratory infections, current knowledge regarding the antigens common among pneumococcal strains and improvements to

the system for delivering these antigens across the mucosal barrier must be integrated. We developed a pneumococcal vaccine that combines the advantages of pneumococcal surface protein A (PspA) with a nontoxic intranasal vaccine-delivery system based on a nanometer-sized hydrogel (nanogel) bearing a cationic cholesteryl-group-bearing pullulan (cCHP). The efficacy of the nanogel-based PspA nasal vaccine (cCHP-PspA) was tested in murine pneumococcal airway infection models. Intranasal vaccination with cCHP-PspA provided cross-protective immunity against lethal challenge with Streptococcus pneumoniae, reduced colonization and invasion by the bacteria in the upper and lower respiratory tracts, and induced systemic and nasal mucosal Th17 responses, high levels of PspA-specific serum immunoglobulin G (IgG), and nasal and bronchial IgA antibody responses. Importantly, there was no sign of PspA delivery by nanogel to either the olfactory bulbs or the central nervous system after intranasal administration. Moreover, in vivo blocking of CCL28 during cCHP-PspA immunization suppressed the induction of IgA-positive plasmablasts in the nasal passages and consequent nasal PspA-specific IgA production showing CCL28 was required for the recruitment of IgA+B220+CD138+ plasmablasts. These results demonstrate the effectiveness and safety of the nanogel-based PspA nasal vaccine system as a universal mucosal vaccine against pneumococcal respiratory infection and provide the mechanistic evidence for effective induction of nasal mucosal IgA immune responses by nasal vaccination using cCHP nanogel vaccination system.

P6.10.11
The protective activity of the bacterial antigens in mucosal route of immunization in mice

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The antigens of the opportunistic bacteria are active stimulators of the mucosal innate immune system effectors. The purpose: to investigate the ability of bacterial antigens (multicomponent vaccine Immunovac-VP-4) administered orally to stimulate adaptive immunity in mice against *K.pneumoniae, P.vulgaris, S.pneumoniae.* Mice were immunized orally twice with vaccine at a dose of 2000 mg, then mice were challenged with lethal doses of bacteria. When mice were immunized with vaccine Immunovac we observed a protective effect against *K.pneumoniae* with the index of efficiency (IE) of 2.3 to 5.7. If the mice were challenged of *P.vulgaris*, the IE was 2.7. It is interesting the data on the extent of mice protection, immunized against *S.pneumoniae* type 3, which antigens are not included in the vaccine. In this case, EI ranged from 12 to 16. Perhaps an important role in the protection of mice against Salmonella infection is the presence of common *K.pneumoniae* and pneumococcal antigens, included in the vaccine. *S.pneumoniae* is important etiologically significant factor in chronic inflammatory diseases of the respiratory system. The activation of the mucosal immunity effector cells and generalization of this process in the lymphoid organs, distant from the route of vaccine administration is a highlight, providing protection against pneumococcus. Therefore, mucosal vaccine constructs based on antigens of opportunistic microorganisms, provide the activation of innate as well as adaptive immunity. This work was supported by RFBR grant №11-04-96037r_ural_a and administrative body of Perm Region.

P6.10.13
T-cell priming characterisation through a systems biology approach

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T-cell priming at mucosal sites is a key event in the development of vaccination strategies. T-cell priming after vaginal and nasal immunization was studied in vivo analyzing CFSE proliferation data through a multi-type branching process. Immunization studies were conducted using a model vaccine formulation constituted by ovalbumin (OVA) plus the mucosal adjuvant CpG ODN 1826. Primary activation and dissemination of Ag-specific T cells was analysed in vivo after adoptive transfer of OVA-specific transgenic T-cells. Antigen-loaded dendritic cells were detected only within the respective draining lymph nodes where a rapid recruitment of activated antigen-specific T cells was observed. The enhanced number of T-cell within draining lymph nodes suggested an antigen-specific T-cell recall from distal lymph nodes and spleen. T cells were activated by the antigen encounter and acquired homing molecules essential to disseminate towards distal lymphoid organs as confirmed by the modulation of the expression of CD45RB, CD69, CD44 and CD62L molecules. A multi-type Galton Watson branching process was used to model in vivo CFSE proliferation data, in order to calculate the probabilistic decisions of a cell to enter in division, rest in quiescence or migrate/dye. This mathematical model, previously used for in vitro analysis of T-cell proliferation, has been here successfully applied to study in vivo T-cell clonal expansion following mucosal immunization.

P6.10.14
Evaluation of the potential of dry thermostable Hepatitis-B mucosal vaccine as a global strategy for cold-chain and needle free vaccination

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Introduction: Thermostable mucosal vaccines will reduce needle borne infection, deprived mucosal immunity, dependency on cold chain facilities while facilitating easy distribution, cost reduction and stockpiling of vaccines, confirming immediate availability in routine and pandemic immunization.
Methods: In present study Hepatitis-B surface antigen loaded chitosan particulates were prepared by ionic gelation method and dried (spray drying and lyophilization) using bland of stabilizers. Formulations were characterized in-vitro on basis of morphology, structural integrity, crystallanity, in process stability and change in Tg. Optimized formulations were assayed for their in-vivo immunogenicity in female Balb/c mice to evaluate protective humoral and cellular immune responses.
Results & Discussion: Developed vaccines were found more stable revealed by in-vitro immunogenicity testing, SDS-PAGE and CD spectra. Aerodynamic diameter of 2.5 and 1.4 µ for lyophilized and sprays dried formulation respectively, was suitable for pulmonary immunization. Developed formulations were later assayed for their in-vivo immunogenicity in female Balb/c mice and found to elicit protective humoral and cellular immune responses revealed by serum IgG, IgG2a, IgG1. IgM and mucosal IgA levels in different body fluids.
Conclusion: An ideal vaccine candidate should elicit both cellular and humoral responses. It should provide protective mucosal immunity to clear infection at site of entry. Developed vaccines were found to elicit protective immune responses and were also stable for sufficient time at normal storage temperature thus confirming an immediate availability and simple distribution of vaccines in routine and pandemic immunization in future. However the work still needs a deep research to be employed at commercial scale.

P6.10.15
Encapsulation of antigens in whole plant cells functions as a combined production and delivery platform for oral vaccines

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Oral delivery of transgenic plant material was heralded as a safe, rapidly produced, and economical platform for the development of vaccines. However, as with conventionally produced oral vaccines, proteolytic degradation during digestion and the subsequent heterogeneous immune response limits what should be a synergistic match of production platform and delivery method. We are currently investigating the effect of sub-cellular localisation of antigenic proteins on the rate of release and immunogenicity following oral administration. Using genetic fusions of plant-cell trafficking signal sequences and the B-subunit of the heat-labile toxin (LTB) from

enterotoxigenic E.coli, we have successfully shown LTB is sequestered to different sub-cellular compartments of the Nicotiana benthamiana leaf cell. Correctly folded LTB is localised to the apoplast, endoplastic reticulum, non-lytic vacuole, and protein bodies. An in vitro simulated gastric fluid assay shows LTB is released at different rates depending on the sub-cellular encapsulation. An in vivo feeding trial in C57Blk6 mice provides evidence that sequestering LTB within non-lytic vacuoles improves the IgG1-domiant humoral, and sIgA mucosal response. Optimisation of release kinetics during digestion may facilitate improved delivery of antigenic proteins and other orally delivered biologics, and may harness the intrinsic capacity of plant cells as both production and delivery vehicles.

P6.10.16
Establishment of murine norovirus S7 infection system for vaccine development

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Norovirus (NV) belongs to the Caliciviridae family with non-enveloped, positive-strand RNA. Over 60% of non-bacterial gastroenteritis outbreaks are due to human NV (HNV), which is a major public health concern. The lack of HNV cell culture system and animal infection model hampers the studies in HNV vaccine development. The discovery of murine NV (MNV) has provided a great volume of biological information of NV. In this study, we aimed to establish the MNV infection mouse model by using MNV-S7 isolated in Japan, as an alternative approach for HNV vaccine development. When Balb/cA and C57BL/6J were orally inoculated with 7.5log10 TCID50/dose of MNV-S7, MNV-RNA and the viral titers could be detected in fecal samples from 1 day post infection (dpi). The excretion of MNV-S7 in feces persisted for at least 2 weeks, with peaks between 1 to 5 dpi. After 14 dpi, MNV-RNA and viral titers decreased, which might be attributed to antigen-specific host immune responses. Using newly established MNV-S7 infection model, we vaccinated Balb/cA nasally with MVN-S7 recombinant VP1 P-domain [rVP1(P)], together with or without cholera toxin. VP1 specific antibodies in both systemic and mucosal compartments, particularly with high levels of serum IgG, were induced in both groups. After MNV-S7 challenge, vaccinated mice showed a slight decrease in MNV-RNA and the viral titers at 5 and 7 dpi, indicating that rVP1(P) exhibited a limited protection. These results will be a support in improving the researches in host infection and immunity on NV and the vaccine development of NV.

P6.10.17
Maternal antibodies enhance systemic and local immune responses after early mucosal immunization in a porcine model

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In newborn mammals, maternal antibodies (MatAb) confer protection against antigenic challenges, although it has been also reported a suppressive effect on neonatal immunization. We evaluated the immune response to ovoalbumin (OVA) after mucosal immunization on 7 and 14 days (7d, 14d) old, SPF Vietnamese minipigs, from negative (OVA-) or previously immunized (OVA+) sows. Sows were intramuscularly immunized to OVA 30 days before farrowing. Maternal anti-OVA IgG and IgA were detected in colostrum and serum and were intestinally absorbed by the offspring during the first three days of life. These antibodies were detected in piglets' serum, but not in mucosal secretions (saliva, nasal secretion). The systemic (serum) anti-OVA IgG and IgA responses were significantly higher in piglets immunized at 7d of life, from OVA+ sows, compared with piglets from OVA- sows and similar to piglets immunized at 14d of age. Also the anti-OVA IgA in nasal secretion was higher in OVA+ 7d piglets and in saliva of OVA+ 14d piglets. In our hands, the suppressive effect of MatAb, reported by others at early immunization, was not detected. On the contrary, a stimulating effect was found with MatAb in one week old piglets and this effect disappeared in 14d old piglets. These results may open the possibility to induce effective early systemic and mucosal immune responses in the young through early immunization, even in the presence of MatAb. Supported by grants from CONACyT (234097) and ICyTDF (323/09). Thanks to DVM Manuel Flores and Daniel Cortes for technical assistance.

P6.10.18
M-cell targeting strategy improved the immunogenicity of viral myocarditis-specific mucosal DNA vaccine

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Induction of mucosal T cell immune responses is a priority of many vaccine strategies against mucosal invaded pathogens such as coxsakievirus B3 type (CVB3). We previously developed a chitosan-pVP1 vaccine and intranasal immunization could induce both systemic and mucosal immunity which provided a moderate prevention of CVB3-myocarditis. How to further improve its immunogenicity and immunoprotection becomes the center of our focus. Considering that M (microfold) cells are specialized epithelial cells responsible for mucosal antigen sampling, targeting M-cells will be an attractive way to elicit a strong immune response toward mucosal antigens. In the present work, we showed that an efficient mucosal vaccination against CVB3-induced myocarditis could be accomplished by incorporating the chitosan-pVP1 vaccine with an M-cell targeting CPE30 peptide. Following chemical conjugation with CPE30, chitosan was coacervated with pVP1 to form vaccine particles. Mice were intranasally vaccinated with CPE30 conjugated chitosan-pVP1 at 2-weeks interval for 4 times. 2 weeks following the last immunization, CVB3-specific T cell immune responses in spleen and mesenteric lymph node (MLN) were carefully determined. We found that compared with chitosan-pVP1, CPE30 conjugated chitosan-pVP1 significantly promoted systemic and mucosal T cell proliferation, increased the percentages of specific IFN-γ+ T cells and robustly enhanced CTL immune responses. Accordingly, less severe myocarditis with limited immune cell infiltration was evidenced in the heart tissues following CVB3 challenge, indicating the enhanced resistance to CVB3 induced myocarditis. These data suggests that M cell-targeting CPE-30-chitosan-pVP1 vaccine might represent a promising vaccine candidate to protect against CVB3 infection.

P6.11 Vaccination and immunotherapy for infectious diseases

P6.11.01
Induction of the activation of innate and adaptive immunity by lipidated peptide vaccine: robust and enduring protective memory Th17 cells against Mycobacterium tuberculosis

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In tuberculosis-endemic population, the main reason for the failure of BCG vaccine is the obstacle caused by non-tuberculous mycobacteria and helminths in its processing and presentation. Usually, peptides do not require extensive antigen processing since they can bind to major histocompatibility complex molecules and therefore can be directly presented to T cells. As a result, peptide vaccines can surmount the problems associated with BCG failure. It is well-established fact that not only adaptive but also innate immunity plays a crucial role in protection against tuberculosis. Hence we have constructed a novel lipopeptide vaccine by linking promiscuous CD4 and CD8 epitopes of Mycobacterium tuberculosis to Pam2Cys, a Toll Like Receptor-2 agonist. This lipopeptide has unique property of self-adjuvanting and concurrently activating both innate and adaptive immunity. The vaccine binds directly to MHC I and MHC II molecules and TLR-2. It stimulates dendritic cells to secrete cytokines, upregulates the expression of costimulatory molecules and significantly augments their ability to present antigen to T cells.

Further, the vaccinated animals impart robust and enduring memory Th1 and Th17 response. The protection observed is significantly better than BCG. This lipidated-peptide vaccine is unique since it overcomes MHC barriers and evokes immune response irrespective of HLA polymorphism in human. This vaccine has enough potential to induce long-lasting protection against Mycobacterium tuberculosis. Therefore can be a potent future vaccine candidate for controlling tuberculosis.

P6.11.02

Pre-clinical isolation and expansion of CMV specific T-cells for adoptive immunotherapy based either on IFN-γ production or activation-dependent expression of CD154

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Introduction. Cytomegalovirus (CMV) reactivation after allogeneic hematopoietic stem cell transplantation (HSCT) counts for up to 30% of deaths. In 2012, in the Hematology of Vicenza Hospital 53% of patients underwent HSCT experienced CMV reactivation. The capture of T-cells secreting cytokines (γ-catch) or expressing activation markers after viral antigens stimulation allows isolation of CMV-specific T-cells (CMV-T) to be used in Adoptive Cellular Therapy.
Aim. We explored the T-cell response of the CMV-T obtained with either γ-catch or CD154 selection upon stimulation with pp65, IE1 or both CMV peptides.
Methods. PBMC were isolated from CMV(+) donors and cultured in standard conditions. For the isolation of CMV-T, cells were either magnetically enriched by the γ-catch or by CD154-microbeads. CMV-T were cultured in the presence of feeder cells for three weeks and then stained for immunophenotyping (IF), intracellular cytokine staining (ICC), CFSE-based proliferation assay by FACS.
Results. Purity and enrichment of CMV-T resulted comparable between CD154+ and IFN-γ+ cells and virus epitopes. The IFN-γ secreting CD4+ T cells have been enriched up to 66%. Although isolation of CD154+ CMV-T cells greatly favours enrichment of CD4+ T cells, a number of CD8+ T cells was expanded depending on the type of peptide and of the simultaneous use of them. CFSE staining on day 21 evidenced that isolated and expanded CMV-T underwent division after stimulation.
Conclusions. Both methods and epitopes elicited CD3+ CMV-T responses but resulting in different T-cell populations, potentially leading to divergent in-vivo T-cell responses.

P6.11.03

Broad and cross-clade CD4+ T-cell responses elicited by a DNA vaccine encoding highly conserved and promiscuous HIV-1 M-group consensus peptides

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Broad, polyfunctional and cytotoxic $CD4^+$ T-cell responses have been associated with control of HIV-1 replication and may represent a beneficial component of an efficacious HIV vaccine. Also, strategies to cope with virus and host genetic diversity are urgently needed. We rationally designed a multiepitopic DNA vaccine in order to elicit broad and cross-clade $CD4^+$ T-cell responses against highly conserved and promiscuous peptides from the HIV-1 M-group consensus sequence. We identified 27 conserved, multiple HLA-DR-binding peptides in the HIV-1 M-group consensus sequences of Gag, Pol, Nef, Vif, Vpr, Rev and Vpu using the TEPITOPE algorithm. The peptides bound *in vitro* to an average of 12 out of the 17 tested HLA-DR molecules and also to HLA-DP, -DQ and murine IA^b and IA^d molecules. Sixteen out of the 27 peptides were recognized by PBMC from HIV-1-infected patients and 72% of such patients recognized at least 1 peptide. Immunization with a DNA vaccine (HIVBr27) encoding the identified peptides elicited IFN-γ secretion against 11 out of the 27 peptides in BALB/c mice; $CD4^+$ and $CD8^+$ T-cell proliferation was observed against 8 and 6 peptides, respectively. HIVBr27 immunization elicited cross-clade T-cell responses against several HIV-1 peptide variants and polyfunctional $CD4^+$ and $CD8^+$ T cells against the pool of HIVBr27 peptides. In addition, HIVBr27 immunization led to lower viral load in mice challenge with recombinant Vaccinia virus encoding HIV-1 Gag and Pol. We believe that our concept may cope with HIV-1 genetic diversity, provide increased population coverage and elicit protective T-cell immunity against HIV-1/AIDS.

P6.11.04

Long-term expression of interferon alpha fused to apolipoprotein A-I allows the development of vaccine-induced adaptive immune responses

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Interferon alpha (IFNα) is a key cytokine for the development of adoptive immune response due to its role as signal 3 in $CD8^+$ activation. However, the high and frequent doses used for the treatment of chronic viral hepatitis and cancer subvert the immunomodulatory capacity of IFNα, boosting the NK cell activities and abrogating the $CD8^+$-mediated immune responses.
In this work, we modeled the long-term treatment with IFNα expressing in the liver of mice this cytokine by a recombinant adenoassociated virus.
The sustained production of IFNα exerted a potent antiviral effect in hepatitis B transgenic mice and in mice infected by a lethal dose of encephalomyocarditis virus. However, these antiviral effects were associated with a profound hematological toxicity, and with a chronically activated immune system that presented an exhausted phenotype. In this setting, the efficacy of vaccines designed to boost the adaptive immune responses were hampered.
Interestingly, the long term expression of IFNα fused to apolipoprotein A-I exerted a similar antiviral effect but minimized the hematological toxicity and the exhaustion of the immune system. In this case, the activity of vaccines was preserved.
In conclusion, IFNα fused to apolipoprotein A-I maintains the potent antiviral activity of IFNα and the capacity of the adaptive immune system to respond to other immunotherapies designed to boost cytotoxic T cell responses able to eradicate established tumors or chronic viral infections.

P6.11.05

HLA-G5 expression in isolated adipose derived stem cells (ASCs) from breast cancer patients compared to normal breast tissues

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Background: Adipose derived stem cells (ASCs) are detected in tumor microenvironment which play an immunomodulatory effects on both innate and adaptive immune responses. ASC-mediated immunesuppression mainly induced through the secretion of soluble molecules such as IDO, NO, PGE2 and HLA-G5.
Aim: to assess the expression of HLA-G5 in adipose derived stem cells isolated from breast cancer patients compared to those isolated from normal breast tissue after cosmetic mamoplasty.
Methods: ASCs were isolated from 15 breast cancer patients and 10 normal adipose tissues using collagenase digestion. HLA-G5 was assessed by q-RT- PCR method.
Result: There was no significant difference in the expression of HLA-G5 in ASCs of breast cancer patients in comparison with those isolated from normal individuals.

Conclusion: Although recent studies showed an immunomodulatory effect for HLA-G5 associated on ASC in cancer tissues, in the current study expression of HLA-G5 was not significantly different between breast cancer and normal breast tissue.

P6.11.06
Immunogenicity of a Clostridium-difficile DNA based anti-toxin vaccine in an aging mouse model

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Clostridium difficile infection (CDI) is a major source of morbidity and mortality in the United States, costing over $1.1 billion/year. More than 90% of CDI-related deaths occur in individuals >85yrs. Protection against C. difficile-associated disease is mediated by host antibody responses against C. difficile toxins. Our goal is to determine the immunogenicity of a DNA based anti-toxin vaccine in an aging mouse model. Thus, young (4 mo.) and old (>18mo.) mice were immunized with our optimized DNA vaccine encoding toxin A and toxin B receptor-binding domain (A-RBD; B-RBD). Day 12 post-first immunization the younger mice had significantly higher IgG titers against A-RBD, with 25 ug/ul versus 5 ug/ul anti-toxin serum IgG. However 10 days post-second immunization the old and young mice had comparable IgG titers (~500 ug/ul IgG against A-RBD). Interestingly, anti B-RBD IgG responses in older mice remained lower (1000 ug/ul vs 2000 ug/ul). Differences in anti-toxin IgG and IgA in fecal extracts between aged and young mice were also observed. Therefore, our DNA vaccine platform generated an antibody response against C. difficile toxin-based DNA vaccines in older mice but defects in vaccine priming occur leading to lower titers. Two weeks post-second immunization higher frequencies of CD4+/CD44+ (activated) T cells were detected in older mice but not in younger cohorts. We also observed a higher frequency of toxin-specific CD4+ T cells that secrete pro-inflammatory cytokines (IFN-g, IL2) and Th2 cytokines (IL4, IL10) in older mice, supporting the notion of a deleterious “inflamm-aging” response.

P6.11.07
Targeting the non-structural protein 1 from dengue virus to a dendritic cell population confers partial protective immunity to lethal virus challenge

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Dengue is the most prevalent arboviral infection and the development of a vaccine is a World Health Organization priority. Among the vaccine candidates being tested in preclinical settings is the nonstructural protein 1 (NS1). In the present study, we tested the immune responses generated by targeting the NS1 protein to two different dendritic cell (DCs) populations. DCs are important antigen presenting cells and targeting proteins to maturing DCs has proved to be an efficient means of immunization. Antigen targeting is accomplished by the use of a monoclonal antibody (mAb) directed against a DC cell surface receptor fused to the protein of interest. We used two mAbs (αDEC205 and αDCIR2) to target two distinct DC populations, expressing either DEC205 or DCIR2 endocytic receptors, respectively, in mice. The fusion mAbs were used to immunize BALB/c mice in the presence of the Toll-like receptor (TLR) 3 ligand poly (I:C), as a DC maturation stimulus. We observed induction of strong anti-NS1 antibody responses in both groups. When we tested the induction of cellular immune responses, the number of IFN-γ producing cells was higher in αDEC-NS1 immunized animals. Also, mice immunized with the αDEC-NS1 mAb were significantly protected from a lethal intracranial challenge with the DENV2 NGC strain when compared to mice immunized with αDCIR2-NS1 mAb. We conclude that targeting the NS1 protein to the $DEC205^{+}$ DC population together with poly (I:C) is a promising approach to induce protective immunity to dengue virus infection.
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P6.11.08
Expression and biological characterization of a recombinant monoclonal antibody, scFv, against BaP1, a P-I metalloproteinase from *Bothrops asper* snake venom

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BaP1 is a metalloproteinase relevant in the local tissue damage associated with envenomations by *Bothrops asper*. Our aim is to express a recombinant scFv antibody against BaP1 with neutralizing ability. For this, the construct was cloned into pMST3 vector in fusion with SUMO protein and expressed in cytoplasm of C43 (DE3) bacteria. Both scFv and SUMO (control) were analyzed by SDS-PAGE to confirm their purity. The ability of monoclonal antibody (MaBaP1) and the scFv to recognize total *B.asper* venom and BaP1 was assessed by ELISA. The capacity of scFv to neutralize fibrin degradation induced by BaP1 was evaluated using agarose gel substrate containing fibrin. The hemorrhage neutralization in skin mice and gastrocnemius muscle necrosis were estimated by incubating BaP1:scFv at 10:1 molar ratio. Purified scFv and SUMO presented bands of 38.9 and 13.6 kDa, respectively. ELISA showed that scFv was able to recognize BaP1 as well as whole venom, but not SUMO. Fibrinolysis induced by BaP1 was significantly neutralized (73.8% and 46.7%) in a concentration-dependent manner by scFv but not by SUMO. ScFv, as well as MaBaP1 completely neutralized hemorrhage and muscle necrosis induced by BaP1. Moreover, the production of inflammatory mediators induced by BaP1 in peritoneal cavity was neutralized by scFv. Our data showed that scFv specifically recognized and neutralized biological effects of BaP1, suggesting its potential use for snake poisoning therapy. Financial support: FAPESP, PRODOC-CAPES, CNPq and INCT-TOX program of CNPq and FAPESP.

P6.11.09
Leishmania major large Rab GTPase (LmlRab) protects BALB/c mice against a L. major challenge and is highly immunogenic in individuals immune to cutaneous and visceral leishmaniasis

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Leishmania major large Rab GTPase (LmlRab) is a new atypical RAB protein displaying a high homologies with the Rab GTPase protein family and highly conserved in leishmania species, it plays a key role in intracellular transport. In order to identify leishmania vaccine candidates, we analysed the protective effect of the recombinant LmRab in the BALB/c mouse model. The cellular immune responses induced by LmRab and it divergent carboxy-terminal part LmRabC, were also evaluated in individuals with healed CL (n= 33) or with asymptomatic infection to L. major (n= 18) (HCLm and AsCLm) and asymptomatic to L. infantum (n=26) for visceral leishmaniasis (AsVLi).
LmRab with CpG were able to confer a strong protection in vaccinated mice against a virulent L. major challenge (2 10^{6} metacyclics), this protection were observed up to 20 weeks post challenge. In immune individuals, LmRab and LmRabC proteins were able to induce a specific significant levels of IFNγ in CL and VL immune groups compared to control group ($p<0,05$). Both proteins induced significant amounts of IL-10 in all groups with no significant difference. Granzyme B, the known cytotoxic mediator of CD8+ T cells, was also observed in individuals with immunity to L. major and controls, with no significant difference. Our results showed that the LmRab protein was able to induce a dominant Th1 immune response in individuals with immunity to L. major or L. infantum infection. These results indicate that LmRab may constitute a potential vaccine candidate against cutaneous and visceral leishmaniasis.

P6.11.10
Recombinant lipidated dengue-2 envelope protein domain III induces durable neutralizing antibodies and reduces the risk of antibody-dependent enhancement

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Vaccines are considered a cost-effective way to control infectious diseases. To rationally design vaccines, antigens and, frequently, adjuvants must be selected to trigger appropriate immune responses against a specific pathogen. We selected dengue-2 envelope protein domain III as a dengue vaccine candidate and expressed this candidate in the lipidated form in an *Escherichia coli*-based system. Dengue envelope protein domain III mediates binding of the dengue virus to the host cellular receptor. The lipid moiety of the bacterial-derived lipoprotein can activate the innate immune system to elicit an appropriate adaptive immune response. We demonstrated that lipidated dengue-2 envelope protein domain III is more immunogenic than nonlipidated dengue-2 envelope protein domain III. Most importantly, the lipidated dengue-2 envelope protein domain III alone triggered a durable neutralizing antibody response with a low risk of severe side effects. The safety profile of the lipidated subunit vaccine is superior to that of live attenuated vaccines because it lacks infectious RNA. In addition, lipidated subunit vaccines are non-replicating and thus may be less susceptible to replication interference than live attenuated vaccines. Our study suggests that the lipidated subunit vaccine approach could be applied to other serotypes of dengue virus as well as other pathogens.

P6.11.11
Human monoclonal ScFv that blocks sodium ion activity of tetrodotoxin

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Tetrodotoxin (TTX) intoxication is commonly found in Asia-Pacific regions mainly from eating seafood containing the toxin, such as puffer fish, eggs of horseshoe crabs. TTX blocks sodium ion channel of nerve cell membrane causing paresthesia, paralysis, respiratory failure and rapid death, if not treated promptly and properly. Currently, there is no specific anti-dote and the TTX intoxicated patients received only palliative treatment. In this study, human monoclonal single chain antibody fragments (HuScFv) that block the TTX activity for treatment of the TTX intoxication was invented. Phage clones displaying HuScFv that bound specifically to the TTX was selected from a human ScFv phage display library; using TTX as the phage bio-panning antigen. TTX specific HuScFv were produced and purified from the phage transfected E. coli. HuScFv from two E. coli transformants: s16 and s35 could neutralize the TTX toxicity to neuroblastoma cells ex vivo as well as in the TTX intoxicated mice. Computer homology modeling and molecular docking enlightened the molecular mechanisms of the HuScFv in neutralization of the TTX activity. The HuScFv invented in this study have high potential for developing further for treatment of human TTX intoxication.

P6.11.12
Broadly neutralizing human monoclonal antibodies: a novel resource against influenza viruses

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Influenza virus still represents a serious threat for human health, causing pandemics and annual epidemics. From 1918, four influenza A pandemics occurred, caused by H1N1, H2N2, H3N2 and vH1N1 subtypes. Moreover, the recurrent concerns deriving from the possible adaptation to humans of influenza viral isolates circulating in animal reservoirs, the potential arising of resistance to the currently available anti-viral drugs and the lack of an effective prevention make influenza virus a relevant menace for the world population. In this scenario, the identification of human monoclonal antibodies endowed with strong neutralizing activity and directed against viral isolates belonging to different influenza subtypes can raise hope for the development of monoclonal antibody-based immunotherapy. Even more interestingly, they can play a pivotal role in the development of new vaccines. Here, we describe two human mAbs directed against influenza haemagglutinin (HA), named PN-SIA28 and PN-SIA49, featuring such characteristics. They can be effectively considered as suitable candidates for antibody-based immunotherapy due to their low IC50 (half maximal (50%) inhibitory concentration) and to the protection of animal models against lethal influenza virus challenges conferred by PN-SIA49. At the same time, the fine characterization of the conserved structural motifs on the HA, recognized by these mAbs, can be the key to success in the development of new epitope-based vaccines.

P6.11.13
Use of Rodent Models to Identify Components of the Immune Response Compromised by Prior Exposure to Adenovirus: Filling in the Missing Pieces with Formulation Development for an Ebola Vaccine

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Development of a rapidly acting vaccine against Ebola is needed for natural outbreaks or those that result from bioterrorist activity. Recombinant adenovirus serotype 5 (rAd5) an advanced vector system to deliver Ebola antigens for immunization. However, Ad is common in the environment and many have anti-Ad antibodies that may compromise its efficacy as a vaccine.
Early studies established pre-existing immunity (PEI) to Ad by intramuscular (IM) injection of Ad 30d prior to immunization. Since Ad is a respiratory pathogen, IM injection does not accurately reflect natural infection. To address this issue, we test the hypothesis that the manner by which PEI is induced will significantly impact the type of immune response generated by an Ad-based vaccine and that this will vary with immunization route.
Multiparameter flow cytometry analysis revealed that, when PEI is induced by the same route as immunization, antigen-specific CD8+ IFN-γ producing T cells and polyfunctional antigen-specific cells producing IFN-γ, IL-2 and TNF-α are significantly reduced. The antigen-specific effector/effector memory CD8+ T cell proliferative response followed the same trend. Th2-type antigen-specific antibody responses were significantly reduced 28d after vaccination. This correlated with poor survival after lethal challenge.
Several novel formulations improved transduction efficiency in the presence of neutralizing antibodies. Formulations that improved multi-functional $CD8^+$ T cell and Th2 type responses increased survival. Unlike current strategies to improve T cell quality in vaccines, we use pharmaceutically acceptable excipients that do not target TLRs or other PPRRs, eliminating unwanted side effects from over-activation of the immune system.

P6.11.14
Recombinant Vesicular Stomatitis Virus-based Vaccine Protects Mice against Viral Myocarditis

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Objective: Coxsakievirus B3 type (CVB3) is a major cause of viral myocarditis and currently there is still no effective vaccine available in clinic. To develop a mucosal vaccine against CVB3 infection, we generated a recombinant vesicular stomatitis virus (VSV) -based vaccine and its protection role against viral myocarditis was examined in Balb/c mice infected with CVB3.
Material and methods: We constructed a recombinant VSV expressing CVB3 capsid protein VP1 using VSV reverse genetic system. Western blot and Immuno-fluorescence staining indicated that the VP1 was successfully expressed upon VSV infection. Mice were intranasally vaccinated with 10^5~10^6 PFU recombinant VSV at single does. A DNA mucosal vaccine (Chitosan-pVP1) previously developed in our lab was used as a control and intranasally immunized at 2-weeks interval for 4 times. Two months later, mice were challenged with $3X10^5$ TCID50 lethal dose of CVB3. Additionally, CVB3-specific T cell mediated and humoral immune responses were carefully determined.
Results: Recombinant VSV-based vaccine elicited robust cellular immune response as well as humoral immune response as evidenced by enhanced T cell proliferation, increased specific IFN-γ^+ T cells, better CTL immune response and elevated sIgA secretion comparing with chitason-VP1. Meanwhile, we noticed that the survival rate increased with less severe myocarditis in heart tissues upon viral challenge.
Conclusion: The VSV-based vaccine expressing CVB3 VP1 might be a promising candidate that protect against CVB3 infection.

P6.11.15
Cell penetrable human ScFv specific to influenza A virus matrix protein, M1, mitigates influenza severity

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Matrix protein-1 (M1) is highly conserved across type A influenza viruses. This protein has many important functions in the viral replication cycle; thus it is one of the targets of novel anti-influenza.To study therapeutic efficacy of M1 specific, cell penetrable human single chain antibody (HuScFv) in mitigating severity of influenza in infected mice.E. coli derived-human single chain antibody fragments (HuScFv) specific to recombinant M1 of influenza A virus H5N1 (clade 1) was produced using a human antibody phage display library. HuScFv from selected huscfv-phagemid transformed E. coli clones were linked molecularly to a cell penetrating peptide, penetratin (PEN) and PEN-HuScFv were produced and purified. BALB/c mice were infected intranasally with mouse adapted-avian H5N1 virus (clade 2.3). They were then treated with M1 specific-PEN-HuScFv. Control infected mice received PBS treatment. Internal organs (lung, brain, spleen, liver and kidney) were collected. Tissue viral loads were determined by real time RT-PCR. Histopathology of the tissues was also examined. Infected BALB/c mice that received M1 specific-PEN-HuScFv had reduced viral loads and histopathological features in tissues compared to the controls.M1 specific-PEN-HuScFv could mitigate severity of influenza in mice infected with A H5N1 of the heterologous clade. Cross therapeutic efficacy of the transbodies on influenza caused by different virus subtypes remains to be evaluated.

P6.11.16
Phylogenetic study of BTV in Morocco since 2004 : determination of new reassortants

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The bluetongue (BT) is a non-contagious disease transmitted by blood-sucking arthropods of the genus Culicoides. The disease affects small ruminants, especially sheep which are very sensitive.
Since 2004 two serotypes circulating in Morocco BTV-1 and 4, several samples were collected during different epidemics, the strains were isolated but no thorough phylogenetic study has been established to characterize and reveal different reassortants have been able to generate from parental strains.
The first year of our study involved a serological survey of sheep and cattle to define the epidemiological situation with respect to this arbovirus, also reveal the rate of coinfection by differents serotypes.
Out of the 429 sera that were collected, 216 are from the cattle of the Gharb region, 109 were collected in 2009 from different regions of Morocco, and the rest was collected in 2012 from sheep of other regions of the country. All the sera collected were tested by neutralization using reference viruses of serotype 1 and 4 and the result is that the rate of co infection was high especially among cattle. Hence the results prove the importance of a molecular study involving BTV isolates that represents different regions of Morocco and different years since the first outbreak.
The next step is mainly based on the sequencing of BTV isolates to better understand the evolution of this virus through the years, then a study of the pathogenesis and immune response of animals would also assess whose goal is to classify different reassortants in Morocco.

P6.11.17
IFN-α improves TAPCells-based immunotherapy by inducing a highly activated dendritic cells in vitro

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Introduction: IFN-α allows a cross-talk between the innate and adaptive immunity, promoting TH1 differentiation and inducing the generation and activation of CTLs. On monocytes, induces differentiation into highly activated DCs. We set to evaluate the effect of IFN-α on our already clinical effective TAPCell immunotherapy on both prostate and melanoma patients.
Materials and Methods: Monocytes were incubated with rhIL-4 and rhGM-CSF, stimulated at day 2 with a melanoma, prostate tumor lysate (TRIMEL and TRIPRO respectively) or lipopolysaccharide and harvested at day 3. Maturation markers were tested by flow cytometry. Cytokine determination was performed by ELISA and by FlowCytomix® Technology. DCs were cultured with allogeneic CD4+ T cells and proliferation, effector and regulatory T cells profiles were determined by flow cytometry. Delayed type hypersensitivity reaction (DTH) of a prostate cancer patient treated with this modified vaccine was evaluated and compared with a prior immunization with the standard DC-based vaccine.
Results and Discussion: IFN-α increased DCs maturation markers. Also, IFN-α treated DCs secrete more IL-12 while secreting lower levels of IL-10. Despite we found no difference on the induction of effector T helper profiles, the amounts of IL-10 secreted by those T cells was significantly lower. We observed that the DTH reaction was significantly larger than observed previously with our standard vaccine.
Conclusion: The use of IFN-α ex vivo on DCs used in cancer immunotherapy could avoid the high rate of adverse reactions of the systemic treatment while delivering the adequate signals to the proper cell types, conferring improved anti tumour immunity.

P6.11.18

Directed evolution of human single-chain Fv antibody fragment (scFv) anti-crotoxin, the main toxin of Crotalus durissus terrificus snake venom

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The WHO incorporated, in 2009, snakebite envenoming in its list of neglected tropical diseases. Single chain (scFv) antibodies consist of variable heavy and light domains joined by short flexible linker and can be used as therapeutic agents. In a previous work, crotoxin (CTX), the main component of *C. d. terrificus* snake venom was used to select human neutralizing recombinant scFvs by phage display technology from a naive library of more than 10^{10} scFvs. Among all ScFvs isolated one of them, named ScFv6, showed the best perfomance. Our aim is to enhance ScFv6 affinity by site-directed mutagenesis viewing a possible therapeutic alternative for envenoming. In order to clarify the mechanisms of neutralization, docking and energy minimization calculations of the antibody-CTX were also conducted. From these simulations, three single changes were chosen to be mutated. Mutants S30A and Y31F had aminoacid changes in CDR H1 while R103H in CDR H3. The mutants were codon optimized, cloned into pET20b+ vector for bacteria expression. Protein expression was induced with IPTG and periplasmic fractions were isolated through osmotic shock. Purification by immobilized metal affinity chromatography and SDS-PAGE analysis showed that all scFvs were expressed in soluble form. Circular dichroism revealed that scFvs had preserved secondary structure. Sequencing confirmed the desired mutations. Preliminary results show that all mutants presented similar expression levels. ScFvs will be now analysed regarding their affinity to CTX by surface plasmon resonance assay.
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P6.11.19

Data-driven approach reveals molecular and physiological events underlying synergistic effects of DNAhsp65 immunotherapy associated to conventional chemotherapy in experimental tuberculosis

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Tuberculosis was declared a global emergency by WHO. New strategies to treat the disease are urgently needed. Immunotherapy, as adjunct of chemotherapy, kills the pathogen and boosts the immune system, shortening the conventional treatment.
We applied a data-driven approach to understand the molecular events underlying the combined effects of chemotherapy plus DNAhsp65 immunotherapy against tuberculosis and to define the real value of this strategy, envisioning their evaluation in clinical trials.
Infected BALB/c mice were treated with DNAhsp65, or isonizid and rifampicin or both. Microarray assay, covering the whole mice genome, was performed from RNA lung samples. The combination of therapies specifically changed the expression of 867 genes ($p<0.05$, fold change>1.5), indicating it produces synergistic and not just addictive effects.
Functional analysis showed that these genes belong to important physiological functions to overcome an infection. Pathways related to drugs side effects had their scores reduced by the combined therapies. Together, these observations indicated infection and inflammation controls and fibrosis reduction. Real-time PCR, ELISA, ELISPOT, CFU assay, histological analysis and collagen deposition measurements validated microarray data and functional analysis.
So, by using a data-driven approach, we showed for first time that the association of chemotherapy and DNAhsp65 immunotherapy in tuberculosis has synergistic effects that ultimately are reflected in the host immune response improvement and lung preservation. Next steps include identify short biosignatures allowing robustly discriminate all groups and translate the therapeutic efficacy.
This strategy has major implications regarding to application of Systems Immunology in therapeutic strategies development as gene based medicines.

P6.11.20

Efficient neutralization of Respiratory Syncytial Virus activity via novel anti-F/G bispecific antibodies concepts: treatment paradigm for severe RSV infections

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Respiratory syncytial virus (RSV) is a highly contagious virus which primarily affects young children and individuals who have underling lung or heart diseases or are immunocompromised. RSV accounts for about 70-90% of all viral bronchiolitis and pneumonia and 2.8-4.3 million hospitalizations annually in children younger than 5 years old. Currently there is no vaccine or effective treatment for RSV infections. The only prophylactic drug available, Palivizumab, is a neutralizing antibody targeting RSV F protein. Severe RSV infections are usually associated with overwhelming inflammation in the lung characteristic of massive immune cell infiltration. RSV G protein has been suggested to be involved in this pathogenesis, and monoclonal antibodies targeting G protein demonstrated potent anti-inflammatory and antiviral effects in cultures and in animal models. In the current study, we generated a bi-specific antibody (Bs-Ab) with high affinity to RSV G and F envelope proteins, aiming for better therapeutic efficacy through blocking both RSV infection and RSV G protein-mediated inflammation. These tetravalent Bs-Abs retained binding specificity and high affinity to their respective antigens. In the plaque and cytopathic effect assays, the Bs-Abs demonstrated more potent antiviral activity than the parental F and G mAbs alone or in combination. In the cotton rat RSV challenge experiment, the Bs-Abs also exhibited better antiviral efficacy than the individual mAbs or combinations. Further studies using appropriate RSV disease models are needed to determine the therapeutic potential of these anti-F/G bs-Abs for severe RSV infections.

P6.11.21

A mucin-like peptide induces protection against Fasciola hepatica infection.

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Fasciola hepatica is a worldwide distributed helminth pathogen that causes great economic losses in sheep and cattle. The appearance of *F. hepatica* populations that are resistant to common flukicidal drugs, along with the fact that these drugs do not avoid the hepatic damage in animals, means that new methods of treatment are required. The future prospect for the development of anti-liver fluke vaccines is optimistic and given their consumer acceptability and environmental friendliness, offer the best way forward.
Our group has recently identified a putative mucin-like protein, Fhmuc, expressed in the invasive newly excysted juvenile (NEJ). Mucins are highly O-Glycosylated proteins that are associated with evasion of immune response and adhesion of parasite and hence important for parasite survival in harmful host environment. Thus, this mucin might be a good vaccine candidate against fasciolosis
In this work we present data regarding the immune response induced by the mucin-like peptide Fhmuc. We observed that Fhmuc has pro-inflammatory properties both *in vitro* and *in vivo*, leading to the production of high levels of pro-inflammatory cytokines. When tested in a model of fasciolosis in mice, Fhmuc has proven to protect from infection, especially when administrated together with dendritic cells. These results open new horizons in the development of vaccines against *Fasciola hepatica* infection.

P6.11.22
Immunological characterization of non-responsiveness to routine vaccination - role for regulatory immune cells and IL10

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Non-responders (NR) lack a sufficient protective immune-response after vaccination, yet underlying immunological mechanisms remain widely unknown. Thus we performed a study with NR to tick-borne encephalitis (TBE) or Hepatitis-B and compared their immune-responsiveness after TBE and Influenza vaccination at humoral and cellular level.
In TBE-NR TBE titers remained low but vaccinees mounted protective Influenza titers. Hepatitis-B-NR reacted with adequate titers to both vaccines as did controls, i.e. TBE high-responders. Cellular immune-response (cytokines) correlated with ab-titers in both TBE groups and flow-cytometric analysis of PBMC revealed that in TBE-NR compensatory mechanisms on T-cell level seem to be present. In Hepatitis-B-NR cellular immune-responses to TBE and Influenza were diminished despite protective ab-titers; low IFNy and IL-2 production was accompanied by high IL-10 levels already present in un-stimulated PBMC. Certain Hepatitis-B associated HLA class II subtypes were over-represented and high IL-10 levels seem linked to these subtypes.
To identify the source of IL-10, regulatory immune cell populations were characterized in selected donors. This revealed that FOXP3+ T-regs (and their IL-10 production) increased post booster in both NR groups yet decreased in high-responder vaccinees. Similarly CD19/24+/38+ immature transitional B-cells (B-regs) were highest in Hepatitis-B-NR, increased from lower pre-booster numbers in TBE-NR and decreased in TBE high-responders. In contrast, CD19/24+/27+ B10 B-cells, FOXP3- Tr1 cells and tolerogenic DCs (DC10) were not present in a conclusive manner in the three groups.
Our data indicate that different types of NR are governed by different immunological mechanisms which involve IL-10 production and regulatory T- and B-cell populations.

P6.11.23
Epitope mapping of the human antibody response to filamentous hemagglutinin of Bordetella pertussis

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Filamentous hemagglutinin (FHA) is an important immunogenic antigen of Bordetella pertussis and a major component of acellular pertussis vaccines. To study the immunodominant epitopes of this antigen we investigated the human antibody response to FHA using four overlapping recombinant fragments spanning the whole sequence of FHA. The recombinant FHA fragments were expressed in Ecoli and purified through His-tag. The antibody response to these fragments was assessed in serum samples collected from healthy adults vaccinated with an acellular pertussis vaccine. Serum antibody levels were measured by an indirect ELISA.The highest levels of antibody were directed against a fragment of FHA (FHA3) spanning amino acid residues 1877-2250 of the FHA molecule in all samples tested. No or little antibody was detected against the other recombinant fragments of FHA. Our results suggest that the human antibody response to FHA is restricted to immunodominant epitopes located within residues 1877-2250 of the FHA molecule. This recombinant fragment of FHA might be employed to replace the native FHA molecule in acellular pertussis vaccines.

P6.11.24
StreptInCor a S. pyogenes candidate vaccine increases the number of natural regulatory T cells in peripheral blood from rheumatic fever patients

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Group A Streptococci (GAS) diseases remain as a major public health problem in development countries, reaching 600 million registered cases with 233,000 deaths for year. If not treated properly can lead to autoimmune post-streptococcal sequels, such as Rheumatic Fever (RF) that affects mainly children and teenagers. Rheumatic heart disease (RHD) is the most serious sequel of RF, leading to progressive and permanent valvular lesions. We are developing a vaccine model against S.pyogenes. After identified protective epitopes of C-terminal portion of the M protein, a peptide with 52 aminoacids named StreptInCor (medical identity) was designed. The vaccine epitope induced systemic protective immune response in murine models. Here, we evaluated the capacity of regulatory T cells induction by StreptInCor, through identification of specific cellular markers (CD3, CD4, CD25, CD127 and Foxp3) for this subset of T cell by gene expression and flow citometry. Peripheral blood mononuclear cells (PBMC) of RHD and controls were stimulated with 10µg/ml of StreptInCor synthetic peptide. We observed that StreptInCor induced the increase of natural T regulatory cells (nTreg) in PBMC in both groups (P=0.0043 and 0.0006, respectively). The number of nTreg cells in PBMC from DRC patients before the stimulus is lower than that observed in PBMC from controls (P=0.0341). These results are very important and indicated that the vaccine peptide may have therapeutic potential to control both inflammatory and autoimmune response in RF/RHD patients.

P6.11.25
The monovalent pandemic influenza A/H1N1vaccine induces a significant CD8+T cells and antibodies response did not reach the threshold necessary for successful vaccination in Mexican hospital employees.

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After the pandemic influenza registered in 2009, the development of a new pandemic influenza vaccine to provide protection to high-risk groups is necessary. With the aim of evaluating the immune responses elicited by the monovalent pandemic influenza A/H1N1vaccine, we investigated employees of a Mexican hospital for respiratory diseases. Methods. A paired blood samples were obtained before and one month post-vaccination (n=60). Antibodies anti-A/H1N1 pandemic influenza virus were determined by hemagglutinin inhibition and microneutralization. The frequency of IFN-[[Unsupported Character - Symbol Font γ]] producing T cells and $CD4^+$ and $CD8^+$ T cells proliferation in response to specific peptides of pandemic A/H1N1, seasonal H1N1 or seasonal H3N2 were determined by ELISPOT and flow cytometry respectively. Results. We observed a significant increase of the geometric mean titers (GMT) of antibodies against pandemic A/H1N1 post-vaccination, with a seroconversion rate of 48.3% and seroprotection rate of 66.7%. Nearly half of our study population had basal levels of IFN-[[Unsupported Character - Symbol Font γ]]-producing T cells and proliferation of $CD4^+$ in response to peptides of pandemic A/H1N1 virus that did not change after vaccination. The proliferation of $CD8^+$ T cells was significantly increased post-vaccination. Conclusions. The monovalent pandemic influenza A/H1N1 vaccine elicited a humoral response that did not reach the threshold necessary for successful vaccination but induces a significant increase of $CD8^+$ T cells proliferation, which could be used as a parameter of protective immunity induced by vaccination.
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P6.11.26
Viral vector-based vaccines encoding multiple epitopes of Hepatitis C virus induce robust and protective T cells responses

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Hepatitis C virus infection contributes to approximately one third of liver cirrhosis and hepatocellular carcinoma cases worldwide. Most cases cannot be cured by standard treatment with pegylated interferon-α and ribavirin and no vaccine is available yet. An absolute prerequisite for a therapeutic vaccine against HCV infection is the potency to induce vigorous and broad spectrum CD8 and CD4 T cells responses. Vaccines based on viral vectors are ideal candidates for therapeutic HCV vaccines as they elicit robust humoral and cellular immune responses. We generated HCV vaccines based on Semliki Forest virus (rSFV) expressing all- or parts of the non-structural (NS) proteins of HCV. Immunizations of mice with rSFV expressing all NS proteins induced strong and long-lasting NS3-specific CD8 T cell responses. The strength of the responses was similar to those induced with rSFV expressing only two NS proteins. Immunizations furthermore resulted in a significant delay of tumor-growth of HCV-expressing EL4 tumor cell. To our knowledge, this is the first study showing that the size of transgene does not affect the activity of rSFV particles even when a transgene as large as 6 kb is inserted. As broad spectrum T cell responses in general are only seen in patients that clear an HCV infection, vaccines based on rSFV, allowing expression of all NS proteins, and inducing robust T cell activity as well as the essential broad spectrum T cell response have a high potential for the treatment of HCV infections.
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P6.11.27
DNA vaccine encoding Hantavirus glycoprotein N-terminal, targeted to the major histocompatibility complex II compartment by lysosome-associated membrane protein, significantly elicits both specific humoral and cellular immune responses and induces immune protection against Hantavirus challenge in Balb/c mice

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Lysosome-associated membrane protein (LAMP) can target and bind the endosome/lysosome, the most important components of the MHC class II-processing compartment (MIIC) in the exogenous antigen-processing pathway. LAMP-targeting could greatly enhance the immune response against a number of antigens and DNA plasmids encoding endogenous antigen could take advantage of LAMP and be carried directly into MIIC. Hantavirus glycoprotein N-terminal, named Gn, could induce neutralizing antibody production, though a low serum titer as natural infection. To analyze the influence of LAMP on Hantaan virus (HTNV) Gn vaccine
potency and develop a novel effective vaccine against HTNV, we constructed three eukaryotic vectors as naked DNA vaccine named pVAX-Gn, pVAX-LAMP and pVAX-LAMP/Gn, respectively. Balb/c mice were immunized with those plasmids, and the specific humoral and cellular responses elicited against Gn were measured by ELISA, cytotoxicity assays and ELISpot assay. To measure the protective efficacy, virus challenging in vivo and neutralizing antibody valence were conducted by viral load detection (qRT-PCR and sandwich ELISA) and the cell microculture neutralization test. We found that HTNV Gn showed a strong immunogenicity to elicit both humoral and cellular responses with LAMP as a chimera. Results of protection assay in vivo indicated the immune response established was HTNV specific and protective. These findings not only demonstrate that the LAMP as a trafficking molecule can introduce Gn to MHCII-presenting pathway and significantly enhance HTNV specific immune response, but also suggest that the pVAX-LAMP/Gn as DNA vaccine has potential application on clinic for HTNV infection immunoprophylaxis.

P6.11.28
Antibody-driven vaccine design: a human cytomegalovirus pentameric vaccine selectively eliciting potent neutralizing antibodies

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The use of neutralizing antibodies to identify novel target antigens has been proposed as an effective strategy to identify candidate vaccines capable of eliciting protective B cell immunity. We previously showed that human neutralizing human cytomegalovirus (HCMV)-specific antibodies can be divided in two groups: (a) antibodies recognizing glycoproteins B (gB) and H (gH) able to neutralize viral infection at nanomolar concentrations, and (b) antibodies recognizing the viral gH/gL/UL128-131 complex able to neutralize viral infection at picomolar concentrations. In this study, we produced a recombinant gH/gL/UL128-131 pentameric complex which preserved all the functional antigenic sites targeted by human neutralizing antibodies and when formulated with a non-denaturing polyanionic carbomer adjuvant was found to induce strong neutralizing antibody response in mice. The pentamer was superior to gB, which represents a candidate vaccine in ongoing clinical trials, both in terms of neutralizing antibody titers and protection of different cellular targets. Through a clonal analysis of the response in vaccinated mice and infected humans, we found that most of the antibodies elicited by the pentamer were neutralizing and effective in the picomolar range, while most antibodies elicited by gB were not neutralizing. These results illustrate how potent neutralizing antibodies can be used to identify, within a complex pathogen, the most effective antigens capable of eliciting a protective response.
Our research project was supported by a grant from Cariplo Foundation, Italy.

P6.11.29
Human alpha-1-antitrypsin therapy blocks multiple organ dysfunction syndrome and diminishes bacterial load in a mouse bacterial sepsis model

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Background: Severe bacterial infection can cause sepsis, multiple organ dysfunction syndrome (MODS) and death. Human alpha-1-antitrypsin (hAAT) is a circulating serine-protease inhibitor that rises during acute phase responses and possesses anti-inflammatory, immune-modulating and tissue-protective properties. Evidence suggests that hAAT may also have the potential to block microbial activity. hAAT is clinically available and currently evaluated for individuals that are not genetically-deficient in hAAT. Aim: Examine whether hAAT improves prognosis in bacterial infection and sepsis model. Methods: Mice transgenic for hAAT (hAAT+/+) and hAAT-injected wild-type mice were compared to untreated wild-type mice in a cecal ligation and puncture (CLP) model. Animal survival, bacterial load and liver and pancreas function were determined, lungs were examined by histology and cytokine and immune cell profiles were obtained. Results: hAAT significantly reduced mortality, as well as liver and pancreas injury, and diminished apoptosis in lung cells. Stimulated circulating IL-1β levels were reduced 358.9±185-fold. Immune cells exhibited a phenotype of reduced activation; surface CD86, CD40 and MHC II on peritoneal cells were 10.05±1.801-fold, 16.50±1.371-fold and 6.925±4.739-fold lower in hAAT+/+ mice compared to wild-type mice. Surprisingly, bacterial load in peritoneal lavages was markedly lower in the hAAT group. Conclusions: hAAT therapy affords a significant reduction in bacterial burden after live bacterial infection. Its ability to reduce organ damage and death rates render it an attractive approach for individuals prone to infections. Since bacteria can readily proliferate in hAAT-containing media, the mechanism of action appears to be host-dependent, suggesting a lower risk for the emergence of bacterial resistance.

P6.11.30

In silico prediction of promiscuous Leishmania infantum KMP-11, H1, LeIF, CPA, CPB peptides and experimental validation of eliciting CD4+ and CD8+ T-cell specific responses

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Previous studies on leishmaniasis have demonstrated that both IFNγ-producing $CD4^+$ and $CD8^+$ T lymphocytes are significant players in protection mechanisms against visceral disease. Recent trend on vaccine designing has been shifted to peptide vaccines based on computational prediction of immunodominant T-cell epitopes that are more specific, safe, and easy to produce. *Leishmania infantum* KMP-11, H1, LeIF, CPA and CPB were screened for antigenic epitopes by using online bioinformatics tools (BIMAS, NetCTL, NetMHCII, SYFPEITHI, EpiJen) on their ability to bind promiscuously to mice and human MHC class I and II molecules. Peptides were selected only if were selected by the most of programs used according to their predicitive scores. From the predicted epitopes, 10 peptides were synthesized that contained both MHC class I and II specific epitopes for each protein, characterized as “polyepitope” peptides. To validate polyepitope peptides immunogenicity experimentally, BALB/c mice were immunized subcutaneously 3 times with two different peptide pools. Cellular responses were detected by proliferation assays, flow cytometry and ELISA. Six out of ten (60%) induced proliferative responses in lymph nodes, whereas only three peptides (30%) induced spleen cells proliferation. These peptides were also potent inducers of $CD4^+IFN\gamma^+$ and/or $CD8^+IFN\gamma^+$ T cells as confirmed by flow cytometry. Finally, four peptides were selected for further vaccination experiments against visceral leishmaniasis, CPA_2, H1_1, LeIF_6 and CPB_1, based on their ability to induce strong T cell responses, suggesting that promiscuous T-cell epitopes can be identified using reverse vaccinology.

P6.11.31

Recombinant polyepitop-forming chimera protein derived from Borrelia burgdorferi OspC variants induces specific antibodies recognizing wide spectrum of antigenic variants of pathogenic Borreliae

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Lyme disease is the most frequent zoonosis in Europe and in the United States. It may progress into a chronic form and could lead to nervous or cardiovascular system, joints, skin or eyes affections. Outer surface protein C (OspC) is 23 kDa, lipoprotein antigen of spirochete Borrelia burgdorferi species complex - causative agent of Lyme disease. OspC is one of the most promising candidates for vaccine design. Due to high sequence variability of OspC it is not possible to induce protective immunity to broader range of Borrelia strains by single variant of OspC protein.
In this study, we created a fusion chimera recombinant protein (chOspC) composed of OspC immunodominant domains from most frequent infective strains of B. burgdorferi. Protein was expressed in E. coli in fusion with His-tag allowing metalloaffinity chromatography purification. Identity of protein was confirmed by MALDI-TOF.
Recombinant chOspC was used for the immunization of experimental mice. Hyperimmune sera were tested to evaluate their reactivity with several OspCs using Western blot and human diagnostic line-blot stripes. Mice immunized with B31 variant of OspC served as a control.
Sera of chOspC-vaccinated mice reacted specifically and strongly with whole B. burgdorferi bacteria lysates on Western blot forming single band at position corresponding to OspC as well as with all three OspCs from selected pathogenic subspecies on line blot. In contrast, control sera reacted to only subspecies-specific OspC. Described properties are promising for further testing of chOspC protein as potential Lyme disease vaccine component.
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P6.11.32

Microparticulate-encapsulated antigen with split topical CpG oligodeoxynucleotide adjuvant as a single-injection immunization strategy

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Antigen-presenting cells (APCs) are responsible for presenting foreign antigens to, and activating T cells to fight off infection. Manipulation of APCs to modulate immune responses to immunization has been a focus in vaccinology. Due to high frequencies of APCs in the skin, we are interested in the skin as a site for immunization. Keratinocytes and skin-resident plasmacytoid dendritic cells can be activated via binding of their Toll-like receptor 9 to CpG oligodeoxynucleotides (ODN) – synthetic DNA that mimic unmethylated CpG bacterial DNA. Here, we encapsulated the ovalbumin (OVA) antigen in polylactide co-glycolide microparticles (PLG-OVA) and show that mice immunized subcutaneously in a prime-boost regime with PLG-OVA generate antigen-specific cytotoxic T cells when CpG ODN is used as an adjuvant. Using flow cytometric analysis, 2.3% antigen-specific cells are detected within the $CD8^+$ T cell population in the blood when CpG ODN is administered topically ($P<0.05$ compared to PLG-OVA alone). In addition, 0.8% of $CD8^+$ splenocytes produce interferon-gamma when re-stimulated with OVA peptide (SIINFEKL) *in vitro* ($P<0.001$). The microparticles show a triphasic release of OVA which simulates two doses of antigen injected separately. Using this release property and a second topical application of adjuvant, we enhance the generation of antigen-specific cytotoxic T cells in the blood from 0.4 to 0.9% ($P<0.05$) and OVA-specific total IgG and IgG2c production, effectively generating a single-injection vaccine with split topical adjuvant administration that induces robust cytotoxic T cell and antibody responses. Generating single-injection vaccines may enhance vaccination compliance and may also decrease the cost of administration.

P6.11.33

An animal model serum cytokine profile (Th1 & Th2) for new formulation of Leishmania major post challenging

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Leishmania is diffused worldwide, resulting in estimated 12 million new cases each year. In the case of cutaneous leishmaniasis effective protection against infection has been largely attributed to the development of potent CD4+ Th1 type immune response, characterized by the production of IL-12 and IFNγ, which subsequently mediates macrophage activation, nitric oxide production and parasite killing. The effect of BCG on the course of established Murine leishmaniasis was examined before. Although to date there is no vaccine against leishmania; several of the vaccine preparations spend their advanced stages of clinical testing. Up to know, several different methods of vaccine preparation have been used which is plus or without adjutant. We had prepared crude antigen combinations by five different methods using antigens from L.major parasites with five injection doses, before. Also, its safety, toxicity, efficacy and immunogenicity evaluated pre challenge in animal models. In this study examined both BCG and new adjuvant (Teucrium polium (T.P), three injection groups Λ(LT, LB, LBT) and two injection doses (100, 200 µg/.0.1ml) in Balb/c mice post challenge.In six injected groups Th1 (IFN-γ, IL-12) and Th2 cytokines (IL-4, IL-10) were measured. Our results showed that in three injection groups (LB, LT and LBT) that obtained one of two adjutants; BCG/TP and or both of them and 100, 200µg/0.1ml with two booster doses: LBT group has lowest IFNγ and highest IL-12 value, LT and LB groups have equal IL-12, but LB have more IFNγ and IL-10 but less than IL-4 in LT group

P6.11.34
HIV infected individuals demonstrate significantly reduced polysaccharide-specific B cell response to T-cell independent vaccination compared to non-infected individuals

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The peripheral blood of newly diagnosed HIV infected individuals is subjected to high uncontrolled viral titers. The decimation of both T and B cells thus leaves these individuals highly susceptible to opportunistic infections, including Streptococcus pneumoniae, despite the availability of vaccines. We have previously shown using directly labeled fluorescent pneumococcal polysaccharides that the majority of pneumococcal polysaccharide-specific B cells in healthy individuals were IgM+ memory B cells. Using this method, we identified specific deficiencies in B cell populations responding to pneumococcal polysaccharide vaccination in HIV infected individuals. Namely, a significant decrease in the pneumococcal polysaccharide-specific IgM+CD27+CD43+CD5+/-CD70- B cell population compared to healthy individuals. This population has recently been described as human B1 cells. Consequently, HIV infected individuals showed reduced polysaccharide specific antibody responses and opsonophagocytic titers compared to healthy individuals. Furthermore, we correlated changes in pneumococcal polysaccharide-specific B cell populations post-vaccination with individual CD4+ T cell counts. We observed a decreasing trend in pneumococcal polysaccharide-specific IgM+ memory B cells with lower CD4+ T cell counts despite the T-independent type II nature of the polysaccharide vaccine antigens. These data indicate that the loss of pneumococcal polysaccharide-specific IgM+CD27+CD43+CD5+/-CD70- B cells in HIV infected individuals correlates with their CD4+ T cell counts and results in poor responsiveness to pneumococcal vaccination.

P6.11.35
Characterization of CMV-specific T-cell receptors for clinical application

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Adoptive therapy with specific T cells can control cytomegalovirus (CMV) reactivation and disease in immunocompromised patients. However, many donors of an allogeneic stem cell transplantation are CMV-negative and thus cannot provide CMV-specific T memory cells. Such T cells can be artificially produced for therapy by T-cell receptor gene transfer. Important criteria for selection of suitable TCRs for therapy include (1) absence of alloreactivity and (2) efficient recognition of infected cells.
It was previously found that many CMV-specific T cells exhibit alloreactivity. We determined the TCR sequences of various CMV-specific T cell clones specific for two pp65-derived epitopes (YSE, HLA-A*0101 and NLV, HLA-A*0201), and have analyzed their reactivity against a panel of B-cell lines that cover 41 of the most frequent HLA allotypes. We identified three NLV-specific and eight YSE-specific TCRs without alloreactivity against frequent HLA allotypes, including allotypes that are cross-recognized by other NLV-specific T cells.
T cells specific for the CMV antigen IE-1 are associated with control of CMV reactivation in immunocompromised patients, but IE-1 appeared to be poorly presented by infected cells. We recently identified IE-1- specific CD8 T cells that overcome these immunoevasive effects. They recognize a novel immunodominant HLA-C-restricted epitope (CRV, HLA-C*0702). We show that these T cells express a multitude of different TCRs, some of which are shared between different donors. Widely shared ("public") TCRs may be preferable for therapy because of their compatibility with various HLA allotypes. We currently analyze the CRV-specific TCR repertoire at high resolution to identify public TCRs for therapy.

P6.11.36
Long-term follow-up study to the Fiji Pneumococcal Project: Investigation of immune hyporesponsiveness following a 12-month pneumococcal polysaccharide booster

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Background & Aim: The Fiji Pneumococcal Project (FiPP) examined the immunogenicity of reduced dose schedules comprising pneumococcal conjugate (PCV) and polysaccharide (PPS) vaccines. Following a micro-PPS (20%) challenge dose at 17 months, children that received the PPS at 12 months age exhibited immune hyporesponsiveness in terms of serotype-specific IgG and opsonophagocytic (OPA) levels. The 12-month PPS dose also had no impact on vaccine-type carriage.
Methods: This was a follow-up study to examine the long-term immunological impact of the 12-month PPS booster. Blood samples were taken pre- and 28-days post-PCV13 immunisation from the first 101 children (now 4-6 years old) re-consented into this study. To date, analysis includes specific IgG measurement to all 23 serotypes in PPS as well enumeration of the memory B cell response to serotypes 1, 3, 4, 6B, 14, 19F and 23F.
Results: Significantly lower pre-PCV13 IgG levels for 6B (p=0.003), 22F (p=0.024) and 23F (borderline p=0.053) were detected in children that received the 12-month PPS; after PCV13, only serotype 22F IgG was lower in PPS-vaccinated children compared to those that weren't (p=0.009). Memory B cell responses were not different before PCV13, however a significantly lower number of 23F-specific B cells (p=0.021) post-PCV13 were found in children given PPS at 12 months of age.
Conclusion: With the exception of a few serotype, immune hyporesponsiveness was no longer observed before or after PCV13 vaccination. Analysis is ongoing to evaluate the impact of the PPS booster in this study.

P6.11.37
Improvement in clinical signs and cellular immunity of dogs with visceral leishmaniasis using the immunomodulator P-MAPA

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This study investigated the immunotherapeutic potential of the protein aggregate magnesium-ammonium phospholinoleate-palmitoleate anhydride immuno-modulator (P-MAPA) on canine visceral leishmaniasis. Twenty mongrel dogs presenting clinical symptoms compatible with leishmaniasis and diagnosis confirmed by the detection of anti-Leishmania antibodies were studied. Ten dogs received 15 doses of the immunomodulator (2.0 mg/kg) intramuscularly, and 10 received saline as a placebo. Skin and peripheral blood samples were collected following administration of the immunomodulator. The groups were followed to observe for clinical signals of remission; parasite load in the skin biopsies using real-time PCR, the cytokines IL-2, IL-10 and IFN-γ in the supernatant of peripheral blood mononuclear cells stimulated in vitro with either total promastigote antigen or phytohemagglutinin measured by capture ELISA, and changes in CD4+ and CD8+ T cell subpopulations evaluated by flow cytometry. Comparison between the groups showed that treatment with the immunomodulator promoted improvement in clinical signs and a significant reduction in parasite load in the skin. In peripheral blood mononuclear cell cultures, supernatants showed a decrease in IL-10 levels and an increase in IL-2 and IFN-γ. An increase in CD8+ T cells was observed

in peripheral blood. In addition, the in vitro leishmanicidal action of P-MAPA was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and no leishmanicidal activity was detected. These findings suggest that P-MAPA has potential as an immunotherapeutic drug in canine visceral leishmaniasis, since it assists in reestablishing partial immunocompetence of infected dogs.

P6.11.38
A novel tool for induction of specific T-cell immune responses: Complexes of streptavidin-fused antigens with biotinylated antibodies targeting receptors on dendritic cell surface

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For various diagnostic and vaccinal applications, it is important to stimulate antigen-specific immune responses of T lymphocytes. This remains a technical challenge, as it depends on targeted delivery of antigens into cytosol or endosomes of professional antigen presenting cells (APCs), such as dendritic cells, which process antigens and present them to T cells in complex with MHC class I and II molecules, respectively.
Here we describe a novel system for antigen delivery into APCs that offers high flexibility of targeting of various endocytic receptors. The system is based on genetic fusion of the antigen of choice with streptavidin that in its tetrameric form binds a biotinylated targeting antibody recognizing an endocytic receptor on the surface of APC.
As a proof of principle, chicken ovalbumin or the mycobacterial antigens CFP-10 and ESAT-6, as well as antigen pp65 of cytomegalovirus were used as model antigens that were genetically linked to N- or C-terminal ends of the streptavidin core protein. Purified tetrameric antigen-streptavidin (Ag-SA) complexes with antibodies against surface receptors CD11b, CD11c, MHC II and DEC 206 (manose receptor) were formed and used for antigen delivery into mouse dendritic cells or human PBMC *in vitro,* injected or administered intranasally into mice for induction of systemic and mucosal immune responses *in vivo*. Analysis of the capacity of such-treated APCs to stimulate specific T cell response revealed that efficient epitope presentation on MHC class I and II molecules can be achieved *in vitro* already at antigen concentrations of 0.1 - 1 nM.

P6.11.39
Vaccine-mediated B cells responses to *S. aureus* hemolysin, and effect of different boosting intervals on functional humoral responses

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Although antibody titers are usually considered the best readout for vaccine efficacy, the importance of memory B-cell responses in conferring protection has remained controversial. Primary exposure to an antigen generates, along with antibody-secreting cells (ASCs), a heterogeneous B-cell memory compartment that changes over time and that may respond in different ways when re-encountering the cognate antigen. The goal of this study is to characterize B-cell responses after priming and the effect of different boosting intervals on the differentiation and function of ASCs and memory B cells. We used a vaccine model based on the alpha-Hemolysin (Hla) protein from *Staphylococcus aureus*, adjuvanted with Alum. This vaccine induced high antibody titers and was protective in sepsis and pneumonia animal infection models. We performed a time course analysis following one or two immunizations, two weeks apart: mice immunized twice had higher anti-Hla IgG titers compared to mice immunized once. ASCs measured by Elispot were increased in spleen 10 days after boosting and remained elevated in bone-marrow for at least 4 months. A similar trend was observed between the two groups when looking at the frequencies of Hla-specific memory B cells. Next steps will address the phenotypic and functional characterization of Hla-specific memory B cells to define a correlation between different boosting intervals and the protective humoral response. This work aims at providing new insights in the memory B-cell compartment that might benefit the design of new vaccination strategies.

P6.11.40
Efficacy of Flu SAM (HA) vaccine against Influenza infection in mice

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Influenza (Flu) is a vaccine preventable disease but remains a major problem worldwide and vaccine production remains suboptimal. SAM vaccines, developed from self-amplifying RNA encoding an antigen and delivered with a non-viral delivery system, could potentially address current vaccine production limitations during a new pandemic influenza outbreak.
In this study, we demonstrated that a pandemic H1-SAM vaccine induced humoral immune responses comparable to non-adjuvanted subunit vaccine, both in terms of total antibody titers and functionality in mice. Antibodies induced by immunization with a SAM (HA) vaccine were able to neutralize virus infectivity and inhibit HA activity. In conclusion, if the SAM vaccine platform proves safe, potent, well-tolerated and effective in humans, fully synthetic vaccine technologies could provide a rapid response platform to influenza outbreaks.

P6.11.41
CXCL10 is critical for the generation of protective CD8 T cell response induced by antigen pulsed CpG-ODN activated dendritic cells

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The visceral form of leishmaniasis is the most severe form of the disease and of particular concern due to the emerging problem of HIV/visceral leishmaniasis (VL) co-infection in the tropics. Till date miltefosine, amphotericin B and pentavalent antimony compounds remain the main treatment regimens for leishmaniasis. However, because of severe side effects, there is an urgent need for alternative improved therapies to combat this dreaded disease. In the present study, we have used the murine model of leishmaniasis to evaluate the potential role played by soluble leishmanial-antigen-pulsed-CpG-ODN stimulated dendritic cells in restricting the intracellular leishmanial growth. We found that mice vaccinated with a single dose of SLA-pulsed DC stimulated by CpG-ODN were protected against a subsequent leishmanial challenge and had a dramatic reduction in parasite burden along with the generation of parasite specific cytotoxic T lymphocytes. Moreover, we demonstrate that the induction of protective immunity conferred by SLA-CpG-DCs depends entirely on the CXC chemokine IFN-c-inducible protein 10(CXCL10). CXCL10 is directly involved in the generation of a parasite specific CD8+ T cell-mediated immune response. We observed significant reduction of CD8+ T cells in mice depleted of CXCL10 suggesting a direct role of CXCL10 in the generation of CD8+ T cells in SLA-CpG-DCs vaccinated mice. CXCL10 also contributed towards the generation of perforin and granzyme B, two important cytolytic mediators of CD8+ T cells, following SLA-CpG-DCs vaccination. Together, these findings strongly demonstrate that CXCL10 is critical for rendering a protective cellular immunity during SLA-CpG-DC vaccination that confers protection against Leishmania donovani infection.

P6.11.42

DNA vaccine encoding hepatitis C virus proteins NS3-NS5B induces more effective immune response in comparison with DNAs expressing individual nonstructural genes

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Currently, there are several DNA vaccines against hepatitis C virus (HCV) being tested, but the optimal composition of HCV genes is not yet defined. The goal of study was to evaluate the effectiveness of genetic immunization of mice with DNA pcNS3-NS5B encoding five hepatitis C virus (HCV) nonstructural proteins: NS3, NS4A, NS4B, NS5A and NS5B in comparison with plasmids containing genes of the same individual nonstructural proteins. The HCV DNA constructions with gene GM-CSF as adjuvant were injected intramuscularly in DBA mice three times. Humoral immune response was assessed with ELISA; cellular immune response - in blast transformation reaction, by quantitation of CD4+ and CD8+ T cell proliferation using flow cytofluorimetry, by intracellular synthesis and secretion of IFN-γ and IL-2 in ELISpot and ELISA. It was found that the functionally active T cell response was achieved to antigens presenting NS3, NS4, NS5A and NS5B epitopes of different HCV genotypes in response to pcNS3-NS5B plasmid and was stronger than that to plasmids carrying individual genes. Injection of pcNS3-NS5B induced high CD4+ T cells proliferation, IL-2 and IFN-γ secretion, anti-NS3 and anti-NS5B IgG2a production. These findings indicate that genetic immunization with pcNS3-NS5B is promising approach for development of hepatitis C vaccine.

P6.11.43

Vaccination with recombinant empty capsids of foot-and-mouth disease virus triggered an immune response that surpassed the viral challenge in a mice model

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The use of recombinant empty capsids against foot-and-mouth disease virus (FMDV) has been reported to be a promising alternative to the current inactivated vaccine.
The encoding sequences of the capsid precursor P12A and the protease 3C were used to generate the expression vectors pTT5-P12A3C and pTT5-P12A. When cells were transfected with pTT5-P12A3C, the protease 3C cleaved the capsid precursor P12A into the structural proteins VP0, VP1 and VP3 that assembled into empty capsids.
To evaluate the immunogenicity of the recombinant proteins, mice were immunized with a low amount of virus that elicits an immune response in all animals and the equal amount of recombinant proteins.
Mice inoculated with the lysates of cells transfected with pTT5-P12A3C and with inactivated FMDV induced high antibody serum titers at 14 days post vaccination that increased after booster immunization and were protected against viral challenge. Mice inoculated with the lysates of cells transfected with pTT5-P12A induced low antibody serum titers that did not increase after booster immunization.
A significant difference was observed in the immune response elicited in the animals vaccinated with P12A3C or P12A recombinant proteins, confirming that the correct cleavage of P12A by protease 3C and assembly of the structural proteins is critical to achieve a protective immunity. Furthermore, the structures produced by transient gene expression in mammalian cells with pTT5-P12A3C induced high levels of antibody and protection against viral challenge in mice suggesting that the strategy is a suitable alternative for vaccine production.

P6.11.44

A novel HPV-16 L1 based chimeric virus-like particle containing HPV 16 E6 and E7 epitopes elicits a persistent specific antibody immune response and tumor regression

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HPV L1-based virus like particles vaccines (VLPs) are efficient to induce temporary prophylactic activity through the induction of neutralizing antibodies. However VLPs that can provide prophylactic as well as therapeutic properties for a long time are necessary. We generated a novel HPV 16 L1-based chimeric virus like particle (cVLP) that was produced in plants and that contains a string of T-cell epitopes from HPV 16 E6 and E7 fused to its C-terminus. In the present study we analyzed the persistence of specific IgG antibodies with neutralizing activity induced by the immunization with cVLPs, as well as their therapeutic potential in a tumor model of C57BL/6 mice strain. Interestingly, we obtained that these cVLPs induced persistent IgG antibodies for over 12 months, with cross-reactivity and cross-neutralizing activity for VLPs composed of HPV-16 L1 protein. Efficient prevention and rejection of tumors induced by TC-1 tumor cells expressing HPV-16 E6/E7 oncoproteins was observed in mice immunized with these cVLPs. In addition, in mice bearing tumors, important tumor regression (57%) was observed after immunization with cVLPs. The chimeric particles of the type described in this work may potentially be the basis for developing HPV prophylactic and therapeutic vaccines with high efficacy. Support: CONACYT (82827 and 8373); FIS-IMSS/PROT (60 and 617 and 876) grants.

P6.11.45

TELL ME project for an effective communication during infectious outbreaks

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During infection outbreaks, one of the major problems has always been to communicate with the population in order to influence behaviors, reduce the spread of disease and avoid panic. TELL ME (Transparent communication in Epidemics: Learning Lessons from experience, delivering effective Messages, providing Evidence) is a 36 month Collaborative Project, funded by the European Commission (FP7), which aims to provide evidence and to develop models for improved risk communication during infectious disease crises. TELL ME combines public health, social sciences, behavioral scie nces, political sciences, law, ethics, communication and media, in order to develop original communication strategies regarding complicated messages and advice based on uncertainties, also addressing the issue of vaccine-resistant groups.
TELL ME aims to identify the most appropriate tools to raise awareness about pandemic risk and to deal with complexity, uncertainty and misinformation on this topic. Amongst the project's main focuses there are also the new opportunities offered by social media for monitoring and epidemiologic surveillance; the narratives underlying the different reactions from different groups; the big issue of stigmatization and discrimination caused by communication mistakes; the cases of resistance to vaccines and the best strategies to support vaccinations.
Since communication is a multifaceted framework, such an effort is directed to all the stakeholders involved, from healthcare professionals to representatives of public health institutions, from researchers to local authorities, from journalists to the general population.
The research leading to these results has received funding from the European Union Seventh Framework Programme ([FP7/2007-2013] [FP7/2007-2011]) under grant agreement n° [278723].

P6.11.46
Selection of neutralizing scFv antibodies against glycoprotein B of Herpes simplex virus type1

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Introduction: Herpes simplex virus type 1 (HSV-1) is responsible for a wide range of human diseases from the localized infection such as orolabial or corneal lesions to lifethreatening encephalitis, neonatal disease and pneumonia in the immunocompromised individuals. Glycoprotein B (gB) play a critical role in the entry of the virus into target cells. Isolation of specific single chain antibodies (scFv) by antibody phage display has become a popular method in recent years. In this study, a phage display library was used to isolate specific neutralizing scFvs against gB of HSV-1.
Materials and Methods: Panning was performed by using human phage display library against the immunodominant epitope of gB. Fingerprinting was carried out on PCR-amplified scFvs. The reactivity of isolated clones to the peptide was determined by phage ELISA. The neutralising activities of scFvs were evaluated using plaque reduction assay.
Results: After four rounds of panning, PCR and DNA fingerprinting of clones, two specific clones for epitope of gB were selected. The frequencies of clones were 45% and 20% which showed neutralizing effects 59% and 55%, respectively.
Conclusions: There are some reports of identification of the neutralizing epitopes of gB by monoclonal antibodies. In this study, we selected two specific neutralizing scFvs against gB of HSV-1 which can be used as a promising immunotherapeutic tool for preventing neonatal herpes infection whenever passive immunization against HSV-1 is needed. Further investigations in vivo is needed to show the effects of soluble forms of the selected scFvs

P6.11.47
Vaccination with MenAfriVac, a serogroup A conjugate meningococcal vaccine, induces salivary antibodies that could prevent pharyngeal colonization

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Background. MenAfrivac, a conjugate vaccine to combat the devastating serogroup A meningococcal disease in sub-Saharan Africa, was first introduced in Burkina Faso in 2010. We have previously shown that the vaccine can eradicate meningococcal colonization. We therefore investigated if the vaccine could stimulate mucosal antibodies that prevent colonization.
Methods. A longitudinal study was conducted in 198 school children aged 10-14 years in Burkina Faso. Saliva was collected using OraSure collection device®, before vaccination with MenAfriVac and 9 weeks after. The concentrations of specific IgA and IgG (specific for serogroup A meningococcal capsular polysaccharide, PsA) and total IgG were determined by in-house bead-based multiplex assays (Bio-Plex) and ELISA. Total IgA was determined by a Bio-Plex kit (Bio-Rad).
Results. Specific IgG and IgA concentrations were significantly higher after vaccination (P<0.0001, paired t-test) while total IgG was lower and IgA concentrations remained stable. The proportion of individuals with detectable anti-PsA IgG increased from 22% (Geometric Mean Titer, GMT=1.5 ng/mL) before vaccination to 60% (GMT=18.1 ng/mL) after vaccination. This increase was likely due to IgG leaking from serum onto the mucosa. PsA-specific IgA was found in higher concentration than specific IgG and was detected in 99% of participants before vaccination (GMT=158 ng/mL). An increase in specific IgA titers was seen in 69% of vaccinees (GMT=255 ng/mL).
Conclusions. Immunization with MenAfriVac induced a significant increase of salivary IgG and IgA antibodies against serogroup A polysaccharide that could act as a first-line defence against meningococci and be a possible mechanism for the observed eradication of colonization.

P6.11.48
Interferon- and immunotherapy in immunocompromised children with associated viral-viral infections: recurrent acute respiratory viral infections (ARVI) and various herpesviral infections

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The treatment of immunocompromised children with high-frequency of recurrent associated viral infections is very difficult. We had examined 27 children 5-8 years suffering from recurrent ARVI with a frequency of 17,2±1,01 episodes per year. The mono-and mixed infections (HSVI/II types, EBV, CMV, HHV6) were identified in 100% of cases. The control group included 20 healthy children. Functions of neutrophilic granulocytes (NG) and interferon (IFN) system were tested. Significant defects of phagocytic activity, killing and microbicidal (NADPH-oxidase) function of NG ($p<0.001$; $p<0.05$; $p<0.05$), the deficit of serum IFNα ($p<0.05$) and IFNγ ($p<0.05$) were present in all children. Patients were randomized into 2 groups. The group 1 received: a) basic systemic and local therapy viferon (recombinant IFNα2) with gradual reduction of doses,-2.5 months; b) for elimination herpes infections - izoprinozin, - three 10-days break courses. The group 2 received the same course of drugs as group 1: viferon, izoprinozin and additionally for the correction of NG - licopid - two 10-days break courses. In both groups clinical efficacy of the therapy during the study and in catamnesis were tested. We saw significant reduction in the frequency of ARVI to 2-3 times a year, prolongation of clinically successful period from 7-10 to 100-150 days. Adequate doses of recombinant IFNα2 had promoted significant growth of serum levels of IFNα ($p<0.001$) and IFNγ ($p<0.05$). Application of licopid had restored disturbed functions of NG in the group 2 ($p<0.001$; $p<0.05$; $p<0.05$), but not in the group 1. Interferon- and immunotherapy had a high clinical-immunological effectiveness, more pronounced in the group 2.

P6.11.49
Influence DNA-vaccines encoding antigens of CCHFV on immune system in mice

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Crimean-Congo hemorrhagic fever virus (CCHFV) - a tick-borne virus that is transmitted to humans through infected tick bites or by direct contact with viremic humans and animals. This is a nairovirus in the family Bunyaviridae causes, highly-contagious hemorrhagic disease with a fatality rate of approximately 30%, it distributed widely in many parts of World. The number of reported cases has increased in recent years, climatic change and human perturbations of biocenoses may have led to the migration of tick. There is currently no commercially available vaccine for CCHF.
The aim of this work was to evaluate efficiency as well as the type of immune response induced by candidate DNA-vaccines (pV3N and pV3GN) carrying the genes of structural proteins CCHFV in mice.
Various parameters including antibody and cytokine responses (INF-g, IL-2 and IL-10), proliferation assay were used to evaluate the type of immune response. To evaluate nonspecific influence the DNA-vaccines on immune system of mice lymphoproliferation in responses on T- and B-cell mitogens were measured. Our results have confirmed the immunogenicity of both individual plasmid pV3N and pV3GN in eliciting cellular Th1-immune response characterized by dominance of IgG2a, high level of INF-g. Moreover immunization with a mixture of these plasmids can enhance responses compared with the individual plasmid, so decreased the effect of immunosuppression.
Our results suggest that recombinant plasmids pV3N, pV3GN may be a candidate DNA vaccine, so they induce both cellular and humoral

immune responses, and the use of a mixture of plasmids is the most promising variant.

P6.11.50
Adeno-associated virus (AAV) based Mycobacterium tuberculosis (MTB) vaccine induces humoral and cellular immune response in mice

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The current tuberculosis epidemic continues to call for the development of new and effective vaccines. Prompted by the recent findings that viral vector based vaccines are effective in boosting immune activation and protection by *bacillus Calmette-Guérin* (BCG) vaccination, we designed recombinant adeno-associated viral vectors encoding the mycobacterial antigen Ag85a. The presence of different serotypes allows various vaccination and booster strategies. Two serotypes were tested for both, humoral and cellular immune responses in Balb/c mice: rAAV2:Ag85a and rAAV5:Ag85a. A single intramuscular injection in Balb/c mice was well tolerated and lead to a strong and sustained humoral immune response towards Ag85a for both constructs under investigation. Furthermore, an intramuscular injection with AAV2:Ag85a followed by a booster injection with AAV5:Ag85a at week 4, induced a specific anti-Ag85a cellular immune response in isolated and cultivated splenocytes of vaccinated mice upon stimulation with Ag85a at week 8. To further enhance immunogenicity, capsid modifications were introduced. The AAV2 capsid was engineered as scaffold for Ag85a display providing a prime contact to the immune system, while the genome served as template for antigen expression thereby boosting the initial response (Ag85a-AAV2:Ag85a). Mice vaccinated with this single shot prime-boost vaccine developed antigen-specific immune responses significantly faster and at higher levels than mice treated with protein-based or conventional gene-based vaccines therefore offering earlier protection. Furthermore antigen-VP2 fusion is conceivable for a variety of applications such as vaccination with multiple antigens using a single construct. These findings demonstrate the potency of capsid modified AAV vectors in vaccine development.

P6.11.51
Evaluation of E. coli DNA vaccine expressing OmpT and Ag43 gene in BALB/c mice

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E. coli is part of the normal microbiota of the gastrointestinal tract of birds and mammals, however, some strains have been associated with gastrointestinal infections that affect humans and animals. Of these, exist a subset of Shiga toxin-producing E. coli (STEC), are capable of producing severe infections in humans as hemorrhagic colitis and hemolytic uremic syndrome (HUS). STEC infections can vary from asymptomatic infection, watery diarrhea to severe illness with bloody diarrhea or hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). The HUS primarily affects children. This study aimed to evaluate two DNA vaccines carry the gene coding for an outer membrane protease T (OmpT), fundamental to the degradation of antimicrobial peptides produced by the host, and the gene for Ag43, outer membrane proteins of highly conserved in STEC. Genes were cloned in an expression vectors pVAX1 and pVAX-Flag, which were used to immunize animals and purification of recombinant proteins respectively. Animals inoculated with DNA vaccine pVAX-OmpT and pVAX-Ag43 developed post-immunization antibodies specific to systemic and mucosal level. In vitro stimulation of splenocytes from vaccinated mice with either recombinant proteins or total Coli protein extracts resulted in a T-cell proliferative response. These findings indicate that whith the DNA vaccine immunization carrying the OmpT and Ag43 gene from E. coli have potential for use as a vaccine to induce protection against infections.
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P6.11.52
An unbiased genome-wide *Mycobacterium tuberculosis* gene-expression approach to discover new antigens for human T cells that are expressed during pulmonary infection

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Mycobacterium tuberculosis (*Mtb*) causes almost 2 million deaths annually. BCG, the only vaccine available against tuberculosis (TB), induces highly variable protection and better TB vaccines are urgently needed. A prerequisite for vaccine-targeted antigens is that they are expressed during *Mtb* infection in the lungs of susceptible individuals. In search for new TB-vaccine antigens, we followed a genome-wide unbiased discovery approach to investigate the *in vivo* expression of 2170 *Mtb* genes (all first genes of all predicted Mtb operons) during *Mtb* infection in the lungs of mice. Four genetically related mouse strains were studied, representing a spectrum of TB susceptibility controlled by the super-susceptibility to tuberculosis 1 locus. We selected the most upregulated sets of *in vivo* expressed *Mtb* (IVE-TB) genes, and analyzed their expression patterns in distinct disease phenotypes such as necrosis and granuloma formation. While a significant subset (44%) were known as environmental stress response genes, the majority of the *Mtb* genes identified has been largely unstudied so far.
To validate their immunogenicity we analyzed antigen induced immune responses in the blood of tuberculin skin test positive (TST+) individuals. Several *Mtb* IVE-TB proteins were recognized by TST^+ESAT6/CFP10-responsive individuals, indicating that these antigens are presented during natural *Mtb* infection. IVE-TB antigens induced strong IFN-γ^+/TNF-α^+ $CD8^+$ and TNF-α^+/IL-$2^+$$CD154^+$/$CD4^+$ T-cell responses in long-term latently *Mtb* infected individuals. In conclusion, these IVE-TB antigens are expressed during pulmonary infection *in vivo*, are immunogenic, induce strong T-cell responses in long-term latently *Mtb* infected individuals and represent attractive antigens for new TB vaccines.

P6.11.53
Cationic Poly-D, L-lactide-co-glycolide (PLG) Microparticles Enhance the T Cell Response to Naked DNA and Protect Mice from Tumor Challenge

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Cationic poly-D, L-lactide-co-glycolide (PLG) microparticles have shown to target to antigen presenting cells efficiently and become an attractive vaccine delivery system. In this study, a DNA plasmid expressing ovalbumin (pOVA) in the presence of the cationic surfactant CTAB could bind onto the surface of PLG microparticles and form PLG/pOVA DNA vaccine. To evaluate the potential of PLG formulated DNA vaccine in vaccine development, we tested the immune stimulating effect of PLG/pOVA and pOVA DNA vaccine in vitro and in vivo. An increase of CD86 expression in a mouse leukemia macrophage RAW.264 was observed after PLG/pOVA stimulation. In addition, mice immunized with PLG/pOVA elicited a higher IFN-γ production than pOVA alone, especially in CD8+ T cells. Polyfunctional T cell responses were also measured and found the percentage of OT-1 specific polyfunctional T cells was higher in PLG/pOVA immunized mice than pOVA alone (8.24% vs. 6.55%). To test the protection of PLG/pOVA, we challenged immunized mice with OVA-expressing EG7 tumor cells. PLG/pOVA and pOVA immunized mice but not control group showed an inhibition in tumor cell growth. In summary, cationic PLG formulated DNA vaccine could be a powerful tool to enhance T cell response and protect from tumor challenge. Furthermore, this PLG microparticle delivery system can carry both plasmid DNA and protein in the same PLG microparticle that might improve the weak antibody production of formulation with DNA alone.

P6.11.54
Production of human monoclonal ScFv that bind to different functional domains of M2 and inhibit H5N1 influenza virus replication

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Novel effective anti-influenza agent that tolerates influenza virus antigenic variation is needed. Highly conserved influenza virus M2 protein has multiple pivotal functions including ion channel activity for vRNP uncoating, anti-autophagy and virus assembly, morphogenesis and release. In this study, fully human monoclonal single chain antibodies (HuScFv) specific to recombinant and native M2 proteins of A/H5N1 viruses (both adamantane sentivie and resistant) were produced from four *huscfv*-phagemid transformed *E. coli* clones (no. 2, 19, 23 and 27) selected from a HuScFv phage display library. HuScFv of all clones show different amino acid sequences particularly at the CDRs. Influenza virus infected cells exposed to the HuScFv had reduced virus release and intracellular virus. Phage peptide mimotope searching revealed that conformational epitopes of HuScFv2 located at the residues important for ion channel activity, anti-autophagy and M1 binding; epitope of HuScFv19 located at the M2 amphipathic helix and cytoplasmic tail important for anti-autophagy, virus assembly, morphogenesis and release; epitope of HuScFv23 involved residues important for the M2 activities similar to HuScFv2 and also amphipathic helix residues for viral budding and release while HuScFv27 epitope spanned ectodomain, ion channel and anti-autophagy residues. Molecular docking results conformed to the epitope identification by phages. While the molecular mechanisms of the HuScFv await experimental confirmation, the small human antibody fragments have high potential for developing further as a safe, novel and mutation tolerable anti-influenza agent especially against drug resistant variants.

P6.11.55
Vaccination using recombinants influenza and adenoviruses encoding amastigote surface protein-2 are highly effective on protection against Trypanosoma cruzi infection

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There is no human vaccine for Chagas' disease and its treatment presents some adversities. ASP2 protein from the agent *Trypanosoma cruzi* is a conserved immunogenic protein that reaches high protection levels in susceptible mice by specific $CD8^+$T cells stimulation being an interesting candidate for vaccine antigen. Recombinant viruses emerge as promising tools for testing heterologous prime-boost vaccination protocols. In the present study, we generated and evaluated recombinant influenza viruses carrying sequences coding for polypeptides corresponding to medial and carboxi-terminal portions of *Trypanosoma cruzi*'s ASP2. Those recombinant viruses were used in vaccination of C57BL/6 and C3H/He mice in sequential immunization with recombinant adenovirus encoding the complete sequence of ASP2 (Ad-ASP2; heterologous prime-boost protocol). This immunization protocol was able to elicit strong specific anti-ASP2 cellular immune response in vaccinated animals. Also, on susceptible mice strain C3H/He, a higher degree of protection could be found in mice primed with recombinant influenza virus and boosted with Ad-ASP2. By intracellular staining of CD8+ T cells obtained from spleen of vaccinated C3H/He mice, we observed that heterologous prime-boost protocol elicited more CD8+ T cells specific for the immunodominant epitope as well as higher number of specific $CD8^+$ T TNF-alfa and IFN-gamma producers that mobilized CD107a protein. Our results suggest that immunodominant specific CD8+ T cells elicited after immunization could be directly related to degree of protection achieved by different immunization protocols using different viral vectors and the usefulness of recombinant influenza viruses as vaccine vectors against infectious diseases.
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P6.11.56
Designing and modeling super evolutionary polytopic constructs as minor and complement vaccine for Human Immunodeficiency Virus (HIV) based vaccines

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Introduction: Feline Immunodeficiency Virus (FIV), Bovine Immunodeficiency Virus (BIV) and Simian Immunodeficiency Virus (SIV), except some differences, are closely similar to the Human Immunodeficiency Virus (HIV) in genomic organization, morphology, physical and biochemical features and manifestation of disease. Evolutionary patterns and different compatibility of this virus to its host, help designing vaccine and stimulate of immune system more effectively.
Method: In this study twofold approaches include experimental known epitopes and modeling by computer were adopted for designing. After basic study, protein sequences of SIV, FIV and BIV retrieved from Genbank. Then sequences were aligned, analyzed by wide array of bioinformatics software and online servers. Then, all sequences tested for prediction of epitopes that have immunogenicity for T cell and B cell epitopes and potency to attachment to some alleles in HLA. Selected epitopes attached to each other in different patterns and evaluated for best statuses of proteasomal cleavage sites and then Codon optimization was done for compatibility with different expression systems.
Results: Ten unique polytopic constructs (3 SIV, 2 FIV, 1 BIV and 4 mixtures of HIV SIV FIV and BIV) with different patterns of attachment were obtained. So many details were considered in process of designing effective vaccine.
Conclusion: Our designed constructs could be used as both Protective and Therapeutic (boost the immune system of an already-infected person). Specially, as minor and complement vaccine beside Human Immunodeficiency Virus (HIV) based vaccines. Although these constructs are designed for humans, they may be used as Feline vaccine .

P6.11.57
The TB vaccine recombinant BCG-LTA-K63 induces higher Th1 immune responses in mice

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Mycobacterium bovis BCG has been widely used as a tuberculosis vaccine, despite waning efficacy in adults. It is considered that BCG is poor in inducing Th1 immune responses for efficient protection against TB. Adjuvant properties have been described for the Escherichia coli heat-labile toxin (LT), composed of an enzymatically active A and nontoxic B subunits. rBCG expressing a nontoxic mutant subunit A (rBCG-LTA-K63) induced protection against TB challenge in BALB/c mice. In the present work we analyzed the cellular immune responses induced by this construct. Groups of mice were immunized s.c. with 106 cfu of BCG or rBCG-LTA-K63 and after 60 days the spleens were placed in culture and stimulated with PDS. After incubation, the cells were collected for intracellular cytokine staining with FITC-, PE-, and PE-Cy7-conjugated monoclonal antibodies against: CD4, CD8a, IFN-γ, IL-4, IL-17, TNF-α and IL-2. The cytokine secretion was also evaluated in the supernatant of splenocyte culture. A proportion of CD4 T cells produced IFN-γ and TNF-α (47.1 and 34.6% respectively), significantly higher in mice immunized with rBCG-LTA-K63 than with BCG (21.4 and 18.9%) or saline controls (13.0 and 8.4%). IFN-γ production was significantly higher in rBCG-LTA-K63 cells (1231+/- 120 pg/mL) when compared with BCG (862+/- 259 pg/mL). IL-17 and IL-2 production was comparable in immunized groups and IL-4 was not detected. In conclusion, we demonstrated that immunization of mice with rBCG-LTA-K63 induces a Th1-shifted immune response characterized by increased production of IFN-γ and TNF-α, significantly higher than BCG, correlating with a higher protection against TB.

P6.11.58
Influence of different adjuvant formulations in humoral responses after immunization with a HIV envelope glycoprotein trimer

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An effective HIV vaccine is urgently needed and is still a scientific challenge. It is widely accepted that robust humoral and T cell responses are required for the control of virus transmission and disease progression. However, the induction of broadly neutralizing antibodies against the virus has proven hard to achieve. One of the most promising antigens is the envelope glycoprotein, but vaccine candidates based on the monomeric envelope protein gp120 failed to induce protection in efficacy trials. One strategy to overcome this caveat is the use of the stable envelope protein trimer gp140 that contains both gp120 and gp41 ectodomains.
Adjuvants are known to improve the immunogenicity of subunit vaccines, and in the last years several TLR and NOD ligands have entered clinical trials and proved their efficacy. They can also modulate the type of immune response induced by the antigen towards a specific profile (Th1, Th2, etc). For the present work, we used different adjuvant formulations (Poly I:C, CpG ODN, MPL, MDP, Imiquimod, Resiquimod) and analyzed their influence on the anti-gp140 antibody titers, IgG isotypes and germinal center formation after immunization in the presence of the gp140 trimer. Robust Env-specific antibody titers and germinal center formation developed after three doses of gp140 in the presence of different adjuvant formulations. The adjuvants MPL, Poly I:C and CpG ODN induced the highest humoral immune response. These observations have implications for gp140 subunit vaccine design and may be relevant for optimal responses in nonhuman and human primates.

P6.11.59
Immunogenic properties of the lettuce-derived C4(V3)6 multiepitopic HIV protein

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Elicitation of broad humoral immune responses is a critical factor in the development of effective HIV vaccines. As an effort to advance in the development of a candidate vaccine based in a multiepitope recombinant protein, this study describes the immunogenic properties of a previously reported C4(V3)6 multiepitopic protein containing V3 loops corresponding to five different HIV isolates MN, IIIB, RF, CC, RU, using *E. coli* as conventional expression host and lettuce cells as a convenient production and delivery platform. Both *E. coli*- and lettuce-derived C4(V3)6 elicited local and systemic immune responses when orally administered to Balb/c mice. Importantly, the lettuce-derived C4(V3)6 showed a higher immunogenic potential than the *E. coli*-derived one. Broad responses were evidenced when compared the reactivity of sera from mice immunized with C4(V3)6 with those elicited by a chimeric protein carrying a single V3 sequence. The lettuce-derived C4(V3)6 elicited antibodies with positive reactivity against V3 loops from isolates MN, RF and CC. In addition, splenocyte proliferation assays indicated that significant T helper responses are induced by the C4(V3)6 immunogen. Taken together, these findings accounts for the concept of eliciting broader humoral responses trough the C4(V3)6 multiepitopic protein and encourage on the production of multiepitopic vaccines produced in plant cells which serve not only as low cost expression host but also as an effective delivery vehicle for oral administered immunogens.

P6.11.60
Efficacy and safety of a nanoproteoliposomic vaccine candidate against epidemic serovars of Leptospira spp

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Leptospirosis constitutes the zoonosis with highest impact in veterinary and human health. The vaccines available are fundamentally made of inactivated whole cells and most of them for veterinary use. These formulations although effective have multiple limitations, among them the narrow margin of crossed protection against serovars included in the same formulation, low immunologic memory, absence of cellular response and several safety issues. The present study evaluated in the Golden/Syrian hamster the efficacy and safety profile of a new nanoparticulated vaccine formulation. This new vaccine candidate protected up to 100% of the immunized animals (schedule of two doses with a three week interval of) against the homologous and heterologous lethal challenge with 100 000 DL50 of the serovars: Canicola, Ballum, Mozdok and Cophenagen. In all the cases the elimination of carrier state was also verified in kidneys, liver and lung. The immunization generated high levels of IgM and IgG (IgG1 and IgG2a) after the first and second dose, respectively. Also, high levels of IFNγ and IL-1 were observed, and in all the groups the animals survived the lethal challenge up to a year after immunization, with high expression of CD45+ memory marker. Toxicity assays demonstrated absence of damage at immunological and vital organs. These results demonstrate the effectiveness of this new vaccine formulation, it is recommended in future studies to enlarge the evaluation of heterologous protection to other strains of epidemic interest.

P6.11.61
The effect of papilloma virus vaccine in the Th17 differentiation

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The cervical cancer had been associated with the persistent infection of certain genotypes of human papillomavirus (HPV). Actually, there are two commercial vaccines against oncogenic genotypes infection of HPV: the bivalent vaccine Cervarix® (GarsoSmithKline) and the quadrivalent vaccine Gardasil® (Merck). The Th17 cells are a subpopulation of lymphocytes important in the elimination of certain pathogens. However, the increase in their expansion cause different pathological conditions and is associated with the development of cancer. The importance as part of the protective response generated after the vaccination against HPV has not been determined. In this work, we evaluated if change in the response of differentiation T CD4+ lymphocytes is promoting due Gardasil® vaccine. 23 young healthy women were vaccine and blood samples were collected at three times: before vaccination and two weeks after the second and third doses. The Th17 differentiation was induced *in vitro* using the vaccine as stimulus and percentage positive cells and IL-17 production were evaluated. In this study, we show that the cells of women which have been vaccinated with Gardasil® have less response to Th17 differentiation in culture. Hence, the results suggest that vaccine protection is probably given to mediate by other type of response like Th1 and Th2.

P6.11.62
Engraftment of plasma membrane vesicles into liposomes: A new method for designing of liposome-based vaccines

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Immunization with plasma membrane vesicles (PMVs) engrafted into liposomes not only preserves the real biological structure of plasma membrane constituents, but also can potentiate the immune responses against a wide range of antigens present in the PMVs. Therefore, in the present study five different techniques have been investigated to engraft the PMVs derived from a characterized

leishmania parasite into liposomes. The percentage of engraftment was determined by two-color flow cytometry. Among the investigated techniques, freeze-drying method with 91 ± 2% of engraftment is preferred over other methods due to its simplicity and stability of particles during storage. In addition, the immunogenicity of leishmania-derived fused particles obtained by this method was checked *in vivo*. The results showed that the serum levels of anti-leishmania IgG2a antibodies (an indicator of Th1 responses) were significantly higher in mice vaccinated with fused particles than mice injected with PMVs alone (0.5 ± 0.02 OD and 0.2 ± 0.02 OD, respectively; p=0.05), while the total anti-leishmania IgG levels did not show significant difference. Therefore, freeze-drying method is an efficient technique for preparation of liposome-based artificial cell like particles with the ability to maintain the native conformation of proteins as well as the induction of Th1-deviated immune responses.

P6.11.63

Protective activity of antibodies generated by the immunization of the mixture of LPPG from E. histolytica with the OmpC porin from S. typhi, as a bivalent vaccine against amebic liver abscess by E. histolytica and infection by S. typhi

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Entamoeba histolytica and Salmonella typhi are high prevalence pathogens in undeveloped countries, causing every year more than 300,000 deaths around the world. Currently, there is no vaccine against E. histolytica and the vaccines against S. typhi, the Germanier and the Vi polysaccharide do not generate long-term immunity and there has being S. typhi outbreaks without antigen Vi. Therefore, a more effective vaccine that can provide protection against both pathogens must be developed. In the Unit of Medical Research in Immunochemistry, we have isolated and characterized key molecules for both pathogens, the lipopeptidophosphoglycan (LPPG) from E. histolytica and the OmpC porin from S. typhi. In this study, we evaluated the protective activity of antibodies generated by the immunization of the mixture of LPPG with the OmpC porin against amebic liver abscess (ALA) by E. histolytica and infection by S. typhi. Our results showed, that two immunizations of LPPG with OmpC porin induced high-titer long-lived of IgG and IgM specific antibodies to both antigens and also had high affinity. The antibodies binded to trophozoites membranes of E. histolytica and had opsonophagocytic activity against S. typhi. Furthermore, the passive transference of only 100µL of immune serum protected mice in 60% against the development of ALA and also in 100% and 60% against 5 and 20 LD50 of S.typhi respectively.These findings demostrate that the administration of LPPG with OmpC porine, generate protective antibodies against ALA development by E. histolyica and infection by S. typhi.

P6.11.64

Broadly anti-hepatitis C virus (HCV) cross-neutralizing monoclonal antibodies (mAbs) directed against the HCV/E2 glycoprotein and not subjected to Ab-mediated interference

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Hepatitis C virus (HCV) is one of the major cause of chronic liver disease worldwide. There is evidence that neutralizing anti-HCV monoclonal antibodies (mAbs) may find potential applications in novel prophylactic and therapeutic strategies against this infection. However, several HCV escape mechanisms from the immune response have been described. One of them consists in the elicitation of Ab subpopulations interfering with the neutralizing activity of other Abs. In particular, it was reported that Abs directed against the 436-447 region (epitope II) of HCV/E2 glycoprotein may interfere with the neutralizing activity of Abs directed against the 412-423 region (epitope I). Here we describe the potent neutralizing activity and the unique biological features of two broadly cross-reactive and cross-neutralizing anti-HCV/E2 human mAbs (e20 and e137). In particular, we investigated on the molecular features of the interference phenomenon describing an anti-HCV/E2 mAb (e509), whose epitope is centred on epitope II. Interestingly, e509 influenced the potent neutralizing activity of one of the best characterized anti-HCV/E2 murine mAb (AP33), whose epitope encompasses epitope I. On the other hand, e509 did not show any interfering activity against e20 and e137, whose epitopes partially overlap with that of e509 and which possibly displace it from the antigen.
These data give a possible clue to interpret the conflicting studies published to date on the mechanism of Ab-mediated neutralization interference and contribute to the comprehension of this phenomenon that may be of importance in the set up of novel mAb-based anti-HCV therapeutic strategies as well as in immunogen design.

P6.11.65

Severity of Epstein-Barr Virus (EBV)-induced infectious mononucleosis (IM) correlates with the frequency of crossreactive influenza A virus-M1 and EBV-BMLF-1-specific CD8 T cells

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During EBV-associated IM influenza A-specific crossreactive memory T cells are activated and play a role in disease severity. In HLA-A2+ IM patients, influenza M158 (IAV-M1)-specific CD8 memory T cell responses cross-reacted with two different EBV lytic epitopes, BMLF-1280 (17/29) and BRLF-1190 (19/20). Furthermore, 11/22 IM patients demonstrated some intra-viral cross-reactivity between EBV-BRLF1 and -BMLF1 responses. Disease severity of IM did not correlate with viral load, but instead directly correlated with significantly increased frequencies of crossreactive IAV-M1/EBV-BMLF-1-specific CD8 cells, as well as IAV-M1, EBV-BMLF-1 specific CD8 cells, but not BRLF1 responses. When severity of IM was scored and patients were assigned to either mild or severe groups, disease severity correlated with specific TCR Vb usage in all three tetramer-positive populations suggesting that TcR selection is driving disease outcome. Consistent with IAV-M1 and EBV-BMLF1 responses driving increased immunopathology was the observation that patients with severe disease had significantly more IAV-M1 and EBV-BMLF1 cells producing IFNg/MIP1-b in response to antigen as compared to patients with mild disease. These results suggest that T cell crossreactivity can impact T cell selection and function and ultimately disease outcome. Insights on these issues are important for the intelligent design of vaccines and to develop effect therapeutic interventions for virally induced disease.
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P6.11.66

Differential proteomics approach to identify putative protective antigens of Mycobacterium tuberculosis presented during early stages of macrophage infection

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There is an urgent need for an effective vaccine against tuberculosis as the currently available BCG vaccine has failed to accomplish its target of controlling the TB epidemic. Waning of BCG efficacy over time and poor induction of MHC class I restricted responses have prompted research for an alternative that can tide over these shortcomings. In this study we employed differential proteomics to obtain a list of potential vaccine candidate antigens. Bacterial epitopes being presented at early stages on MHC class I and class II molecules of macrophages infected with *Mycobacterium tuberculosis* (*M.tb*) were identified using iTRAQ labeling and reverse phase LC-MS/MS. The putative vaccine candidates thus identified were tested as plasmid DNA vaccines in mice to ascertain their protective efficacy

against a challenge of aerosolized *M.tb*. The protection was evaluated as the ability of the vaccine candidates to reduce the bacterial CFU counts compared to empty vector control in the lungs of infected mice at 8 weeks post challenge. Of the seventeen DNA vaccine constructs we evaluated, two vaccine candidates Rv0143c (chloride channel gene) and Rv1384 (carbamoyl phosphate synthase), both presented in context of MHC class I, induced levels of protection comparable to those observed with BCG. This proof of concept study demonstrates the rationale of our strategy to find novel T-cell epitope based TB vaccine candidates and shortlist the promising ones for further, more stringent evaluation in the guinea pig model.

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P6.11.67

Skewing of SAG mediated therapy for a predominant Th1 during Visceral Leishmaniasis on triggering CD2 epitope

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We have evaluated the effect of combining CD2 with conventional antimonial (sb) therapy in protection in BALB/c mice infected with either drug sensitive or resistant strain of Leishmania donovani with $3x10^7$ parasites via-intra-cardiac route. Mice were treated with anti CD2 adjunct SAG sub-cutaneously twice a week for 4 weeks. Assessment for measurement of weight, spleen size, anti-Leishmania antibody titer, T cell and anti-leishmanial macrophage function was carried out day 0, 10, 22 and 34 post treatments. The combination therapy was shown boosting significant proportion of T cells to express CD25 compared to SAG monotherapy. However, the level of IFN-γ was not statistically different between combination vs. monotherapy ($p = 0.298$) but CD2 treatment even alone significantly influenced IFN-γ production than either SAG treatment ($p = 0.045$) or with CD2 adjunct SAG treatment ($p = 0.005$) in Ld-S strain as well as in Ld-R strain. The super-oxide generation began enhancing very early on day 10 after SAG treatment with CD2 during which SAG action was at minimum. Unlike SAG treatment, treatment of SAG with CD2 also led to production of nitric oxide and TNF-α, resulting in most effective clearance of L. donovani from infected macrophages. Our results indicate that CD2, which can boost up a protective Th1 response, might also be beneficial to enable SAG to induce macrophages to produce leishmanicidal molecules and hence control the infection in clinical situation like Kala-azar along with overcoming drug resistance.

P6.11.68

Analysis of Brucella abortus mutant strains Δbmfp and Δomp19 as potential live vaccines against brucellosis

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Brucella abortus, a facultative intracellular bacterium, is the etiological agent of a zoonotic disease termed brucellosis, which causes serious economic losses in livestock and represent a public health threat. Current commercial vaccines strains against brucellosis have several drawbacks, as residual virulence and variable efficacy. Therefore, research efforts have focused on developing improved vaccines that combine safety and efficacy. Herein, we evaluated the role in pathogenesis of the *Brucella* Outer Membrane Protein 19 (OMP19) and *Brucella* Membrane Fusogenic Protein (BMFP). Additionally, we also tested the vaccine potential of the *Brucella* mutant strains Δ*omp19* and Δ*bmfp*. In this study, we showed that the B. abortus Δ*omp19* and Δ*bmfp* mutant strains have reduced persistence in mice and defective intracellular multiplication in bone marrow derived macrophage. The mutant strains are attenuated and confer protection after challenge with *B. abortus* virulent strain in immunocompromised IRF-$1^{-/-}$ mice as follows: Δ*bmfp* (100%), S19 (87,5%), RB51 (63,5%), Δ*omp19* (59,2%). In immunocompetent C57Bl/6 mice, the Δ*bmfp* mutant strain induces superior protection than that conferred by commercial strain RB51 and similar to vaccine S19, while the Δ*omp19* mutant strain induced lower protective immunity than vaccine S19, but similar to vaccine RB51. In conclusion, *B. abortus* OMP19 and BMFP are critical for full bacterial virulence and the Δ*bmfp* mutant strain is a potential vaccine candidate. Moreover, the absence of the protein BMFP in *Brucella* Δ*bmfp* mutant strain may serve as a molecular marker useful as a diagnostic tool to differentiate infected from vaccinated animals.

P6.11.69

Inhibition of the entry of *Streptococcus pyogenes* in epithelial and endothelial cells by disintegrins

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Group A *Streptococcus* (GAS, *Streptococcus pyogenes*) is an important human pathogen that cases acute pharyngitis, superficial skin infections, sepsis and necrotizing fasciitis in humans. This bacterium uses adhesins (ex. M protein and streptococcal fibronectin binding proteins) to enter mammalian cells by using fibronectin (Fn) as bridge to interact with integrins. This may help GAS to evade antibiotics killing and host immune responses. Snake venom disintegrins are the nature antagonists against integrins. We hypothesized that disintegrin may disrupt the entry process of GAS into mammalian cells to enhance the elimination of GAS by antibiotics. GAS could be internalized into epithelial and endothelial cells in the presence of FBS or Fn. Disintegrin rhodostomin (Rho) and its mutants (ARLDDL and KG) did not influence the growth and viability of GAS and mammalian cells. Further, we use the Rho, ARLDDL and KG to treat cells while NZ131 infecting epithelial and endothelial cells in the presences of FBS or Fn. Rho and KG reduce GAS invasion into epithelial and endothelial cells under both FBS and Fn conditions. In addition, both Rho and KG also influence the adhesion of GAS onto epithelial and endothelial cells under Fn condition while almost no effects on adhesion under FBS condition. However, ARLDDL had no effects on adhesion or invasion of NZ131 in both epithelial and endothelial cells. These results suggest that disintegrins (Rho and KG) may enhance the efficiency of antibiotics in treatment of GAS infections and potentially agent to inhibit GAS infection.

P6.11.70

Immunophenotypic features of peripheral blood in case of severe adverse event associated to yellow fever vaccination

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Introduction and aim: Yellow fever (YF) infection remains an important public health problem. The vaccination constitutes the most effective means to control the disease. Although YF-17D vaccine has proven to be very safe, cases of serious adverse events (YF-SAE) associated to the vaccine have been reported. Herein, we report immunological features in case of severe adverse event, 15 days after yellow fever vaccination.
Material and Methods: Ex vivo analysis of leukocyte subsets was performed by flow cytometry. Data from 10 healthy volunteers, 15 days after YF-17DD vaccination were considered as reference values (RV).
Results: Our data revealed that the impact of YF antigens on monocytes induced a deficient expression of CD32 (117.3/215.0±47.0). In addition, on the adaptive immunity compartment, this YEL-SAE case were characterized by activation events in T and B cells demonstrated by increased frequency of CD4+CD69+(7.2/3.0±1.8), CD4+HLA-DR+(12.7/4.0±2.0), CD8+HLA-DR+(40.5/5.0±2.4), and CD69/CD19+(49.1/12.0±3.6) cells, as well as an increased expression of CD54 in CD4+ and CD8+ cells (28.4/4.0±2.2; 74.4/6.0±4.0, respectively). On the other hand, it was

observed a decreased expression of CD32 in B-cells (75.0/160.0±32.0), CXCR3 in T-cells (11.6/26.68±4.7;16.6/32.73 ± 6.50), besides lower frequency of CD62L+CD8+ cells (5.8/48.0±6.4).
Conclusion: Analysis of this YF-SAE demonstrated a deficiency innate immune response characterized by down modulation of Fc-receptors in monocytes. On the other hand, in adaptive microenvironment we observed a specific induction of T and B cell responses in peripheral blood, 15 days after vaccination. These results corroborate data of YF-SAE case reported previously by our group.
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P6.11.71
E2 multimeric scaffolds displaying HIV-1 Envelope regions elicit V3 and MPER-specific neutralizing antibody and T cell responses when combined with gp160 plasmid DNA

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It is widely believed that induction of both broadly neutralizing antibodies (bNAbs) and T cells are crucial for a successful HIV-1 vaccine. To elicit immune responses against HIV-1 we generated multimeric scaffolds, based on the E2 component of the pyruvate dehydrogenase complex from Geobacillus stearothermophilus, displaying antigens from the HIV Envelope (Env) glycoproteins gp120 and gp41. E2 self-assembles into a 60-mer particle with 60 copies of an antigen on each scaffold. The HIV-SF162 V3 and MPER regions were expressed as N-terminal fusions to the E2 core. To increase solubility, Env-E2 scaffolds were refolded from inclusion bodies with equimolar amounts of E2wt and purified by size exclusion chromatography. Purified Env-E2 multimers were tested alone or in combination with HIV-SF162 Env(gp160) plasmid DNA in rabbits and mice. We also tested DNA expressing Envelope gp160 with deletions in variable regions V2, V3, and V1-V3. Immunization with V3-E2 co-administered with DNA elicited strong V3-specific NAb response after two immunizations in 6 weeks in rabbits and V3-dextramer specific CD8+ T cells producing IFN-gamma in mice. Rabbits immunized with DNA plus MPER-E2 rapidly generated NAbs targeting the broadly neutralizing 2F5 as well as the 4E10 epitopes, confirmed using HIV-2/MPER chimeric viruses. CD4+ T-cell response and cytokine polarization were also investigated. Rabbits immunized with MPER-E2 had NAbs against both Tier 1 and Tier 2 HIV-viruses. Our results indicate that this novel strategy of co-immunization with E2 scaffolds and DNA could represent an efficient platform by which to direct immune responses, including bNAbs, toward HIV-1 conserved regions.

P6.11.72
***A S. agalactiae* protein of heterologous expression, an interesting alternative for vaccine development**

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Streptococcus agalactiae, also known as Group B streptococcus or GBS, is a β-hemolytic Gram-positive streptococcus which is one of the leading causes of neonatal sepsis and postpartum infection. Genitourinary tract colonization reaches 20% of pregnant women and is considered a sexual transmission disease.
In the world there is no vaccine against GBS, only a phase 2 study based on conjugated polysaccharides. An interesting alternative is to use proteins as the basis for vaccine development. In our laboratory, we obtained the SIP protein (rSIP) by the recombinant DNA techniques and formulated as a prototype vaccine with AbISCO 100 ® adjuvant. Protective immunity in murine model was evaluated against a challenge with GBS; bacterial infection and then genitourinary tract washings was also tested. High levels of antibodies type IgA, IgG and IgG2a were observed, an increased secretion of cytokines IL-2 and INF-γ was detected in the immunized experimental groups with rSIP. Besides, there was a decrease in the colony forming unit (CFU). These observations increased when the protein was formulated with the adjuvant AbISCO 100 ®.
Moreover, a passive immunity transfer was analyzed, detecting that infused animals with a mixture of CD8/CD4 cells purified from rSIP/AbISCO-100 immunized animals showed a decreased of CFU count in animals infected with GBS.
The strategy of using conserved immunodominant proteins is an interesting alternative for the development of vaccines against GBS

P6.11.73
Passive immunotherapy with modified intravenous immunoglobulins (IVIg) improves survival in experimental sepsis by attenuating inflammatory and coagulation pathways

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The exposure of some IgG antibodies to ferrous ions is known to enhance their polyspecificity that includes the ability to bind at least one pro-inflammatory cytokine (J.Biol.Chem.2006:281,439). Sepsis is a medical disaster that responds poorly to available drugs including intravenous immunoglobulin (IVIg) preparations. IVIg, pre-exposed *in vitro* to Fe(II) ions was used for passive immunotherapy of mice with sepsis induced by the injection of LPS, of live *E.coli*, of zymosan or by the CLP technique. A single dose of 50 to 250 mg/kg of the modified immunoglobulin preparation, but not of the native, commercially available IVIg significantly increased survival in all sepsis models.
The mechanisms of the protective activity of modified IVIg were studied in LPS sepsis. Its therapeutic effect was not due to a more efficient LPS neutralization and was still present when administered 6 hours after LPS. In the treated animals the serum levels of pro-inflammatory cytokines were decreased, IL10 levels were increased, the coagulation abnormality was overcome and the complement exhaustion was prevented. The exposure to Fe(II) ions induced structural changes in the IgG molecules as demonstrated by fluorescent spectroscopy, kinetic and thermodynamic analyses. These changes did not result in their denaturation as the modified preparations still met the strict Pharmacopoeia requirements for IVIg. We suggest that modified IVIg with additionally enhanced polyspecificity, induced by a brief exposure to pro-oxidative ferrous ions, has a clinical potential in sepsis and other variants of the SIRS syndrome (post-traumatic, in avian flu, etc.).

P6.11.74
Protection in mice by administrating antibodies against C-terminal modified nonstructural protein 1 in dengue virus infection

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Thrombocytopenia and plasma leakage are important symptoms in severe dengue patients, which are related with dysfunction of platelets and endothelial cells. Previous studies in our laboratory showed that antibodies (Abs) against dengue virus (DENV) nonstructural proteins 1 (NS1) cross-react with human platelets and endothelial cells leading to their dysfunction. Based on sequence homology analysis, the C-terminal region of DENV NS1 protein contains cross-reactive epitopes. For safety in vaccine development, the cross-reactive epitopes of DENV NS1 protein should be deletion or modified. In this study, we tested the protective effects of Abs against full-length DENV NS1, NS1 lacking the C-terminal aa 271-352 (designated delta C NS1) and chimeric DJ NS1 consisting of N-terminus of DENV NS1 (aa 1-270) and C-terminus of JEV NS1 (aa

271-352). DENV NS1 immunization caused a prolonged bleeding time in mice, but delta C NS1 and DJ NS1 immunization did not. The anti-delta C NS1 and DJ NS1 Abs showed lower binding activity to endothelial cells and platelets than that of anti-DENV NS1 Abs. Using a DENV-induced hemorrhage mouse model, anti-delta C NS1 and DJ NS1 Abs reduced DENV-induced mouse tail prolonged bleeding time, MCP-1 production, and macrophage infiltration. Treatment with anti-delta C NS1 and DJ NS1 Abs controlled the viral load of DENV2 infection in vivo and synergized with complement to inhibit viral replication in vitro. According to these findings, modified NS1 proteins, delta C NS1 and DJ NS1, may provide a safer strategy for vaccine development against DENV infection.

P6.11.75
Single cell cloning of antigen specific monoclonal antibodies from macaque plasmablasts

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In spite of a renewed interest in the induction of neutralizing antibody responses in the field of HIV research over the last few years, the majority of our knowledge about these responses is limited to the analysis of serum antibodies or monoclonal antibodies generated from memory B cells during chronic infection. Relatively little is known about the early cellular responses that eventually will give rise to the long lived plasma cells and memory B cells both in a chronic infection as well as in a vaccine setting. We and others have found that the vast majority of the plasmablasts appearing in blood after acute viral infection or vaccination are specific for the inducing pathogen and thus represent an excellent source for generating recombinant monoclonal antibodies from single cells. Using this approach we can interrogate the epitope specificity and the repertoire breadth of the individual responding B cells. Herein we have adapted these strategies for use in ongoing SIV and HIV studies in macaques and thus established a novel and very useful tool to analyze antibody responses in macaques at a single cell level, and also generate a large panel of virus specific monoclonal antibodies. A thorough understanding of plasmablast responses induced by vaccination or infectious challenge, how they are modified by the use of different adjuvants and finally how they correlate with protection after SIV challenge will be important in designing better HIV vaccine candidates for use in humans.

P6.11.76
A clinical trial on anti-HBV-DC vaccine combined with thymosin-α1 in the HBeAg negative patients of chronic hepatitis B virus infection

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Aims: To observe the clinical efficacy of anti-HBV-DC vaccine, the dendritic cells originating from peripheral blood mononuclear cells(PBMC) sensitized by HBsAg, in combination with thymosin-α1, in the HBeAg negative patients of chronic hepatitis B virus(HBV) infection.
Methods: 24 inactive HBsAg carriers and 25 chronic hepatitis B(CHB) patients were recruited in the trial. PBMCs obtained from 50ml of heparinized peripheral blood through density gradient centrifuge and adherence method were proliferated under the induction by GM-CSF and IL-4, and sensitized with the 50μg HBsAg. Anti-HBV-DC vaccine was harvested on day 7 and injected, half hypodermically and half intravenously, to the patient once every two weeks for 12 practices applications totally. Thymosin-α1 1.6mg was injected hypodermically twice a week. Quantitative HBVM(TRFIA) and HBVDNA and hepatic functions were evaluated at week 0, 4, 12, and 24.
Results: At week 4, 12 and 24, The HBsAg negative conversion rate were 8.00%(2/25), 12.00(3/25) and 20.00%(5/25) respectively, the HBVDNA negative conversion rate were 63.64%(7/11), 72.73%(8/11) and 72.73%(8/11) in HBeAg negative CHB patients.
The HBsAg negative conversion rate were 12.50%(3/24), 25.00%(6/24) and 37.50%(9/24) respectively in inactive HBsAg carriers.
Conclusions: anti-HBV-DC vaccine in combination with thymosin-α1 can be considered as a safe approach with high efficacy for HBeAg negative patients of chronic HBV infection, which can effectively inhibit the viral replication, decrease rapidly and eliminate the HBsAg, and may eliminate the HBsAg for partial patients.

P6.11.77
A clinical study on anti-HBV-DC-MTL in the HBeAg negative chronic hepatitis B virus infection

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Aims: To observe the clinical efficacy of anti-HBV-DC-MTL, the dendritic cells(DC) and mixed T lymphocyte(MTL) originating from PBMC sensitized by HBsAg, in HBeAg negative chronic hepatitis B virus(HBV) infection.
Methods: 19 inactive HBsAg carriers and 19 chronic hepatitis B(CHB) patients were recruited in the study.PBMCs were obtained from 50ml peripheral blood. Anti-HBV-DC was proliferated from adherence PBMCs under the induction by GM-CSF and IL-4, and sensitized with 50μg HBsAg. Anti-HBV-MTL was proliferated from no-adherence PBMCs under the induction by IL-2, IL-12 and anti-HBV-DC. Anti-HBV-DC was harvested on day 7 and injected, half hypodermically and half intravenously, to patient once every two weeks for 12 times totally. Anti-HBV-MTL was harvested on day 14 and injected intravenously to patient once every two weeks for 12 times totally. Telbivudine was taken 600mg daily for 10 CHB patients those HBVDNA>10000 copy/ml. Quantitative HBVM(TRFIA) and HBVDNA were evaluated at week 0, 4, 12, and 24.
Results: Mean of HBsAg and HBVDNA decreased significantly at week 4, 12 and 24, HBsAg negative conversion rate were 10.53%(2/19), 15.79(3/19) and 21.05%(4/19) respectively, HBVDNA negative conversion rate were 69.23%(9/13), 76.92%(10/13) and 92.31%(12/13) in HBeAg negative CHB patients.
Mean of HBsAg decreased gradually along the time from week 4, 12 to 24,in inactive HBsAg carriers. At week 4, 12 and 24, HBsAg negative conversion rate were 15.79%(3/19), 26.32%(5/19) and 36.84%(7/19) respectively.
Conclusions: Anti-HBV-DC-MTL can effectively inhibit the viral replication, decrease rapidly and eliminate the HBsAg, and may eliminate the HBsAg for partial patients.

P6.11.78
A clinical study on hepatitisi B vaccine triple-therapy in chronic hepatitis B virus infection

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Aims: To observe the clinical efficacy of the admixture of hepatitis B vaccine, rhGM-CSF and BCG polysaccharide ucleic acid(for short: hepatitis B vaccine triple-therapy) combined with adefovir dipivoxil in the patients of chronic hepatitis B virus(HBV) infection.
Methods: 18 HBeAg positive chronic hepatitis B(CHB) patients, 11 HBeAg positive chronic HBV carriers, 5 HBeAg negative CHB patients, 2 HBeAg negative chronic HBV carriers and 12 inactive HBsAg carriers were recruited in the study. Hepatitis B vaccine containing 20μg HBsAg, rhGM-CSF 100μg and BCG polysaccharide nucleic acid 2ml were mixed. And then the admixture injected hypodermically to the patient once every two weeks for 12 practices applications totally. Adefovir dipivoxil was taken 10mg daily for 33 patients those HBVDNA>10000 copy/ml. Quantitative HBVM(TRFIA) and HBVDNA were evaluated at week 0, 12, and 24.
Results: At week 12 and 24, HBeAg seroconversion rate were 13.79%(4/29) and 27.59%(8/29) respectively, HBVDNA negative

conversion rate were 37.14%(13/35), and 60.00%(21/35), HBsAg negative conversion rate were 2.08%(1/48) and 6.25%(3/48), HBsAb positive conversion rate were 16.67%(8/48) and 37.50%(18/48), HBsAg seroconversion rate were 2.08%(1/48) and 6.25%(3/48).
The rate of adverse effect was 50.17%. The adverse effect include fever, headache, ache all over, bellyache, urticaria and hives, dyspnea, and tumefaction ache in the injection site after injected the admixture.
Conclusions: The hepatitis B vaccine triple-therapy in combination with adefovir dipivoxil can be considered as a efficient approach for patients of chronic HBV infection, which may effectively inhibit the viral replication, improve the production of HBsAb and HBeAb, and increase HBeAg seroconversion rate.

P6.11.79
Human monoclonal ScFv specific to NS1 protein inhibits replication of influenza viruses across types and subtypes

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Currently, there is a need of new anti-influenza agents that target influenza virus proteins other than ion channel M2 and neuraminidase. Non-structural protein-1 (NS1) is a highly conserved multifunctional protein which is indispensable for the virus replication cycle. In this study, fully human single chain antibody fragments (HuScFv) that bound specifically to recombinant as well as native NS1 proteins were produced from three different huscfv-phagemid transformed E. coli clones selected from a human ScFv phage display library. Results of Western blot analysis, mimotope searching/epitope identification, homology modeling and molecular docking and phage mimotope ELISA inhibition indicated that HuScFv of one E. coli clone reacted with NS1 R domain important for host innate immunity suppression while HuScFv of the other two clones bound to E domain at the sites necessary for NS1 binding to the host eIF4GI and CPSF30, respectively. The HuScFv could enter the influenza virus infected cells and interfered with the NS1 activities leading to replication inhibition of viruses belonging to various heterologous A subtypes and type B. HuScFv treated influenza virus infected cells had up-expression of IRF3 and IFN-beta genes in comparison with the controls, indicating that the antibodies could restore the host innate immune response. The fully human single chain antibodies have high potential for developing further as a safe (adjunctive) therapeutic agent for mitigating, if not abrogating, severe symptoms of influenza.

P6.11.80
3-dimensional laser structured Si scaffolds improve macrophage adherence and antigen-specific response

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Aim: The aim of the present study was to investigate whether 3D micro and submicron laser textured transplantable Si scaffolds with tunable morphology and chemistry could support macrophage adherence, antigen presentation and specific antibody production.
Methods: Differences of the morphological features of Si substrates were advantageously achieved by tuning the laser structuring parameters resulting in scaffolds of gradient micro-geometries, 3D porosity and hydrophilicity. Macrophages were isolated from BALB/c mice and cultured on 3D substrates seeded with Human Serum Albumin (HSA) and subsequently syngeneic T and B lymphocytes. . The adherence of macrophages and their interaction with T_H cells on the 3D substrates was revealed using SEM and Confocal Microscopy analysis. Functional assays included the measurement of interleukins IL-2, IL-4 and specific antibody production in cell culture supernatants by ELISA.
Results: SEM and confocal microscopy analysis showed that less rough 3D hydrophilic scaffolds were more favorable to macrophage adherence as compared to flat or high roughness scaffolds. T cell activation was demonstrated by detecting IL-2 and IL-4 in culture supernatants, while the development of antigen specific response was proved by detection of HSA-specific antibody production.
Conclusions:
The 3D scaffolds displaying the capacity to exquisitely control pore size, microtopography and surface chemistry support development of humoral immune response more effectively as compared to conventional flat surfaces and could therefore provide useful materials for *in vitro* and *in vivo* applications.

P6.12 Organ transplantation

P6.12.01
The effect of atorvastatin on skin allograft survival and growth of B16F10 melanoma cells in mice

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Earlier we reported that atorvastatin (Lipitor) suppressed IL-4, interferon-γ and antibody levels in mice immunized with egg albumin. It was also observed that acute rejection episodes were less, and duration of graft survival was longer in kidney transplant patients at the American University of Beirut Medical Center who received a statin in addition to their immunosuppressive regimen. The aim of this study was to evaluate the immunomodulatory effect of atorvastatin on skin allograft survival and on tumor growth in mice.
BALB/c mice were transplanted with skin allografts from C57BL/6 mice and given either atorvastatin alone or in combination with immunosuppressive agents. Average survival days of skin allografts was recorded and serum levels of interleukin-1β and interferon-γ were quantified. BALB/c mice and C57BL/6 mice were challenged intraperitoneally with B16F10 melanoma cancer cells and were then treated with atorvastatin. They were observed regularly for tumor growth.
The results indicated that in transplanted mice, atorvastatin given alone or in combination with immunosuppressive agents prolonged allograft survival time in spite of a non-significant change in serum cytokine levels. Furthermore, atorvastatin treatment enhanced tumor growth in C57BL/6 mice and promoted tumor growth in BALB/C mice. The results obtained are supportive of atorvastatin being an immunosuppressive agent.
It is hypothesized that the immunosuppressive effect of atorvastatin could be related to its effect on membrane cholesterol of antigen presenting cells (APC) resulting in hindering the ability of APCs to present antigen to T-lymphocytes.

P6.12.02
The -318C/T, -1722T/C and +49A/G polymorphisms at the CTLA-4 gene in kidney allograft tolerance

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Kidney rejection remains as a major cause of morbidity associated with successful engraftment. The CTLA-4 molecule is expressed on activated T cells and binds to B7 molecules on antigen-presenting cells, delivering negative signals to T lymphocytes. We investigated whether single nucleotide polymorphism (SNP) -318C/T, -1722T/C and +49C/G of the CTLA-4 gene were associated with susceptibility to acute or chronic kidney allograft rejection. DNAs were extracted from peripheral blood cells of 114 kidney transplant patients (acute rejection n=17, chronic rejection n=51 and no rejection n=46) and 196 healthy controls and the CTLA-4 polymorphisms were successfully genotyped using allelic discrimination method on the StepOnePlus instrument (Applied Biosystems). No statistically significant association was found between the groups at the -318C/T CTLA-4 polymorphism. When compared to healthy controls, the frequency of the CTLA-4 -1722TT genotype, responsible for increased transcription of the CTLA-4, was marginally decreased in the rejection group (acute + chronic) (p=0.0609) and the CTLA-4 -1722CT genotype was increased in the chronic rejection group (p=0.0158) and in the whole rejection group (acute + chronic) (p=0.0066). The CTLA-4 +49AG genotype was underrepresented in chronic rejection group when compared to no rejection (p=0.0018) and to healthy controls (p=0.0022) and also in rejection group (acute + chronic) when compared to no rejection (p=0.0111) and to healthy controls (p=0.0153). In conclusion, the CTLA-4 -1722TT and CTLA-4 +49AG genotypes were associated with protection against development of kidney allograft rejection, while CTLA-4 -1722TC genotype was associated with susceptibility to kidney allograft rejection.

P6.12.03
KIR & their HLA ligand and aGVHD incidence post HSC TX

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Introduction: Killer Ig-like receptor (KIR) play an important role in the outcome of HSC Tx and GVHD incidence .**Material and methods:**The study population was comprised of 86 donor and recipient siblings transplanted with hematopoietic cells during March 2010 to June 20111. The patient group consisted of: 29 patient with ALL, 27 patients with AML and 30 with Thalassemia. All the patients and donors were examined for KIR and KIR HLA Ligand by multiplex PCR-SSP method using commercial Olerup KIR and HLA ligand genotyping KIT.**Results:**The results of this study showed that the presence of 2DL2 in donors is related to aGVHD incidence with a Hazard ratio is 4.21 (P.value =0.001.) by contrast, presence of 2DS4 (o.125 Hazard ratio and 0.000 P.value) and 3DS1 (Hazard ratio= o.248 and P.value=0.000) is associated with reduction of aGVHD incidence. **Inconclusion:**Accordingly donor NK cells can destroy their alloreactive targets. If malignant cells remained after chemotherapy targeted by NK cells and prevent relapsing the disease. The result of this study is in accordance with Ruggeri`s study which found that alloreactivity prevents aGVHD. Therefore, Nk cells alloreactivity by destroying APC prevent recipient antigen from presenting to T cells which are the most important cells in aGVHD and consequently preventing disease occurance .

P6.12.04
Clinical Relevance of HLA and MICA Antibodies in Live Related Renal Transplantation

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The presence of HLA and non-HLA antibodies has been associated with rejection and poor allograft outcome following renal transplantation. The present study was undertaken to evaluate the clinical role of HLA and MICA antibodies in live related renal transplantation. Sera from 64 renal transplant patients before and after 7-14 days post transplantation were taken and analysed by Luminex XY platform for the presence of HLA and MICA antibodies. Presence of both of these antibodies correlated with poor graft survival. A comparison of well functioning grafts (WFG) with the rejection cases in the presence/ absence of all the three types of antibodies was done. The 30% of the acute rejection cases had all the three types (HLA Class I, II and MICA) of antibodies present at the pre-transplant stage. However, none of the well functioning graft showed the presence of all the three types (*P<0.001*). 10% of the acute rejection cases showed *de novo* development of HLA antibodies. On the other hand 50% of the ATN cases showed the presence of all the three types of antibodies (*P<0.0001*). Interestingly, 33% of the cases which developed ATN were detected to have no HLA class I or HLA class II antibodies but anti-MICA antibodies in both pre and post transplant serum samples. Although 5% of the patients with WFG showed the presence of HLA class I and class II antibodies none were detected to have the pre-transplant or post-transplant MICA antibodies. The study highlights the relevance of MICA antibodies in poor graft survival.

P6.12.05
Kidney donor MICA antigens and MICA antibody specificities detected in renal transplant recipients

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Purpose/Objective: Despite recent advances in solid organ transplantations, an antibody mediated rejection caused by donor specific antibodies represents still a major problem in kidney graft survival. Besides HLA-induced humoral response, anti-MICA antibodies have recently attracted attention because of their possible role in a graft rejection.
Patients, material and Methods
The aim of our study was to establish if MICA antibodies are induced following the kidney transplantation and if are specific for MICA antigens of their donors. 20 patients were enrolled to the study and anti-MICA antibodies were detected by a multiplex technology. MICA alleles were typed by a sequence analysis.
Results: Anti-MICA positive sera from renal transplant patients evaluated for antigen specificities showed mostly more complex reactivity patterns as 20% of the tested sera reacted with a single MICA antigen only. The spectrum of anti-MICA antibodies gradually enlarged: six months after the transplantation, antibodies specific for other than original MICA antigens were identified. Evaluation if the recipient′s anti-MICA antibodies are donor′s MICA antigens specific has disclosed that it is not the case. Most of patient′s sera (65%) recognised not only the donor MICA antigens, however also others. The match between antibody specificities and MICA antigens was observed in 35% of patients only.
Summary: We didn't find a correlation between the specificities of the kidney recipient′s MICA antibodies and MICA antigens of the donor.
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P6.12.06

Detection of lymphocyte subsets in kidney transplant patients receiving induction with basiliximab or thymoglobulin: impact on kidney function

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Induction therapy with basiliximab or thymoglobulin after kidney transplantation modifies the expression of lymphocyte subsets, including the Treg cells (CD127lowCD25high).Though this may influence the renal graft function, it has barely been studied using fine needle aspiration cytology (FNAC). In a cross-sectional study of 27 kidney transplant recipients(aged 53.5±15 year; 19men) with no acute rejection we studied by flow cytometry the expression of the receptors CXCR3 and CCR4 in CD127highCD25low and Treg cells of the graft obtained six months post-transplant. Overall, the patients who received basiliximab (as compared with thymoglobulin) had a greater percentage of CD4 T cells (31.4±10.3% vs. 19.5±12.3%; *P*=0.033) and a lower proportion of CD8 T cells (22.7±12.5 vs. 43.1±23.7%; *P*=0.020) in the FNAC and peripheral blood samples. As previously reported, thymoglobulin was associated with an increased percentage of Treg cells as compared with basiliximab (13.2±10.7 vs. 9.5±6%), though this trend was not seen in the FNAC samples (9.1±6.7 vs. 9.5±7%).The patients with post-transplant delayed graft function had increased CD8 T cells (39.5±21 vs. 19.6±14.5%; *P*=0.029) and expression of the receptor CCR4 on the Treg cells (14±22.4 vs. 9.6±17%) as compared with those who had immediate graft function. Likewise, a worse renal function (Crs≥2 mg/dL) was associated with a similar result after the FNAC (CD8: 44.6±14.6 vs. 24.5±20, *P*=0.06 and CCR4:24±27 vs. 7.6±14.7%; *P*=0.09). In summary, monitoring the expression of the chemokine receptors CCR4 and CXCR3 by flow cytometry in the conventional CD4 Tcell and Treg subsets obtained by FNAC may be a useful tool to study the course of the immune response in transplant patients with delayed graft function and worsening graft function.

P6.12.07

Evolution in kidney transplant recipients of the expression of skin-homing receptors in the different CD4+ T cell subsets

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Monitoring the CD4+Tcell subsets, including the Treg cells(CD4 CD25highCD127low)and the conventional CD4+Tcells (CD127highCD25low), may help optimize the management of kidney transplant recipients. The expression of the chemokine receptors CXCR3 and CCR4 in these cells may provide valuable information about the functionality of these cells in the kidney graft. In a prospective study of 27 kidney transplant recipients (aged 53.5±15years;19 men) with no acute rejection we measured by flow cytometry the expression of the receptors CXCR3 and CCR4 in Treg cells and conventional CD4+T cells in samples obtained from both fine needle aspiration cytology (FNAC) of the graft and from the peripheral blood at 1 and 6 months posttransplantation. Whereas in peripheral blood samples we detected a significant increase in Treg cells from the first to the sixth month post-transplant (5.8±10versus11.6±6.6%;*P*=0.040),this increase was more obvious in the Treg using the FNAC samples(3.3±4versus12.3±7%; *P*=0.002).Likewise, at 6 months post-transplant we noted an increase in the percentage of Treg with a high expression of the skin-homing CCR4 in peripheral blood (1.6±2.5 versus 4.4±6.6%).This receptor (binding CCL2) is related with chemokines involved in inflammatory processes unrelated to rejection. This finding was more notable in the kidney graft samples obtained by FNAC (CCR4 1 month: 0 versus 6 months:9.7±16.4%;*P*=0.055). No significant changes were seen in the expression of the chemokine receptor CXCR3 or in theTreg cells or the conventional CD4+T cells. In summary, post-transplant monitoring of Treg cells and the chemokine expression (CXCR3 and CCR4) by FNAC may be a useful tool for following up the immunological response in kidney transplant patients.

P6.12.08

Pretransplant interferon-gamma secretion by CMV-specific CD8+ T cells informs the risk of cytomegalovirus replication after transplantation

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OBJECTIVES: We analyzed pretransplant CMV-specific CD8+ T cells secreting IFNγ by CMV-specific CD8+ T cells to determine whether this assessment provides prognostic value of CMV replication after transplantation.
MATERIALS AND METHODS: This longitudinal study was carried out in two centers of the REIPI network (Reina Sofia Hospital, Cordoba, and Cruces Hospital, Bilbao, Spain). Fifty-five adult patients awaiting lung or kidney transplantation were evaluable for the study. IFNγ production by CMV-specific CD8+ T-cells was assessed pretransplant using the QuantiFERON®-CMV (QF-CMV) assay. A result was considered "reactive" when the CMV antigen response minus the negative control response was greater than 0.2 IU/mL of IFNγ. CMV load was monitored for 24 months after transplantation.
RESULTS: All CMV-seronegative recipients were QF-CMV "non-reactive", QF-CMVNR, (11/11). However, in CMV-seropositive recipients, R(+), 30/44 (68.2%) were QF-CMV "reactive" (QF-CMVR) and 14/44 (31.8%) were QF-CMVNR. Within R(+) recipients 7/14 (50%) of R(+)QF-CMVNR recipients developed CMV replication after transplantation, whereas the virus replicated only in 4/30 (13.3%) of R(+)QF-CMVR patients. In kidney transplant recipients, CMV replicated earlier post-transplant, reached a higher peak CMV load, replication episodes were longer and the frequency of CMV disease was higher in R(+)QF-CMVNR patients than in R(+)QF-CMVR recipients.
CONCLUSIONS: One third of R(+) transplant candidates are QuantiFERON-CMV "non-reactive" and they are at higher risk for CMV replication after transplantation. Thus, determining pretransplant IFNg production by CMV-specific CD8+ T cells may be useful in predicting the risk of post-transplant CMV replication in solid-organ transplantation and it might aid in reclassifying transplant candidates.

P6.12.09

Role of toll like receptors in sensing endogenous danger signals released during hepatocyte cold storage or upon warming-up

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Cell death such as upon tissue injury precipitates release or exposure of intracellular molecules some of which are immune stimulatory damage-associated molecular patterns (DAMPs). They bind to specific pattern recognition receptors (PRRs) - the same receptors that detect pathogen-associated molecular patterns - of the innate immune system. Therefore, pathogen induced and non-infectious inflammatory responses do not differ principally and a distinction between self and non-self is not operative at this level. Specific PRRs such as toll like receptors (TLRs) have been implicated as sensors of specific DAMPs released from damaged tissue such as extracellular matrix components. Ischemia/reperfusion injury elicits inflammatory response while not involving microbial products but nonetheless posing a potentially fatal threat to organ allograft hosts profoundly influencing acute as well as medium and long-term graft function. Both hypothermic storage as well as heat shock (such as upon

reperfusion) induced cell death are being considered as triggers of immune activation and primary graft non-function upon transplantation. We evaluated the stimulatory potential of homogenates of cold stored or/and heat shocked primary hepatocytes by challenging macrophages of various genotypes. We observed that neither overnight hypothermic (4°C) preservation nor rewarming of the hepatocytes induced a measurable stimulatory capacity in our experimental system. In contrast, 45°C heat shock (HS) followed by incubation at regular cell culture conditions (cult) resulted in profound cell stimulation upon hepatocyte homogenate challenge. Moreover, lack of activation of Myd88/Trif / macrophages in response to HS-cult treated hepatocytes suggested an involvement of TLRs. We will report our work in progress.

P6.12.10
Relationship between human leukocyte antigen genotypes and cytomegalovirus infection in kidney transplantation

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The aim of our study was to analyze the association of HLA alleles and the occurrence of CMV antigenemia in kidney transplantation Romanian recipients. The HLA types were performed in 115 patients by polymerase chain reaction with the sequence specific primer method by low resolution Olerup SSP KIT. DNA was extracted from whole blood using innuPREP Blood DNAMini kit. The CMV antigenemia test was performed by immunofluorescence assay for detection of CMV pp65 antigen in circulating peripherial blood leukocytes. The HLA type were compared between the patients who had undergone and those who had not undergone antigenemia. 24 Recipients were CMV antigenic positive. As a result, the frequency of HLA-32 was high in patients with antigenemia, the OR value was 5.24 ($P<0.05$). Also, in our study, the HLA-DQ8 might was more frequent in patients with antigenemia compared to patients compared to patient without CMV antigenemia ($p=0.046$, OR=4.25, CI=1.21-18.47). In conclusion HLA-A32 and DQ8 might represent a potential risk for CMV infections after kidney transplantation.

P6.12.11
Mesenchymal stem cell (MSC) therapy to improve nerve function in a rat limb transplant model

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Introduction: Optimizing nerve regeneration and preventing immune rejection are vital to successful outcomes in hand and limb transplantation (Tx). As a first step, our objective was to investigate whether MSC can improve nerve regeneration and function. **Methods:** We performed orthotopic syngeneic right hind-limb transplants in Lewis ($RT1.A^l$) rats. Following Tx rats received syngeneic MSC (5×10^6; passage ≤7) or vehicle locally around nerve, bone and vascular anastamoses sites, and 5×10^6 MSC intravenously on the day of surgery; MSC injections (i.v.) were repeated at weekly intervals for four weeks. **Results:** Rat MSC expanded *ex vivo* were $CD29^+$, $CD90^+$, $CD34^-$, $CD31^-$, $CD45^{low}$, MHC Class I^+, Class II^-, $CD80^{low}$, and $CD86^-$. MSC were pluripotent and differentiated in to adipocytes, osteocytes and chondrocytes in *ex vivo* cultures under specific conditions. At 4 weeks post-Tx sensory nerve function as determined by cutaneous pain reaction test was <0.6 on a scale of Grade 0-3 (0=No function; 3= Normal function). However, by 8 weeks in vehicle treated animals (n=7) it was 2.2±0.7 (tibial), 1.1±0.5 (peroneal) and 1.5±0.9 (sural), and in MSC treated animals (n=6) it was 2.6±0.5 (tibial), 1.9±0.5 (peroneal) and 1.9±0.7 (sural); peroneal ($p<0.05$), tibial ($p>0.05$) and sural ($p>0.05$) nerve function was higher in stem cell treated group compared to vehicle group. Walking track analysis did not produce clear foot prints to calculate Sciatic Function Index (SFI). Laser doppler analysis revealed normal vascularization and limb transplant survival was >90%. **Conclusion:** The limb transplant procedure was highly successful, and MSC therapy appears to promote nerve function recovery.

P6.12.12
Monoclonal Antibody Therapy does not Abrogate Rejection Risk in Renal Transplant Recipients

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Monoclonal antibodies are being increasingly used as therapeutic agents in medicine. Rituximab (anti-CD20) and Daclizumab (anti-IL2Rα) are two such monoclonal antibodies used to prevent organ rejection, but are not fail-safe. We have analyzed the pre and post-transplant antibody profile in serum of renal transplant recipients receiving Rituximab and /or Daclizumab.
Study Group: Kidney recipients with acute rejection and having PRA ≥ 10% pre-transplant were selected for the study (n=11). Those with well-functioning grafts served as the control group (n=15). Serum from these recipients was analyzed retrospectively by LABScreen kits for anti-HLA class-I, class-II and anti-MICA antibodies.
Results: Patients undergoing graft dysfunction showed the presence of either anti-HLA or anti-MICA antibodies or both. Acute antibody-mediated rejection was preferentially associated with the presence of pre-transplant anti-HLA and/or anti-MICA antibodies (9/11 cases, $p \leq 0.05$). Pre-transplant anti-MICA antibodies alone led to hyperacute rejection in two cases and pre-transplant DSA led to hyperacute rejection in one case. Two cases that had acute rejection within 10 days post-transplant revealed anti-HLA and anti-MICA antibodies in the pre-transplant serum.
Conclusion: A negative CDC cross-match following rituximab/daclizumab therapy does not imply absence of anti-HLA or anti-MICA antibodies in the recipient. Antibodies may still be detectable by more sensitive methods like Luminex and presence of pre-transplant anti-HLA and/or anti-MICA antibodies correlates with poor graft outcome in renal transplant. Further analysis on Luminex has shown that de-novo antibodies post-transplant, to HLA class-I/II or MICA indicate poor prognosis and may lead to accelerated graft rejection in such cases.

P6.12.13
Related organ transplantation - how voluntary is the act?

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In spite of the significant advances in transplantation technology and immunossupression procedures for a successful grafting, a few issues concerning emotions and feelings projected over the potential live donor still demand considerations. It is the purpose of this essay to approach the conflicts and dilemmas inherent to related donor organ donation. The methodology consisted on a search on current literature on the subject of organ donation and bioethics. Live donation brings an inherent internal conflict once it is consensus that donor safety is important which means that, for him or her, the best choice is not donating. However, there is a continuous tension between personal autonomy and the expectation in the family environment that wishes the well being of all its members, in special of those in vulnerability, the sick member in need of an organ and the potential donor, seen as the ultimate chance of restoring the equilibrium in the family environment and bringing back one´s health. In biomedical area knowledge and new technologies incorporated to transplant procedures follow international safety criteria; however, under bioethics and family optics, a few gaps demand reflections. In the approach by the doctor and his staff, the technical, legal and bioethical issues must be present and equally valued during the dialogue with the family. In this scenario, in which so many individualities prevail, the challenge relies on how to minimize the conflicts and dilemmas in order to reach a harmonious and balanced decision reducing coercion to a negligible issue.

P6.12.14
Dysregulated innate immunity is associated with Post-Transplantational Diabetes Mellitus in renal transplantation

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Toll-like receptors (TLRs) are key innate immune receptors that mediate the inflammatory responses in transplantation and are related to the pathogenesis of insulin resistance. Post-transplantation diabetes mellitus (PTDM) is an important metabolic complication after renal transplantation. In this study, we ascertained whether single nucleotide polymorphisms (SNPs) in TLRs have any association with PTDM in Korea population.
A total of 305 patients who had received kidney transplants were included. We analyzed the association between the PTDM development and the 6 SNPs (TLR1 rs 4833095, TLR1 rs 5743557, TLR2 rs 3804099, TLR6 rs3775073, TLR6 rs3821985, TLR6 rs1039559).
The overall incidence of PTDM in this study population was 16.7% (51 of 305 patients). The percentage of steroid-treated acute rejection episodes were no significant different between the two groups. In PTDM groups, there were more patients using tacrolimus, but the difference was also not statistically significant. The patients with PTDM had significantly higher allele frequency compared to those without PTDM for the TLR2 rs3804099*T, TLR6 rs3775073*A, TLR6 rs3821985*C, TLR6 rs1039559*C alleles. The TLR6 rs 1039559 were significantly associated with the development of PTDM after adjust with age. Then we tested whether any particular TLRs' halpotype was associated with PTDM. The frequency of the GGT haplotype of TLR6 was significantly higher in the non-PTDM group compared to the PTDM group.
Our study demonstrates a significant association between SNPs of TLR6 and PTDM, and this suggests that activation of the innate immune system via TLR activation might have an essential role in the pathogenesis of PTDM.

P6.12.15
Mechanisms of HLA class I antibody in inducing endothelium inflammation

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The formation of donor-specific HLA antibodies post-transplantation is associated with acute and chronic allograft rejection episodes in most types of solid organ transplantation. In this study we examined the pathways responsible for the expression of adhesion molecules and chemokines by endothelial cells in response to HLA class I antibodies. The potential of locus specific HLA antibodies to induce endothelial cell activation was also studied.
Stimulation of human microvascular endothelial cells (HMEC-1) with mouse HLA class I antibody (W6/32) induced significant expression of adhesion molecules; VCAM-1 andICAM-1 by a mechanism sensitive to PI3K/Akt inhibition (P<0.001). W6/32 antibody significantly induced the expression of CXCL8 which was reduced by 50% and 35% in the presence of Akt and PKA pathway-specific inhibitors, respectively. Silencing of CREB using siRNA induced 50% reduction in the expression of CXCL8 compared to control, p<0.001. W6/32-treated endothelial cells induced a significant monocyte adhesion and migration compared to control, p<0.001. Allospecific antibodies from sensitized kidney patients significantly enhanced the phosphorylation of CREB and expression of VCAM-1, ICAM-1 and CXCL8. Monoclonal human antibodies targeting specific HLA-A68 or HLA-B58 antigens also induced CREB phosphorylation. Down-regulation of HLA-B locus antigens using siRNA significantly reduced CREB phosphorylation in response to HLA-B58 antibodies.These data show that antibodies formed against HLA class I antigens are able to induce activation of microvascular endothelial cells. Monoclonal antibodies targeting only HLA-A or -B antigens also induced endothelial activation, demonstrating that antibodies to either locus can act independently to activate cells.

P6.12.16
Dynamic changes of T-regulatory/dendritic cell subsets and IL-17/TGF-β gene expression in stable kidney transplanted patients

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Introduction: We aimed to identify some cellular and molecular biomarkers that would allow clinicians to assess the graft acceptance. Patients and Methods: This prospective study considered 57 consecutive stable graft patients during one year after transplantation. Flow-cytometric frequencies of circulatory Tregs ($CD4^+CD25^+FoxP3^+$ and $CD8^+CD28^-$), myeloid Dendritic cells (mDC type 1, mDC type 2), plasmacytoid DCs (pDC) and real-time PCR-evaluated gene expression of IL-17 and TGF-β of peripheral blood mononuclear cells were investigated before transplantation, 2 weeks, 1, 3, 6 months, and one year after transplantation.
Results: The mean number of Tregs and PDCs decreased 2 weeks after transplantation (P<0.001) and then increased till the end of follow up as they reached their values before transplantation within one month in the case of pDCs (P=0.16) and 6 months in the case of $CD4^+CD25^+FoxP3^+$ Tregs (P=0.338). The mean number of mDC1s and the gene expression of TGF-β increased 2 weeks after transplantation (P=0.002) and then decreased till the end of follow up as they reached their values before transplantation within 6 months in the case of mDC1s (P=0.714) and 3 months in the case of TGF-β gene expression (P=0.18). There were no significant changes in the mean number of mDC2s till the 6^{th} month (P=0.041). Despite the expression of internal control of IL-17, there was not any expression of IL-17.Conclusion: There may be a clinical utility for Treg/DC and IL-17/TGF-β gene expression monitoring as predictors of stable grafts.

P6.12.17
Crosstalk between inflammation, ADAM10 proteolytic activity and Notch signaling in antibody-mediated rejection and endothelial dysfunction

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A Disintegrin And Metalloproteinases (ADAMs) regulate key proteolytic events involved in inflammatory cytokine and chemokine release and in Notch signaling. ADAM10 is constitutively and preferentially expressed in endothelial cells (EC) whereas ADAM17 is usually induced by inflammation. This study investigates the contribution of ADAMs and Notch signaling to vascular injury associated with donor-specific antibody-mediated rejection (AMR) in cardiac allografts. Regulation of ADAM10, -15, -17, Notch receptors (Notch1, 2, 3, 4) and ligands (Jagged1, Dll4), and VCAM1 was analyzed by quantitative PCR and by immunohistochemistry in cardiac biopsies from patients with stable graft (n=4) or with AMR (n=9), non failing heart (n=8) and dilated cardiomyopathy (n=9). Crosstalk between inflammation, ADAM10 proteolytic activity and Notch pathway was further investigated in cultured EC from donor transplants. We found that AMR induced by donor-specific anti-HLA is characterized by an upregulation of both ADAM10 and ADAM17 mRNAs (respectively 4.3 and 3.4-fold increase *versus* controls, p<0.01) without any change in ADAM15 mRNA. ADAM10 is located in graft EC and in infiltrating CD68+ macrophages and some CD3+ T cells. AMR is also associated with a significant increase in Notch ligands Jagged1 and Dll4 and drastic downregulation of the endothelial Notch4. In cultured EC, TNF recapitulates ADAM10-dependent Dll4/Notch4 imbalance. ADAM10 blockade also efficiently decreases the production of the pro-inflammatory cytokines and chemokines IL6, IL8, MCP1, CXCL16 and CX3CL1. To conclude, our findings suggest that ADAM10 is a major metalloproteinase driving proteolytic events involved in inflammatory responses and immune cell recruitment during AMR through the Notch pathway.

P6.12.18
Calculated Panel-Reactive Antibody: comparison of different approaches to calculate it

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Background: Solid Phase Immunoassays, as Single Antigen Luminex (SA), enable to identify antibody specificity with high sensitivity and therefore increase the detection of unacceptable antigens for patients who have antibodies against HLA antigens. The Calculated Panel-Reactive Antibody (cPRA) is a parameter that estimates the percentage of donors who would be non acceptable for a recipient that have donor specific antibodies (DSA).
Methods: A cPRA calculator tool has been developed with a spreadsheet application based in HLA genotypes from 2260 deceased donors and 543 receptors of hematopoietic progenitor transplantation and the direct upload of the SA software output. Different ways to approach the algorithm (cut-off values, inclusion of HLA loci C* and DQB1* and resolution) have been compared. 85 subjects were selected from the renal transplantation (TX) waiting list according to Complement Dependent Cytotoxicity PRA (PRA-CDC) reactivity (>50%).
Results: The modification cutoff values (MFI>1500, MFI>3000, MFI>6000) produce no relevant changes in cPRA results (less than 12%). Inclusion of DQB1 confers a mean increase of 20% in the cPRA value while C* only 1%. There is no relevance between the results calculated using either high resolution typing or low resolution. A group of patients (47%) with PRA-CDC between 40% and 80% but cPRA>80% is identified.
Conclusion: Low resolution typing of deceased donors is adequate to calculate cPRA and the inclusion of DQB1 typing should be considered. cPRA based on local population can be helpful to mathematically asses the difficulties of sensitized patients to be transplanted if DSA negativity is required.

P6.12.19
Antibody Mediated Rejection with a Negative C1Q Assay

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We present a 20-year-old female with orthotopic heart transplant secondary to congenital heart disease who developed late antibody mediated rejection with negative C1q staining. At the time of the transplant she was highly presensitized. She underwent a series of desensitization measurements. She had a negative retrospective crossmatch. She did well over the first 10 years of her transplant. At her 10 year mark, steroids were discontinued secondary to low bone density. The following year, she developed a low-level Class I HLA antibody. She continued to do well until 6 months later when she presented in antibody mediated rejection with hemodynamic compromise (endothelial activation with 1+C3d and 2+C4d staining). She had developed multiple new Class I and Class II donor specific antibodies. C1Q testing was negative for both Class I and Class II antibodies. She was hospitalized for over a month for aggressive treatment for antibody-mediated rejection which included plasmapheresis, IVIG and bortezomib. She improved clinically with significant reduction in her DSA levels. One year later she presented with a rise in antibody titers and trace focal C3d but negative C4d. Again her C1q staining was negative. She was started on cyclophosmadie and DSA levels regressed. Six months later she presented in AMR with hemodynamic compromise for a second time with patchy diffuse C4d and negative C3d with endothelial activation. Again, her C1q staining was negative.
Conclusion: We present a case where AMR with hemodynamic compromise presented twice in the same patient with multiple negative simultaneous C1q assays.

P6.12.20
Relationship between the number of NK cell subsets and Chronic Allograft Nephropathy (CAN)

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Background: NK cells have two key effector functions, which are the cytotoxic lysis of target cells and the release of inflammatory cytokines that amplify the immune response. Around 90% of human peripheral blood NK cells are $CD56^{dim}$ and display a high level of cytotoxicity, while the remaining 10% are $CD56^{bright}$ and display greater cytokine secretion. It has been reported that expression of the NK cell activating receptor NKG2D is increased with acute and chronic nephropathy after human kidney transplantation. In this study we have shown that the number of NK cells is not different between biopsy proven CAN patients and healthy controls, particularly at early time-points. **Methods** A case-control study was carried out on 25 patients with CAN and 25 matched healthy controls. NK cell numeration was determined by flow cytometry of PBMC, and NK cells that were $CD56^{bright}$ discerned with anti-CD127 from NK $CD56^{dim}$ cells that don't have CD127. **Results** No significant difference was observed in NK cells numeration between controls and patients (P value>.05); although, the number of NK $CD56^{dim}$ cells was higher than NK $CD56^{bright}$ cells in CAN patients and controls. **Conclusion** We have evaluated the effect of natural killer cells frequency and stage of maturity among CAN patients. Our results revealed that there is no difference between the number of NK cells of CAN patients and healthy controls.

P6.12.21
Cardiomyocyte and P19 embryonic stem cell

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Introduction: Three types of pluripotential stem cell lines have been established from mammalian embryos: embryonic stem (ES), embryonic germ (EG), embryonic carcinoma (EC) cells. P19 cells are a line of pluripotent embryonal carcinoma cells. They are feeder cell independent and can differentiate into cells of all three germ layers. The purpose of this study is differentiation of these cells to cardiac cells in culture medium.
Material and Methods: In this research, we used dimethylsulfoxide(DMSO) (Sigma) for inducing P19 cells into cardiac cells. Undifferentiated P19 cells were cultured under adherent condition in growth medium, Dulbecco Modified Eagles Medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (Gibco), penicillin (100 U/ml) and streptomycin (100µgr/ml) (Gibco). The cultures were maintained in 5% CO2 atmosphere at 37˚C.
Results: We treated P19 cells with 2 concentrations of DMSO and formed Embryoid Bodies (EBs) via liquid suspension culture in bacterial- grade dishes method. 5×104 cells/ml was aggregated for 4 days in 60 mm non adhesive bacteriological-grade Petri dishes containing 5 ml of the growth medium, in the presence of 0.5% and 1% DMSO.
Discussion: Optimization of DMSO concentration is necessary for differentiation to cardiac cells. It is concluded that P19 line cells are sensitive to dilution and concentration of inducing medium supplements.

P6.12.22
Clinical relevance of cytokine gene polymorphism on the development of acute rejection episodes and chronic allograft nephropathy following live donor renal transplantation

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Genetic variability in cytokines, which results in a shift of the cytokine milieu, could significantly impact the outcome of immune response, and thus the incidence of rejection and graft survival. In this study, 22 single nucleotide polymorphisms (SNPs) in 13 cytokine genes namely interleukin (IL) 1-alpha (T/C -889), IL1-beta (C/T -511, T/C +3962),

IL12 (C/A -1188), interferon-gamma (A/T UTR 5644), transforming growth factor-beta (C/T codon 10, G/C codon 25), tumor necrosis factor-alpha (G/A -308, G/A -238), IL2 (T/G -330, G/T +160), IL4 (T/G -1098, T/C -590, T/C -33), IL6 (G/C -174, G/A nt 565), IL10 (G/A -1082, C/T -819, C/A -592), IL1R (C/T pst1 1970), IL1RA (T/C mspa1 11100) and IL4RA (G/A +1902) in182 patients undergoing renal transplantation from their live related and unrelated donors. Of these, 50 patients experienced acute rejection episodes (ARE), 42 experienced chronic allograft nephropathy (CAN) (25 patients who had previous ARE and 17 without any ARE), while the remaining 115 patients had well functioning grafts (WFG). The high producer genotype TT of IFN- showed a strong association with development of ARE; while the AA genotype conferred protection from ARE (p=0.000) and CAN (p=0.007). Similarly, genotype GG at codon 25 of TGF-, was associated with ARE (p<0.05) and CAN (p<0.05). Further, TT genotype of IL-1 -511 and IL1RA mspa1 11100 was also associated with AR episodes (p<0.001 and p<0.01). The study highlights the clinical relevance of CGP as an important biomarker to predict acute rejection episodes and chronic allograft nephropathy following kidney transplantation.

P6.12.23
Short term survival of the related alive donor's renal implant and the compatibility HLA

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Introduction: The separation of the lymphocytes T and B for magnetic pearls are a highly effective technique (compared with the technique of Fiber of Nylon) for the serological typing and the different modalities of tests crusades for the transplant. Objective: The magnetic pearls are covered with specific antibodies that recognize specific markers in the surface of the membrane of the lymphocytes T and B. When the lymphocytes have been separated, HLA typing is doing and later on the couple's donor-receiver selection**.** Results: To all they the protocol of the related alive donor's donating selection was applied settled down in the department of Immunology of the Institute of Nephrology: cross-match and auto reaction of lymphocytes to 4 °C, 22 °C and 37 °C, study of sensitization and the HLA typing class I and class II by means of the microlymphocitotoxicity test. The final cross-match carries out 48 hours before the transplant with the selected donor. It uses little quantity of blood, quick, a viability of a 98% of alive cells, without contamination and the concentration of cells required is obtained to carry out the HLA typing and other histocompatibility tests. Conclusion: With this technique we are able to introduce the typing HLA DR and HLA DQ for serology in the Donating couple's selection Lives Related in the Renal Transplant. Of the 100 studied receivers, 9 were identical HLA with their donor (siblings) with a survival of 100% and the rest HLA haploidentical (siblings and parents) with a survival of 96%.

P6.12.24
Relationship of Serum Soluble Interleukin-2 Receptor (IL-2) & CD 30 with the Renal Allograft Rejection- A Hospital Based Experience in Renal Transplant Patients of Kashmir Valley

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Background: Renal allograft rejection is mediated by T lymphocytes, expressing interleukin-2 receptors and their concentrations have been suggested as a marker of acute rejection episodes. Soluble CD30 released following T cell activation can be used also as biomarkers for the prediction of allograft rejections. Thus, irreversible damage caused by rejection could be relieved and avoided by early diagnosis and treatment.
Objective: The purpose of this study was to investigate the relationship between serum sIL-2R & sCD30 levels with the rejection episodes in recipients.
Methods: Serum soluble IL-2R & CD 30 levels were measured with ELISA in recipients and in controls using the IL-2R &s CD 30 ELISA kits.
Results: Serum sIL-2R levels in patients with the rejection group were evidently higher (Mean ±SD: 329.85±59.22 pgs/ml) as compared to the Non- rejection group (Mean ±SD: 18.12±11.22 pgs/ml P<0.05). The higher values of sIL-2R were markedly reduced by adequate antirejection therapy. The healthy control group showed lower sIL-2R concentrations (Mean ±SD: 14.67±7.53 pgs/ml).
Our study demonstrate that the sCD30 levels in patients before transplantation was higher to that of healthy controls (Mean ± SD: 25.63±13.84 ng/ml vs Mean± SD: 13.44± 5.01 ng/ml; P=0.0001), 95%CI (6.96-17.41). On comparing pre and their corresponding post transplant samples, sCD30 values were not found to be significant, P= 0.89, 95%CI (-19.1-16.8).
Conclusion: Our results suggest that the serum sIL-2R concentrations showed significant correlation with the acute rejection episodes. The data does not support the idea of sCD30 being used as a potential marker for allograft rejection.

P6.12.25
Frequencies of CTLA4^{+}, FoxP3^{+} and CD127^{low} T cells within the CD4^{+}CD25^{high} population early after lung transplantation predict the incidence of chronic rejection after two years

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Regulatory T cells (Treg) regulate alloantigens and thus may counteract the development of chronic rejection (i.e. bronchiolitis obliterans syndrome, BOS) in lung transplantation. BOS affects over 60% of lung transplant recipients within five years. Here, we analyzed Tregs in peripheral blood of 120 lung recipients prospectively during the first 2 years and correlated with the onset of BOS.
In consecutive lung transplant recipients, the number of circulating Treg was detected by flow cytometry before transplantation and 3 weeks, 3, 6, 12, 18 and 24 months after transplantation. Treg were defined as CD4^{+}CD25^{high} T cells and were further analyzed for relevant markers such as, amongst others, CTLA4, CD127, FoxP3 and IL-2. Lung function and protocol biopsy results at the time points 3 weeks, 3, 6, 12, 18 and 24 months after transplantation were analyzed. We defined two groups by the development of BOS stage 1 or higher versus BOS stage 0. Repeated measures ANOVA were performed to compare differences of Treg sub-populations between the groups.
38/120 patients developed a BOS stage 1 or higher at 2 years after lung transplantation. The other 82/120 patients showed a course with stable lung function. A significant positive correlation was detected between the frequencies of CD4^{+}CD25^{high}CD127^{low}, CD4^{+}CD25^{high}CTLA-4^{+}, CD4^{+}CD25^{high}FoxP3+ and CD4+CD25highIL-2+ T cells and the absence of BOS 2 years after lung transplantation (p<0.05).Higher frequencies of CD4+CD25highCD127low, CD4+CD25highCTLA-4+, CD4+CD25highFoxP3+ and CD4+CD25highIL-2+ T cells as early as 3 weeks after transplantation are associated with protection against BOS during the initial 2 years after lung transplantation.

P6.13 Bone marrow transplantation and graft versus host disease

P6.13.01
Correlation between reconstitution of differentiated effector, effector memory and regulatory T cells populations and insulin independence in type 1 diabetes patients after autologous hematopoietic stem cell transplantation

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Introduction: High dose immunossuppression (HDI) followed by autologous hematopoietic stem cell transplantation (AHSCT) has emerged in last past years as a therapeutic alternative for newly diagnosed type 1 diabetes mellitus (T1D) patients, however the mechanisms involved in this setting remained unclear. Objective: To address the correlation between immune reconstitution and insulin independence in two clinical groups, based in duration of insulin independence and time of remission (less or more than 3 years insulin-free), after HDI/AHSCT. To evaluated the reconstitution of effector, effector memory and regulatory T cells after treatment. Methods: Peripheral blood was collected from 21 T1D patients (group A: 11 insulin-free for at least 3 years; group B: 10 insulin-free less than 3 years), at pre-transplantation and different time points after transplantation. Peripheral blood mononuclear cells were immunophenotyped by flow cytometry analysis. Results: We found significant increased numbers of $CD4^+CD27^-CD45RO^-$ differentiated effector T cells at months +24 and +54 post-AHSCT in group B when compared with the group A in the same periods. We also detected significant increase of $CD4^+CD27^-CD45RO^+$ effector memory T cells at months +2, +3, +6, +9 and +30 post-AHSCT in group B when compared with the group A in the same periods. The $CD4^+CD25^{hi}FoxP3^+$ regulatory T cell numbers only increased in group A at months +18, +30 and +60 after transplantation when compared with pre-transplantation period. Conclusions**:** Our results suggest an improvement of the peripheral immunoregulatory mechanisms after HDI/AHSCT, which may contribute to reestablishment of self-tolerance and control of autoimmunity on these T1D patients.

P6.13.02
Immunotherapy of xeno-graft versus host disease with ICOS monoclonal antibodies in immunodeficient mice

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ICOS (CD278), which belongs to the CD28 superfamily, is a powerful co-stimulatory molecule for T cells and is highly expressed on effector/activated T cells. Thus, targeting ICOS could dampen an unwanted immune response. To address this question using human cells, we determined the impact of a novel anti-human ICOS monoclonal antibody on the xeno-Graft vs Host Disease (GVHD) that develop after transfer of human PBMC into immunodeficient $NOD.SCID.\gamma c^{-/-}$ mice. Sixty-five percent of the mice injected with the anti-ICOS mAb (n=12) survived GVHD compared to controls, which all died by day 50 (n=12; median survival of 19 days). Injected mice were analyzed for numbers and phenotype of human cells in the blood, and in lymphoid and non-lymphoid organs by flow cytometry and histology. Expansion of human T cells in the blood of xenografted aniamls was greatly diminished by the antibody. Moreover, a significant reduction in mononuclear cell infiltrates was observed in the peripheral organs of treated animals. However, protection from GVHD did not correlate with lower activation of effector T cells, nor with increased numbers of regulatory T cells. In anti-ICOS treated animals, human $ICOS^+$ T cells were observed 3 months after treatment with no or moderate signs of GVHD. Protection from GVHD by the antibody might be due to a defect in T cell survival/expansion, related to blockade of ICOS-LICOS co-stimulatory signal. Thus, targeting ICOS may represent a new therapeutic strategy to treat GVHD.

P6.13.03
Identification of hematopoietic minor H antigens using leukemia reactive T cells and genetic linkage analysis

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A successful effective treatment of leukemia patients by allogeneic hematopoietic stem cell transplantation depends on graft-versus-leukemia effect mainly mediated by donor T cells. GvL is frequently associated with alloreactivity of T cells to epithelial organs, graft-versus-host disease, and can be sometimes too weak resulting in leukemia relapse after transplantation. Overcoming these limitations adoptive transfer of donor T cells specifically recognizing leukemia cells as targets is a promising strategy. Minor histocompatibility antigens play a crucial role as targets for GvL/GvHD reactive donor T cells. Knowledge of mHag with specific or preferential expression in hematopoietic tissue may therapeutically induce selective GvL responses in the absence of GvHD.
For identification of human hematopoiesis-specific mHag recognized by cytotoxic T lymphocytes, genetic linkage analysis can be used. Leukemia-reactive CTL were isolated by in vitro stimulation of naïve CD8+ T cells from healthy individuals with fully HLA class I-matched primary acute myeloid leukemia blasts. The recognition pattern of two CTL to a panel of previously genotyped B-LCL were analyzed by IFN-γ-ELISPOT assay. B-LCL originated from three large pedigrees from the CEPH reference family collection (Corielle Institute/New Jersey/USA). Based on the recognition patterns all individuals were divided into either mHag-positive or mHag-negative, respectively. Genetic linkage analysis using genotypes of these individuals, which are online available at the CEPH database, was performed to identify linked regions and to narrow down the region for final gene identification coding the mHag peptides. Of particular interest for future immunotherapy trials are those mHag expressed specifically or at least preferentially in hematopoiesis.

P6.13.04
Monitoring early CMV-specific CD8+ T-cell responses after allogeneic stem cell transplantation by HLA-multimers

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We present the results of a pilot study using pentamer (PM) and streptamer (ST) multimer complexes for monitoring CMV-specific CD8+ T-cells (CTLs). We analysed 14 patients that underwent allogeneic Stem Cell Transplantation (allo-SCT). All patients and donors were positive for the HLA-A*02:01 allele. PM and ST were directed against the epitope NLVPMVATV (495-503) of the CMV phosphoprotein 65 (pp65). Samples were obtained at 15-day intervals until day +90 and monthly thereafter.
Three patterns were observed. In 2 patients (14%) no CMV-specific-CTLs could be detected despite several CMV reactivations, requiring prolonged cumulative antiviral therapy (67 and 136 days each).
In 6 patients (43%) CMV reactivation occurred at a mean of 36 days (10-74) and triggered a rapid increase of CMV-specific-CTLs with a median of 6.9×10^5/L (range 0.02-279.7). The CMV-PCR became immediately negative and antiviral therapy was stopped promptly after a median of 15.3 days (8.7-23).
Finally, 6 patients (43%) showed an early immune reconstitution with CMV-specific-CTLs detected with a median of 1.53×10^5/L (range 0.2-54.72) in the absence of CMV-PCR reactivation at a median of 20.5 days (10-34) post-SCT. No CMV-PCR reactivation was observed in this group with a median follow-up of 8 months (3-14).
Monitoring CMV-specific-T-cells might be able to distinguish patients at higher risk of recurrent virus reactivation and in need of prolonged antiviral therapy. Patients with increasing CMV-specific-CTLs detectable at the time of CMV-PCR reactivation may only need a short course of antiviral therapy, while those with early CMV-specific-CTLs may be protected from CMV reactivation.

P6.13.05

Mesenchymal Stem Cells as an Immunotherapy for Chronic graft-versus-host disease

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Background and Aims
Mesenchymal Stem Cells (MSCs) have immunomodulatory properties in vitro and in vivo. Chronic graft-versus-host disease (cGVHD) is an important problem in long-term survivors of allogeneic stem cell transplantation, it increases mortality and morbidity, and causes impaired functional status. cGVHD is a TH-2 mediated disease. In this study, TH1 and TH2 cytokines and lymphocytes subsets were analysed in cGVDH patients after MSC infusion .
Methods
Patients with cGVHD newly diagnosed, were treated with intravenous infusion of MSC, obtained from adipose tissue. We evaluated lymphocyte subsets and cytokines after 7, 20, 42 and 56 weeks using Flow Cytometer.
Results
Differences were observed on cytokines evaluated, especially on IL-10, IL-2 and TNFa. Also we found difference between control and MSC patients in the lymphocyte subsets, especially on week 20 after therapy.
Conclusions
MSC have an impact on cytokines and lymphocytes population. MSC infusion may be an effective therapy for patients with cGVDH.

P6.13.06

Acute myeloid leukemia (AML)-reactive CD4+ T-cell clones isolated from 10/10 HLA match donors recognize HLA-DP mismatch antigens and eliminate leukemia blasts in NSG mice in vivo

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In allogeneic hematopoietic stem-cell transplantation, donor-derived T cells mediate beneficial graft-versus-leukemia (GvL) effects but also graft-versus-host disease (GvHD). As $CD4^+$ T cells recognize peptides presented by HLA-class-II molecules that are not expressed on non-hematopoietic cells under non-inflammatory conditions, they might have a more favourable safety profile than $CD8^+$ T cells in terms of avoiding GvHD. In this project we have developed a reliable protocol for the *in vitro* generation of AML-reactive donor $CD4^+$ T cells that could be used for adoptive transfer experiments in AML-engrafted NOD/SCID-IL2Rγ(null) (NSG) mice.
Naive $CD4^+$ T cells were sorted from healthy donor PBMC. They were stimulated in 96-well mini-mixed lymphocyte-leukemia cultures (mini-MLLC) with irradiated primary AML blasts with complete HLA-A/B/C/DR/DQ match. AML-reactive mini-MLLCs were expanded in six different donor-patient pairs. They showed strong IFN-γ and TNF-α production as well as lysis (up to 60% at E:T = 20:1) upon incubation with AML blasts or patient-derived EBV-B lines in ELISpot and ^{51}Cr-release assays. Several $CD4^+$ CTLs were of clonal origin according to TCR-Vβ chain analysis. Nearly all of them recognized patient-derived mismatched HLA-DP, as determined by using HLA-DP transfectants as targets. NSG mice engrafted with AML blasts and then infused with AML-reactive $CD4^+$ CTL showed complete disappearance of AML blasts in bone marrow, spleen and blood. Homing of T cells was confirmed as they could still be detected in these organs one week after transfer. Our data suggest to further investigate $CD4^+$ T cells as potent cytolytic effector cells in adoptive immunotherapy of leukemias.

P6.13.07

Fetal membrane cells promote regulatory T cells and suppress alloreactivity in a cell contact dependent manner

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We introduced the use of stromal cells from term fetal membrane (FMCs) in patients with steroid refractory Graft-versus-Host Disease. FMCs have many advantages compared to bone marrow-derived mesenchymal stromal cells (BM-MSCs), including an increased allosuppression, unlimited supply, great expansion potential and high expression of integrins. In this study, we are elucidating factors of importance in the reduction of alloreactivity by FMCs. We found that FMCs require cell-cell contact in order to mediate suppression in mixed lymphocyte reactions (MLR) (p=0.01, n=11). The frequency of CD4+CD25highFOXP3+ regulatory T-cells (Tregs) was increased (median 2.3-fold), whereas the proportion of total CD25+ T-cells was decreased (median 1.4-fold) when the FMCs were added to the MLR cultures (p=0.03, n=10 and p=0.03, n=6, respectively). This was only observed when cell-cell contact was allowed between the MLR and FMCs. Blocking activity of indoleamine-2,3-dioxygenase (IDO) (median 2.6-fold increase in proliferation compared to MLR+FMC-control, p=0.01, n=8), IFN-gamma (median 2.0-fold increase, p<0.01, n=11), Prostaglandin E2 (median 1.26-fold increase, p=0.01, n=12) and PD-L1 (median 1.23-fold increase, p=0.03, n=7), significantly impaired the anti-proliferative ability of the FMCs in MLRs. Inactivation of IDO reduced the frequency of Tregs. In contrast to BM-MSC, pre-treatment of the FMCs with IFN-gamma (100 U/ml) decreased suppression of alloreactivity (p<0.01, n=16), but stimulation of FMCs with MLR supernatants containing low levels of IFN-gamma had no effect on the suppressive capacity in the MLR.
To conclude, FMCs differs in several aspects from MSCs and needs proximity to allo-reactive lymphocytes to mediate a suppressive effect and increase the frequency of Tregs.

P6.13.08

Circulating human Thymic Seeding Progenitors expand after allogeneic HSCT in parallel with a decrease of CXCR4 expression

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Long term T-cell reconstitution after allogeneic hematopoietic stem cell transplantation (HSCT) is dependent on patient thymic function and affected by acute graft-versus-host disease (aGVHD). T-lineage reconstitution after HSCT is limited by progenitor supply to the thymus. In humans, a subset of circulating Thymic Seeding Progenitors (TSP) with a CD34+lin-CD10+CD24- phenotype, exhibits a very low myeloid but a B, T and NK lymphoid potential. We analysed by flow cytometry this population in the peripheral blood of 39 patients, 3 months after HSCT, in comparison to 15 healthy donors (HD). Patients without aGVHD (n=11) and patients with a grade I aGVHD (n=7) had significantly (p=0,005) more TSP than patients with severe aGVHD (grade II, III and IV (n=21)) or HD. TSP from patients and HD both generated mature T cells in vitro using limiting dilution assay on OP9/DL1 cells. Moreover, patients and HD cell-sorted TSP displayed a similar global expression by quantitative RT-PCR of 22 genes involved in progenitor T cell differentiation and homing.
Noteworthy, we observed an important reduction of CXCR4 expression in TSP from patients without aGvHD compared to HD (p=0.002), consistent with an enhanced exit from the bone marrow. CXCL12, the CXCR4 ligand, was decreased in patient's plasma, especially in case of aGVHD. In conclusion, at 3 months after HSCT, we observed in patients without severe aGVHD, a rebound of circulating functional TSP associated with a modulation of the CXCR4/CXCL12 axis. These data identify TSP as a novel therapeutic target to enhance immune reconstitution after HSCT in humans.

P6.13.09

In vivo rapamycin-induced Tregs do not traffic to the bone marrow: preservation of the GVL effect after T-cell replete HLA-haploidentical HSCT

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Introduction: we have recently completed a Phase I/II study of T-cell replete hematopoietc stem cell transplantation (HSCT) from an HLA-haploidentical donor in 113 pts with hematological malignancies followed by GVHD prophylaxis with rapamycin. Incidences of grade III-IV acute GVHD and chronic extensive GVHD were 21% and 35%, respectively. There was expansion of circulating CD4+/CD25+/FoxP3+/IL-7Rα- Tregs (median 6.5% range 0.2-37.2, P<0.01 compared with controls), which were suppressive ex vivo. Tregs frequencies correlated with GVHD severity. One-year PFS was 44% in pts with early disease (n=18) and 30% in pts with advanced disease (n=95).
Aim: A long-standing question is whether Tregs-based strategies may interfere with the GVL effect. We thus aimed at verifying if rapamycin-induced Tregs traffic to the bone marrow (BM) suppressing effector T cells locally.
Results: The BM of rapamycin-treated pts was depleted of Tregs (at day 30, BM Tregs frequencies: median 0.3% range 0.0-2.2, P<0.01 compared with PB Tregs). High-level CXCR4 expression on circulating Tregs suggested a specific antimigratory effect of rapamycin. On the contrary, the BM was heavily infiltrated by CD45RA-/CD62L- effector memory CD8+ T cells that expressed VEGFR-2. Circulating T cells co-cultured with VEGF-producing BM-resident stromal cells promptly up-regulated VEGFR-2, resulting in a dose-dependent suppression of proliferation. Interestingly, VEGFR antagonists, such as sorafenib and sunitinib, but not dasatinib, partially reverted the antiproliferative effects of stromal cells.
Conclusions: While protecting from GVHD, rapamycin-induced Tregs do not traffic to the BM, thus leaving the GVL effect unharmed. The potential immunosuppressive effect of BM-resident stromal cells merits further investigation.

P6.13.10

Basophils as regulators of CD4 T cells in graft versus host disease

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Until recently basophils were considered mainly as effector cells of an innate immune response linked to allergy and parasite infection. Only in the past years they were recognized as important regulators of adaptive immunity.
T cell proliferation is an important part of immune regulation, as well as in malignant disease.
We have found that non-activated, as well as activated basophils are able to inhibit T cell proliferation in vitro. This effect is independent of Fas and MHCII, but dependent on the soluble factors IL 4 and IL-6.
Based on these findings we analysed the role of basophils in the model of acute graft-versus-host-disease (GvHD) and found a significant increase in the GvHD score of mice after depletion of basophils. Adoptive transfer of basophils leads to a significant reduction in the GvHD score compared to control. Mice depleted of basophils showed an increased CD4+ T cell count in spleen and mesenteric lymph nodes as well as an increase in total CD45+ cells in the GvHD model. Depletion of basophils also led to more IL-17+ CD4+ T cells in the mesenteric lymph nodes but not in the spleen compared to controls.
To analyse the in vivo effects of IL-4 and IL-6 in the GvHD model we administered the cytokines or PBS. IL-4 led to a significant lower GvHD score und better survival compared to controls and the IL-6 treated group. Injections with IL-6 on the other hand significantly increased the GvHD score and reduced the survival compared to IL-4 and controls.

P6.13.11

Innate memory CD4 T cells suppress autoimmune graft-versus-host disease

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Chronic graft-versus-host disease (cGVHD) is the major complication post hematopoietic cell transplantation. Major histocompatibility complex class II (MHCII) mediated $CD4^+$ T cell differentiation and function plays a critical role in cGVHD, and the lack of hematopoietic MHCII (H-MHCII) causes cGVHD in syngeneic irradiated murine recipients. We have studied this process using murine bone marrow transplant of MHCII deficient or sufficient donors. We find that irradiated WT recipients that received $MHCII^{-/-}$ bone marrow exhibited a systemic decrease in IL-10 and increase in TNF-α production, and defective development of H-MHCII dependent innate memory phenotype (IMP, $CD44^{hi}/CD62L^{lo}$) $CD4^+$ T cells. Transfer of IMP $CD4^+$ T cells, but not conventional regulatory T cells, abrogate the pathogenesis and suppress the activation of pro-inflammatory intraepithelial lymphocytes. The presence of MHCII on dendritic cells (DCs) is sufficient to rescue IMP $CD4^+$ T cell development and reverse subsequent pathogenesis. Furthermore, the absence of the Tec kinase ITK rescues the development of IMP $CD4^+$ T cells independent of MHCII expression on donor bone marrow, and resultant $Itk^{-/-}$ IMP $CD4^+$ cells can partially prevent the pathogenesis. We conclude that IMP $CD4^+$ T cells can act as a suppressive population during cGVHD, and that DC expression of MHCII is critical for the development of such IMP $CD4^+$ T cells. In addition, ITK regulates the development of IMP $CD4^+$ T cells partially independent of DC expression of MHCII. This work provides a mechanism for the development of cGVHD, and suggests an approach of suppressing such responses in syngeneic bone marrow graft.

P6.13.12

Establishment of a protocol for evaluating human mesenchymal stem cell efficacy on GvHD treatment

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Mesenchymal stem cells (MSCs) exist in almost all adult tissues and organs. MSCs are multi-potent and can differentiate into distinct cell types such as chondrocytes, osteocytes, and adipocytes. Since the discovery of the immunomodulation property of MSCs about a decade ago, it has been actively investigated whether MSCs can be used for the treatment of immune related diseases such as graft-versus-host disease (GvHD). However, how to evaluate the MSC efficacy for the clinical trial is still unclear since MSC showed controversial results between in vitro and in vivo. Therefore, we established a protocol for evaluating the human MSC efficacy on GvHD treatment using a mouse MHC-unmatched B6 -> BALB/c GvHD model system. Human clonal MSCs (hcMSCs) were isolated from human bone marrow according to our subfractionation culturing method. To establish a mouse model, we administrated two different mouse clonal MSCs. One of them showed significantly reduced the GvHD-related mortality of the murine recipients and the other one did not, although both lines can significantly suppress the allogeneic proliferation of T-cells in vitro. It indicates that each MSC line possesses different immune response in vivo. Using this mouse model, we investigated whether hcMSC can reduce the GvHD mortality. Surprisingly, xenogeneic hcMSC could prolong the survival of murine recipients and hcMSC also significantly inhibited the murine T-cell proliferation in vitro. Based on these results, we successfully established a MHC-unmatched B6 -> BALB/c GvHD model which might be useful for the evaluating human MSC's efficacy before the clinical trial.

P6.13.13
HLA-G: a possible biomarker for Graft versus Host Disease

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Introduction: Graft versus host disease (GvHD) is the most common cause of morbidity and mortality after Hematopoietic Stem Cell Transplantation (HSCT) and identifying biomarkers for prediction of GvHD is important. HLA-G, an immunosuppressive molecule has been identified as a key mediator of tolerance, however its impact on stem cell transplantation outcome is under investigation. The exon-8, 14bp insertion/deletion in 3' UTR influences the HLA-G surface expression and soluble levels (sHLA-G). Objective: To understand the clinical relevance of HLA-G in HSCT, the present study was performed on recipients who underwent HSCT at our centre. Methodology: We evaluated the 14bp polymorphism (by PCR-SSP) and serum sHLA-G levels (by ELISA) in 120 healthy controls and 28 recipients at pre and post transplant stage (day 15, 30, 90 and at time of GVHD). Results: Seven (25%) recipients manifested GvHD. The +14bp allele was observed more frequently in recipients than controls. The sHLA-G levels correlated with 14bp polymorphism. Amongst controls, the subgroup with +/+14bp genotype had higher sHLA-G level in comparison to groups with -/- or -/+14bp genotype. Majority of the recipients carrying high secretary +/+14bp genotype (85%), did not develop GvHD. sHLA-G levels decreased in patients at the time of GVHD as compared with their pre-transplant levels. It is likely that higher sHLA-G levels may have influenced the alloreactivity and inhibited manifestation of GvHD. Conclusion: Our preliminary findings indicate that HLA-G might have influenced the occurrence of GvHD and evaluating larger cohort may confirm the role of HLA-G as a predictor of transplant outcome.

P6.13.14
Expression profile of candidate immune response genes in patients after haematopoietic stem cell transplantation

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Graft-versus-host disease (GVHD) is a severe complication of allogeneic haematopoietic stem cell transplantation (aHSCT). The main effector cells of GVHD are donor T-lymphocytes that are addressed to the target tissues.
The aim of this study was therefore to determine the expression profile of genes for activating markers that characterise Th1 and Th2 type of immune response (TBX21 and GATA3, respectively), and surface lymphocyte integrins (ITGA4 and ITGB7) in patients after aHSCT.
The series of peripheral blood (PB) samples were collected from 16 aHSCT patients at 7 events (before conditioning, before aHSCT, 14, 21, 28, 100 and 180 days after aHSCT). Quantitative real time PCR was used to determine mRNA relative expression of candidate genes (TBX21, GATA3, ITGA4, ITGB7).
Conditioning regimen before aHSCT decreased the relative expression of ITGB7 and GATA3 (p =0.003; p=0.0001)). The number of TBX21 mRNA transcripts was up-regulated by conditioning (p=0.015) and decreased after transplantation (p=0.035). The mRNA expression of ITGB7 and GATA3 restored to the state before chemotherapy in samples 100 and 180 days after transplantation. By contrast, TBX21 expression was increased in late samples (100 days, p=0.005; 180 days, p=0.022). The expression of ITGA4 was not significantly affected after aHSCT within the study period.
In conclusion, our data indicate that the expression of Th1 marker (TBX21) in PB increases after conditioning and in later period after aHSCT. On the other hand, Th2 marker (GATA3) and integrin ITGB7 were strongly downregulated by conditioning regimen. Grant support: IGA MZCR NT12454, IGA UP 2013_009 and CZ.1.05/2.1.00/01.0030.

P6.13.15
KIR-ligand mismatch on allograft outcome

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Introduction: It has studied the possible effect of graft-versus-leukemia mediated by donor killer cell immunoglobulin-like receptor (KIR)-ligand HLA incompatibility between groups C1 and C2.
Patients: We included 31 patients that underwent an allogeneic UCBT: Group A: receptor is homozygous C2 and donor is C1/C2 or C2/C2, Group B: patients without anti-leukemic effect because they are C1/C2 or C1/C1 or C2/C2 with donor also C1/C1 or C2/C2, respectively and Group C: patients C1/C1 or C2/C2 whose donor lacked C2 or C1 respectively.
Results**:** With a median follow-up post-UCBT of 53 months, KIR ligand incompatible patients in the GVH direction (A) showed a lower actuarial probability of relapse at 5 years (33.3±17%vs68.5±18.1%vs61.1 ±28.4% p=.11). Thus, the GS is better in A than B and C (57.1±18.7%vs13.6 ±11.7%vs24.3±18.7%;p=.31). In A complete donor chimerism was reached in 85.7% of patients, with a median time of 37 days to achieve it, in 54.5% of B in a median time of 45 days and in 53.8% of C in a median time of 55 days. The median time to myeloid engrafment was 18 days for A, 24 days for B and 18 days for C (p=.42). Transplant related mortality at day 100 and at 1 year were 12.9% and 32% respectively for entire group, being 0% and 42,9% for A, 9.1% and 36.4% for B and 23.1% and 23.1% for C.
Conclusions: KIR/ligand incompatibility based on HLA C1/2 missmatch in the GVH direction is associated with a trend to lower probablility of leukemia relapse

P6.13.16
Non-myeloablative bone marrow transplantation for mdx mice muscular dystrophy therapy

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The replacement of the mutant stem cells for wild type stem cells is seen as radical approach to the treatment of monogenic diseases. Bone marrow (BM) transplantation is used to treat monogenic diseases such as β-thalassemia. We present the results of treatment of muscular dystrophy of mdx mice, the counterpart of human Duchenne muscular dystrophy, by C57BL/6 mice BM transplantation. Reason of mdx mice muscular dystrophy is absence of dystrophin synthesis by mutant muscle fibers (MF). Like another authors we did not observed a growth of dystrophin synthesis after 5 - 11 Gy irradiation of mdx mice and subsequent isogenic BM transplantation of C57BL/6 mice. The nuclear of transplanted BM stem cells was shown to occupy sacroplasma of mdx mice MF without following dystrophin gene expression. To overcome the silence of wild dystrophin gene we used X-ray irradiation of mdx mice in dose 3 Gy with next step of wild type C57BL/6 BM transplantation. Non-myeloablative bone marrow stem cells transplantation induced gradual rise of dystrophin positive striated MC fibers of diaphragm from 4.1 % at 2 months after irradiation and BM transplantation up to 27.7±6.7 % at 6th months after BM transplantation. There are also increase of striated MF without central nuclei up to 22 %. Accumulation of dystrophin positive MF are accompanied by reparation of structure of nerve-muscle junctions (NMJs) and of NMJs electrical resting membrane potential. Practically we cured mdx mice of muscular dystrophy because 20-30% of dystrophin positive MF is enough to cure Duchenne disease.

P6.13.17
Comparison of immunosuppressive effect on lymphocytes of bone marrow, umbilical cord blood and placenta mesenchymal stromal cells

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Bone marrow mesenchymal stromal cells (BM-MSCs) display immunoregulatory properties and have been used to treat steroid-refractory acute graft-versus-host disease (GVHD) in hematopoietic stem cell transplant patients. In this study, we have obtained MSCs from umbilical cord blood (UCB-MSCs), placenta (PL-MSCs) and BM and compared their immunosuppressive effect on activated CD3+ T-cells. We co-cultured activated CD3+ T-cells (by using CD3 and CD28 antibodies) and MSCs from the three sources, in absence and presence of cell-cell contact. Stimulated CD3+ T-cells cultured alone served as positive control. T cell proliferation (bromodeoxyuridine uptake of CD4+ populations) and CTLA-4 expression was evaluated by flow cytometry analysis on day 4 of culture. MSCs from all three sources significantly diminished the proliferation of CD4 lymphocytes only in cell-cell contact condition, however such effect was increased in presence of BM- and UCB-derived MSCs than in PL-MSCs. Furthermore, we observed an increase in CTLA-4 expression on CD4+ T-cells only in cell-cell contact co-cultures with BM- and UCB-derived MSCs. Our results indicate a higher capacity to decrease T cell proliferation by MSCs from BM and UCB compared with PL, indeed such capacity was similar in the formers two cells. Moreover, increase in CTLA-4 expression on T cells, could be involved in the mechanism through which BM- and UCB-derived MSCs decrease T cell proliferation. In conclusion, UCB-MSCs may prove useful in the development of cellular therapy protocols related with immunosuppression activity.

P6.13.18
In vitro **effect of rhG-CSF on cytokine production of cultured mononuclear cells**

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Granulocyte colony stimulating factor (G-CSF) is used therapeutically for the treatment of neutropenia and mobilization of hematopoietic stem cells. It has been reported that G-CSF also has effect on other cell types such as monocytes and lymphocytes. *Ex vivo* studies have shown that recombinant human G-CSF (rhG-CSF) induces changes in the immune response in healthy donors, modifying T lymphocyte function and modulating cytokine production, thereby changing the balance between Th1 and Th2 responses. Here we report an *in vitro* model that allowed us to study the effect of rhG-CSF on cultured peripheral blood mononuclear cells (PBMN) from healthy donors. To establish optimal time and concentration of rhG-CSF required to induce changes on cytokine production, a five-days kinetic experiment was set up. Our results showed that cultures incubated for six hours with PMA/ionomycin plus 200 ng/mL rhG-CSF produced similar amounts of IFN and TNF by T lymphocytes and PBMN cells, respectively. Results from our *in vitro* model resemble those obtained from *ex vivo* studies, analyzing the effect of rhG-CSF on healthy donors PBMN. This *in vitro* model which allows basic studies will help us to better understand the potential use of rhGCSF as a therapeutic agent for autoimmune diseases.
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P6.13.19
Mobilization effect on CD8+ T cells and their contribution in controlling the development of GVHD

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Allogeneic hematopoietic stem cell transplantation is a therapy that can restore bone marrow function. The success of this procedure depends greatly on having an adequate number of stem cells (SC). To mobilize SC from bone marrow to peripheral blood the donor is subjected to a treatment with granulocyte colony stimulating factor (G-CSF). SC are collected by and subsequently infuse into the patient. Some of the drawbacks of this therapy include the graft versus host disease (GVHD), relapses, and infections caused by opportunistic microorganisms. The mobilization process promotes preferentially a Th2 phenotype. The aim of this study was to search for the effect of G-CSF on CD73 and CD39 expression and cytokine production by CD8+ T cells, and their correlation with the clinical course of the patients. Thirteen patients and their SC donors were studied. Seven healthy individuals were included as controls. The G-CSF induced the expression of regulatory molecules and cytokines such as CD73, CD39, IL-10 and TGF-β, by CD4+ and CD8+T cells. It was shown that CD8+ cells are stable, 80% expressed the anti-apoptotic protein BCL-2 and up to 5% expressed active proapoptotic protein caspase 3. Moreover, the low production of regulatory cytokines in response to polyclonal stimulus correlated with the development of GVHD. We conclude that the G-CSF promotes a low percentage of CD8+T cells with regulatory phenotype, which could contribute to the control of GVHD, whereas the lack of response of CD4+ and CD8+T cells to polyclonal activators is of poor prognosis.
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P6.13.20
The Nlrp3-inflammasome regulates acute graft-versus-host disease

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The success of allogeneic hematopoietic cell transplantation is limited by acute graft-versus-host disease (GvHD), a severe complication accompanied by high mortality rates. Yet, the molecular mechanisms initiating this disease remain poorly defined. Here, we show that after conditioning therapy (irradiation), intestinal commensal bacteria and the damage-associated-molecular pattern uric acid contribute to Nlrp3-inflammasome-mediated IL-1β production, while gastro-intestinal decontamination and uric acid depletion led to reduced GvHD severity. Early blockade of IL-1β or genetic deficiency of the IL-1 receptor in dendritic cells and T cells improved survival. The Nlrp3-inflammasome components Nlrp3 and Asc, which are required for pro-IL-1β cleavage, were critical for the full picture of GvHD. In transplanted mice, IL-1β originated from multiple intestinal cell compartments and exerted its effects on dendritic cells and T cells, the latter being preferentially skewed towards Th17. Compatible with these data from the mouse model, increased levels of active caspase-1 and IL-1β were found in circulating leukocytes and intestinal GvHD-lesions of patients. Thus, the identification of a crucial role for the Nlrp3-inflammasome sheds new light on the pathogenesis of GvHD and opens a new mechanism-based avenue for the targeted-therapy of this severe complication

P6.13.21
Reduced serum levels of soluble Interleukin-7 receptor during graft-versus-host disease (GVHD) in children and adults.

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Interleukin 7 (IL-7) signals via the IL-7 receptor (IL-7R) and drives homeostatic T-cell proliferation in patients after allogeneic hematopoietic stem cell transplantation (aHSCT); the role of IL-7R/IL-7 axis in driving graft-versus-host disease (GVHD) is debated. We performed a prospective study in adults (n=33) and children (n=29) undergoing aHSCT, by measuring plasma IL-7 and soluble CD127 (sIL-7R) concentrations in five time points between 1 and 12 months after aHSCT. In all patients sIL-7R, but not IL-7 was increased with time after HSCT. In adults, plasma IL-7 levels were elevated at 6 months ($p<0.05$) if donor age was <30 years. Low sIL-7R was associated with any grade of acute graft-versus-host disease (GVHD) at 2 and 6 months ($p=0.02$) and with CMV infection at 2 months after aHSCT ($p<0.05$). Patients with cytomegalovirus (CMV) reactivation had increased IL-7 values at 2 and 3 months ($p=0.02$) after HSCT. In multivariate analysis, high sIL-7R levels were associated with acute GVHD (relative hazard (RH): 0.70, $p>0.01$) and sibling donor (RH: 2.23, $p=0.004$). Recipients of sibling grafts showed high levels of IL-7 (RH: 1.38, $p<0.05$) and bone marrow recipients had low IL-7 levels (RH: 0.73, $p=0.04$). Measurement of the sIL-7R/IL-7 axis will help in guided immune monitoring after HSCT and guided interference with sIL-7R may be explored in GVHD management.

P6.13.22
Accelerated B cell recovery characterizes haploidentical T-repleted bone marrow transplantation

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T-repleted, haploidentical bone marrow transplantation (BMT) with post-transplant cyclophosphamide expands the pool of potential bone marrow donors for the treatment of certain haematological diseases. As revealed by multiple clinical trials, the incidence of bacterial and fungal infections is significantly lower compared to other haploidentical strategies. In search for a cellular immune mechanism responsible for this effect, we analysed B cell recovery and B cell subsets by polychromatic flow cytometry in 10 patients treated with the mentioned protocol and followed the patients for 1 year post BMT. Here we show that B cell counts normalize faster compared to classical haploidentical protocols, i.e. within 8-10 weeks after transplant. In the first three months the B cell ontogeny is recapitulated. CD10+ CD38 bright transitional B cells are first observed: within this subset, CD21 low precede CD21 high transitional B cells. CD10- CD38 dim/- mature, naïve B cells subsequently occur and comprise the vast majority of B cells. Conversely, IgG+ memory cells are undetectable during the first 2 months post BMT, develop only 1 month later and remain at low frequency over the following months. Our results suggest that the fast B cell reconstitution process observed in these patients is associated with a decreased susceptibility against infections in T-repleted, haploidentical bone marrow transplantation compared to other clinical protocols. Further analyses are required to investigate whether fully competent B cell memory is recovered in this particular BMT setting.

P6.13.23
Cell Subset Chimerism after HSCT - Clinical case

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Allogeneic Hematopoietic Stem cell transplant (HSCT) is potential curative option for recurrent lymphomas. Early prediction of relapse, graft rejection or graft versus host (GvHD) can change patient management. Routine chimerism tests include quantification of polymorphic Indels as a group of informative markers by qPCR method. Cells from blood or marrow are frequently used for chimerism testing, however, lineage-specific populations increase sensitivity and diagnostic accuracy.
Here we describe a clinical case of 24 years -old female with Hodgkin Lymphoma. Samples were collected during 193 days after double cord HSCT. Patient's Hodgkin-Reed-Sternberg (HRS) cells were positive for CD3 and CD15, but negative for CD20. Chimerism was analyzed in Peripheral blood, and in enriched CD3, CD14, CD15, CD19 cell populations. On day 15 post HSCT, we could detect both cords, one of them less than 1% which later disappears. Even though % DNA from other cord2 reached 42% on day 42, only 9% was detected on day 193 (PB results). Similar trend was observed for CD14 and CD15, suggesting no engraftment contrary of CD3 and CD19 cells. Cord 2 CD3 cells were 11% on day 15, which on subsequent days (15-193) was 86-95%. Cord2 CD19 cells were 95% on the 2 time points measured (Day 93, 193). The highest cord 2 % DNA was observed on day 42 analyzing each subset: 42%PB, 96%CD3, 29%CD14, 25%CD15.
The final diagnosis was non-engraftment, However, CD3, and CD19 from Cord 2 have been stimulated. This scenario is suggestive of GVHD and/or GVL reactivity

P6.13.24
Chimerism patterns of long-term stable mixed chimeras post-HSCT in patients with non-malignant diseases

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Introduction: Long-term stable mixed chimerism (MC) is a rare phenomenon after hematopoietic stem cell transplantation (HSCT) characterized by 5-95% residual recipient hematopoietic cells. The mechanisms directing hematopoietic recovery to MC in some patients and to full donor chimerism (DC) in others are poorly understood. In this study we compared DC patients with long-lasting stable MC patients for a median of 9.5 years (range 5 - 16.5) post-HSCT in patients with non-malignant diseases.
Methods: Chimerism analysis was performed for all patients at regular intervals post-HSCT on peripheral blood samples. The cell lineages analysed were T-lymphocytes (CD3), B-lymphocytes (CD19) and myeloid cells (CD33).
Results: Several factors significantly associated with the likelihood of stable MC development were identified by univariate analysis, e.g. younger donor age, sibling donor and conditioning regimen. Despite a limited patient cohort, our multivariate analysis could confirm that a sibling donor was associated with stable MC development. Furthermore, development of acute-graft-vs.-host disease (aGvHD) and blood stream infection (BSI) was significantly more prevalent in the DC patient group. Additionally, significant fluctuations in the recipient:donor chimerism ratio decreased over time after HSCT in MC patients.
Discussion: Sibling donor cells are a closer HLA and minor histocompatibility antigen match than unrelated donor cells and will thus be less prone to induce immune-mediated killing of recipient cells. Additionally, milder conditioning regimens may also stimulate MC development. Furthermore, MC was not linked to a worse prognosis, as both aGvHD and BSI development were more prevalent in the DC group.

P6.13.25
Changes in the TCR repertoire in type 1 diabetes patients after autologous hematopoietic stem cell transplantation

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Introduction and objective: Type I diabetes mellitus (T1D) is a autoimmune disease resulting from the immune-mediated destruction of β-cells. High dose immunosuppression followed by autologous hematopoietic stem cell transplantation (HDI/AHSCT) has emerged in last years as a therapeutic alternative for recent-diagnosed T1D patients. To address the immune mechanisms by which HDI/AHSCT induces remission in T1D patients, we evaluated the reconstitution of T cell repertoire diversity by the TCRBV CDR3 Spectratyping method in T1D patients treated with HDI/AHSCT. Methods: Peripheral blood mononuclear cells from healthy controls (N=5) and T1D patients (N=16) at pre-transplantation and at various time points after transplantation were isolated by Ficoll-Hypaque. Total RNA was isolated and used to analyze the T cell receptor Vβ repertoire by the TCRBV CDR3 Spectratyping method. Results: The overexpression of 4 Vβ families (Vβ 5, 6, 13, 22) was observed in T1D patients before AHSCT. However, in some cases, these overexpressed families were found in high frequency after transplantation. There were changes in the composition of the T cell repertoire after transplantation, evidenced by qualitative and quantitative alterations in the CDR3 peaks and in the frequency of the 24 Vβ families analyzed. Furthermore, it was identified four patterns of repertoire reconstitution after transplantation and the pattern that consisted of reconstitution of diversity from a normal diverse repertoire was the most dominant in the T1D patients analyzed. Conclusions: Our results suggest that the HDI/AHSCT induced changes in the composition of the TCR repertoire that might explain the induction of insulin-independency in the T1D patients.

P6.13.26
Analysis of the circulating Th1/Th17 lymphocyte repertoire after hematopoietic stem cell transplant may reveal opportunistic fungal infections rather than GvHD reaction

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Human Th17 lymphocytes have been suggested to play a role in the pathogenesis of Graft versus Host Disease (GvHD). However, their actual involvement has not been clarified so far. We analysed the T cell recovery in the peripheral blood of 30 paediatric patients at different time intervals after allogeneic hematopoietic stem cell transplantation (HSCT).
We investigated the Th1 (CD4+CD161-CXCR3+CCR6-), Th1/Th17 (CD4+CD161+CXCR3+CCR6+) and Th17 (CD4+CD161+CXCR3-CCR6+) cell subsets repertoire in relation with the GVHD onset, the occurrence of opportunistic infections and/or viral reactivation. In the first 90 days after HSCT, we found no significant difference between patients developing or not acute GVHD. On the other hand, patients affected by chronic GVHD display a sharp reduction of circulating CD4+ lymphocytes. In addition, they were significantly enriched in CD4+CD161+. Analysis of cytokine production revealed that chronic GVHD patients display an increased production of IFN-gamma and IL-17. In patients with acute GVHD, increases of IFN-gamma and IL-17 production were statistically significant, however, no correlation could be found between surface phenotype and cytokine production. On the other hand, in four patients a clear correlation could be observed between the onset of Candida infection and increases of circulating IL-17-producing Th17 cells. Th17 increases were transient and paralleled the course of infection.
Our data suggest that chronic GvHD might involve Th1/Th17 cells. More importantly, they show that monitoring Th17 cell populations may provide an useful information on the onset and the severity of opportunistic fungal infections.

P6.13.27
Nonmitogenic form of acidic fibroblast growth factor protects against graft-versus-host disease without accelerating leukemia

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Acid fibroblast growth factor (aFGF) has been shown to prevent epithelial damage under various conditions, suggesting its potential to inhibit graft-versus-host disease (GVHD). However, because aFGF receptors are expressed on tumor cells, it may possibly offset the graft-vs.-tumor (GVT) effects of allogeneic bone marrow transplantation (allo-BMT). Here, we addressed these questions in a B6-B6D2F1 allo-BMT model. Although aFGF administration attenuated GVHD in non-leukemic recipients, aFGF treatment markedly accelerated death in mice that received recipient-type tumor (P815) cells along with allo-BMT. Similar protection against GVHD was also achieved by administration of a non-mitogenic form of aFGF (naFGF). Importantly, GVT effects were fully preserved in naFGF-treated recipients. Furthermore, aFGF, but not naFGF, significantly enhanced P815 cell proliferation in vitro. Our data indicate that the tumor-promoting, but not GVHD-protecting, effect of aFGF largely depends on its mitogenic activity, and suggest that naFGF may provide a safer approach to inhibiting GVHD in patients with malignancies.

P6.13.28
Post-Transplant Lymphocyte Reconstitution and Viral Reactivation in Paediatric Patients with Leukaemia

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Method: We retrospectively reviewed kinetics of lymphocyte reconstitution and viral reactivation in 48 patients with acute leukaemia after Bone Marrow Transplant (BMT) from 2005 through 2012. Lymphocyte subsets were evaluated at 30, 100, 200, 365 days after BMT and correlated with viral reactivation.
Results: Median age at BMT was 11.0 (range, 1.2 to 20.7) years for indications of acute lymphoblastic leukaemia (ALL) (n=23) and non-ALL (n=25). Pre-BMT cytomegalovirus (CMV) serostatus was donor-patient positive in 35% and donor negative-patient positive in 23%. Majority (71%) of patients received unrelated donor transplants. Stem cell sources were from bone marrow (BM, n=24), peripheral blood (PB, n=11) and cord blood (CB, n=13). HLA was 8/8 allele matched in 48% and > 2 mismatches in 19%. Acute graft versus host disease requiring treatment occurred in 58%. At 100 days post-BMT, 13% of CB transplant patients achieved CD4>200 vs. 28% of BM and 45% of PB patients (p=0.27). CD8 reconstitution was of similar trend. At 1 year post-BMT, 95% achieved CD4>200. Viral reactivation occurred in 48%. Viral associated mortality was 4% and occurred in CB patients within 200 days after BMT; with 1 death each from adenovirus and CMV.
Conclusion: In our cohort, viral reactivation correlated with recipient CMV seropositivity (p=0.02) and delayed CD4 reconstitution while outcome was influenced by the source of stem cells reflecting the interplay of donor-derived immune reconstitution and post-BMT immunosuppression.

P6.13.29
Human T cell progenitors generated in vitro from stem cells facilitate thymus engraftment

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The thymus provides the necessary environmental cues for the differentiation and generation of T-cells. The thymus is continuously colonized by a rare subset of bone-marrow derived progenitors, which

in humans are characterized as CD34+CD45RA+CD7+ cells. Further characterization and directed expansion of this important progenitor subset would represent an important step in developing T-cell and thymus regenerative approaches. Here we show the induced generation of human progenitor T-cells (pro-T) with a thymus-colonizing phenotype from umbilical cord-blood CD34+CD38-/lo hematopoietic stem cells (HSCs) cocultured on OP9-DL1 stromal cells. To determine whether pro-T cells generated in vitro from human HSCs possess an intrinsic ability to home, engraft and reconstitute a thymus in vivo, sorted CD34+ CD7++ pro-T cells were injected into immunodeficient mouse strains, which can support human multilineage differentiation from CD34+ HSCs. Our results show high levels of human chimerism in the thymus of immunodeficient mice. Furthermore, our findings showed that T-lineage progenitors generated in vitro from HSCs exhibit key properties of being able to home to, settle, and effectively reconstitute the thymus of immunodeficient mice. Additionally, in vitro-generated pro-T cells, when transferred together with purified HSCs, were able to dramatically enhance the thymus reconstituting ability of HSC-derived progenitors in vivo. Taken together, the generation of humanized mice reconstituted with in vitro derived progenitor T-subsets offers a new means of therapeutic evaluation and the potential to rapidly restore the T-cell compartment for the treatment of immunodeficiencies.

P6.14 Immunosuppressive and tolerogenic treatments

P6.14.01
Hsp70 inhibits the direct allorecognition, downregulating MHC class II through IL-10 driven MARCH-I expression via TLR2-ERK-STAT3 pathway

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The Hsp70 has a protective and anti-inflammatory role in several animal models of inflammation. We have previously demonstrated that incubation of skin allografts with Hsp70 can delay acute rejection and improve graft survival in mice. However, the pathways involved in this phenomenon have yet to be elucidated. We performed a skin allograft model which allografts from B6 mice were immersed in a solution containing OVA or Hsp70, and then grafted on BALB/c mice. Treatment with Hsp70 led to decreased expression of MHC II in donor DC that migrated to the host DLN. This MHC II downregulation was mainly on donor CD103+ DCs subset. Also, a diminished T CD4+ and CD8+ proliferation in host DLN was observed. Next, we determined that Hsp70 can induce both IL-10 mRNA and protein expression on mature DCs. IL-10 production and MHC II downregulation were dependent on TLR2-ERK-STAT3. We verified that donor expression of TLR2 and IL-10 were required for inhibition of acute rejection induced by Hsp70. Next, we elucidated which mechanism triggered by IL-10 was leading DCs to MHC II downregulation. We observed that Hsp70-treated DCs had a increase in MARCH-I mRNA expression related with untreated DCs. Hsp70-driven MARCH-I expression was dependent on TLR2-ERK-STAT3 pathway. Finally, IL-10 KO DCs treated with Hsp70 had no MARCH-I expression and no MHC II downregulation compared with WT DCs. Altogether, our results indicated that Hsp70 induces IL-10 in DCs via TLR2-ERK-STAT3 pathway. This IL-10 production leads to MARCH-I expression and MHC II downregulation, inhibiting allograft rejection.
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P6.14.02
Glucosamine modulates T helper cells differentiation via the blockade of N-linked glycosylation pathway

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Glucosamine is an amino sugar that has been widely used as supplement for patients with osteoarthritis. Previous studies have demonstrated that it can attenuate acute experimental autoimmune encephalomyelitis by inducing the secretion of Th2 cytokines and improve atopic dermatitis-like skin lesions by inhibition of Th2 development.
To evaluate the effect of glucosamine on effector T helper cell differentiation, we sorted and cultured naïve CD4 T cell from NOD mice on different T helper cell culture condition for 3 days with glucosamine (5mM) treatment. Glucosamine strikingly impeded the Th1, Th2 and iTreg cell differentiation but promoted Th17 cells differentiation. Furthermore, we found that glucosamine slightly attenuated STAT3, 4, and 6 phosphorylation, but significantly inhibited STAT5 phosphorylation during different T helper cell differentiation. We also found that the surface expression of IL-2 receptor subunit α (CD25) on T helper cells was significantly inhibited, but IL-2Rβ and γc were minimally affected by glucosamine treatment. However, we found that glucosamine blocks the N-linked glycosylation of CD25 without affecting CD25 gene expression. Therefore, our data suggested that N-linked glycosylation is required for CD25 surface expression to regulate IL-2 signaling pathways to modulate T cell differentiation. Interestingly, we found that 2DG and 2FDG (glycolytic pathway inhibitors) have similar effect to modulate T helper cell differentiation through blockade of N-linked glycosylation of CD25. Our data suggested that glucosamine might modulate T helper cell differentiation via blockade of the N-glycosylation throguh limiting IL-2 signaling.

P6.14.03
Dextromethorphan modulates the immune function of dendritic cells

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Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) with a unique capacity to prime naïve T cells and connect innate to adaptive immunity. Accordingly, DCs are considered as a major target in the development of immunomodulators. Detromethorpahn (DXM), a clinical medicine for cough-suppression with a high safety profile, has been reported to possess anti-inflammation and immunomodulatory activities. In this study, we examined the effect of DXM on the immune function of DCs. In a non-cytotoxic condition, DXM significantly reduced the maturation of mouse bone marrow derived DCs (BMDCs) after LPS stimulation. DXM also impaired the amounts of reactive oxygen species (ROS), pro-inflammatory cytokines, and chemokines produced by LPS-stimulated BMDCs. Furthermore, the treatment of DXM decreased the T cell activation induced by LPS-stimulated BMDCs, as determined by the reduction of proliferation and IFN-γ secretion in mixed lymphocyte reaction. In the molecular approach, blockage of LPS-induced MAPK activation, IκB degradation and the translocation of NF-κB could be involved in the suppressive effect of DXM on BMDCs activation. In addition to mouse DCs, we also confirmed the suppressive activity of DXM on the activation of human monocytes-derived dendritic cells (MoDCs). In conclusion, we have identified the immunomodulatory role of DXM in attenuating the function of DCs, which suggests that DXM may have potential in the treatment of DC-related autoimmune and chronic inflammatory diseases.

P6.14.04

Inhibition of human allogeneic skin graft inflammation by ex vivo expanded human Treg in a humanized PBMC-SCID/Skin allograft model

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Treg cell-therapy is of interest for therapeutic intervention in transplant rejection and autoimmunity. Although clinical Treg-therapy trials have started, the *in vivo* behavior of ex-vivo expanded human Treg is unclear. Therefore, we investigated the effect of ex-vivo expanded CD4+CD25+CD127low human Treg-cells on the inflammatory response of human skin allograft in a humanized-SCID mouse model. First, we demonstrated that ex-vivo expanded human Treg-cells maintained suppressive capacity *in vitro*. Next, these expanded Treg were studied *in vivo* in our humanized mouse model, in brief human skin-grafts were transplanted on immunodeficient SCID/beige mice, after healing mice were infused (i.p.) with allogeneic hu-PBMC with/without Treg-cells. After 3-weeks inflamed skin-grafts, spleen and peripheral blood were harvested and analyzed by histology and/or flowcytometry. Analysis of inflamed human skin-grafts revealed that Treg infusion restored the inflammation related aberrant K10/K16 epidermal marker expression and influx of hu-CD8 T-cells. The Infusion of Treg also affected the systemic response, they inhibited human CD4+ and CD8+ T cell activation and proliferation as indicated by reduced Ki67. We showed that under the conditions tested, ex-vivo expanded Treg-cells reduce but do not fully prevent hu-PBMC induced skin inflammation *in vivo*. The observed reduction of skin transplant inflammation by human Treg encourages the use of Treg-therapy in transplantation.

P6.14.05

An increase in B cell and cytotoxic NK cell proportions in peripheral blood of Natalizumab-treated patients with relapsing-remitting multiple sclerosis

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Background Multiple sclerosis is an autoimmune disease affecting the CNS, traditionally treated with immunomodulatory drugs, and the monoclonal anti-VLA-4 antibody natalizumab is the most effective drug currently in use. By VLA-4 binding, natalizumab supposedly prevents passage of lymphocytes across the blood-brain barrier
Materials and methods A panel of markers for blood lymphocyte populations was assessed by flow cytometry in 40 patients with relapsing MS before and after one-year natalizumab treatment. In addition, whole blood cultures were set up, and the CD4+ and CD8+ T-cell response to antigens and mitogens was assessed. For comparison of T-cell responsiveness, 20 healthy controls were included.
Results Absolute numbers of all investigated major populations of lymphocytes increased after treatment, most markedly for NK-cells ($p<0.001$) and B-cells ($p<0.001$). The fraction of both memory (CD27+, $p<0.0005$) and presumed regulatory B cells (CD25+, $p<0.005$) increased, as did CD3-CD56dim cytotoxic NK-cells ($p<0.005$), whereas CD3-CD56bright regulatory NK-cells decreased ($p<0.005$). The fraction of OX40L+ T-cells decreased ($p<0.005$). Responsiveness to recall antigens and mitogens was restored for both CD4+ (influenza $p<0.005$; PPD $p<0.005$) and CD8+ T-cells (PPD $p<0.01$; PWM $p<0.005$; CMV $p<0.01$).
Conclusions Our data confirm that natalizumab treatment has a pronounced effect on the peripheral blood lymphocyte composition. The expected main mode of action, reduction of lymphocyte extravasation, is likely not only to involve CNS trafficking, given the profound increase in lymphocyte numbers in blood and given the general expression of VLA-4/VCAM-1/MadCAM adhesion molecules. The intricate changes in proportions of subpopulations suggest that cell-signaling effects through VLA-4 may be operative.

P6.14.06

Refractory amyopathic dermatomyositis (CAMD) with interstitial lung disease (ILD) successfully treated with Rituximab

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Background: CADM is a unique subset of dermatomyositis (DM) with typical skin manifestations of DM but no evidence of myositis.
Herein, we describe a case of CAMD with interstitial lung involvement successfully treated with Rituximab.
Medical History and Presentation: A Caucasian, 60 y-old woman, was diagnosed with CAMD because of arthralgia, fever, heliotrope rash, Gottron's papules in the absence of muscular symptoms. Blood tests showed normal value of CPK, myoglobin and aldolase; hyperferritinemia, ANA and anti-SSA positivity; anti-Jo1 were negative. There was no evidence of malignancy at PET-CT and MRI-scans.
During steroid and cyclosporine association therapy patient developed dyspnea on moderate efforts. Chest HRCT documented diffuse sub-pleural interstitiopathy. Patient was then treated with pulses cyclophosphamide (500 mg/m^2 weeks, 4 administrations) without clinical improvement. Considering the risk of fatal ILD progression, we administered anti-CD20 monoclonal antibody Rituximab (375 mg/m^2, weekly, 6 courses) with gradually resolution of respiratory and systemic symptoms and disappearance of skin lesions. After 30 days, we started maintenance therapy with Rituximab (375 mg/m^2 monthly) and azathioprine; prednisone was successfully tapered. Patient condition is currently good and there are no signs of CADM and ILD recurrence.
Conclusion CADM is a rare condition at risk for progressive fatal ILD, often resistant to conventional immunosuppressant therapy. Some reports described the efficacy of B-cell depleting treatment in refractory cases of DM, overlap myositis and ILD associated with immune disorders. Rituximab could be a well-tolerated and safe option for induction and maintenance therapy in resistant cases of CADM with or without ILD.

P6.14.07

mTOR inhibitors therapy after liver transplantation allows a sustained increase in regulatory T cells while preserving their suppressive capacity

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The mammalian targets of rapamycin (mTOR) inhibitors (sirolimus [SRL] and everolimus [EVR]) are used in liver transplantation for their immunosuppressive activity. Evidence indicates that CD4+CD25+CD127-FoxP3+ regulatory T cells (Tregs) have a crucial role in immune tolerance. Furthermore, mTOR inhibitors have been demonstrated to preserve Tregs and in contrast to Tacrolimus (Tac).
The aim of this study was to evaluate Tregs number and function, in liver transplanted recipients before and after conversion from Tac to mTOR inhibitors.
Twelve patients with stable graft function where converted from Tac to SRL (n=5) or EVR (n=7). We prospectively analysed, at day 0, 30 and 90 after conversion, using flow cytometry, Treg population (CD4+CD25+FoxP3+CD127-) on blood cells and then performed a functional assay to test Treg ability to suppress CD4+ Tcell activity.
mTOR inhibitors were well tolerated, no acute rejection was observed. All patients displayed sustained rise in Treg levels after the introduction of mTOR inhibitors, in the two groups, SRL or EVE (mean peak Treg level at 3 months: 6,18±1.17% of CD4 T cells, vs a mean baseline level of 3.49±1,06%, p=0.0004; mean fold increase=2,01±0.63); for SRL group: mean peak Treg level: 6.01±0.92 vs 3.79±0.94, p=0.019, and in the EVE group: 6.23±1,31 vs 3.24±1,106, P=0.0053. Moreover, Tregs preserved their functional ability to suppress activated CD4+ T cells.
Our results suggest that mTOR inhibitors introduction after liver transplantation induced sustain and significant increase in Tregs, and

the suppressive capability of these cells was maintained. We thus confirmed their potential role in graft tolerance.

P6.14.08
Cyclophosphane effect on peripheral blood leukocytes and organs of immune system in experimental animals

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The aim of investigation was the analysis of cyclophosphane (CP) effect on the immune system organs, on peripheral blood leukocytes in experimental animals.
The study was conducted in 20 male rats (284±20 g). CP was administered intraperitoneally at a concentration of 100 mg/kg of body weight, 4 fold with 24h-interval. Blood samples were taken from the tail vein to determine the total leukocyte count and leukocyte formula. Pathomorphological changes in bone marrow, thymus, spleen and lymph nodes were analyzed through 48 h after final CP injection.
It was shown that leukocyte number reduced by 50% on the 2nd day after the first CP injection, and by 99% - in subsequent two days. After the final CP injection the recovery of initial leukocyte number was not detected. Following the first CP introduction there was noted statistically significant increase in segmented neutrophils that were sharply declined after the 2nd cytostatic injection and were not further recovered. The amount of monocytes and lymphocytes was lowered 24 h after the first injection of cyclophosphane and subsequently continued to reduce. The alteration in the functional activity was determined in phagocytic cells. The statistically significant reduction in thymus, spleen and lymph node weight, was detected, as well as degenerative changes therein and decrease nucleated cells in bone marrow.
Thus as a result of investigation the development of induced immunosuppression was revealed that involved the lowering in blood leukocyte count, alteration of their composition and functional activity, progression of degenerative changes in the organs of immune system.

P6.14.09
Influence of immunosuppressive drugs on regulatory T cells

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Background and aims: Regulatory T-cells (Tregs) can promote tolerogenic immune responses after liver transplantation. But liver transplant recipients also receive a variety of immunosuppressive drugs to avoid rejection. The aim of this study was to assess the effect of immunosuppressive drugs on the Treg population.
Methods: Peripheral blood mononuclear cells from healthy donors were incubated with immunosuppressive drugs (Cyclosporin A (CsA), Tacrolimus, MMF, Everolimus, Prednisolon) in different concentrations over various time points. Phenotyping of these cells was performed using flow cytometric analysis. Cytokines in cell culture supernatant were analysed using Cytometric Bead Array (CBA). Suppression assays after incubation of Tregs with CsA were performed.
Results: FoxP3 and CTLA-4 expression on Tregs decreased after 48 hours of incubation with CsA in a dose dependent manner, but not with any other drug. TGFb expression showed no change. Incubation with Tacrolimus, another Calcineurin inhibitor, did not lead to any distinct phenotypic change. Pre-incubation of Tregs with CsA did not inhibit the suppressive function of Tregs but improved it. Additionally, production of MIP1a and MIP1b was reduced.
Conclusions: Cyclosporin A, but none of the other immunosuppressive drugs, induced consistent phenotypical alteration of Tregs by decreasing FoxP3 and CTLA-4, but enhanced Treg suppressive function. Our results suggest that CsA could improve Tregs inhibitory function being additionally beneficial in avoiding graft rejection.

P6.14.10
Oral tolerance improves transgene persistence in gene therapy setting

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Gene therapy represents a promising strategy to treat monogenic diseases by providing a functional copy of an otherwise defective gene. This can be efficiently achieved in vivo using viral vectors such as recombinant Adeno-associated Viruses (rAAV). It is however well documented that immune responses can lead to destruction of transduced cells thereby limiting long-term transgene expression. Immunosuppressive treatments may be used to improve therapeutic efficacy but they are unspecific and may cause side effects. Here, we evaluated a strategy to inhibit antigen-specific immune activation using a protocol of oral tolerization.
We showed that intramuscular injection of rAAV2/1-Ova leads, as expected, to robust immune responses. Indeed, 14 days after Ova-transgene transfer we detected conspicuous anti Ova IgG humoral response and about 15% CD8+ T cell direct against the OVA-derived immunodominant peptide. Kinetic studies showed undetectable levels of Ova in serum after day 14 and absence of corresponding mRNAs in muscle at day 80. In striking contrast, Oral tolerization for 7 consecutive days completely abolished specific cellular and humoral immune responses and allowed long-term persistence and expression of Ova-transgene. This further correlates with the inability of lymphocytes from tolerized animals to reject Ova-bearing tumors. Mechanistically, tolerance was not exclusively mediated by Tregs as their depletion prior to rAAV2/1-Ova injection did not restore immune responses. Importantly, induction of tolerance by antigen feeding was efficient only in naïve but not in primed mice suggesting that this protocol may be suited to improve tolerance in gene therapy settings but not in autoimmune diseases.

P6.14.11
Targeting PKC in human T cell using sotrastaurin (AEB071) stabilizes regulatory T cells and prevents IL-17 production

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Regulatory T-cells (Treg) are crucial for immune homeostasis and prevention of immune pathology. Yet, Treg may lose Foxp3 expression and start secreting IL-17, dependent on environmental cues. Our previous data revealed that Treg from severe psoriasis patients are particularly prone to such conversion. Thus, the question of how to maintain Treg stability in the context of inflammation awaits immediate resolution. Recently, the pan-PKC inhibitor Sotrastaurin was successfully used in clinical trials of psoriasis. Here, we show that Sotrastaurin inhibited effector T cell responses, whereas the regulatory response was enhanced. Sotrastaurin prevented TCR/CD28-induced conventional T cell (Tconv) activation characterized by pro-inflammatory cytokine production and expression of activation markers. In contrast, Sotrastaurin preserved a stable Treg phenotype as evidenced by maintenance of suppressive capacity, high Foxp3/CD25 expression, and lack of IL-17A and IFNγ production, even when stimulated with the Th17-enhancing cytokine IL-1β. Moreover, ex vivo incubation of CD4+ T cells from psoriasis patients with Sotrastaurin enhanced Foxp3 expression and prevented IL-17A and IFNγ production. In psoriatic Treg, Foxp3 expression was maintained even when stimulated with IL-1β. Thus, pharmacological inhibition of PKC may serve as a powerful tool to concurrently inhibit effector T cells and to facilitate Treg.

P6.14.12
Searching for an adenosinergic loop in immune privileged human corneal tissues

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Corneal cells are epithelial stem-derived cells with immunoregulatory features. The aim of the present study was to demonstrate that corneal cells are endowed with the ability to produce an immunosuppressive soluble factor that modulate a number of physiological processes (e.g., signal transduction, immunological response, proliferation, wound healing, among the others).
The results obtained indicate that cells isolated from the human limbus, a compartment characterized as a niche for a corneal SC population, coexpress surface CD38 (NADase), CD39 (diphosphohydrolase, NTPDase), CD203a (pyrophosphatase, PC-1) and CD73 (5'-nucleotidase). These molecules are functionally connected and metabolize ATP and NAD^+ for the generation of the nucleoside adenosine (ADO), a powerful pleiotropic molecule involved in the modulation of immunity and inflammation. The ectoenzymes shaping the pathway have been analyzed by cell cytometry, and the signals implemented were confirmed by molecular cell profiling. The enzymatic activities were essayed by *ad hoc* constructed HPLC assays.
The results indicate that corneal limbal cells express CD38, CD39 and CD203a and metabolize extracellular adenine nucleotides, originating AMP. In sequence, this is converted to ADO by CD73, present in the corneal crypts.
These results demonstrate that a so far unreported ectoenzymatic signaling pathway(s) constitute an adenosinergic axis, which exerts key roles in corneal physiology.

P6.14.13
Immunomodulatory properties of new conjugates muramyldipeptides derivatives with adenosine

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Muramyl dipeptide (MurNAc-L-Ala-D-isoGln; MDP) is the minimal biological structure of bacterial peptidoglycan with adjuvant activity[1]. MDP acts through intracellular NOD2 receptor expressed in immune cells [2]. Modifications of MDP structure gave derivatives with immunomodulative, antiviral or antitumor activity [3]. Interestingly, analogues were D-alanine was replaced with L-amino acid exerted immunosuppressive action. Adenosine is a purine nucleoside, an important homeostatic modulator in all cells. It acts through the four types of adenosine receptors: A1, A2A, A2B and A3, belonging to the G-protein-coupled receptor family. A2A receptors are expressed ubiquitously in the body, but they can be found mainly in the immune system [4].
We synthesized a series of muramyl dipeptide and nor-muramyl dipeptide derivatives conjugated with adenosine. MDP were modified in a peptide part, where L-Ala was replaced with D-amino acid (D-Ala, D-Ser, D-Val, D-Pro). Conjugates were evaluated as inhibitors of a leukemia cell line Jurkat and human peripheral blood mononuclear cells from healthy donors.
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P6.14.14
New potent IMPDH inhibitors

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Organ transplantation is a well-established method for the therapy of end-stage organ failure, increasing life expectancy and improving quality of life. The long-term outcome of these patients is now challenged by life-threatening complications such as cardiovascular disease, infections and post-transplant malignancies [1]. In modern transplantology inosine-5'-monophosphate dehydrogenase (IMPDH) is a major therapeutic target.
Mycophenolic acid (MPA), an inhibitor of IMPDH, reduces the availability of guanine nucleotides, especially GTP. The morpholinoethyl ester of MPA, mycophenolate mofetil (MMF), has potent immunosuppressive properties and is used worldwide in transplantation medicine to prevent organ rejection [2].
Although nomerous compounds were received and examinated as potentantial immunosuppressants, the risk of graft rejection has been not eliminated so far.
We continue our project concerning amino acid derivatives of mycophenolic acid. First, mycophenolic acid was coupled with amino acid esters via amide bond formation. At this stage we compared antiproliferation activity of compounds bearing methyl ester group with bulky benzyl esters. Secondly, we investigated influence of the additional polar group in amino acid moiety on cytotoxic activity of target compounds. Thus, mycophenolic acid was coupled with threonine, arginine methyl esters followed by hydrolysis to products having free carboxylic group. Their biological activity was evaluated against lymphoid cell line Jurkat and activated peripheral blood mononuclear cells (PBMC) as in vitro model of immunosuppression. The obtained results will be presented.
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P6.14.15
The response to anti-TNF ab therapy is variable in patients with psoriasis

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Psoriasis is a proliferative epidermal disorder with immunological background. While some monoclonal antibody therapies against immune mediators are available for the patients, efficacy of the therapy is highly variable. We describe the diversity of anti-TNF ab effects against the psoriasis. Seven psoriasis patients were treated by anti-TNF ab. The therapy was effective for 5 patients, while it was not effective for 1 patient. One patient developed pneumocystis carinii pneumonia after the therapy. The TNF resistant was successfully treated by IL23 blocker. There was no sign or specific clinical findings in this patient when compared to other patients who were improved by TNF therapy. Although it was unclear in this study, the identification of genetic and/or disease variants has the potential to improve the selection of the drug. A suitable approach in implementing such a strategy could potentially reduce medical costs and improve success of drug therapy. Since ~20% of patients do not respond to TNF therapy, we would focus on these patients and TNF related molecules. Further, the evaluation will include experimental/preclinical studies, using patient own skin/immune cells and candidate drugs. Thus, personalized medicine will mandate special physicians capable of understanding molecular medicine in addition to genetics and biomarkers for diagnosis, evaluation of the effect of drugs, and overall prognosis.

P6.14.16
Interleukin-35 mediated supression on neutrophilic airway inflammation with the IL-17/IFN-γ dominant phenotype

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Interleukin (IL)-35 is a heterodimeric cytokine that is composed of Epstein-Barr virus-induced gene (EBI) 3 and IL-12p35 subunit. IL-35 is a member of the IL-12 family cytokines and has been found to have potent
immuno-supressive activities. In this study, we delivered a major dust mite allergen, Blo t 5, intranasally into Blo t 5-specific T cell receptor transgenic mice and found an neutrophil and lymphocyte dominant airway inflammation phenotype with upregulated IL-17 and IFN-γ. By pulmonary delivery of a construct expressing fusion EBI3 and IL12p35 subunit, we examined the effectiveness of pVAX1-IL-35 on the modulation of the neutrophilic and IL-17/IFN-γ dominant airway inflammation in the experimental model. Intra-tracheal administration of pVAX1-IL-35 was found to effectively reduce the infiltration of neutrophils, lymphocytes and total cell numbers in bronchoalveolar lavage fluid after intranasal Blo t 5 instillation. In addition, pro-inflammatory cytokines and chemotactic chemokines such as IL-17, IFN-γ, IL-1α, IL-6, LIF, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, KC/CXCL1, MIG/CXCL9 and IP-10/CXCL10 were significantly attenuated in bronchoalveolar lavage fluid from mice in the experimental pVAX1-IL-35 group as compared to that from the control pVAX1 group. These data suggested that IL-35 could be a promising therapeutic target in controling neutrophilic and IL-17/IFN-γ dominant airway inflammation.

P6.14.17
Immunohistochemical characterization of leukocyte infiltrates in minimally invasive gastric cancer

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The aim of investigation was to study the immunohistochemical characteristics of leukocyte infiltrates of gastric mucosa in minimally invasive cancers.
To visualize and make photography of micropreparations of gastric biopsies from 14 patients with adenocarcinoma and mucinous carcinoma the Scope Photo program was used. The analysis of images was realized via ImageJ program. To reveal T-lymphocyte (CD3, CD8) and B-lymphocyte (CD20) marker expression the avidin-biotin method was applied (Novocastra, Switzerland) according to C.R. Taylor, R. Cote. [1994] and L.A. Sternberger [1979] procedures. Calculation of positively- and negatively-stained cells was made in square closed test-system. Statistical data processing was implemented with Biostat 5 program.
During the study of cell composition in leukocyte infiltrates of gastric mucosa affected with tumor process while using the hematoxylin- and eosin-stained preparations it was revealed that lymphocytes, plasmocytes and macrophages prevailed therein. The density of CD3-positive cells was nearly similar in focal and diffuse leukocyte accumulations (44,9 and 45,4%, respectively). Meanwhile, among T-lymphocytes the number of $CD8^+$ cells significantly differed between 1-type and 2-type infiltrates (38,1 and 50%, respectively). In contrast, B-lymphocyte ($CD20^+$) concentration was significantly higher in focal infiltrates as compared with diffuse ones (80,9 and 69,6%, respectively).
Thus, results obtained demonstrate significant differences in T- and B- lymphocyte subset distribution within the focal and diffuse leukocyte infiltrates of gastric mucosa in minimally invasive cancers. These data could be valuable under the development of immunocorrection methods for malignancy biotherapies.
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P6.14.18
Influence of Stimforte on the immunologic characteristics of mice against induced immunosuppression

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The aim of study was the analysis of Stimforte effect on immunological parameters in mice against induced immunosuppression. Investigation was carried out with 80 BALB/c mice there were injected either with cyclophosphane (CP) and Stimforte in different combinations or with physiological solution (control group). The immunophenotype of mononuclear leukocytes (ML) isolated from animal spleen and killer activity against YAK-1 tumor line were defined. Morphological investigations of Romanosvky-Giemsa-stained smears were administrated. CP administered (100 mg/kg) within 3 days evoked immunosuppression that was manifested in nearly 7-fold lowering of ML number in mice spleen as compared with control group. ML population in CP-received animal group was represented by mature lymphocytes, whereas control group demonstrated considerable amount of young lymphoid cells in spleen. Animals against CP introduction exhibited significant decrease in natural killer (NK) and pronounced reduction in their NK-activity. Stimforte injection against CP-induced immunosuppression increased more than by 2-fold the ML content in mice spleen, when immature forms such as pro-lymphocytes were detected. In addition, Stimforte favored the normalization of NK content in spleen ML and their NK activity increment nearly to the initial values. Peripheral blood of animals under study also demonstrated the increase in granulocyte number. Thus, CP resulted in marked immunosuppression manifesting in lowering in total lymphocyte amount in mice peripheral blood and spleen, as well as declining in NK content in lymphocyte population as compared with intact animals. Stimforte administration against cytostatic-induced immunosuppression resulted in significant increase in lymphocyte number and normalization of their immunophenotype and function characteristics.

P6.14.19
Triptolide ameliorates autoimmune diabetes and prolongs islet graft survival in nonobese diabetic mice

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Triptolide (TPL) possesses profound immunosuppressive effects and has potential in allograft transplantation. We investigated whether TPL treatment prevents autoimmune diabetes in nonobese diabetic (NOD) mice and prolongs the survival of islet grafts against autoimmune attack or allograft rejection. In this study, diabetic incidence was monitored in TPL-treated NOD mice. NOD or BALB/c islets were transplanted into diabetic recipients treated with TPL. Different T cell subsets in grafts or spleen were analyzed. The proliferation, apoptosis, cytokines and activities of AKT, NFκB, caspases 3, 8 and 9 of T cells were determined. Our results revealed that diabetic incidence was reduced and inflammatory cytokines were decreased in islets and spleen under TPL treatment. T cell proliferation was reduced and the survival of syngeneic or allogeneic grafts was significantly increased in TPL-treated mice. The populations of CD4, CD8, CD4CD69, CD8DC69 and IFN-γ-producing T cells in islet grafts and spleen were reduced. TPL treatment increased the apoptosis of T cells in the spleen of recipients. Levels of p-AKT and p-IκB in splenocytes were reduced and caspase-3, -8 and -9 were increased in TPL-treated mice. In conclusion, TPL treatment not only reduced the diabetic incidence in NOD mice but also prolonged the survival of syngeneic or allogeneic grafts.

P6.14.20

Highly suppressive and non-toxic Trichosanthin-derived peptide induces skin transplantation tolerance via expanding $CD4^+CD25^+Foxp3^+$ Tregs

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Trichosanthin (Tk), a component isolated from a Chinese medicinal herb *Trichosanthes kirilowii,* is active in abortifacient, anti-tumor, anti-HIV, as well as functions as an immune down-regulator. To separate its toxic property, a Tk-derived peptide Tk-PQ, containing forty C-terminal amino acids, was synthesized. We found that 10~50 μg/ml of Tk-PQ inhibited cell proliferation in a dose-dependent way without cytotoxicity as compared with native Tk. In the Tk-PQ-treated allogeneic mixed lymphocyte reaction, there were an increased production of IL-10/TGF-β and a decreased secretion of IFN-γ/IL-17A. Moreover, Tk-PQ promoted the activation of $CD4^+CD25^+Foxp3^+$ Treg subset and remarkably increased the expression of interferon-regulatory factor 4 (IRF4). Pearson correlation analysis shows a significant positive correlation of IRF4 and Foxp3 expression (r=0.99; p=0.0003, n=5). Using murine full-thickness trunk skin allo-grafting model, we were able to show that Tk-PQ remarkably increased the survival of the allografts in the recipients from 9.0±1.1 days (control) to 22.2±2.8 days. Histological analysis of the allografts on day 9 post transplantation showed that the Tk-PQ treatment reduced the infiltration of mononuclear cells. The in vivo ratio of $CD4^+CD25^+Foxp3^+$ Tregs was expanded in both spleen and peripheral blood of the recipients. Intracellular staining indicated that the percentages of IL-10^+ cells were also increased in splenocytes and the $CD4^+CD25^+Foxp3^+$ Treg population, while the IFN-γ^+ cell percentages were decreased. Furthermore, Tk-PQ inhibited the phosphorylation of IκBα and the nuclear translocation of NF-κB. These results indicate that Tk-PQ may have potential therapeutic values for the delay of graft rejection and the treatment of other inflammatory diseases.

P6.14.21

Effects of cyclosporine A and rapamycin on the ability of dendritic cells to activate T cells

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Introduction: The most important antigen-presenting cells are dendritic cells (DCs), which present antigen to T cells. The state of maturation of DCs is crucial for induction of a T-cell lymphocyte response. It was noted that immature DCs play an important role in peripheral tolerance, whereas mature DCs induce immune response.
We have studied the effect of immunosuppressive agents: rapamycin and cyclosporine A on dendritic cells cytokine production and the ability to induce the proliferative T cells activity.
Methods: Human peripheral blood monocytes were induced by using cytokines: IL-4 and GM-CSF, in the direction of DCs in the presence of rapamycin (Rapa-DCs) and cyclosporine A (CsA-DCs) or without drugs. Then these immature DCs were stimulated with LPS to create mature DCs. The supernatants have been collected and measurements of DC pro-inflammatory cytokine (IL-6, IL-12) levels were performed by ELISA. Mature DCs were then incubated with T cells in mixed leukocyte reaction and the proliferative T cells activity was determined by measuring the fluorescence intensity of CFSE dye using flow cytometry.
Results: We have noted that the percentage of proliferating T cells do not change significantly after incubation with Rapa-DCs and CsA-DCs. We have also observed a diminished production of IL-6 by immature DCs and LPS-activated DCs, when these cells were differentiated in the presence of immunosuppressive agents. Production of IL-12 was notably undetectable in all culture conditions.
Conclusions: We have shown that the immunosuppressive agents: rapamycin and cyclosporine A do not change the ability of dendritic cells to activate T cells.

P6.14.22

Mir-142-3p target sequences reduce transgene directed immunogenicity following intramuscular AAV vector-mediated gene delivery

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Appearance of immune responses against the transgene product after adeno-associated virus (AAV) vector-based gene therapy is one of the concerns that limit efficacy of the treatment. Use of microRNAs (miRNAs) that are specifically expressed in antigen-presenting cells (APCs) is a promising approach to avoid those immune responses. Cellular mir-142-3p, which is APC-specific, is able to repress translation of its target cellular transcripts by binding to a specific target sequences.
We explored the potential of mir-142-3p specific target sequences to reduce or abolish immune responses directed against ovalbumin (OVA), a highly immunogenic protein, expressed as transgene and delivered by AAV vector administered intramuscularly.
The occurrence of humoral and cellular immune responses against OVA transgene following intramuscular delivery by AAV have been previously described and resulted in loss of OVA protein expression. In our study we demonstrate that OVA protein expression was maintained when mir-142-3pT sequences were incorporated in the expression cassette. The sustained expression of OVA protein over time correlated with a reduced increase in anti-OVA antibody level. Furthermore, no cellular infiltrates were observed in the muscle tissue when AAV1 vectors containing 4 or 8 repeats of mir-142-3p target sequences after OVA sequence were used.
Our data provides the evidence that humoral and cellular immune responses against OVA protein after intramuscular delivery can be efficiently reduced by use of mir-142-3p target sequences. Overall, this study identifies a promising approach for gene therapy applications as it could be applied as a “safety lock” for any intramuscular AAV vector based therapeutic gene delivery.

P6.14.23

Induction of antigen-specific regulatory T cells as a therapy for autoimmune diseases

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Regulatory T (Treg) cells play a fundamental role in maintaining peripheral tolerance to self-antigens. Defect in Treg cells can result in uncontrolled effector T cell responses to autoantigens and development of autoimmune disorders. Conversely, approaches that enhance the generation of autoantigen-specific Treg cells have potential to prevent or treat of autoimmune disease. The induction of Treg cells is enhanced by anti-inflammatory cytokines, with IL-10 and IL-27 promoting the generation of IL-10 secreting Tr1-type Treg cells and TGF-β with retinoic acid inducing peripheral conversion of naïve T cells into conventional $CD4^+CD25^+Foxp3^+$ Tregs. Finally, certain immunomodulatory molecules form bacteria or helminths can activate dendritic cells (DCs) to promote induction of Treg cells *in vivo.* The aim of this study was to examine the capacity of IL-10, TGF-β or IL-27 or pathogen-derived molecules that induce IL-10 and TGF-β and suppress IL-12 from DCs to act as adjuvants to promote the induction of Treg cells against self-antigen and thereby prevent or reduce the clinical course of an autoimmune disease in a mouse model. The results to date have shown that TGF-β enhanced survival of $CD4^+$ T cells and upregulated expression of Foxp3, which was enhanced by IL-10 and IL-27. *In vivo* studies demonstrated that a low molecular weight fraction from the helminth, *Fasciola hepatica*, enhanced the frequency of $Foxp3^+$ Treg cells, whereas supernatant from homogenate of liver fluke enhanced IL-10-secreting T cells. These helminth-derived fractions are currently being tested as adjuvants to induce Treg cells against autoantigen, MOG, and to prevent experimental autoimmune encephalomyelitis.

P6.14.24
PD-1 dependent exhaustion of autoaggressive effector T cells following tolerogenic peptide administration

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The clinical requirement for antigen-based therapy is to target and switch-off the activated antigen-experienced T cells involved in ongoing disease pathogenesis. Various experimental models of both autoimmune and allergic disease have demonstrated that prophylactic administration of soluble auto-antigenic peptides can be effective at inducing tolerance in naïve antigen-reactive T cells and protecting from subsequent disease induction. However, antigen-experienced T cells possess distinct activation requirements to those of naïve T cells and there exists the potential for soluble peptide administration to enhance activation of effector T cells and exacerbate ongoing disease. Using traceable TCR transgenic T cells in a model of experimental autoimmune encephalomyelitis (EAE) we demonstrate that tolerogenic peptide administration can result in a substantial expansion of antigen-experienced effector T cells (Teff). Despite the considerable increase in the number of antigen-reactive Teff cells, these cells were no longer pathogenic and were unable to drive CNS autoimmune disease. Phenotypic analysis of the Teff cells following infusion of soluble peptide revealed a lack of IL-2 and effector cytokine production and high level expression of the co-inhibitory molecule PD-1. PD-1 expression was maintained for at least 16 days following peptide treatment and was required for the induction of tolerance in Teff cells, as PD-1 deficient T cells retained the ability to induce EAE following peptide treatment. Furthermore, epigenetic modifications were detected within the PD-1 promoter of Teff cells post-peptide administration, which may provide a potential mechanism for the long-term expression of this co-inhibitory molecule and the enduring tolerant phenotype observed in these cells.

P6.14.25
Role of calcineurin/NFAT signalling in myeloid cell haematopoiesis and function

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Myeloid cell homeostasis, a complex process regulated by multiple factors, plays a major role in innate immune responses as these cells present the first line of defense by recognition and elimination of pathogens. We have recently reported a novel role for calcineurin/NFAT signalling as a negative regulator of myeloid haematopoiesis. Here we uncover the underlying mechanisms by which calcineurin/NFAT signalling regulates innate immune responses and the steady state renewal of myeloid cells and their progenitors. Our data provides evidence that specific inhibition of NFAT signalling during differentiation of bone marrow progenitors in vivo results in preferential expansion of myeloid cells as well as increased proliferation of granulocyte-macrophage precursors. This phenomenon can be explained through the interaction of NFAT with cell cycle-regulating genes as shown by global gene expression analysis of c-kit-enriched progenitors impaired in NFAT signaling by treatment with cyclosporine A or tacrolimus. Analysis of FACS-sorted individual progenitor subsets impaired in calcineurin/NFAT signaling confirmed changes in the expression of cell cycle-regulating genes. To address the role of NFAT signalling in vivo, different mouse models are used to study the frequency, differentiation and proliferation of myeloid progenitors during steady state and inflammatory conditions under treatment inhibiting calcineurin/NFAT signalling. Conditional deletion of calcineurin in mouse haematopoietic cells reveals which progenitor subsets are involved in the enhancement of myeloid development after calcineurin/NFAT inhibition. Our findings may have important clinical applications since immunosuppressive drugs that target the calcineurin/NFAT pathway (e.g. cyclosporine A) to inhibit T cell functions may also impact myeloid cell homeostasis.

P6.14.26
CD6-targeted therapy validation with Itolizumab in autoimmune diseases

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CD6 is a membrane glycoprotein implicated in lymphocyte activation and trafficking, through its membrane-proximal domain (Scavenger Receptor Cysteine-Rich, SRCR3) interaction with ALCAM (Activated Leukocyte Cell Adhesion Molecule, CD166). We found that CD6 synergizes lymphocyte costimulation in vitro, enhancing the intrinsic activity of the CD3 activation pathway and boosting the IL-2 sensitivity of recent activated cells, skewing the response to a pro-inflammatory commitment. Unexpectedly, generated CD6 membrane-distal domain 1 (SRCR1) specific monoclonal antibody (mAb) modulated in vitro CD6-ALCAM induced lymphocyte effector differentiation, without blocking their binding and, in vivo disease control without peripheral lymphocyte depletion in autoimmunity models. Itolizumab is an anti-human CD6 SRCR1 specific non-agonistic humanized IgG1 mAb. It recognizes with an intermediate affinity constant, a conserved conformational epitope independent of N-glycosylation. Itolizumab inhibits CD6-ALCAM mediated co-stimulation, reducing lymphocyte proliferation, pro-inflammatory cytokines production i.e.: IL-17A, IFNγ, IL-6, TNFα; intracellular activation pathways i.e.: AKT, MAPK, STAT3 and cell adhesion associated molecules. Dose-finding, single-blind, randomized studies were performed in active moderate-to-severe Psoriasis and active Rheumatoid Arthritis patients, administering Itolizumab as monotherapy, or in combination with Methotrexate in the latter. Itolizumab is poorly immunogenic and well-tolerated; mild adverse events were observed mainly associated to the first infusion, reducing afterwards. Itolizumab promotes a significant clinical response persisting after the last administration, without rebound effect or immunosuppression. These results have been validated in a randomized, double-blind, placebo-controlled, pivotal phase III clinical trial in Psoriasis. Current preclinical and clinical findings in more than 400 patients support CD6 as a novel therapeutic target for autoimmune immunopathology modulation.

P6.14.27
Erythrina abyssinica reduces astrocytosis in T.brucei chronic mouse model

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Human African trypanosomiasis is prevalent in Sub-sahara African countries that lie between 14° North and 29° south of the equator.Trypanosoma brucei gambesience occurs in West and Central Africa while Trypanosoma brucei rhodesience occurs in East and Southern Africa. In this region, close to 60 million people are at risk of infection. The neurological stage of the disease is characterized by neuroinflammation and 10% of patients treated with the recommended drug develop PTRE (Post treatment reactive encephalopathy) which is fatal. Our study aimed at screening medicinal plants used by local communities for potential activity in reducing these side effects. Erythrina abbysinica was selected based on its wide use by different communities in Kenya and other parts of Africa. We used histology, GFAP immunohistochemistry and electron microscopy (TEM and SEM) to study the pathogenesis and grading neuroinflammation.We also used SDS-PAGE electrophoresis to compare the protein profiles of the different test groups. Data was analyzed by one way ANOVA to compare difference between treatment groups. Results indicated water extract ameliorates neuroinflammation and conserves some high molecular weight proteins. AKNOWLEDGEMENT This work was supported by a fellowship grant from Carnegie Newyork through RISE-AFNNET.

P6.14.28

Intravenous immunoglobulins in the treatment of toxic epidermal necrolysis due to omeprazole in a patient with systemic lupus erythematosus

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Toxic epidermal necrolysis (TEN) may supervene during systemic lupus erythematosus (SLE). Omeprazole has been reported as a drug inducing TEN.
We present a 19-year-old girl with a history of discoid lupus erythematosus since the age of 12. She was treated with antimalarials and topical corticosteroids. She was in remission up to March 2012, when she developed fever, arthralgia and myalgia. On the basis of 6 ACR criteria (malar rash, photosensitivity, polyserositis, leucopenia, ANA, anti-dsDNA) the diagnosis of SLE was established. Two weeks after the commencement of systemic prednisone and omeprazole, numerous erythematous target lesions rapidly appeared on the face, trunk and extremities. Two days later, extensive erythema, widespread bullae and erosions of the lips, face, neck, trunk, oral and conjunctival mucosa developed, with positive Nikolsky sign, accompanied by high fever and malaise.
Except high ESR, routine laboratory analyses and renal function tests were normal. Cardiac echography showed pericardial effusion. Skin histology showed basal and suprabasal epidermal cell necrosis with dermo-epidermal separation. DIF test was negative. The diagnosis of TEN was made. SCORTEN was 2.
The patient received IVIg 2 g/kg, divided in two days. One week after starting the IVIg, erosions completely epithelized. The prednisone dose was gradually tapered and azathioprine was added.
According to our patient's history and the course of the disease, omeprazole was the most probable cause of TEN. IVIg was very efficacious in our patient.

P6.14.29

Salivary evaluation of pediatric patients with cancer, before and after antineoplastic treatment

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This study evaluated the salivary biochemical and immunological status of children with cancer undergoing to antineoplastic treatment in an attempt to identify alternatives for a less invasive and less painful monitoring of these patients. Materials and methods: Unstimulated whole saliva samples were obtained from 115 children without cancer (control) and 32 children with cancer (CA). Children with cancer were also evaluated after antineoplastic treatment (CAT, n = 17). The salivary concentrations of glucose, triglycerides, total cholesterol, alkaline phosphatase, gamma-glutamyltransferase (GGT), urea, insulin, thyroid-stimulating hormone (TSH), triiodothyronine (T3), levothyroxine (T4), and immunoglobulin A (IgA) were determined. Results: Acute lymphocytic leukemia, acute myeloid leukemia, and Hodgkin's lymphoma were the most frequent cancers, although cases of non-Hodgkin's lymphoma, medulloblastoma, ependymoma, osteosarcoma, nephroblastoma, Ewing's sarcoma, and endodermal sinus tumor were also observed. The salivary concentration of cholesterol, triglycerides, or GGT did not differ between groups. Instead, the concentrations of alkaline phosphatase and T4 were higher in patients with cancer, irrespective of treatment. TSH levels were higher in the CA group and urea concentration was lower in the CAT group. T3 was undetectable in all groups. Antineoplastic treatment increased the glucose level and decreased the insulin concentration. Salivary concentration of total IgA was lower in children with cancer, irrespective of treatment. Conclusions: Cancer and antineoplastic treatment affected biochemical and immunological parameters in the saliva of children, shedding new light on the potential usefulness of saliva for monitoring children with cancer, especially to patients undergoing immunosuppressive therapy.

P6.14.30

IVIg suppresses allogeneic mixed lymphocyte reactions by induction of anti-inflammatory monocytes ($CD14^{LOW}$, $HLA\text{-}DR^{HIGH}$) with $CD80^{LOW}$ and $PDL1^{HIGH}$ expression

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Intravenous immunoglobulin (IVIg) is a therapeutic preparation of human IgG isolated from thousands of plasma donations and has been proposed as a therapy to improve the rate of graft survival in patients with a high risk for antibody-mediated-rejection. Blocking of anti-HLA antibodies by anti-idiotypic IgG present in IVIg was proposed to explain the rapid anti-inflammatory effect of IVIg. However, peripheral tolerance beyond the half- life of IVIg was observed in IVIg-treated patients, suggesting that IVIg modulates the functions of immune cells. In the present study we showed, using the allogeneic mixed lymphocyte reaction (MLR) as an in vitro model of allograft rejection and GvHD, that IVIg strongly inhibits IL-2 secretion (T cell activation) and modulates the level of other pro- and anti-inflammatory cytokines secretion (IL-6, INF-γ, TNF-α, IL-1RA). To determine the mechanisms underlying the inhibition of T cell activation in MLR, we studied the effect of IVIg on the phenotype of the cells involved in MLR (mainly T cells and monocytes). Our results revealed that MLR inhibition by IVIg correlates with the induction of anti-inflammatory monocytes ($CD14^{LOW}$, $HLA\text{-}DR^{HIGH}$) with a low CD80 and high PDL1 expression. To evaluate the importance of PDL1 on the MLR inhibition, anti-PDL1 was added during the MLR. Blocking of PDL1 restored the MLR, as evaluated by IL-2 secretion by activated T cells. We thus propose that PDL1 plays a central role in the inhibition of MLR. Our results help to better understand how IVIg induces long-term peripheral tolerance and improves graft survival in transplanted patients.

P6.14.31

A comprehensive and quantitative analysis of the specificities in rabbit antithymocyte globulin preparations

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Antithymocyte globulins (ATGs) are the polyclonal IgG fraction of sera from rabbits that are immunized with human thymocytes or Jurkat cells. Their polyclonal composition is reflected by various immunomodulatory activities and therefore ATGs are used as strong immunosuppressants for the treatment of graft rejection, graft versus host disease and aplastic anaemia. In order to better control the outcome but also the side effects of an ATG treatment, it is important to determine the qualitative and quantitative aspects of antibody specificities in ATGs. Therefore, we developed a method based on retroviral expression cloning for the identification of novel ATG specificities directed to antigens on human leukocytes. Furthermore, we established methodologies that allow for the quantification of ATG antibodies to a given antigen. The functional consequences of the ATG antigen binding interaction on pBMCs in vitro, were investigated. In this study, ATG antigens directed to human leukocytes that have not been described in the literature could be identified. A profile of about 90% of the major antibody specificities directed to leukocyte antigens of ATG-Fresenius and Thymoglobulin was calculated. In addition, the role of specific ATG antibody binding to target antigens involved in T cell activation was analysed.This study gives for the first time comprehensive and quantitative information on the composition of both rabbit ATG preparations that are in clinical use. Furthermore, the data contribute to a more complete understanding of the immunosuppressive and immunomodulatory effects exerted by ATGs and can be used for the establishment of a more patient orientated regimen.

P6.14.32
CD38 and PC-1 pathway leading to the production of adenosine: a rescue pathway for the immune system at the maternal-fetal interface

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Pregnancy is considered to be an in vivo transplant and the interactions taking place at the maternal-fetal interface have been the focus of several studies in recent years. Maternal and fetal immune defenses establish a condition of reciprocal tolerance which lasts throughout the pregnancy.
Beside dominant immunogenetic components, the ectoenzyme network contributes with a chain of surface molecules mainly operating in closed environments. The products of the reactions provide inhibitory or activating signals. Adenosine (ADO), a purine nucleoside that plays multiple roles in almost all tissues and organs, is one of the soluble products of the ectoenzyme network with immune suppressory potential.
The working hypothesis of this work, i.e., that the adenosine generating network contributes to immune tolerance in pregnancy, has been studied in women with recurrent pregnancy loss (RPLs), which remain unexplained in over 50% of cases. We analyzed the expression of CD39 (ectonucleoside triphosphate diphosphohydrolase-1) and CD73 (ecto-5'-nucleotidase), the main pathway for adenosine generation. The study included the evaluation of the expression of TNF-α (a pro-inflammatory cytokine) and of an alternative pathway of adenosine generation run by CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) and PC-1/ENPP1 (ectonucleotide pyrophosphatase/phosphodiesterase 1).
Comparison of the gene expression patterns between RPL and control group suggested that the ectoenzyme network may provide an important contribution to the generation of local tolerance and may significantly influence the clinical outcome in RPL.
These findings may directly prove useful in the design of protective therapies for women at risk for RPL.

P6.14.33
Regulatory T cell epitopes in IgG (Tregitopes) suppress immune responses without skewing toward TH2

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Immunoglobulins (IgG) have been shown to be excellent tolerogenic carriers, although the basis for this tolerogenicity is not clear. Five years ago, De Groot and colleagues reported that IgG contains epitopes, termed Tregitopes, that stimulate T regulatory cell expansion and suppress T cell responses in vitro and in vivo. In this study, we asked whether these Tregitopes triggered detectable T cell responses or skewed immune responses per se even when delivered in complete or incomplete Freund's adjuvant. While administration of Tregitopes in IFA led to an increase in CD25+ FoxP3+ cells, there was no proliferation and minimal cytokine production detected in response to those peptides, and no increase in transcription factors typifying a TH2 response. Based on our results, we suggest that Tregitopes can be used to expand Tregs and that these epitopes may represent a new paradigm for treatment of undesirable immune responses in autoimmunity and adverse drug reactions. (Supported by NIH grants AI035622 [DWS] and DK081261[ADG])

P6.14.34
An immunotherapy study to compare immunomodulatory properties of Mesenchymal Cells derived from bone marrow and adipose tissues for application in non-human primate kidney transplantation

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Multipotent non-hematopoietic progenitor mesenchymal stem cells are originally isolated from bone marrow (BM) as well as adipose tissues and they are capable of differentiating into various mesodermal lineages. Several studies have reported low immunogenicity for MSCs as they appear to have great potential to be used as modulators of immune responses in a variety of diseases related to alloreactive immunity in addition to organ transplantation. In vitro studies have shown immunosuppressive effect of MSCs on T cells, B cells and natural killer cells as well as in vivo prolong skin graft survival. Studies have proposed human adipose tissues (hAD) as an unlimited source of MSCs which showed to have very similar immunological properties to MSCs derived from hBM. As MSCs isolation from BM is an aggressive method, therefore in this project we aim to isolate MSCs from adipose tissues and then study in vivo immunomodulatory effect of hAD-MSCs on immune responses in kidney xenografts. In addition, we aim to compare the immune regulation of hAD-MSCs in kidney transplantation in nonhuman primates with hBM-MSCs and identify any possible immunomodulatory differences between hAD-MSCs and hBM-MSCs. The understanding of immune modulation consequences by hMSCs is required for their use as derivatives of adipose tissues which provide cells for immunotherapy in clinical applications.

P6.14.35
Oral Tolerance through Treg cells promotes reduction of inflammatory lesions in mice infected with *Leishmania amazonensis*

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The Oral Tolerance is a physiological mechanism of systemic hyporeactivity to an immunogen previously ingested and bystander suppression is a inhibited response to a second immunogen when it is presented along with the immunogen for which oral tolerance was established. Previous studies with mouse strains, extreme phenotypes to susceptibility (TS strain) and resistance (TR strain) to Oral Tolerance, showed that when infected with *Leishmania amazonensis*, TR strain develops an exacerbate lesion while a regulatory activity of the TS strain depresses the inflammation and avoid acute lethal response. The bystander suppression reduced the inflammatory lesion in TR strain, similarly to the swelling of infected TS strain. Mice previously tolerized and immunized showed an increase of CD4+ CD25+ FoxP3+ cells, increase of IL-10 and decrease of IFN-g in draining lymph nodes of the bystander site when compared with naïve or immunized mice. The transfer of regulatory T cells (CD4+ CD25+), from tolerized to infected mice, resulted in reduction of inflammatory lesion. These results suggest the involvement of Treg cells in the bystander suppression mechanism and demonstrate their importance in the reduction of inflammatory lesions in animals infected with *Leishmania amazonensis.*

P6.14.36
Immunosuppressive circular plant peptides

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Background: The immune system is constantly detecting and removing endogenous and exogenous threats, but sparing the organism`s own cells and tissues. In case of a dysregulated immune system, like in autoimmunity, this homeostasis is destroyed. Cyclosporine A, a naturally-occurring circular depsipeptide, is routinely used in clinics as immunosuppressant, but provokes many and often severe side-effects. Recently we identified circular peptides from plants, which exhibit promising anti-proliferative effects towards T-cells. These so-called cyclotides are composed of about 30 amino acids with a head-to-tail cyclized backbone. Their six conserved cysteines that are arranged in a typical cyclic cystine-knot motif, confers them with resistance to enzymatic, chemical or thermal degradation, making them attractive pharmaceutical tools.
Aim: The aim of this study is to investigate the immunosuppressive properties and mechanism of native and synthetic cyclotides in vitro and in vivo, and compare their mode-of-action to other immunosuppressive drugs such as cyclosporine A.
Methods & Results: We were able to demonstrate their anti-proliferative characteristics towards human activated primary lymphocytes in vitro. This significant inhibitory effect was dose-dependent and no cytotoxicity was observed in the active concentration range. Furthermore an experimental autoimmune encephalomyelitis mouse model was used to test the therapeutic impact on lymphocyte proliferation in vivo. We could show significantly delayed and minor symptoms of disease in mice treated with cyclotides. Moreover the anti-proliferative effects could be confirmed in isolated restimulated mouse splenocytes in vitro.
Conclusion: Cyclotides have great potential as peptide-based drugs and display promising immunosuppressive properties towards lymphocytes in vitro and in vivo.

P6.14.38
Apolipoprotein E attenuates acute rejection of experimental renal allografts

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Apolipoprotein E (ApoE) is a multifunctional protein, originally described in the context of lipoprotein metabolism and cardiovascular disease. More recently, anti-inflammatory functions of ApoE have been documented. In this study, we test the hypothesis that ApoE attenuates acute renal allograft rejection.
The Brown Norway to Lewis rat strain combinations was used to investigate fatal acute rejection. In addition, Fischer 344 kidneys were transplanted to Lewis rats to study reversible acute rejection. Isograft recipients and untreated Lewis rats were used as controls. ApoE mRNA expression was quantified in intravascular leukocytes accumulating in the blood vessels of renal grafts and in graft tissue. ApoE protein levels were assessed in blood plasma. To test the protective potential of ApoE, recipients of Brown Norway kidneys were treated with ApoE-mimetic peptide. The number of different graft infiltrating leukocytes was evaluated by immunohistochemistry.
Intravascular graft leukocytes and renal tissue obtained from animals undergoing reversible acute rejection expressed increased levels of ApoE mRNA, whereas during fatal rejection, ApoE expression remained unchanged. On the protein level, no changes in ApoE were seen in plasma. However, the local leukocytic ApoE expression could result in increased ApoE concentrations inside graft blood vessels. Treatment of allograft recipients with peptide reversed fatal rejection and significantly improved animal survival. Immunohistochemical analysis revealed reduced number of infiltrating T cells. Furthermore, reduced mRNA expression of granzyme B could be detected.
ApoE plays a protective role in acute organ rejection. This effect is probably mediated by a reduced influx of cytotoxic T cells.

P6.14.39
Production of mmLFA3-Ig with immunosuppressive activity by recombinant expression in Pichia pastoris

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Engagement of lymphocyte function antigen 3 (LFA3) and CD2 can increase the activation of T cells. Blockade of LFA3-CD2 pathway efficiently down-regulated the abnormal immune activation, thus resulting in the development of human LFA3-Ig (hLFA3-Ig) as a treatment for psoriasis. Nevertheless, it is far from to unravel the role of LFA3-CD2 in regulation of immune system. Rhesus monkey macaca mulatta has been widely used as animal models for human immune disorders. Due to specificity of the immune system, *M. mulatta* LFA3-Ig (mmLFA3-Ig) might be required for pathogenesis of immune disorders in rhesus monkey models. The gene encoding mmLFA3 were amplified from peripheral blood mononuclear cells (PBMCs) by RT-PCR. Amino acid sequence analysis demonstrated that the identity between the predicted extracellular domain (ECD) of mmLFA3 and that of hLFA3 is about 86%. To prepare mmLFA3-Ig, the ECD of mmLFA3 was fused to the N-terminus of human IgG1 Fc fragment. The gene encoding mmLFA3 ECD was firstly optimized according to the codon bias of *P. pastoris*. MmLFA3-Ig produced by *P. pastoris* specifically bound CD2-positive cells. Positive staining was detected by flow cytometer in approximately 90% CD2-positive Jurkat cells, compared to that in 5% CD2-negative Raji cells. Moreover, mmLFA3-Ig showed dose-dependent suppression on monkey and human lymphocyte proliferation in both Con A- and allogenic antigen-stimulated systems. Interestingly, mmLFA3-Ig showed stronger immunosuppression on monkey lymphocyte proliferation than that of human. These results suggest that mmLFA3-Ig produced by *P. pastoris* might be a used for pathogenesis of immune disorder in rhesus monkey model.

P6.15 Immunopharmacology

P6.15.01
Production of TNF-α-specific monovalent and bivalent variable domain of llama heavy-chain antibody fragment in transgenic rice

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Tumor necrosis factor-α (TNF-α) plays a pivotal role in chronic inflammation such as Rheumatoid Arthritis and Crohn's disease. Although anti-TNF-α antibody therapy is now commonly used for the treatment of patients suffering from those inflammatory diseases, the cost of treatment remains to be a major concern. We have developed a novel system for the production of a single variable domain of llama heavy-chain antibody fragment (VHH) specific for mouse TNF-α in rice seeds (MucoRice-TNF-VHH). MucoRice-TNF-VHH was produced at high levels in rice seeds using our most recent transgene (Tg)-overexpression system with RNAi technology to suppress the production of major rice endogenous storage proteins but enhances the Tg- derived protein expression. Two molecular forms of TNF-α specifc monovalent (mVHH) and bivalent (bVHH) were expressed in the rice seed of MucoRice. Production levels of mVHH and bVHH were shown to reach an average of 0.82% and 0.47% of rice seed weight, respectively. Further, approximately 85% of mVHH and bVHH were released easily when the powder form of MucoRice-TNF-VHH was mixed with PBS (rice-water). To examine inhibitory activity of MucoRice-TNF-VHH in the rice-water, we used in vitro TNF-α cytotoxicity assay using WEHI164 cells. Although the both forms of MucoRice-TNF-VHH showed the neutralizing activity, bVHH was 50 times higher than that of mVHH. These results show that the rice-expression system (MucoRice) is useful for the production of neutralizing VHH specific for TNF-α. In our ongoing study, we are currently investigating whether purified MucoRice-TNF-VHH can suppress the progression of mouse collagen-induced arthritis or not.

P6.15.02
Involvement of histamine H2-receptors in gastric tissue inflammation and oxidative stress induced after scorpion envenomation

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The activation and release of pro-inflammatory mediators such as cytokines and vasoactive substances (Histamine, eicosanoids···) may play an important role in the pathophysiology of scorpion envenomation. The mechanism of gastric inflammation induced by scorpion venom has not yet been elucidated and was little investigated.
The aim of this study is to investigate the potential role of histamine H2-receptors in gastric tissue inflammation and oxidative stress following experimental scorpion envenomation by *Androctonus australis hector* (Aah) venom. Gastric inflammation was assessed by evaluating the index of increased vascular permeability, by histological analysis and by measuring the myeloperoxydase (MPO) and eosinophil peroxydase (EPO) activities in the gastric tissue. Lipid peroxidation expressed as malondialdehyde (MDA) level, glutathione (GSH) and catalase (CAT) activities were measured to assess the oxidative stress.
Envenomed mice displayed an installation of an inflammatory response marked by neutrophil and eosinophil infiltration. This infiltration was preceded by the increase in the vascular permeability. Severe imbalanced redox status with decreased catalase and GSH activities, but increased MDA level was observed. Pre-treatment of animals with the ranitidine or cimetidine (H2-receptor antagonists), two hours before venom injection, significantly inhibited vascular permeability, edema formation and tissue recruitment of inflammatory cells. Lipid peroxidation products were found to be significantly reduced, but catalase or GSH activities were partially restored.
These results suggest that the histamine by acting with the H2 receptors is involved in the triggering of the inflammatory response and also in the alteration of the oxidant/antioxidant balance in the gastric tissue caused by the *Aah* venom.

P6.15.03
Novel anti-inflammatory strategies through protein engineering of Evasins

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We have identified 3 chemokine binding proteins (ChBP) from a tick salivary gland library which were named Evasin-1, -3 and -4. Evasin-1 and -4 bind CC-chemokines, the first being highly selective whilst the second has a broad pattern of selectivity. Evasin-3 binds the neutrophil chemoattractant ELR CXC-chemokines. In order to create therapeutic modalities for the treatment of chronic inflammatory diseases, the Evasins have been fused to Fc to produce molecules with a long half-life. The fusions have been made both at the N- and the C-terminus of the Evasins. Both Evasin-3 fusions showed similar activity whereas Fc-Evasin-4 was superior to Evasin-4-Fc. We then took advantage of this property to create a bi-specific molecule by fusion of Evasin-4 to Enbrel®, a commercialized anti-TNF in order to inhibit simultaneously cytokine and chemokine pathways. As both Evasin-4 and Enbrel® have been shown to be effective in murine models but still also allow space for improved efficacy, this combination will provide an answer to the potential synergistic effect of the two strategies. We have characterized both their binding properties and their neutralizing activities in vitro. The strategy to test the efficacy of these Evasin/Fc fusions in vivo will be discussed.

P6.15.04
Eugenol bronchodilator potential on il-13-induced hyper-reactivity of murine tracheal smooth muscle

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Introduction:Muscle cells are potentially linked with many features of asthma, including the production of cytokines and inflammatory mediators involved in tissue remodeling. Interleukin-13 has been implicated as a key cytokine in the pathogenesis of allergy and asthma. Eugenol (Eug), an aromatic product, exhibit anti-inflammatory properties and a possible relaxing effect on smooth muscle, so we examined in vitro the bronchodilator potential of this compound in hyper- reactive murine tracheal smooth muscle induced by IL-13. Methods: Animals were euthanized, trachea was rapidly removed and tracheal segments were placed individually in each well of a 48-well plate and incubated at 37°C in the presence or absence of IL-13 at 10ng/mL in supplemented DMEM for 24h. Trachea smooth muscle reactivity was assessed at organ baths containing Krebs-bicarbonate at 37°C and aerated with a carbogenic mixture.Results: We demonstrated the increased contractility in response to CCh in airway smooth muscle induced by IL-13.Our study showed that the effects of IL-13 on contractility were principally to increase Emax, and no significant changes in EC50 were observed, indicating that IL-13 seemed to increase smooth-muscle contractility rather than induce increased sensitivity to contractile agents (CCh), thus demonstrating hyper-reactivity. The effect of eugenol was not altered in hyper-reactive smooth muscle (sensitized with IL-13) when compared to its effect on normal smooth muscle, showing that the bronchodilatory potential of eugenol is not selective for hyperactivity induced by IL-13 pathway. More studies are needed to verify if eugenol may have a selective effect on other mechanisms involved in the hyper-reactivity.

P6.15.05
***In vitro* treatment with ganciclovir restores the functionality of exhausted T cells from cancer patients**

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Human cytomegalovirus (CMV) is a lifelong and often asymptomatic infection, with the virus maintained in latently infected myeloid cells. Patients with cancer on immunosuppressive therapy are at high risk for reactivation of latent CMV, which may further contribute to a poor anti-tumor immune response. Pre-emptive anti-CMV medication may, therefore, serve as an adjunct therapy, preventing deterioration of the patient's immunity during treatment for cancer.
We evaluated ganciclovir treatment's *in vitro* effects on CMV-associated T cell exhaustion and the anti-tumor capacity of cells from cancer patients. Following ganciclovir treatment, there was a redistribution of T cell subpopulations which was clearly distinct from the baseline pattern of T cell exhaustion. Treatment resulted in increases in early differentiated populations, including naïve, IL-7Rα$^+$, and CD28$^+$CD8$^+$ T cells, and a decrease in the proportion of effector memory T cells in cancer patients. Ganciclovir treatment significantly improved tumor killing capacity, with a concomitant downregulation of T cell exhaustion marker PD-1. These findings demonstrate that ganciclovir treatment *in vitro* affects immune function by partially reconstituting T cell subsets and restoring the T cell anti-tumor response.

P6.15.06
Direct and natural killer cell-mediated antitumor effects of CB1 receptor antagonist rimonabant in human glioblastoma

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The failure of conventional cancer therapy renders glioblastoma (GBM) an attractive target for novel combined chemo-immunotherapeutic strategies. In the last years the pharmacological tuning of (endo)cannabinoid system has constituted a promising tool for the management of GBM patients. In this study we investigated the therapeutic exploitation of CB1 inactivation with regard to direct and NK cell-mediated effects against GBM.
We show for the first time that Rimonabant treatment inhibited growth and viability of a panel of human glioma cell lines in both monolayer culture and 3D Minitumour spheroid models by decreasing the expression levels of Bcl-2, Bcl-XL and Survivin anti-apoptotic factors and concomitantly by inducing tumor necrosis factor (TNF)-related apoptosis-inducing ligand death receptor 4 (DR4) and DR5.
Interestingly Rimonabant lead also to functional expression of MHC class I-related chain A and B (MICA/B) on surface of malignant p53mut U251MG and T98G glioma cell lines but not on normal human astrocyte (NHA). This make GBM cells potent targets for natural killer (NK) cell mediated recognition through a NKG2D restricted mechanism, thus stimulating cytotoxicity and IFN-Ɣ production of cocultured NK cells. This last effect relies on the inhibition of STAT3 signaling, whose constitutive activity has been previously seen to promote escape to NKG2D-mediated immune-surveillance.
Our study suggests that, in addition to the direct cytotoxic effect, Rimonabant also can make GBM immunovisible priming it for NK cell antitumor reactivity. Taking into account that the brain tumor microenvironment is more commonly associated to CD56-positive NK cells, GBM patients may benefit from Rimonabant treatment.

P6.15.07
Towards predictive pharmacogenetic profiling for azathioprine treatment: characterization of SNPs in TPMT and XDH genes

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Azathioprine (AZA) is an immunosuppressant drug used for myasthenia gravis (MG) treatment. Intolerance or not responsiveness is a limiting problem for drug treatment in the 30% of the patients. Aim of this study is to determine genotype-phenotype association that can be applied as predictive biomarker for AZA treatment of MG. We analyzed thiopurine S-methyltransferase (TPMT) and xanthine dehydrogenase (XDH) single nucleotide polymorphisms (SNPs) in 124 Italian MG patients in the context of their response to AZA. Haplotype analysis and linkage disequilibrium (LD) of TPMT SNPs detected a new haplotype associated with intolerance to AZA (Psim=0.0026): TPMT*3E, including the intronic T140+114A SNP and the previously reported TPMT*3A allele . TPMT*3E was detected in five of the 23 AZA-intolerant patients and was not observed in unresponsive or responsive patients. Therefore this haplotype should be taken into account when considering AZA treatment. LD analysis of XDH detected different haplotype blocks, one of those was significantly associated with intolerance (Psim = 0.037). Composed by three SNPs located in the 3' untranslated region, it is not clear how this haplotype induces intolerance to AZA. One possibility is that SNPs alter the target sites for microRNAs, thus influencing the expression of the protein XDH.
In this work, we identified new haplotypes that should be taken into consideration in pharmacogenetic profiling for AZA.Work supported by 7th Framework Programme of the European Union FIGHT-MG (Grant no. 242210).

P6.15.08
Molecular characterization and valorization of cuban natural products, in particular Vimang® and its naturally Glucosylxanthone Mangiferin, for therapeutic applications against cancer and Inflammation

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Vimang is a standardized extract derived from Mango bark (Mangifera Indica L.), commonly used as anti-inflammatory phytomedicine, which has recently been used to complement cancer therapies in cancer patients. We have further investigated potential anti-tumour effects of glucosylxanthone mangiferin and indanone gallic acid, which are both present in Vimang extract. We observed significant anti-tumour effects of both Vimang constituents in the highly aggressive and metastatic breast cancer cell type MDA-MB231. At the molecular level, mangiferin and gallic acid both inhibit classical NFκB activation by IKKα/β kinases, which results in impaired IκB degradation, NFκB translocation and NFκB/DNA binding. In contrast to the xanthone mangiferin, gallic acid further inhibits additional NFκB pathways involved in cancer cell survival and therapy resistance, such as MEK1, JNK1/2, MSK1, and p90RSK. This result in combinatorial inhibition of NFκB activity by gallic acid, which results in potent inhibition of NFκB target genes involved in inflammation, metastasis, anti-apoptosis and angiogenesis, such as IL-6, IL-8, COX2, CXCR4, XIAP, bcl2, VEGF. The cumulative NFκB inhibition by gallic acid, but not mangiferin, is also reflected at the level of cell survival, which reveals significant tumour cytotoxic effects in MDA-MB231 cells. Altogether, we identify gallic acid, besides mangiferin, as an essential anti-cancer component in Vimang extract, which demonstrates multifocal inhibition of NFκB activity in the cancer-inflammation network.
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P6.15.09
Study of the immunomodulatory role of heteroglucan on a 3D tumor spheroid and macrophage co-culture milieu

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Over the past several years, glucans have garnered much attention due to their immunomodulatory properties. Till date most studies of glucans has been done in vivo or in two dimensional cultures. A 3D platform may thus serve as a tool to optimize drug effects in tissue environment as well as reduce animal testing and economical wastage. The present study was focussed on establishing a three dimensional tumor spheroid model and its co-culture with glucan (isolated from Pleurotus ostreatus mycelia) stimulated macrophages to mimic the complexity retained in tumor tissues and the immunological phenomena of stimulated phagocytes. Spheroids of HT-29 were incubated with glucan activated human THP-1 macrophages at an effector:target cell ratio of 1:1 and the invasive property of the phagocytes were examined. Interestingly, about 46% increase in the migration and penetration of activated macrophages to the tumor spheroids was observed by confocal microscopy using a two dye staining method. Concomitantly, immunohistological studies further corroborated these facts whereby activated macrophages were found to deeply penetrate the spheroidal layer. Tumor cell death was also found to be increased in the peripheral zones of spheroids incubated with glucan treated macrophages. The degree of distortion of the spheroidal morphology attained higher growth with an increase in the co-culture incubation period. Untreated macrophages apparently exhibited lower invasiveness, death and distortion of the tumor spheroids. Spatiotemporal and micro environmental influences in the behaviour of cells monitored in such 3D system are therefore prospects to be accounted for deeper understanding of the complex immune network.

P6.15.10
Specific targeting of extracellular Cyclophilins for treatment of inflammatory diseases

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Cyclophilin A (CypA) is a ubiquitously distributed peptidyl prolyl cis/trans isomerase (PPIase) which is secreted under inflammatory conditions. Extracellular CypA has been reported to display chemotactic activity and may thus contribute to inflammation-mediated diseases. The objective of this study was to determine the impact of blocking the PPIase activity of cyclophilins using cell-impermeable derivatives of cyclosporin A (CsA) to specifically target the extracellular pool of cyclophilins (eCyp).
We have developed novel selective inhibitors of eCyp PPIases LDC043199 (MM-284) and LDC045325 which display significantly less calcineurin inhibiton and cytotoxicity compared to the parent CsA. No liabilities were detected by early ADME and PK analysis thus qualifying these compounds as lead structures suitable for pharmaceutical drug development. These compounds lack the significant immunosuppressive activity of CsA as demonstrated in primary human T cell proliferation and in vivo mouse T cell proliferation analysis.
We show excellent efficacy of the i.p. administered compounds in an antigen specific (Keyhole limpet hemocyanin) delayed type hypersensitivity ear swelling mouse model as well as significant efficacy in a therapeutically relevant mouse asthma model. Furthermore the compounds displayed complete inhibition of CypA-induced chemotaxis by primary human dendritic cells and neutrophils.
We have developed high quality leads in the field of selective inhibitors of extracellular cyclophilins. Our findings validate the concept that cell-impermeable derivatives of CsA provide strong anti-inflammatory activity without dose-limiting immunosuppressive toxicity as observed with CsA. Specifically blocking the activity of extracellular cyclophilins may provide a novel approach towards a safer and more effective treatment of inflammatory diseases.

P6.15.11
Selective targeting of the immunoproteasome for treatment of autoimmune diseases

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The proteasome is a multicatalytic enzyme complex playing a key role in antigen presentation, cellular protein homeostasis and signal transduction. Two major forms of the proteasome have been described based on different incorporated beta-subunits: while the constitutive proteasome (CPS) is ubiquitously expressed, the immunoproteasome (IPS) is predominantly expressed in hematopoetic cells and is inducible under inflammatory conditions.
We have developed novel selective, non-covalent inhibitors of the IPS specific subunit beta5i (LMP7). LDC compounds were profiled in enzymatic and cell based assays in comparison to the CPS inhibitor Bortezomib and the covalent IPS inhibitor PR-957. LDC compounds are highly potent (nM range) and selective (>1000x over CPS) IPS inhibitors showing a pronounced species specificity (hu>rat>ms). We also found that PR-957 due to its irreversible, covalent binding to the target is not truly selective for the IPS over time. After several hours of incubation PR-957 also inhibited the CPS activity while the non-covalent LDC compounds did not lose the IPS selectivity over time. In line with these findings LDC compounds were significantly less neurotoxic compared to Bortezomib and PR-957 using a SY5Y neuroblastoma cell viability assay. Preliminary data indicate that LDC compounds are effective inhibitors of (auto)antibody secretion.
We have developed high quality leads in the field of truly selective and non-covalent immunoproteaseome inhibitors. Considering the reported toxicities for covalent CPS inhibitors such as Bortezomib, an IPS selective inhibitor may represent a safer therapeutic alternative in the treatment of autoantibody mediated autoimmune diseases such as SLE or Myasthenia gravis.

P6.15.12
Role of protein phosphatases in the functional desensitization of β-adrencoceptor-mediated responses in human lung mast cells

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Adrenoceptor agonists are potent bronchodilators, which have also been shown to inhibit the release of histamine and eicosanoids from dispersed human mast cells. Long-term exposure of mast cellto β-agonists attenuates the subsequent ability of β-agonists to inhibit histamine release. Since phosphorylations of the β_2-adrenoceptors are probably important in inducing desensitization. In the present study the effects of fostriecin, a selective inhibitor of pp2a, and okadaic acid, an inhibitor of pp2a and pp1, on the functional desensitization of β-adrenoceptor-mediated responses in mast cells were investigated.
To evaluate the effects of the pp2a inhibitor fostriecin on the isoprenaline inhibition, cells were incubated for 2 or 4 h or 24 h with fostriecin before incubation with isoprenaline and then challenged with anti-IgE. Treatments with fostriecin had no significant ($p > 0.05$) effect on either the efficacy or the potency of isoprenaline on mast cell which were treated with either buffer or fostriecin and with or without isoprenaline for 24 h. Isoprenaline pre-treatment reduced the maximal response to isoprenaline by about 50% and fostriecin had little effect on the extent of functional desensitization. In addition the effects of okadaic acid on the functional desensitization induced by prolonged exposure to isoprenaline were investigated. Although, long-term treatment with okadaic acid had no effect on the ability of isoprenaline to inhibit histamine release, okadaic acid significantly ($p < 0.05$) enhanced the extent of isoprenaline-induced desensitization.
These data suggest that pp2a has an important role in regulating mast cell β_2-adrenoceptors.

P6.15.13
ImmuDrug platform: high throughput screening for novel immunomodulators

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Immune diseases like rheumatism, diabetes type I, multiple sclerosis, and other autoimmune diseases and allergies are commonly treated by unspecific pharmacologic immunosuppression. However, the treatment effect is only transient and side effects like risk of infections are inherent. Therefore, we want to target endogenous mechanisms of self-regulation in the immune system such as regulatory cells or suppressive cytokines. To discover new drug candidates that address these mechanisms, we have developed a micro cell culture system that allows the simulation of inflammatory conditions in the presence of either synthetic or natural compounds. Using high throughput multichannel flow cytometry, we are able to analyze thousands of substances per week by monitoring changes in reporter protein (GFP) and/or surface molecule expression, viability, and cell number. Here we show results of a screening for regulatory T cell (Treg) inducing compounds using cells from Foxp3-GFP reporter mice. A library of 35.000 compounds provided by the Leibniz-Institut für Molekulare Pharmakologie (FMP) Berlin was screened and a number of promising hits were obtained which are currently validated. The versatile assay setup can easily be adapted to different functional assays and will not only help to discover new candidates for future treatment of immune-mediated diseases but will also enable insights into molecular mechanisms of immunoregulation.

P6.15.14
B7H triggering inhibits the migration of tumor cell lines

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Vascular endothelial cells (EC) and several cancer cells express B7h, which is the ligand of the ICOS T cell costimulatory molecule. We have previously shown that B7h triggering by a soluble form of ICOS (ICOS-Fc) inhibits the adhesion of polymorphonuclear and tumour cell lines to human umbilical vein EC (HUVEC), and we have suggested that it may act as an anti-inflammatory and antitumour agent. Since cancer cell migration and angiogenesis are crucial for metastasis dissemination, the aim of this work was to evaluate the effect of ICOS-Fc on migration of cancer and EC. ICOS-Fc specifically inhibited the migration of HUVEC, human dermal lymphatic EC, and the HT29, HCT116, PC-3, HepG2, JR8, and M14 tumour cell lines, expressing high levels of B7h, whereas it was ineffective with the RPMI7932, PCF-2, and LM cell lines, expressing low levels of B7h. Furthermore, ICOS-Fc down-modulated hepatocyte growth factor (HGF)-induced epithelial to mesenchymal transition in HepG2 cells. Moreover, ICOS-Fc down-modulated the phosphorylation of FAK and the expression of β-Pix in both HUVEC and tumour cell lines. Finally, treatment with ICOS-Fc inhibited the development of lung metastases in NOD-SCID-IL2Rγnull mice injected with the CF-PAC1 luc tumour cell line in the tail vein. In conclusion, the B7h:ICOS interaction may modulate the spread of cancer metastases, which introduces a novel view on the use of ICOS-Fc as an immunomodulatory drug.

P6.15.15
Effect of beta-adrenoceptor agonist and antagonist on immune response under thyrotoxicosis

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It is known that thyroid hormones may increase the expression of beta-adrenoceptors and/or transduction of intracellular signals in target cells. The aim of this study was to investigate the influence of agonist and antagonist of beta-adrenoceptors on immune response under experimental thyrotoxicosis. Rats were injected L-thyroxine (T4) subcutaneously during 14 days in a daily doses of 0.04 or 4 mg/kg of body weight (b. wt.) for modeling mild or severe forms of thyrotoxicosis respectively. It was established that mortality of rats directly depends on the dose of T4. The increase in number of antibody-forming cells (AFC) and the activation of the delayed type hypersensitivity (DTH) response were revealed under mild form of thyrotoxicosis. The level of AFC and DTH response was decreased under severe form of thyrotoxicosis. The stimulation of AFC response under mild form of thyrotoxicosis was canceled in rats which received the agonist of beta-adrenoceptors (hexoprenaline sulfate i.p. in a daily dose of 0,001 mg/kg b. wt. during 14 days). Hexoprenaline sulfate led to decrease in DTH response level under both forms of thyrotoxicosis. The antagonist of beta-adrenoceptors (sotalol hydrochloride i.p. in a daily dose of 10 mg/kg b. wt. during 14 days) inhibited immunosuppression in severe thyrotoxicosis and increased immunostimulation under mild form of thyrotoxicosis. This study was supported by the programs of the Presidium of the Russian Academy of Sciences "Molecular and Cell Biology" and "Fundamental Science for Medicine".

P6.15.16
Effect of hexoprenaline sulphate on the immunoglobulin production in the presence of thyroxine and hydrocortisone in vitro

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Adrenergic compounds, thyroid hormones and glucocorticoids play an important role in the neuroendocrine regulation of the immune system in health and disease. It is known that administration of thyroid hormones and glucocorticoids lead to the increase in expression of beta-adrenoceptors and/or intracellular signal transduction in target cells of various organs. Functional implications of such interaction at the level of the immune system are not fully understood yet. The aim of this work was to investigate the effect of beta-adrenoceptor agonist hexoprenaline sulphate on the production of immunoglobulins in the presence of thyroxine and hydrocortisone in vitro in cultures of human mononuclear cells of practically healthy volunteers. It was established that addition of thyroxine (10^{-8} M) in cultures with pokeweed mitogen (PWM) led to increase in the IgG production. Hexoprenaline sulfate (10^{-6} M) abolishes this effect in cultures with thyroxine, but has no effect on the IgG production in cultures without thyroxine. Hydrocortisone (10^{-6} M) has no influence on production of IgG alone and along with hexoprenaline sulfate (10^{-6} M). All compounds have no effect on production of IgM. This study was supported by the programs of the Presidium of the Russian Academy of Sciences "Molecular and Cell Biology" and "Fundamental Science for Medicine".

P6.15.17
Study of adverse events reported to the center of health surveillance related to the use of intravenous immunoglobulin (IVIG)

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Antibody replacement therapy was first introduced for clinical use in the 1950s, mainly for the treatment of primary immunodeficiency conditions, usually used subcutaneously or intramuscularly, either as an adjunctive or first line therapy. Due to the recognition of its modulator effects, subsequently suitable preparations for intravenous use have been developed. Intravenous immunoglobulin has been, since then, increasingly used in many inflammatory and autoimmune conditions, although only a few have received approval by FDA and EMEA and unlabeled use is now common. Clinical preparations consist of IgG antibodies and all four subclasses, obtained from a pool of thousands of healthy donors, comprising the entire repertoire of antigen binding sites expectedly found in normal human serum. Intravenous immunoglobulin mechanisms of action are complex and include anti-infective, immunoregulatory, and anti-inflammatory properties. However, adverse events due to the use of this immunobiological have been reported. It was the purpose of this study to identify the adverse events most commonly reported to the center of health surveillance related to the use of intravenous immunoglobulin. Notification forms, spreadsheets and database files have been analyzed and data were organized and systematized in order to provide easy access to the information for health professionals and surveillance center. Hypersensitivity reactions, thrombotic events, acute renal failure, aseptic meningitis syndrome, chills, fever during infusion, artralgia, mialgia, abdominal pain and headache are some of the adverse events commonly reported. High cost, supplies demand and safety of the product are some of the concerns related to the use of intravenous immunoglobulin.

P6.15.18
Inflammatory pathogenesis induced by scorpion venom: In vitro and in vivo analysis of immune cells activation

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Scorpion venom is a complex biological mixture of many substances such as toxins, enzymes with a wide spectrum of biological activities. Many effects are induced by this venom, such as inflammation tissue damage and cardiorespiratory distress. This study aimed to evaluate the inflammatory activity promoted by Androctonus australis hector (Aah) venom. The cytotoxic effects were evaluated by several cell viability assays (MTT, Neutral Red and LDH activities). The results obtained showed that Aah venom induced an activation of neutrophils and monocytes revealed by an increased ability of these cells to increase myeloperoxidase into the extracellular medium, lipid peroxidation as measured by thiobarbituric acid-reactive species level. It was also observed that venom induced higher IFNγ, IL-6 and IL-10 production. Nitric oxide and H2O2 were significantly produced, which showed a higher rate of cell death induction when compared with control. Results obtained also showed that venom induced same response in vitro through cell injury, cytokines and H202 productions. The release of LDH in the supernatants of spleen cultures and fragmentation of cellular DNA are the consequence of an oxidative stress responsible for the alterations of the cell membranes and probably to the inhibition of the activity of some enzymatic proteins.

P6.15.19
The immuno-modulatory effects of heparin and LMW-heparin in mite-allergic mice model

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Background: Previous studies have shown (1)the airway anti-inflammatory and anti-allergic activity of heparin (2) the anti-inflammatory potency of heparins are molecular-weight-dependent.We investigate the immuno-modulation effects in mite-induced allergic animal model treated with inhaled heparin and compared the effects of inhaled LMW-heparin.
Method: BALB/c mice were divided into five groups: 1. Control, 2. Mite intratracheal (mIT), 3. Inhaled heparin(hIN), 4. Inhaled LMW-heparin(lmwhIN), 5. Inhaled half dosage of heparin and LMW-heparin. Group 2,3,4,5 were sensitized twice with Der p allergen subcutaneously on day 1 and 8. Der p allergen was administered intratracheally on day 15. Groups 3, 4, and 5 were treated with heparins for 22 days. Splenocytes alone or co-cultured with dendritic cell, stimulated with Der p 16mg/ml were cultured for 72 hours after mice scarification on day 23. Supernatants of splenocyte were collected to analyze the effect of INF-γ, Interleukin (IL)-10, IL-13. Serum was also collected for IgE level on day 23.
Results: Both hIN and lmwhIN groups had lower serum IgE level than mIT group (both p<0.0001. The p values of inhaled hIN and lmwhIN groups compared with mIT group in splenocyte supernant are shown as following: IFN-γ(p=0.002,0.007 respectively), IL-17(both p<0.001), IL-13(p=0.0002, 0.01 respectively). hIN group had lower IL-10 level than mIT group(p=0.03). Supernatant from co-cultured dendriticyte+splenocyte+ Der P 16mg/ml showed decrease in IL-17A/F in lmwhIN group than mIT group(p=0.017).
Conclusions: Intranasal administration of heparin and low molecular weight heparin can also decrease serum IgE level. From cultured splenocyte, IL-17A/F decreased serections; which implied systemic immuno-modulation effects on Th deviation.

P6.15.20
Cimetidine enhances delayed-type hypersensitivity responses and serum interleukin (IL)-2, IL-10, IL-12, and IL-17 levels after thermal trauma in an animal model

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The immunosuppression that occurs after burn injury causes an increase in susceptibility to infection. The aim was to investigate time-related alterations in various cytokines following thermal injury and to modulate cytokines by using cimetidine. Male Balb/c mice anesthetized and given a 10% total body surface area full-thickness burn. Time-dependent changes in delayed type hypersensitivity (DTH) and serum levels of the cytokines IL-2, IL-10, IL-12, IL-17 and TGF-[[Unsupported Character - Symbol Font β]] were then assessed at various post-burn day (PBD) timepoints. Effects of 10 mg cimetidine/kg on DTH responses and cytokine levels were evaluated up to PBD 14. In comparison to healthy non-burned control mice, levels of IL-2 and IL-17 significantly decreased at PBD 3, 5, 10, and 14, those of IL-10 at PBD 1, 3, 5, and 10, and those of IL-12 at PBD 1, 3, 5, 10, and 14. Administration of cimetidine significantly augmented the levels of IL-2 (at PBD 3, 5, and 10), IL-10 (at PBD 1 and 5), IL-12 (at PBD 3, 5, 10, and 14), and IL-17 (at PBD 3 and 14) as compared to those in burned counterparts who did not receive drug. Cimetidine also significantly augmented DTH responses at PBD 5, 10, and 14 as compared to responses in non-drug-treated burned hosts. These results showed significant time-dependent changes in serum cytokines levels after burn injury and that cimetidine was able to significantly augment IL-2, IL-10, IL-12 and IL-17 levels as well as DTH response that are normally suppressed following thermal trauma.

P6.15.21
A single dose of rituximab does not deplete B cells in secondary lymphoid organs, but alters phenotype and function

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A single dose of the anti-CD20 monoclonal antibody rituximab induces a nearly complete B-cell depletion in peripheral blood, but not in secondary lymphoid organs. Modulation of this remaining B-cell population due to rituximab treatment may contribute to the therapeutic effects of rituximab. To assess the *in vivo* effects of rituximab we used lymph nodes collected during renal transplant surgery in patients who had received rituximab four weeks earlier in preparation for an ABO-incompatible transplantation. Rituximab treatment resulted in a lower percentage of naïve (IgD^+CD27^-) and a higher percentage of switched memory (IgD^-CD27^+) B cells. Remarkably, transitional ($CD24^{++}CD38^{++}$) B cells were virtually lacking in the lymph nodes of rituximab-treated patients. Moreover, lymph node-derived B cells from rituximab-treated patients produced different amounts of various Ig-subclasses after anti-CD40/IL-21 stimulation *ex vivo*. Finally, after stimulation of allogeneic T cells with lymph node-derived B cells from rituximab-treated patients, the proliferated T cells showed a decreased production of IL-17. In conclusion, after treatment with rituximab there remains a B-cell population with different functional capacities. Consequently, the effect of rituximab on the immune response will not only be determined by the extent of B-cell depletion, but also by the functional properties of the remaining B cells.

P6.15.22
Morin attenuates airway hyperresponsiveness of allergic asthma via down regulation of immune-inflammatory biomarkers

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Introduction: Asthma is immune-inflammatory airway disorder characterized by airway obstruction and hyperresponsiveness. Morin [2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one] is a flavonoid reported to have inhibition of IgE-mediated allergic response in mast cells.
Aim: Aim of present investigation was to evaluate antiasthmatic activity of morin against ovalbumin (OVA)-induced airway hyperresponsiveness (AHR) in rats.
Materials and methods: AHR was induced in the male Sprague Dawley rats by intraperitoneal injection of OVA. Rats were treated with morin (10, 30 and 100 mg/kg, p.o.) for 28 days followed by OVA-aerosol intranasal challenge. Lung function test was performed. Biochemical and molecular parameters were assessed in blood, bronchoalveolar lavage fluid (BALF) and lung tissue.
Results: Total cell counts, eosinophil counts in BALF and blood increased significantly ($P < 0.001$) in AHR control rats whereas treatment with morin (30 and 100 mg/kg, p.o.) significantly reduced cell counts ($P < 0.001$). Significant decrease ($P < 0.001$) in spirometry flow and peak inspiratory flow whereas peak expiratory flow and force expired volume was significantly increased ($P < 0.001$) in morin treated rats. Increased levels of nitric oxide, malondialdehyde, IgE and histamine were significantly ($P < 0.001$) inhibited by morin. RT-PCR analysis of lung revealed that morin (30 and 100 mg/kg, p.o.) significantly ($P < 0.001$) suppressed mRNA expression of TNF-α, interleukin (IL)-1β, IL-4, IL-6, COX-2 and leukotriene B4 (LTB-4) in a dose-dependent manner.
Conclusion: It is concluded that morin has anti-asthmatic effects mediated by reduction of oxidative stress and airway inflammation via down regulation of IgE, COX-2, IL-4, IL-6 and LTB-4 expression.

P6.15.23
Effect of (E)-2-(3,4-dimethoxyphenyl)-4-oxo -4H-chromen-7-yl-3-(3,4-dimethoxyphenyl) acrylate on the development of atopic dermatitis-like lesions

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In this study, we synthesized a novel chemical, (E)-2-(3,4-dimethoxyphenyl)-4-oxo-4H-chromen-7-yl-3-(3,4-dimethoxyphenyl) acrylate (CSH) and investigated the effect of CSH on atopic dermatitis (AD) by evaluating the anti-inflammatory effect of CSH on immune cells *in vitro* and on atopic dermatitis-like lesions *in vivo*. Human monocytic THP-1 cells and human eosinophilic EoL-1 cells were treated with house dust mite extract in the absence and presence of CSH. Nc/Nga mice were sensitized to 2,4-dinitrochlorobenzne (DNCB) for 5 weeks and then orally and dorsally administered with CSH or dexamethasone for 3 weeks. CSH inhibited the secretion of monocyte chemotactic protein-1 (MCP-1), interleukin (IL)-6 and IL-8 due to house dust mite extract in THP-1 cells. CSH also suppressed the secretion of MCP-1 and IL-8 in EoL-1 cells. *In vivo*, the skin severity score decreased after CSH treatment as compared to the control group. CSH suppressed the inflammatory cell infiltration into the dermis and thickened the epidermis. CSH reduced serum IgE level as compared to the control group. The levels of IL-4, IL-5, IL-13 and eotaxin in mouse splenocytes increased after treatment with concanavalin A and the increase of the cytokines was decreased by pre-treatment with CSH. The inhibitory effects of CSH on atopic lesions of DNCB-treated Nc/Nga mice were similar to those of dexamethasone, despite differing degrees depending on results evaluated in this study. These results may contribute to the development of a therapeutic drug for the treatment of AD.

P6.15.24
Immunostimulatory activity of low-molecular weight components of lysates of probiotic bacteria

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The ability of probiotic bacteria to modulate the innate and acquired immune responses is presumed to be due to complex molecules of their outer membrane. The major representatives of immunobiologically active polymeric components are lipoteichoic acid (LTA) of Gram+ bacteria, lipopolysaccharide (LPS) of G- bacteria, and peptidoglycan (PGN) occurring mainly in G+ species. We investigated possible immunostimulatory functions of other chemical, notably the low-molecular weight (LMW) entities prepared from lysates of probiotic bacteria *Lactobacillus casei* DN-114001 and *Escherichia. coli* strain Nissle 1917. The lysates were prepared by passing bacteria through a French press (1500 psi) followed by lyophilisation. The LMW fractions were obtained by microfiltration of lysates using the 3-, 10-, 30-, 50-, and 100-kDa cutoff centrifugal devices (Amicon®, Millipore). Nitrite and cytokines levels in supernatants of rat peritoneal cells were determined at the 24-h interval of culture, using Griess reagent and ELISA, respectively. All live and dead bacteria, LPS, LTA, and PGN as well as crude lysates activated the cytokine secretion and nitric oxide (NO) production. The effects of LPS, LTA and PGN were completely abolished by the 100-kDa cutoff microfiltration. On the other hand, the high stimulatory activity was retained after the reduction of chemical constituent diversity of crude lysates to LMW fractions. Those containing molecules up to 3 kDa exhibited approximately 80% of the stimulatory potential of the fraction containing molecules up to the 100 kDa.*The research was supported by grant CZ:GA ČR:303/12/0535 from the Grant Agency of the Czech Republic.*

P6.15.25
The efficient use of the anti-inflammatory cyclic peptide T*np* in the treatment of experimental autoimmune encephalomyelitis

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The currently available therapies for the treatment of multiple sclerosis (MS) have a modest effect in reducing relapses and remission. *Tnp* is a described anti-inflammatory cyclic peptide identified in the venom of *Thalassophryne nattereri* Brazilian fish. Here we examine the ability of T*np* to ameliorate the experimental autoimmune encephalomyelitis (EAE) in mice, a representative model of MS. In female C57BL/6 mouse model of EAE induced by sensitization to myelin oligodendrocyte glycoprotein$_{35-55}$ peptide, we found that prophylactic (0-9 days), therapeutic (10-19 days) or continuous (0-19 days) s.c. injections of *Tnp* (3 mg/kg/day) significantly reduced the clinical severity of EAE. The analyses by histology, zymography and flow cytometry allowed us to observe that modulation of EAE by T*np* was related with: 1) suppression of the activation state of conventional dendritic cells (DC) and the emergence of plasmacytoid DC during the induction phase of EAE, 2) blocking the traffic and leukocyte infiltration in the central nervous system (CNS) by suppression of metalloproteinases (MMP-9) activity and synthesis, 3) blocking of the reactivation and retention of Th1 and Th17 pathogenic clones in the CNS, 4) prevention the growth of microglia cells and the infiltrate of macrophages in the CNS, 5) increasing of regulatory cells (pDC, T and B cells) in the secondary lymphoid organs and in the CNS. These results shed light on the role of *Tnp* a small molecule in the regulation of inflammation and provides a new therapeutic opportunity for the treatment of MS. Support by: Fapesp and CNPq.

P6.15.26
GKS284 ameliorates inclusion formation in a primary neuronal model of SBMA

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Spinal and bulbar muscular atrophy (SBMA) is a polygutamine repeat disorder that results in gradual degeneration of the lower motor neurons of the anterior horn of the spinal cord and bulbar muscle of the jaw. Histologically, the disease is characterized by the formation of neuronal nuclear inclusions (NII) of mutant androgen receptor (AR) protein. Androgen ablation has been shown to halt or even reverse symptoms in transgenic mouse models of disease. Thus, selective androgen receptor modulators (SARMs) may provide an effective strategy for treatment of SBMA. PC-12 cells containing a mutant, expanded androgen receptor were treated with variety of SARMs.

P6.15.27
Cyclophosphane-induced in vitro phagocyte activity of peripheral blood leukocytes from healthy donors

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The aim of research was to study the effect of cyclophosphane (CP) on phagocyte activity of blood cells from healthy donors. Investigations were carried out with peripheral blood samples of 14 healthy donors. Blood samples were incubated for 1 h with CP (100; 10; 1; 0,10 and 0,01 µg/ml), then with formalinized sheep erythrocytes in 20 or 60 min at 37°C. All phagocyte parametres of neutrophils and monocytes were counted.
Low CP concentrations are found to increment the relative amount of phagocytizing neutrophils as compared with the control and data obtained at high doses. At CP concentrations of 100 µg/ml the amount of phagocytizing neutrophils comprised 40,00±3,69%, while at CP concentration of 0,01 µg/ml it was 59,88±3,72% (p<0,05). Introduction of 0,01 µg/ml CP also provoked the elevation of the phagocytizing monocyte number. CP applied in high doses resulted in lowering of phagocyte number as compared with the control. The absolute number of non-phagocytizing neutrophils under CP concentration of 100 µg/ml was statistically greater than under the dose of 0,01 µg/ml. Absolute amount of phagocytizing monocytes under the CP concentration of 100 and 10 µg/ml was statistically less than in control samples. While assessing the uptaking ability of phagocytes it was found that index of phagocytosis completeness was less than 1 with all of CP doses analyzed.
Thus, cytostatic cyclophosphane modulates the leukocyte phagocyte activity. High CP doses reduce all known parameters of monocytes and neutrophils.
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P6.15.28
Phycocyanobilin positively modulates inflammatory genes in acute cerebral hypoperfusion in rats

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Stroke causes high mortality rates and serious disability worldwide. Ischemic penumbra rescue is the main objective of acute interventions, such as thrombolysis and neuroprotection, but they show low translational success. Increasing evidence demonstrates that inflammatory response is involved in the stroke cascade. Here we evaluated the effects of Phycocyanobilin (PCB), the C-Phycocyanin linked tetrapyrrole, on gene expression in the anterior cortex of hypoperfused brains of rats. The acute drop of cerebral blood flow in this model is comparable to ischemic penumbra, thus offering a reliable tool for studying molecular mechanisms. After the permanent bilateral common carotid arteries occlusion, the animals were treated therapeutically with saline or PCB. Samples were taken 24h after the surgery. Global gene expression was analyzed with GeneChip Rat Gene ST 1.1 from Affymetrix, and the expression of particular genes was assessed by the Fast SYBR Green RT-PCR Master Mix method. The PCB treatment significantly modulated 190 genes (93 up- and 97 down-regulated) associated to several immunological processes in both the regulatory and effector subsets. Furthermore, PCB was able to positively modulate 13 genes mostly related to a detrimental pro-inflammatory environment. Our results support the view of major inflammatory mediators in acute cerebral hypoperfusion, effectively counteracted by PCB. These results may justify the application of PCB as a new acute disease modifying drug against ischemic stroke.

P6.15.29
Nitro-oleic acid regulates the classical and alternative activation of macrophages

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Nitrated fatty acids (NO2-FAs) are endogenously generated as an adaptive response of organism to oxidative conditions. NO2-FAs were shown to exert significant anti-inflammatory signaling action in immune and vascular cell models; nevertheless, detailed mechanisms of their action in regulation of innate immune responses are not completely understood. Therefore, we investigated the role of nitro-oleic acid (OANO2) in classically and alternatively activated macrophages.
In this study we used the concentration range of 0-2000 nM of OANO2. RAW 264.7 and bone marrow-derived macrophages were treated with OANO2 in the presence of lipopolysaccharide (LPS) and interleukin-4 (IL-4) for different time periods. The following analyses (cytokine array, ELISA, luminol-enhanced chemiluminescence, Griess reaction, Western blot, PCR) were focused on the basic physiological responses of macrophages.
Our results showed that OANO2 significantly regulated the activation of macrophages in a dose-dependent manner. More importantly, changes in the production of pro-and anti-inflammatory mediators were associated with reduced activation of c-Jun N-terminal kinase and expression of nuklear factor-kB and CD36, as well as with increased activation of signal transducer and activator of transcription 6.
In conclusion, NO2-FAs are able to serve as a protective compounds that can effectively down regulate the activation of macrophages. Our results have significant implications since the identification of key regulators of immune functions and information about the mode of their action may provide very important data for future clinical investigations.
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P6.15.30
A non toxic concentration of ethyl acetate extract of Cordia verbenacea D.C. reduces the expression of proinflammatory cytokine IFN-γ in human peripheral blood lymphocytes

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Cordia verbenacea is a native Brazilian plant popularly used as anti-inflammatory. Although this activity has been demonstrated previously, the molecular mechanisms are not yet fully understood. Likewise, few studies have reported the cytotoxic potential of this

plant. Thus, the aim of this study was to evaluate the toxicity of the ethyl acetate extract of C. verbenacea (EACV) on human erithrocytes and peripheral blood mononuclear cells (PBMC) as initial phase of the study about the probable anti-inflammatory activities of the plant. Cell viability and hemolytic activity were evaluated by trypan blue exclusion test. Furthermore, we performed an evaluation of immunomodulatory potential of EACV on proinflammatory cytokines expression. For this propose PBMC cultures stimulated with PMA in the absence or presence of a non-toxic concentration of the EACV was used to evaluate the intracytoplasmatic cytokine profile, using flow cytometry. According to the results, the EACV showed no hemolytic activity, neither cytotoxicity action on PBMC at the concentrations equal to or lower than 50µg/mL, when compared to control cell cultures. Moreover, the extract at 50µg/ml, added together PMA, reduced the percentage lymphocytes-IFN-γ + compared to cell cultures stimulated with PMA alone. These data presented here suggest that the anti-inflammatory effect observed in the folk use of the plant Cordia verbenacea can be partly explained by its inhibitor effect on type 1 cytokine expression.
Support: CAPES, CNPq and FAPEMIG

P6.15.31
Pseudobrickellia brasiliensis (Spreng) R. M. King & H. Rob. (Asteraceae) aqueous extract decreases IFN-gamma and TNF-alpha expression on human peripheral blood mononuclear cells, in vitro

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Pseudobrickellia brasiliensis (Asteraceae), known as “Arnica-do-Campo”, is a native plant from Brazilian flora and its alcoholic preparation has been used as anti-inflammatory in folk medicine. However, the biological mechanisms involved in this biological activity are not well elucidated. In our study we aimed verify the anti-inflammatory potential of P. brasiliensis aqueous extract (AQU) by performing an anti- and pro- inflammatory cytokine expression analysis on human peripheral blood leucocyte surface. The cytotoxicity promoted by the AQU, in vitro, was evaluated using trypan blue assay. For cytokine analysis, humans-PBMC were incubated in medium containing PMA plus Ionomycin as inespecific stimulating agents, in the absence or presence of AQU at the concentrations 25, 50 or 100 µg/ml. According our results, AQU showed no toxic effects on human PBMC and AQU at 100 µg/ml promoted a significant reduction in IFN-γ and TNF-α expression in response to PMA stimulation. These findings suggest that P.brasiliensis anti-inflammatory effect can be, at least in part, due PBMC pro-inflammatory cytokines inhibition.
Support: CAPES/CNPq/FAPEMIG

P6.15.32
Silymarin Normalizes Immune Dysfunction in Beta-Thalassemia Major Patients

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Iron overload conditions in beta-thalassemia major is the principal cause of oxidative stress and cell-mediated immune deficiencies. Despite indicative signs of severe oxidative deficiencies associated with beta-thalassemia major, little is known about intracellular redox status of immune cells. Once glutathione is a primary intracellular antioxidant and plays a fundamental role in the many T cell functions, present study was aimed to investigate intracellular glutathione (GSH) levels as well as proliferation of PHA-activated peripheral blood mononuclear cells (PBMC) in 28 beta-thalassemia major patients and 28 healthy age-matched individuals. In respect to potential benefits of flavonoids in the oxidative stress therapy, the effects of silymarin on the GSH levels and proliferation of PBMC from normal and thalassemia individuals were further examined. Quantitative determination of intracellular GSH and proliferative response of PBMC to PHA were performed before and after 72 h incubation of PBMC with various concentrations of silymarin (0, 5, 10, or 20 mug/ml). Our results revealed a significant reduction of GSH and proliferation in beta-thalassemia major cells; though treatment with silymarin led to restoration of both GSH levels and PBMC proliferation in thalassemia patients. Considerably low levels of GSH and depressed proliferative response of PBMC in beta-thalassemia major may be responsible for the cell mediated immune abnormalities in iron overload conditions. Moreover, the GSH restoration and improvement of PBMC growth by silymarin is a possible explanation for its reported antioxidant and immunostimulatory activities. These data suggest the benefit of using flavonoids to improve immune dysfunction in beta-thalassemia major.

P6.15.33
Establishment of Experimental Autoimmune Encephalomyelitis in Cynomolgus Monkeys, A Model for Multiple Sclerosis

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The current high failure rates of clinical trials for multiple sclerosis (MS) therapeutics impede the correct translation of the new treatments from concept to the patients. One of the major drawbacks leading to the failure is the use of rodent models in the preclinical studies. To improve this, we established the EAE model in non-human primate (NHP) that provide the much closer–to-human immune system for modeling MS. Three different antigens: brain white matter, spinal cord and MOG peptides, were tested for induction of EAE in Cynomolgus monkeys by two injections of antigen-adjuvant followed by daily monitoring of the disease progression. Two weeks after the immunization, monkeys started to show the typical disease related neurological symptoms, such as anorexia, weight loss, ataxia, neck spasticity, and blindness. MRI examinations discovered the multiple hyper-intense areas in the brain of the EAE monkeys at disease active stages, similar to those found in MS patients. The findings of gross autopsy indicated multiple hemorrhagic lesions in the white matter of the affected monkey brains. Both disease incidence and severity of the EAE were antigen-dose dependent. Interestingly, the disease progression induced by brain white matter and spinal cord presented mixed types of the EAE episodes, while only the typical relapsing-remitting disease, representing the major type of MS in patients, was induced by MOG peptide.
Our results demonstrated the successful establishment of the EAE models and, for the first time, evidenced the relapse-remitting EAE induced by MOG peptide in Cynomolgus monkeys.

P6.15.34
Novel S1P1 receptor agonist, CP9531, has desirable characteristics as a next generation agonist

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Fingolimod, a non-selective S1P1 receptor agonist, has been already launched for relapsing-remitting multiple sclerosis, and other agonists are under development for autoimmune diseases. These S1P1 agonists, however, provoke AV blocks and heart rate reductions. GIRK channel activation of cardiomyocytes is considered to be crucial, and it is activated by Gi and inhibited by Gq. We therefore tried to create a compound with fast receptor uptake and high selectivity to Gq as a next-generation S1P1 agonist.
Novel S1P1 agonist, CP9531 showed an ability to internalize 50% of the receptors by 30 minute-treatment at 10 nM. In addition, "EC_{50} of Ca influx" / "EC_{50} of GTP binding" ratio of CP9531 was approximately 2, which is 5-50 times lower than other S1P1 agonists.
Male SD rats were orally administered with CP9531 and the number of blood lymphocytes was counted at 6 and 24 hours after administration. ED_{50} was 0.17 mg/kg at 6 h and 0.65 mg/kg at 24 h. Similar PKPD profile was also confirmed in cynomolgus monkeys.

The preventive and curative effect of CP9531 in a mouse EAE model showed remarkable improvement in the EAE score in either conditions at 1 mg/kg p.o., q.d. The cardio toxicity of fingolimod phosphate in anesthetized rats was observed at 0.01 mg/kg, but not at 0.1 mg/kg of CP9531.
These results suggest that novel S1P1 selective agonist CP9531 with potent receptor uptake activity and Gq biased-activity has high efficacy and safety. We believe CP9531 has desirable characteristics for the next generation of S1P1 agonists.

P6.15.35
Evaluation of toxicity of the dichloromethane and ethyl acetate extracts from Ageratum fastigiatum in human peripheral blood mononuclear cells

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Ageratum fastigiatum is a common Brazilian medicinal plant used by folk medicine as topical anti-inflammatory agent. However, little is known about its anti-inflammatory mechanisms, as well as its cytotoxicity. Thus, before assessing the possible actions of the plant on the immune system, we investigated the toxicity of the dichloromethane and ethyl acetate extracts of A. fastigiatum on human PBMC, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. PBMCs obtained from five healthy volunteers were cultured with supplemented RPMI in the presence or absence of dichloromethane (DCM) and ethyl acetate (EA) extracts of the plant at different concentrations. Cells treated with DMSO or cadmium chloride (CdCl2) were used as solvent control and cytotoxicity positive control, respectively. EA extract from A. fastigiatum did not reduce cell viability at the tested concentrations, when compared to DMSO (EA at 0.8 µg/ml: 89.32 ± 11.49%; EA at 1,5µg/ml: 91.73 ± 9.062%; EA at 3µg/ml: 88.93 ± 7.316%, DMSO: 86.52 ± 5.693%). Conversely, the DCM extract decreased cells viability at the tested concentrations compared to DMSO (DCM at 20µg/ml: 52.12 ± 10.29%; DCM at 60µg/ml: 49.62 ± 5.502%; DCM at 125µg/ml: 52.55 ± 3.158%). These results indicate that ethyl acetate extract from A. fastigiatum, but not the dichloromethane extract can be used in PBMC cultures, at the tested concentrations, for further in vitro analysis.

P6.15.36
Effects of venoms from five Brazilian snake species on the viability of human peripheral blood mononuclear cells

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Snake venoms are complex mixtures of proteins, peptides, amino acids, nucleotides, lipids, carbohydrates, enzymes showing a broad range of biological activities. Venom derived components have been used as a pharmacological drugs in humans. The present work aimed to assess the cytotoxic effects of crude venom from 5 different species of snakes from Central region of Brazil on human peripheral blood mononuclear cells (PBMC) in order to further investigate the biological effects of them. $2x10^5$ cells/well from 12 healthy donors were stimulated with phytohemagglutinin PHA and IL-2 in presence of different concentrations of venoms (50, 5, 0.5, 0.05, 0.005 and 0.0005 µg/mL) from the following Brazilian snake species : *Bothrops pauloensis, Bothrops moojeni, Bothrops jararacussu, Caudisona durissa collilineata, Caudisona durissa terrificus. C*ytotoxic activity, DNA fragmentation were analyzed after 24, 48 and 72 hours of culture. Cell counts were done using hemocitometer chamber and trypan blue, and DNA fragmentation by gel analysis. Our results show that the absence of cytotoxic effects of all 5 venoms at concentrations of 0.05 ug/mL and 0.005 and 0.0005 ug/ml. However at concentration of 5ug/ml and 0.5 ug/ml the venoms killed the cells and a DNA fragmentation were also observed. Cytokine production and the expression of innate and adaptive receptors induced by those venoms on PBMC are under investigation. Our results suggest that at high concentration levels those venoms may induce apoptosis and open the possibility to further investigate the microbicidal, stimulatory and inhibitory effect of those snake venoms. Supported by CAPES (1433/2007-0), FAPEG, CNPq

P6.15.37
Activation of telomerase by novel compounds in human lymphocytes

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Recent studies demonstrate the importance of telomere length and telomerase activation on the viability and functionality of the immune system cells. Telomerase is a ribonucleoprotein that catalyzes the addition of telomeric repeats to the telomeres ends thus enables cell proliferation and controls cell lifespan. The ability of telomerase activation in lymphocytes, as a response to antigen, is decreased with age. Therefore, telomerase activation in human lymphocytes may improve their functionality. We have synthesized novel compounds (AGS) that activate telomerase expression and activity in vitro and in vivo. Here we investigated the effect of treatment of human lymphocytes with AGS in the presence and absence of mitogen on the expression and activity of telomerase. We found that naïve lymphocytes have a detectable level of telomerase protein and activity, both in the cytoplasm and in the nucleus. AGS compounds (25nM-250nM) increased telomerase expression and activity in normal human lymphocytes after treatment of 3h-15, while a significant increase of telomerase in concanavalin A (ConA) treated lymphocytes was detected 60 hrs. after treatment. However, AGS compounds did not increase the level of telomerase beyond that observed with ConA alone and did not cause cell proliferation. Increasing telomerase by AGS in oxidative stressed lymphocytes enhanced their survival up to 80%. These results suggest that pharmaceutical activation of telomerase in lymphocytes may improve their survival under stress conditions and may serve as possible therapeutic strategies in age related diseases.

P6.15.38
HLA-B allele frequencies in the Thai population

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There are 3 classes of *HLA* molecules; *HLA* class I, II and III, different classes each of which has different functions. The most important class for predicting drug allergies is *HLA-B* which belongs to *HLA* class I. Variations in *HLA-B* genes account for drug-response differences among individuals. *HLA-B**1502 has been reported to be associated with Stevens - Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) (SJS/TEN) in patients who received Carbamazepine, Allopurinol, Phenytoin, Amoxicilin and Co-trimoxazole etc. We determined *HLA-B* allele frequencies in Thai population which is useful for estimating the incidence of drug hypersensitivity. Blood samples from seven hundred and thirty nine Thai individuals were collected and DNA was extracted. The *HLA-B* genotyping was performed using bead array technique and we found that the allele frequencies in *HLA-B**4601, *HLA-B**4001, *HLA-B**5801 and *HLA-B**1502 are 12.72%, 8.32%, 8.32%, and 7.51% respectively. The *HLA-B**4601 did not classify as a marker for any drug hypersensitivity. *HLA-B**4001 was associated with Stavudine hypersensitivity, the drug in Acquired Immune Deficiency Syndrome (AIDs) treatment whereas *HLA-B**5801 was associated with Allopurinol hypersensitivity, a rheumatoid arthritis treatment. Moreover, *HLA-B**1502 was associated with response to Carbamazepine hypersensitivity, an anti-epileptic drug. However, the tendencies for drug hypersensitivity besides the HLA-B genotype depend on the drug exposure and pharmacodynamics interaction.

P6.15.39
Cationic liposomes bearing IL -2 induce cytotoxic lymphocytes

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We have recently described a cationic liposome that contains spermidine in its lipidic bilayer, and which is able to bind IL-2 (CL-IL-2) in such a way that this cytokine is expressed on its external surface. We also demonstrated that this liposome binds to INBL, a cervical cancer cell line that expresses IL-2 receptors, thus making the cells themselves IL-2 presenters. In this work, we demonstrated that when the lymphocytes are stimulated with of CL-IL-2, the leukocytes proliferate and become cytotoxic. Almost 50% of the cells were CD4 /CD45 RO, and the maining 50% were CD8/CD45 RO thus hinting that lymphocytes are responsible for the tumor burden reduction. The presence of a large number of lymphocytes and the fact that many had a CD8+ phenotype suggests that these lymphocytes were the cytotoxic cells are responsible for the previously observed antitumor effect. The possible formation of a bridge between the IL-2R receptors on both, the lymphocytes and the INBL cells, by the IL-2 bearing liposomes, and its possible effect on the activation of antitumor cytotoxic lymphocytes is discussed.

P6.15.40
Activity of beta-adrenergic receptors in vascular lesions associated with proteins inflammatory in the periodontal disease

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Introduction:Nervous system may play an important role in regulating inflammatory mediators by adrenoceptors.**Objectives:W**e evaluated the proteins related to vascular damage after the action of β antagonist in an experimental model for Periodontal Disease(PD).**Methodology:**The groups:1) animals that received vehicle without ligature (SPD), 2) with ligature that received vehicle (PD),3) with ligature receiving 0.1 mg of propranolol (P),4) animals with ligature treated with 5mg of P and 6)animals with ligature that received 20mg of P. Thirty days after induction of PD, it was collected the whole blood for a hemogram analysis and serum for measurement of TNF-α and IL-6 and C-reactive protein (CRP).Blood counts were obtained using an automated method.Cytokines were quantified by ELISA and CRP by turbidimetry.The mandibles were evaluation the bone resorption. **Results:**We found changes in the systemic levels of TNF-α and IL-6 when compared the groups SPD and PD as well as in the P-treated animals CRP was bigger in the PD group (67.3%-PD=0.875±0.46) in comparison to SPD(0.52±0.37) Both treated groups 0.1(0.75±0.25) and 5 mg/kg(0.45±0,24) had decreased the CRP in comparison to the group PD, in contrast with the treated group, the group with 20mg/kg increased by 96.15%(1.02±0.63) the amount of CRP related to the group SPD an increase of 16.57% compared to PD-group.Regarding the systematic evaluation of hemogram there was no change.The resorption was confirmed by histological evaluation, in which the group treated with 0.1and 5mg/kg inhibit the bone resorption.**Conclusion:**The data indicate an indirect contribution of the beta-adrenergic blockade in development of vascular changes. **Financial Support:**FAPEMIG 097/09

P6.15.41
A new immunomodulatory therapy to treat pathologies related to a defective cell-mediated immunoresponse

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A new immunomodulatory therapy based on association of intradermal administration and anti-COX2 drug has been used to treat pathologies related to a defective cell-mediated immune response in animals, which represent a good model for similar disease in humans. In particular, we treated canine Leishmaniasis (CL), feline inflammatory induced sarcomas (FIIS), a bird model of Guillame Barrè syndrome (Proventricular dilatation Disease or PDD), and a chronic-granulomatous progressive and lethal pathology of the cat, known as Feline Infectious Peritonitis (FIP). We investigate the changes of lymphocyte subsets, immunological indexes, in vitro macrophage function, IgG1/IgG2 ratio with determination of levels in Th1/Th2 response induction, and clinical data. The study involved 30 dogs with CL, 12 cats with FIIS, 25 parrots with PDD, and 10 cats with FIP. Compared to the control groups, only the patients treated with the immunomodulatory protocol showed an enhancement of cell-mediated immune response, which resulted in remission of symptoms in CL (89%, included PCR negativization), PDD (93%), FIP (52%), and absence of tumor recurrence in FIIS (78%). Usually, in the last three diseases listed above the conventional therapy has always bad prognosis. Combined immunomodulatory therapy appears to yield improved survival for patients with different pathological conditions in which the cell-mediated immune response is altered or depressed; these findings should be verified in larger clinical trials.

P6.15.42
Ageratum fastigiatum essencial oil compounds decrease the expression of proinflammatory cytokine TNF-alpha in peripheral blood leukocytes stimulated with Phorbol Myristate Acetate (PMA) in vitro

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Ageratum fastigiatum (Gardner) R. M. King & H. Rob, a plant also known as "matapasto", belongs to Asteraceae family utilized by folk medicine as a plant with anti-inflammatory and analgesic proprieties. However, little is known about its potential to alter parameters of the inflammatory response. The aim of this study was to charachterize the main chemical compounds present in Ageratum fastigiatum essencial oil (AFOE) and to evaluate the intracelluar TNF-alpha, IFN-gamma and IL-10 profile on human peripheral blood lymphocytes stimulated in vitro with PMA (phorbol myristate acetate) in the presence or absense of AFOE. Addicionally, the nontoxic concentrations of EOAF on PBMC was evaluated by trypan blue exclusion test. The CG/MS analysis revealed the main compound observed in EOAF was α-pinene (7.51%), limonene (5.9%), trans-caryophylleno (2.04%) and α-Humoleno (3.52). According to our results, EOAF at 5x10-3 and 1x10-2µL/mL did not alter the viability of leukocytes when compared to the control group and both concentrations reduced the percentage of lymphocytes-TNF-α+. No changes were observed in percentage of cells stained for the IFN-gamma and IL-10 cytokines. Our results suggest that part of anti-inflammatory activity attributed to Ageratum fastigiatum can be, at least in part, due the action of its constituents in reducing the pro-inflammatory cytokine TNF-α.
Support: CNPq, CAPES and FAPEMIG

P6.15.43
Biochemical and cellular correlates of embelin against bacterial endotoxin induced airway inflammation

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To investigate mechanism of anti-inflammatory activity of embelin against bacterial endotoxin induced acute lung inflammation.
Embelin was isolated from dried seeds of *Embelia ribes.* Female Sprague Dawley rats (180-230g) were divided in six groups. Saline (5ml/kg/day,*p.o.*) Embeline (5,10&20mg/kg/day,*p.o.*), Roflumilast (1mg/kg/day,*p.o.*) were administered for four days. Animals were anesthetised with ketamine-xylazine (80:20 mg/kg,*i.p.*) and administered with sterile solution of LPS (Lipopolysaccharide) intratracheally. After four hours bronchoalveolar lavage was done with icecold phosphate buffer. Bronchoalveolar lavage fluid (BALF) and lung samples were investigated for total cell count, differential cell count and myeloperoxidase, nitrate/nitrite, total Protein, albumin, histopathology respectively.

Data was analysed by GraphPad Prism 5.01 and results were expressed as means ± SEM. P value of <0.05 was considered to be significant.
Saline treated animals showed significant increase in total and differential cell count (p<0.05). Embelin (5,10&20mg/kg) & Roflumilast (1mg/kg) treated animals showed significant decrease in total and differential cell count compared to saline treated animals (p<0.05).
Saline treated animals showed significant increase in myeloperoxidase, nitrosative stress, albumin and total protein in lung samples but embelin (5, 10 & 20mg/kg) and roflumilast (1mg/kg) showed reduction compared to saline treated animals (p<0.05). Mild diffused lesions involving focal interalveolar septal, intraluminal infiltration of neutrophils were observed in embelin (5&10 mg/kg) pretreated while no abnormality was detected in embelin (20 mg/kg) rofelumilast (1mg/kg) pretreated rats.
Embelin showed anti inflammatory and protective effect against LPS induced airway inflammation by reducing nitrosative stress and inhibiting mononucleated cellular infiltration. Further studies on molecular level are in progress.

P6.15.44
Immunotropic drugs for local use in stomatology

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The problem of the treatment of inflammatory periodontal and oral mucosa diseases is one of the important problems in stomatology. A complex therapy using medicines of topical application plays crucial role in the treatment of these diseases. Taking into account that the forming and development of the most of the known pathological processes are determined by the nature of immunopathogenetic changes, development of a new immunotropic drugs for local application is especially relevant.
The number of new pharmaceutical compositions for local treatment of inflammatory oral cavity diseases using immunotropic substances of cytokine agents, phthalhydrazide and piperazine derivatives and zinc-containing components were suggested [1]. Silicon-and silicontitanium-containing glycerohydrogels were proposed as an ointment bases. These hydrogels shown a strong regenerating activity due to the presence of silicon in biologically active form, as well as antioxidant and cytoprotective action in the case of titanium in a biologically active form. In addition, these bases have a high transcutaneous activity, which allows to reduce the concentration of immunotropic substances and leads to a deeper penetration into the affected tissue.
High pharmacological activity (anti-inflammatory, healing and regenerating) and safety of developed medicines were shown in experiments with laboratory animals. The acute local inflammation of the mucous membrane of the lower lip (thermal burns) and mechanical injury of gingival of rats of the Wistar population were used as models.
The work was supported by the Presidium of the Russian Academy of Sciences - programs №№ 12-М-34-2071 and 12-П-3-1030.
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P6.15.45
T-cell Electrophysiology Dynamics

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The presence of T-lymphocytes, in particular T-helper-cells, is in the context of great importance, but in some medical issues their immunological implication must be repressed. For example, the appearance of graft rejection is a major problem in transplantation medicine. In order to suppress the causative immunological reaction, the pharmaceuticals cyclosporin A (CsA) and tacrolimus (FK506) are in use as standard drugs. Both immunomodulators affect electrophysiological processes by blocking the relevant signaling cascade in activated T-cells and in this way also modulating the calcium influx passing through the calcium-release activated channel (CRAC) in the plasma membrane of T-lymphocytes.
With regard to the significance of electrophysiology in coherence with immunological interactions and pharmacological issues, a mathematical modeling approach is demanded in order to quantitatively understand the associated ion dynamics in T-lymphocytes, embedded in a systems immunological context. Building on the results from electrophysiological measurements, the in silico T-cell model includes single transmembrane protein characteristics in order to derive ion dynamics patterns on the whole cell level with a particular focus on calcium. Beside of the most central CRAC, ionic pumps and channels for calcium, sodium and potassium, as well as the endoplasmatic reticulum acting as a calcium store are represented in the model.
Starting with simulations of a naive T-cell, the model will then be used to analyse the electrophysiological changes following T-cell activation. Further, the modeling approach will finally provide a starting point for in silico studies relating to the therapeutic immune modulation of ion-conducting proteins in T-lymphocytes.

P6.15.46
Study of Curcumin anti-inflammatory effects on Astrocyte cell line: relevance to multiple sclerosis

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Background: In recent years, several studies have been focused on the effects of Curcumin, the principal active component of turmeric, on neurodegenerative diseases. Astrocytes contribute to the pathogenesis of Multiple Sclerosis. Therefore, we investigated the influence of Curcumin on LPS-induced astrocytes cell line (U-373 MG) compared with HEK293 cell line as a control.
Methods: After selecting the optimal concentration of Curcumin by MTT assay, cells were treated with purified Curcumin. Then, the activity of MMP-9, involved in the destruction of the Brain Blood Barrier, was measured by gelatin zymography. The expression of different genes including CTLA-4, neurotrophin 3, CCL-2 and insulin-like growth factor 1 were evaluated by Real-Time PCR. At the end, five pro and anti-inflammatory cytokines were assessed with quantitative ELISA.
Results and Conclusion: Curcumin demonstrates anti-inflammatory effects in activated astrocytes.

P6.15.47
Daphanetin prevents acute inflammatory lung injury in mice via a TNFAIP3-dependent mechanism

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Daphnetin, a Chinese herb-derived compound, has been used to treat chronic inflammatory and autoimmune diseases, but its mechanism is not well understood. We here showed that Daphnetin significantly reduced lipopolysaccharide (LPS)-induced production of TNF-α, IL-1β and IL-6 in both RAW264.7 cells and mouse primary peritoneal macrophages, and inhibited LPS-induced activation of p38 and ERK MAPKs. Moreover, Daphnetin exhibited to retard the LPS-induced activation of IκB kinase, IκBα degradation, p65 nuclear translocation and phosphorylation, as well as NF-κB-driven promoter activity. Further study indicated that TNFAIP3 (also A20) was remarkably induced by Daphnetin in macrophages, which appeared

to be largely responsible for the down-regulation of NF-κB-dependent inflammatory gene expression. Accordingly, intratracheal administering Daphentin significantly protected mice from LPS-induced acute lung injury and inflammation, in parallel with the up-regulation of TNFAIP3 levels in lung tissues. Our results indicate that Daphnetin exerts anti-inflammatory effects, at least partially through TNFAIP3-regulated NF-κB-dependent inflammatory signaling in vivo as well as in vitro.

P6.15.48

Proteasome inhibition by bortezomib increases IL-8 expression in ovarian cancer cells by IKK and EGR-1 dependent mechanism

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Interleukin-8 (IL-8) has a crucial role in ovarian cancer progression through its induction of tumor cell survival, proliferation, angiogenesis, and metastasis. IL-8 expression and serum levels are increased in patients with ovarian cancer, and correlate with poor prognosis. Bortezomib (BZ) is the first clinically approved proteasome inhibitor that has shown a remarkable activity in patients with multiple myeloma and other hematological malignancies. One of the main mechanisms of BZ function is the suppressed proteasomal degradation of IκBα, resulting in the inhibition of NFκB activity and expression of NFκB-dependent anti-apoptotic genes. Bortezomib has so far failed to exhibit a significant clinical activity in ovarian cancer patients. However, the mechanisms underlying ovarian cancer resistance to BZ are largely unknown. In this study, we investigated how BZ regulates transcription of IL-8 in ovarian cancer OVCAR-3 cells. Our data demonstrate that BZ markedly increases IL-8 expression, while expression of other NFκB -regulated genes is unchanged or suppressed. The mechanism consists of an increased recruitment of S536-p65 NFκB, IκB kinase (IKK) and the transcription factor EGR-1 to the endogenous IL-8 promoter. In addition, IKK inhibition and EGR-1 suppression significantly inhibit the BZ-increased IL-8 expression in OVCAR-3 cells. Together, our data indicate that combining proteasome inhibition with anti-inflammatory drugs targeting IKK could provide a new effective strategy for IL-8 suppression in ovarian cancer and other tumors characterized by excessive IL-8 expression.
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P6.15.49

Safety, tolerability and pharmacokinetics (PK) of human immune globulin subcutaneous (IGSC), 20%: interim analysis of a phase 2/3 study in patients with primary immunodeficiencies (PI)

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Background: IGSC 20% is a new, ready-for-use, liquid preparation of highly purified human IG for SC administration. We report interim analyses from data cutoffs in June 2012 (safety/tolerability) and September 2012 (PK) of IGSC 20% in patients with PI in Europe.
Methods: This is a phase 2/3 open-label study in patients with PI aged ≥2 years. Epoch 1: IGSC 16% or intravenous IG (IGIV) 10% administered at pre-study doses for 3 months. Epoch 2: IGSC 20% administered once-weekly for 12 months at Epoch 1 doses. IgG trough levels are maintained >5 g/L. The primary endpoint is the rate of acute serious bacterial infections (SBIs). PK was evaluated in patients treated with IGIV in Epoch 1.
Results: At this time, 31 of 43 patients who started the study are in Epoch 2. Interim analysis (n=30) showed no acute SBI episodes or severe infections with IGSC 20%. Overall infection rates/patient-year: 4.6 (IGSC 20%), 8.1 (IGSC 16%) and 7.3 (IGIV). There were no serious adverse events (AEs) considered related to any treatment. All local adverse drug reactions were mild in severity; no severe drug-related systemic AEs were reported with IGSC 20%. Of 385 IGSC 20% infusions, 99.7% required no administration changes. $AUC_{IGSC20\%}/AUC_{IGIV}$ (n=9) was PK-equivalent at 83.9%. Median trough IgG levels (n=13): 8.5 g/L (IGSC 20%) and 7.5 g/L (IGIV).
Conclusion: To date, IGSC 20% has provided a well-tolerated, self-administered therapy, with no dose adjustments needed from pre-study IGIV dose. This study is ongoing to confirm IGSC 20% efficacy/safety/PK over 12 months.

P6.15.50

Tolerability, efficacy and pharmacokinetics (PK) of facilitated-subcutaneous infusion of human immunoglobulin G, 10% (IGSC) and recombinant human Hyaluronidase (rHuPH20) [IGHy] in adult patients with primary immunodeficiency (PI): results from a Phase 3 study

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Background: IGHy, compared with IGSC alone, has reduced the need for multiple infusion sites and weekly IGSC dosing, and has permitted infusion rates and dosing intervals equivalent to intravenous administration (IGIV). We report a data subset, for patients aged≥18 years at study entry, from the final tolerability/efficacy/PK data of a pivotal phase 3 trial of IGHy in pediatric and adult patients with PI.
Methods: Patients were treated with IGIV for 3 months at prestudy doses and frequency. Subsequently, IgG, 10% was administered SC, at a dose that was 108% of the weekly equivalent of the IV dose, preceded by rHuPH20 infused through the same SC needle at a dose of 75U/g IgG. Patients received IGHy for 12 months.
Results: Fifty-nine patients received 985 IGHy infusions, 98% of which were completed without a change in rate due to adverse drug reactions (ADRs). Median infusion sites/month was 1.13. There were no serious ADRs. Local ADR rate was 0.286/infusion. Temporally associated systemic AE rate was 0.20/infusion for IGHy vs. 0.33/infusion for IGIV. The annual rates were 0.00 for acute serious bacterial infections and 3.20 for all infections. AUC_{IGHy}/AUC_{IGIV} was pharmacokinetically-equivalent at 91.8% (90% CI: 89.9-93.7%).
Conclusion: In patients with PI aged≥18 years, IGHy was effective and well tolerated at infusion rates comparable to prestudy IGIV administration. The majority of patients were treated every 3-4 weeks in 1 site without a rate change due to ADRs. Bioavailability (per AUC) was similar to IGIV, and significantly better compared with IGSC without rHuPH20.

P6.15.51

In vitro cytotoxic activity of four plants used in Persian Traditional medicine

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Aim of the study: the aim of this study was to investigate in vitro cytotoxic activity of four methanolic crude of four Persian plant extract against panel cell lines. **Material and Methods:** Methanolic extracts were tested for their possible antitumor activity and cytotoxicity using the 3-(4,5-dimetylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay on six cancer cell line; non-Hodgkin's B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), Human acute myelocytic leukemia (KG-1A) cell lines, Human breast carcinoma cell line (MCF-7 cells), human prostate cancer cell line (PC3) and mouse fibrosarcoma cell line (WEHI-164) and one normal cell line; Human

umbilical vein endothelial cells (HUVEC). **Results:** All species showed dose dependent inhibition of cell proliferation. IC50 values ranging from 25.66±1.2 to 205.11±1.3 µg/ml. The highest cytotoxity activity *Chelidonium majus L> Ferulago Angulata DC> Echinophora platyloba DC> Salvia officinalis L*, respectively. **Conclusion:** all extracts demonstrate promising cytotoxity activity as a natural resource for future bio-guided fractionation and isolation of potential antitumor agents.

P6.15.52
Inhibition of growth and induction of apoptosis in fibrosarcoma cell lines by *Echinophora platyloba* DC: In vitro analysis

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Cancer is a disease that is characterized by too little apoptosis. Understanding apoptosis regulation is a main concern in the development of chemotherapeutic anticancer drugs on malignant cells. *Echinophora platyloba DC* plant is one of the indigenous medicinal plants in Iran. The objective of this study was to examine the in vitro cytotoxic activity and the mechanism of cell death of crude methanolic extracts of *E. platyloba*, on WEHI-164 cell line. Cytotoxicity and viability of methanolic extract was assessed by 3- (4, 5-dimethylthiazol-2-yl) - 2, 5-diphenyltetrazolium bromide (MTT) and dye exclusion assay. Cell death detection was employed to quantify the nucleosome production during apoptosis. The cell death was confirmed as apoptosis using terminal deoxynucleotidyl transferase-(TdT) mediated dUTP nick end labeling (TUNEL) assay and DNA fragmentation (Gel Electrophoresis). Semi-quantative RT PCR was used to evaluate the mRNA expression levels of c-myc, Bcl2, and caspase 3 in WEHI-164 cells. Our results demonstrated that the extract decreased cell viability, suppressed cell proliferation, and induced cell death in a time- and dose dependent manner in WEHI-164 cells when compared with a chemotherapeutic anticancer drug, Toxol. Observation proved that apoptosis was the major mechanism of cell death. WEHI-164 cell death elicited by the extract was found to be apoptotic in nature based a clear indication of DNA fragmentation and TUNEL assay, which are a hallmark of apoptosis. Furthermore, RT-PCR analysis showed that the extract induced apoptosis in WEHI-164 cells by activating Caspase-3 and down-regulating Bcl-2.
Key words: Echinophora platyloba DC; Cytotoxic; Anti-tumor; Apoptosis; Cancer

P6.15.53
Apoptotic and cytotoxic activities of Ferulago Angulata extract on panel cancer cell line by induction of apoptosis

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Ferulago Angulata (FA), also known as Chovir, is an indigenous plant, which has been used in Persian medicinal herb therapy. Although recent studies have demonstrated its anti-tumor activities on cancer cells in vitro, the exact mechanism is not completely explained. Furthermore, the objective of this study was to examine the in vitro cytotoxic activities of methanolic extract prepared from Ferulago Angulata (FA) on prostate cancer (PC3), fibrosarcoma (WEHI-164), B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), Human acute myelocytic leukemia (KG-1A) and Human Umbilical Vein Endothelial (HUVEC) cell lines using a Micro-culture tetrazolium test (MTT) cytotoxicity assay, and to test whether the mechanism involves induction of apoptosis. Cell death ELISA was applied to quantify the nucleosome production subsequent nuclear DNA fragmentation during apoptosis. Results have shown that methanolic extract at 50 to 800 µg/ml dose-dependently suppress the proliferation of WEHI-164, PC3, Raji, U937 and KG-1A cells by more than 80% ($p < 0.01$), with ascending order of IC50 values in 24h : WEHI-164 > PC3 > KG-1A > U937 > Raji. The extract did not exert any significant cytotoxic effect on normal cell line HUVEC (IC50 > 800 µg/ml). Nucleosome productions in WEHI-164, PC3, Raji, U937 and KG-1A cells were significantly increased by 8-fold respectively upon the treatment of FA extract, while no significant nucleosome production was detected in extract-treated HUVEC cells. The FA extract was found to selectively and dose-dependently inhibit the proliferation of lymphoma and leukemic cells possibly via an apoptosis-dependent pathway.

P6.15.54
Studies on inhibitory and cytotoxic activities of Salvia officinalis L. extract on cancer cells by induction of apoptosis

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Ferulago Angulata (FA), also known as Chovir, is one of the indigenous plants, which used in Persian medicinal herbs. Although recent studies have demonstrated its anti-tumor activities on cancer cells in vitro but the exact mechanism is not completely explained. Furthermore, the objective of this study was to examine the in vitro cytotoxic activities of methanolic extract prepared from Ferulago Angulata (FA), prostate cancer (PC3), fibrosarcoma (WEHI-164), B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), Human acute myelocytic leukemia (KG-1A) and Human Umbilical Vein Endothelial (HUVEC) cell lines by a Micro-culture tetrazolium test (MTT) cytotoxicity assay, and to test whether the mechanism involves induction of apoptosis. Cell death ELISA was applied to quantify the nucleosome production subsequent nuclear DNA fragmentation during apoptosis. results were shown that methanolic extract at 50 to 800 µg/ml dose-dependently suppressed the proliferation of WEHI-164, PC3, Raji, U937 and KG-1A cells by more than 80% ($p < 0.01$), with ascending order of IC50 values in 24h : WEHI-164 > PC3 > KG-1A > U937 > Raji. The extract did not exert any significant cytotoxic effect on normal cell line HUVEC (IC50 > 800 µg/ml). Nucleosome productions in WEHI-164, PC3, Raji, U937 and KG-1A cells were significantly increased by 8-foldrespectively upon the treatment of FA extract, while no significant nucleosome production was detected in extract-treated HUVEC cells. The FA extract was found to selectively and dose-dependently inhibit the proliferation of lymphoma and leukemic cells possibly via an apoptosis-dependent pathway.

P6.15.55
Immunosuppressive and anti-inflammatory properties of pyrimidine derivatives

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We synthesized novel C5-substituted pyrimidine analogues belonging to the groups of 2-amino-4,6-dihydroxypyrimidines (I), 2-amino-4,6-dichloropyrimidines (II), 4,6-dichloro-2-[(*N*,*N*-dimethylamino)methyleneamino]pyrimidines (III), and 4,6-dichloro-2-formamidopyrimidines (IV). The C5 substituents were represented by hydrogen (1), isopropyl (2), allyl (3), butyl (4), *sec*-butyl (5), and benzyl (6). The immunobiological properties of compounds were screened under the *in vitro* conditions using mouse and rat peritoneal macrophages ($2x10^6$/ml; 24-h culture in RPMI-1640 medium; the concentration of compounds being 50 µM). The effects were found to be closely dependent on the structure of analogues. Compounds from the groups III and IV proved to interfere with the immune (LPS)-activated production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) that were determined by Griess reagent and ELISA, respectively. Both NO and PGE_2 were inhibited by about 90%. Many derivatives significantly reduced secretion of proinflammatory cytokines IL-1β, IL-6 and TNF-α. The compounds had no effect on the viability of cells. No substantial biological significance of the C5 substituents was observed. The compounds from the group II were less effective,

reducing the production of NO by approximately 50%, and only some of them (II4, II5, II6) significantly reduced the PGE_2 production. The group I compounds were virtually biologically inactive. Compounds from the groups II and IV proved to possess anti-inflammatory activity under conditions *in vivo*. They were effective in alleviation of carrageenan-induced acute paw oedema and in substantial reduction of severity of adjuvant-induced arthritis in rats. *The research was supported by grant CZ:GA ČR:303/12/0172 from the Grant Agency of the Czech Republic.*

P6.16 Biomarkers and clinical profiling of human immune responses

P6.16.01
Slceroderma: from clinical classification to autoantibodies profile

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Background: Scleroderma (SSc) is an autoimmune condition strongly associated with the presence of antinuclear antibodies (ANA) with more than 90% of patients showing seropositivity.
Aim: We had investigated the relationship between SSc-specific autoantibodies (Aab) and clinical phenotype in monocenter patients with SSc.
Method: 97 sera of consecutive patients with diagnosis of SSc were analyzed for anticentromere (ACA), anti-Scl70, anti-RNA polymerase III, anti-U1RNP, anti-Th/To, anti-Pm/Scl, anti-Ku and anti-fibrillarin Aab using the Euroline immunoblot assay.
Results: In sera from 97 patients with scleroderma, ACA was present in 37 (38.1%), Scl70 in 22
(22.6%), RNA Pol III in 4 (4.2%), U1RNP in 20 (20.6%), Th/To in 4 (4.14%), Pm/Scl in 5 (5.3%) Ku in 3 (3.1%) anti-fibrillarin antibodies in 2 patients (2.06%).
Limited SSc (=46) were associated with ACA (n=34,74%, P < 0.001) and Th/To (=3, 6.6%)
antibodies. Diffuse SSc (=28) were associated with Scl70 (=18, 64.2%, P = 0.002) and RNA polymerase III (=4, 14.2%). Overlap syndrome (=23) were associated with U1RNP antibodies (=18, 78.2 %,P<0.001) . Significant associations between Scl70 and interstitial lung disease (9/23, 39.1%,), RNA Pol III and renal crisis (4/4 100%,), U1RNP and pulmonary hypertension (10/20, 50%) were seen. SSc patients that were anti-fibrillarin positive had severe lower gastrointestinal involvement.
Conclusions: SSc-specific ANA are associated with both clinical, organ complications and survival. Autoantibody status could confer relative protection from specific organ disease or an overall good prognosis. Clinical features in SSc are remarkably heterogeneous but grouping patients based on autoantibody profiles can be a useful approach for clinicians.

P6.16.02
Biomarkers associated with treatment failure in Kawasaki disease

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[Background and Purpose] Non-response to intravenous immunoglobulin (IVIG) is a risk factor for developing coronary artery lesions (CAL) in children with Kawasaki Disease (KD). Our DNA microarray analysis on whole blood leukocytes demonstrated that IVIG influenced the gene expressions in a broad functional range favoring down-regulation. Among them, Polycythemia Rubra Vera-1 (PRV-1), a differentiating granulocyte-specific gene, was most abundantly expressed in non-responsive KD patients than in responsive patients and febrile controls. Thus we measured the protein levels of PRV-1 as well as other inflammatory cytokines in KD patients to identify biomarkers associated with IVIG non-response and CAL development.
[Methods] Plasma levels of G-CSF, IL-6, IL-8, IL-10, IL12p40, IL-17, TNF-α, IFN-γ, MCP-1, VEGF, sIL-2R, sTNFR1, sTNFR2 and protein contents of PRV-1 and intracellular phosphorylated-STAT3 were measured before and after IVIG treatment using multiple beads assay and flow cytometry, respectively.
[Results] Among 100 eligible patients, G-CSF, IL-6, sTNFR1, sTNFR2 and PRV-1 expression were significantly elevated in non-responders ($p<0.01$) and in patients who developed CAL ($p<0.01$). Logistic regression analysis showed a combination of sTNFR1 and PRV-1 to be associated with both IVIG non-response and CAL development. Using these two parameters, IVIG non-response can be predicted with 81.3% sensitivity and 78.3% specificity; CAL development with 85.7% sensitivity and 74.4% specificity.
[Conclusion] These results provide evidences for dysregulated immunological pathways in KD patients. Especially, our study suggests that sTNFR1 and PRV-1 are useful biomarkers to predict IVIG non-response and CAL development.

P6.16.03
Is Basophil Activation Test as a Safe Diagnostic Tool for Immediate Drug Hypersensitivity to Proton-Pump Inhibitors?

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Declared immediate hypersensitivity (HS) reaction to widely used proton pump inhibitors (PPIs) are rare but can be life-threatening. For the diagnosis, skin tests have limited sensitivity and the oral provocation test (OPT) is not devoid of risks.
This study assessed the reliability of an *in vitro* test, flow cytometry-assisted basophil activation test (BAT) as a safe diagnostic method for PPIs-induced HS.
The BATs were performed in 7 patients with immediate PPI HS reactions and in 6 specimens from healthy volunteers tolerating these drugs. Heparinized whole blood samples from all subjects were stimulated with diluted various PPI solutions and allergen derivatives. The basophils of samples were labeled with PE conjugated anti-CD203c. The basophils were detected by FACSCalibur. Results of the test are considered positive when the difference within activated basophils of the patient and negative control is greater than or equal to 15%.
Patients with HS to PPIs were predominantly presented with an urticaria or angioedema (%70), the indicated allergy was with lansoprozole for six cases, with esomeprazole with one case. The test remained negative for all the controls tolerating PPIs, but positivities for the culprit PPI (lansoprazole: 33%) were present for allergic patients. BAT results were compared with patients' histories and skin tests.
BAT is a rapid, safe and useful additional method for diagnosis of hypersensitivity reactions and this study is the first, to our knowledge, reporting its use in PPI hypersensitivity.

P6.16.04
HLA-G 14-bp Deletion/Insertion, 3003C/T, 3010C/G and 3027A/C polymorphisms in HTLV-1 infection

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The most of HTLV-1 infected patients remain asymptomatic (HAC) throughout life, and the risk factors associated with the development of related diseases, such as HAM/TSP and ATL, are not fully understood. The human leukocyte antigen-G molecule (HLA-G), is expressed in several pathological conditions, and is related to

immunosuppressive effects that allow the virus-infected cells to escape the antiviral defence of the host. In this work, we evaluated the correlation between HLA-G polymorphisms in symptomatic and asymptomatic HTLV-1 infected patients. Four polymorphisms of the exon 8 of the HLA-G gene were analysed by gene sequencing in 46 HAC and 42 HAM HTLV-1 infected patients and 155 healthy control patients. HTLV-1 infected patients group showed higher frequency of the 14-bp D/I genotype, responsible for increased transcription of the HLA-G, than healthy control group (p=0,0642). HAC and HTLV-1 infected patients groups, compared to healthy control group, showed increased frequency of the 3003TT genotype, responsible for increased transcription of the HLA-G, (p=0,0146 and p=0,0058 respectively) and decreased frequency of the 3003CT genotype (p=0,0142 and p=0,0037 respectively). Still, HAC and HTLV-1 infected patients groups showed increased frequency of the 3003T allele and decreased frequency of the 3003C allele compared to healthy control group (p=0,0147 and p=0,0042 respectively). We found no relation among the groups in studies to 3010C/G and 3027A/C polymorphisms. In conclusion, the 14-bp D/I and 3003TT genotypes were associated with risk factor against HTLV-1 infection and 3003CT genotype was associated with protection factor against HTLV-1 infection.

P6.16.05
Different *In vitro* profiles of biologicals in human PBMC lymphocytes activation assay

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The development of biopharmaceuticals which includes recombinant proteins and monoclonal antibodies, has been on the increase due to the commercial success of many biological products. Regulatory agencies have become interested in potential invitro assays using human cells that may predict immunotoxic and immunostimulatory events in humans especially after the Tegenero incident in London. Aim of this work was to develop *in vitro* assays to characterize and potentially predict the activation of human PBMC lymphocytes induced by different biological compounds. Human PBMC were isolated from different donors and culture in the presence of different biologicals. At different time points, cytokines release involved in the innate and adaptive immunity were measured in medium by quantitative immunoassay and in parallel, the expression of lymphocytes activation markers, CD69, CD25, HLA-DR were also assessed by flow cytometry. Biologicals showed a concentration-dependent release of cytokines in medium with a different profile of response in term of type of cytokine released and the concentration in the medium compared to vehicle treated cells. Some biologicals showed also an activation of lymphocytes activation markers with an effect that was dependent to the time of incubation. In conclusions, different kind of biologicals were tested to human PBMC lymphocytes activation assay. Biologicals showed to induce a release of cytokines and/or an increase of lymphocytes activation markers expression with a profile that reflect their biological interaction with innate or adaptive immunity. This *in vitro* assay was then suitable to predict a possible immunostimulatory effect of biological compounds in human lymphocytes.

P6.16.06
Standardization of ELISPOT counts for accurate assessment of human T cell responses performed by 10 different laboratories

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Introduction: There are a defined number of T cells specific for any given antigen within a PBMC pool. However, an accurate and reproducible method to measure this response, between different laboratories has been controversial. In ELISPOT assays, antigen-induced spots show a broad spectrum of sizes and densities over variable background. Therefore, even for experienced investigators using ELISPOT analysis software, it is difficult to judge the minimal spot size/density to be counted, and the gates for upper threshold to distinguish single cell-derived spots vs. clusters.
Methods: We studied PBMC plated in serial dilutions, with 24 replicates per dilution, to establish the distributional properties of IFN-γ ELISPOTS elicited by antigens with defined HLA-Class I or Class II restriction. We also sent ELISPOT plates, and image files of such, to 10 different laboratories for independent counting. The plates were machine counted by each laboratory relying on the subjective counting parameters assessment by different investigators, and by the statistics-based auto-gating method in conjunction with auto-threshold algorithm.
Results: Both these CD8- or CD4 cell-derived spots were found to closely follow log-normal distribution. These log-normal distributional properties of ELISPOTs permit to set the lower and upper gates for counting by means of statistics, automatically, with a 95% confidence interval.
Conclusions: While counts based on judgment call of the investigator showed considerable variability, the counts obtained in the laboratories by the statistic gating method were comparable to each other and thus establishes the prospect of harmonizing ELISPOT counting.

P6.16.07
A novel mechanism for recognition of a small molecule carbohydarte immunotherapeutic by human myeloid cells: Potential for modulation of efficacy in the clinic

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Imprime PGG® (Imprime), is a soluble β-1,3/1,6 immunomodulatory glucan, being developed in combination with monoclonal antibody therapy for multiple oncologic indications. To date, limited studies exist on the recognition and immunomodulatory activities of soluble glucans on the human immune system. Although yeast β-glucans have been shown to be capable of binding human cells through leukocyte complement receptor 3 (CR3, Mac-1, αM β2 integrin), the key components for this event were not elucidated. Here, we demonstrate that Imprime, a soluble carbohydrate pathogen associated molecular pattern (PAMP), is recognized by only certain human cells expressing CR3 (i.e., bind to CR3-expressing myeloid and T cells, but not to CR3 expressing NK cells) and requires opsonization by complement components such as iC3b. Inactivation of serum complement components resulted in loss of binding and function. Surprisingly, not all healthy donor myeloid cells were capable of binding Imprime despite the presence of complement components. Looking into potential reasons for differential binding led to a strong correlation between being a high-Imprime binder and having a high-natural receptor anti-β glucan titer. Both, in vitro and in vivo feasibility studies in human demonstrate the potential for converting a low-Imprime binder to a high-binder with anti-β glucan antibodies. Given the preclinical requirement for Imprime binding to myeloid cells for antitumor effects, we propose that assessing Imprime binding to blood immune cells could be a predictive biomarker, and enriching for high-binder patients or converting low-binders to high-binders by treating with high-titer plasma or IVIG, would enhance therapeutic efficacy.

P6.16.08
Surface Plasmon Resonance Imagery-based strategy to study hnRNPA2/B1 peptides as specific biomarkers in systemic lupus erythematosus, rheumatoid arthritis and autoimmune hepatitis

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Heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 is one of antinuclear autoantibodies targets in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and autoimmune hepatitis (AIH).

Aim. To monitor molecular interactions between peptides spanning the entire sequence of hnRNP and sera from patients and healthy controls.
Methods. Sera from 8 patients from each pathology and controls were passed across a Surface Plasmon Resonance Imagery (SPRi) surface containing 39 overlapping peptides of 17 mers covering the human hnRNP B1. Interactions involving the immobilised peptides were followed in real time and the dissociation rate constant koff for each interaction were calculated.
Results. Several significant interactions were observed: i) high stability (lower koff values) between P55-70 and the AIH sera compared to controls (p= 0.003); ii) less stability (higher koff values) between P118-133 and P262-277 and SLE sera, P145-160 and RA sera compared to controls (p=0.006, p=0.002, p=0.007). The binding curves and koff values observed after the formation of complexes with anti-IgM and anti-IgG antibodies and after nuclease treatment of the serum indicate that i) IgM isotypes are prevalent and ii) nucleic acids participate in the interaction between anti-hnRNAP B1 and P55-70 and also between controls and the peptides studied.
Conclusions. These results indicate that P55-70 of hnRNP B1 is a potential biomarker for AIH in immunological tests and suggest the role of circulating nucleic acids, (eg miRNA), present or absent according to the autoimmune disorders and involved in antigen-antibody stability.

P6.16.09
A novel cell-based assay for measuring neutralizing autoantibodies against type I interferons in patients with autoimmune polyendocrine syndrome type 1

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Autoimmune polyendocrine syndrome type 1 (APS-1), also known as autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a unique monogenic disorder caused by loss-of-function mutations in the autoimmune regulator (AIRE) gene. The loss of a functional AIRE protein presumably causes defects in central thymic tolerance and the escape of pathogenic autoreactive T cells into peripheral tissues. The most prevalent clinical manifestations are chronic mucocutaneous candidiasis, hypoparathyroidism and primary adrenocortical failure although a wide range of other autoimmune manifestations may occur. An important characteristic feature of APS-1 is the presence of neutralizing serum autoantibodies against type I interferons (subtypes -α and -ω) at frequencies approaching 100 %. These autoantibodies may therefore serve as an important diagnostic tool, complementing genetic mutation analysis of AIRE when APS-1 is suspected. Several techniques for measuring these antibodies are currently available, including fluid-phase binding assays using recombinant antigens labeled with radioactivity or luciferase, and assays measuring the actual interferon-neutralizing capabilities of the antibodies such as the antiviral interferon neutralizing assay (AVINA). We report here on a novel cell-based assay using a commercially available reporter cell-line that provides a simple, rapid and reliable way of assaying autoantibodies against type I interferons.

P6.16.10
Increased circulating levels of leukocytes, lymphocytes and atypical lymphocytes in patients with dengue hemorrhagic fever

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Dengue hemorrhagic fever (DHF) is a severe clinical manifestation of dengue virus infection in tropical areas worldwide. Diverse hematological parameters have an important diagnostic value in case definition. Here we evaluated the potential usefulness of leukocytes (Lk), lymphocytes (Ly) and atypical lymphocytes (aLy) counting in dengue classification and prognosis. Patients (n=554) attending Dengue Medical Service of the UFPB University Hospital between 2009-2011 were prospectively enrolled. They were grouped as Dengue Fever (DF, n=) or DHF (n=) according to WHO1997 classification. Blood samples were collected on days 5, 7 and 10 after start of disease symptoms and proceeded to cell counting. At d5 and 7, patients with DHF I&II presented higher numbers of Lk, Ly and aLy than DF patients. At d10, DHF I&II still showed higher numbers of Lk and Ly but not aLy than DF. Patients with DHF III presented higher levels of aLy at d5 and Lk at d7 than DF patients. No differences were observed between DHF I&II and DHF III patients. Moreover, DF patients showed lower numbers of Ly and aLy at d5 compared to d7 and d10, while DHF patients presented lowest levels of Ly at d5 but the highest levels of aLy at d7. Although significant differences on cell numbers could be observed between DF and DHF during follow-up, this experimental determination seemed not to be useful in dengue prognosis as determined by ROC curves. The differential cell counting observed between DF and DHF patients suggests their important role in disease progression.

P6.16.11
Cancer Testis Antigens Expression: Sex and Multiple Myeloma

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Background: Multiple myeloma (MM) is a cancer of plasma cells wherein aberrant cells accumulate in the bone marrow leading to bone damage and interference with normal blood cell production. Although MM remains incurable, it is treatable, and early detection is the key to avoiding permanent damage. Cancer Testis Antigens (CTAs) are a class of proteins that are expressed in gametogenic tissue but generally not in the somatic tissue of healthy adults. However, a number of cancer types have been shown to produce these proteins and their expression has been correlated with malignancy. Ropporin is a specific CTA shown to be associated with MM, and this protein represents a potential target for MM diagnosis as well as immunotherapy.
Objectives: Here we examine the impact of gender on the expression of the CTA, Ropporin, in MM patients.
Methods: The expression of the CTA, Ropporin, in MM patients was analyzed using PCR, ELISA, and immunohistochemistry.
Results: In a cohort of 34 MM patients (23 male and 11 female, 44% were positive for ropporin. Of the ropporin-positive group, 65% were female while 28% were male. In addition, 90% of the female ropporin-positive group were deceased.
Conclusion: Our results provide for the first time indications that sex can shift the expression of CTA and possibly the prognosis for female MM patients. Further studies in a large cohort of patients are warranted to validate the correlation of CTA/ropporin in MM patients and sex.

P6.16.12
Cancer treatment and diagnosis using cancer testis antigens for multiple myeloma

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Despite recent improvements in standard pharmacological treatments of multiple myeloma (MM), immunotherapy may prove to be more effective due to its higher specificity and lower toxicity. Cancer testis antigens (CTAs) are a family of proteins with testis-restricted expression that are not present, or barely present, in other tissues, but are associated with many tumors. Here we characterized the expression of two novel MM CTA, Ropporin and AKAP4, in tumor plasma cells from patients and cell lines. We showed that Ropporin and AKAP4 are strongly immunogenic and are therefore potential targets for effective MM vaccines. Additionally, because of their preferential expression in tumor cells, Ropporin and AKAP4 are outstanding biomarkers for tumor detection and monitoring in vivo. Recently, a modified NOD strain, carrying disrupted Rag1 and the

IL2-R γ chain genes (NOD-Rag1null/IL2rgnull, NRG), has been reported to tolerate higher levels of radiation compared with NOD/SCID strain and to allow for efficient engraftment of human tumors and HSC. The development of successful animal models for MM also relies on the choice of the biomarkers used to track the disease course and to identify tumor cells. Here we used the NRG strain to establish an innovative model of MM, allowing for the growth and the spread of MM cell lines and primary patients' cells, which can be monitored by using AKAP4 as a tumor biomarker.

P6.16.13
The cancer/testis antigens, SP17, AKAP4, and PTTG1 are expressed in non-small cell lung cancer: new biomarkers and vaccine targets

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Cancer testis antigens (CTA) are a class of tumor associated antigens, showing a restricted expression in cancer, strong immunogenicity, and weak expression in normal tissues. SP17/AKAP4/PTTG1 have been previously investigated, showing promising results as a target antigens (Chiriva-Internati et al, 2001; 2003; 2008; 2010; 2011). Our aim was to investigate the expression of Sp17/AKAP4/PTTG1 in lung cancer patients. We analyzed two lung cancer cell lines, one normal bronchus cell line, a panel of normal tissues and patient's cells by RT-PCR, flow-cytometry, immunocytochemistry (ICC), and immunofluorescence (IF). CTA immunogenicity was investigated by measuring circulating specific antibodies in the sera of lung cancer patients ELISA analyses show the presence of circulating CTA-specific antibodies in the sera of lung cancer patients, indicating the immunogenicity of SP17, AKAP-4 and PTTG1. We showed that CTA, SP17, AKAP-4 and PTTG1 can be detected in both sera and tissue of patients with NSCLC. Furthermore, CTA can elicit an immunogenic response in patients affected with this disease.

P6.16.14
Proteomic analysis of aqueous humor from uveitis patients

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Introduction: Uveitis is an ocular inflammatory condition that is one of the leading causes of irreversible blindness worldwide. However, the study in immunopathogenesis is limited and how overt inflammation arises in this immune privilege site is not clearly understood. The proteomic study could lead to a global understanding of proteins involved in the pathogenesis of the disease. We hereby performed proteomic analysis of aqueous humor to detect proteins involved in uveitis.
Method: Proteome of aqueous humor from 15 uveitis patients were analyzed in comparison to those from 30 non-inflammatory cataract patient controls. Proteins from 2 groups of samples were resolved by two-dimensional gel electrophoresis and the results were compared. The protein spots found in uveitis but not control samples were then further analyzed by tandem mass spectrometry and matched with NCBI database for protein identification.
Results: Five different protein spots were found only in uveitis samples but not in controls. Further analysis by tandem mass spectrometry identified two proteins including uromodulin and plasma serine protease inhibitor.
Conclusion and Perspective: Uromodulin and plasma serine protease inhibitor were identified as proteins found in uveitis but not in controls suggesting their potential roles in the pathogenesis of uveitis. Further functional studies are needed to delineate their roles in this disease.

P6.16.15
Intracellular modulation, extracellular disposal and serum circulating increase of miR-150 upon lymphocyte activation

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Activated lymphocytes release a large amount of nano-sized vesicles (or exosomes), containing microRNA (miRNA) that may be monitored non-invasively in the bloodstream. We have evaluated whether lymphocyte activation in vitro results in selective extracellular release of miRNAs, and consequently whether elicitation of immune responses by vaccination in vivo is followed by changes in the serum levels of lymphocyte-specific miRNAs. We have found that, upon activation in vitro of both human and mouse lymphocytes, miR-150, a key regulator of lymphocyte differentiation and functions, gets dramatically down-modulated at the intracellular level and in parallel accumulates in extracellular vesicles. Upon activation of immune responses in vivo, in both adults and infants vaccinated with an adjuvanted flu vaccine and in mice immunized with adjuvanted ovalbumin, miR-150 serum levels increased significantly. Notably, this increase was enriched in purified circulating nano-sized vesicles, while was not recordable in purified larger vesicles, suggesting an exosome specific phenomenon. The increase in the serum level of miR-150 post-immunization correlated with elevation of antibody titers, and immunizations of immunodeficient mice resulted in little or no elevation of circulating miR-150, demonstrating that increase in the serum level of miR-150 depends on the elicitation of immune responses. We thus provide proof of concept that serum-miRNAs can be searched as non-invasive biomarkers of adaptive immune responses. Finally, the fact that activated lymphocytes reduce the intracellular levels of miR-150 while releasing it in the external milieu suggests that extracellular disposal of selected miRNAs can be an additional way to regulate gene expression during lymphocyte differentiation.

P6.16.16
Identification of biomarkers associated with treatment for latent tuberculosis by microarray

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Latent tuberculosis (LTB) is characterized by a positive tuberculin skin test (TST) or IFN-γ release assay (IGRA). The risk for TB reactivation is estimated as 10% for lifetime, but the risk is greater in immunocompromised persons. Treatment with isoniazid (INH) is recognized as the standard therapeutic agent in LTB infection. However, the effect of treatment on immune response genes potentially involved in reactivation or protection is not known. With the aim of assessing biomarkers associated with treatment of LTB, we investigated 27 TST positive individuals with documented contact with TB cases who received INH treatment using microarrays. Genome-wide transcriptional profiles were generated from whole-blood RNA obtained before, 1 month and after INH treatment. A distinct 202-transcript signature was defined in individuals with INH treatment using a combination of expression-level and statistical filters and hierarchical clustering. Normalizing across arrays, from 202-transcript signature, 124 genes were downregulated while 78 upregulated as compared to baseline (14.3% (at 1 month post-treatment); 30.19% (at 6 months post-treatment); 55.5% throughout treatment, fold change ≥1.2, p value 0.01. Our study provides a broad range of transcriptional biomarkers with potential as treatment prognostic tools. The biomarkers candidates include: CCR8, CXCR5, CXCL-10, IFNα, IFN-γ, IL-6, IL-13, IL-17C and IL-23R. Biomarkers candidates were validated by qRT-PCR. INH modifies the LTB

signature, it potentially indicating a reduction in risk of active disease. These data improve our understanding of LTB-immune host genes interactions and may offer future leads for biomarkers of treatment efficacy.

P6.16.17
Novel monoclonal antibodies against human Carbonic Anhydrase XII, a potential biomarker of tumor cells

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Carbonic anhydrases (CAs, EC 4.2.1.1) are zinc enzymes that catalyse the reversible hydration of carbon dioxide to bicarbonate. CAs have important physiological and pathological functions in human organism. CA IX is well-recognized tumor marker. Recently, the association of CA XII with human cancers has been demonstrated. The CA XII is a transmembrane protein with an extracellular catalytic domain. It is involved in tumor progression by acidification of the extracellular milieu and regulation of intracellular pH. Recent studies indicate that CA XII is aberrantly overexpressed in breast, cervix, brain cancers, renal carcinomas. Therefore, CA XII might be considered as a useful biomarker of tumor cells and a promising target for specific therapies. The aim of the current study was to develop new monoclonal antibodies (MAbs) against human recombinant CA XII and evaluate their diagnostic potential.
Seven stable hybridoma cell lines producing IgG antibodies against human CA XII were generated. The MAbs were highly specific to CA XII and did not cross-react with other human recombinant CAs. In order to demonstrate the diagnostic value of the MAbs, they were employed for the IHC staining of CA XII in human tumor tissue specimens. The MAbs demonstrated a strong and specific reaction with colon and renal carcinoma specimens and did not show any unspecific background staining of the respective normal tissues. Flow cytometry analysis revealed a specific immunostaining of CA XII in human tumor cell lines.
The newly developed MAbs represent a promising diagnostic tool for the immunodetection of CA XII-expressing tumor cells.

P6.16.18
Tertiary lymphoid structures have features of an ongoing humoral immunity with a benefit for lung cancer patients

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It is now well established that immune responses can take place at distance of secondary lymphoid organs, in tertiary lymphoid structures (TLS). We have previously shown that such structures can develop in human lung cancer (LC), and may represent an activation site for tumor-specific T cells. The aim of this study was to determine whether a protective humoral immunity can take place within TLS.
We showed that B-cell follicles of TLS present the same cellular composition and organization as in lymph nodes, with all B-cell differentiation stages. The somatic mutation and isotype switching machineries were activated in these B-cell follicles. Intra-tumoral PNAd+ high endothelial venules were exclusively associated with TLS. The PNAd+ ligand, CD62L, was selectively detected on TLS B cells except by germinal center B-cells, as reported for lymph nodes. CXCR5 expression decreased on fully differentiated B cells, a known key regulatory process that allows effector cells to leave CXCL13+ germinal centres. In a retrospective study, the density of TLS B cells was associated with a favorable clinical outcome in patients with early-stage LC. More interestingly, combination of both markers of APC (mature dendritic and B cells of TLS), indicated that they are strongly correlated with long-term survival for patients with early-stage LC and patients with advanced-stage of LC treated by neo-adjuvant-chemotherapy.
Altogether, TLS may represent an active site for the initiation of a protective humoral immunity. Preliminary data on the specificity of antibodies secreted locally by intra-tumoral B cells will be discussed in the physiopathology of human LC.

P6.16.19
Assessment the immune responses in the microenvironment of breast cancer tissues

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Introduction: Breast cancer is the most prevalent cancer among women. Several therapeutic approaches such as immunotherapy have been suggested for cancer therapy. However, immunotherapy approach is not very effective in activating immune responses against tumor antigens. It is believed that clarifying the tumor microenvironment and illustrating the type of infiltrated cells could be helpful for increasing the effectiveness of cancer immunotherapy. Therefore, this study was done to illustrate the tumor microenvironment in human breast cancer tissues.
Methods and Materials: Fifty five human breast cancer tissues from 4 stages and 11 normal tissues were collected. Subsequently, mRNA was extracted from the tissues and cDNA was synthesized. Then, the mRNA expression of 9 genes related to T helper 1(T-bet), T helper 2(GATA3), T helper17(RORC) and T regulatory(FOXP3) cells and also some inhibitory mediators of immune system (IDO,VEGF,IL-10,FASL,TGF-betta) were determined by Real time PCR method. In this study betta-actin was measured as housekeeping gene. Results: The results showed that the expression of genes related to Th1 and Th17 decreased significantly along with tumor progression. In contrast, the genes expression assessment of Treg, Th2 and also inhibitory mediators showed that the expression of these genes significantly increased in advanced stages. Conclusion: Based on the achieved results, we conclude that considerable changes in tumor microenvironment were seen during tumor progression. These changes can be used to improve the efficacy of therapeutic methods and to introduce novel biomarkers.

P6.16.20
Molecular allergology : Component resolved diagnostics for managing cow's milk allergy in West Algeria

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IgE-mediated allergies affect more than 25% of the world's population. Diagnostic tests based on natural allergen extracts are composed of relativelyill-defined mixtures of non-allergenic materials, major allergens, and cross reactive allergens, with the result that it is difficult and often impossible to precisely identify the disease-eliciting allergen, particularly in patients sensitized to more than one allergen source like milk protein. The term component-resolved diagnosis has been coined to designate diagnostic tests based on pure allergen molecules which are either produced by recombinant expression of allergen-encoding cDNAs or by purification from natural allergen sources. Materials & Methods: 64 milk allergic young infants were 7,7 ± 8,2 month old. Specific IgE toward purified milk allergens was explored with Fluorescent immune-enzymatic test (F-ELISA) and by component resolved diagnostics using milk allergen microarray: pure natural and recombinant allergen molecules have been used for this purpose. Diagnostic was approved clinically by oral challenge and with a convincing history of reactions on exposure. Positif specific IgE cutoff value measured by F-ELISA was 0,35 kUA/L.

The results obtained after IgE exploration shown that most allergic patients were ploysensitized to more than one milk allergens. Bovine milk allergen microarray demonstrates that lactoferrin present in milk in very low quantity could be a strong allergen. Thus, microarray test allow us to assess the IgE binding ability of IgE to milk allergen using reduced amount of clinical material. Component resolved diagnostics tests could be efficient and reliable test to manage, and helping in vitro diagnosis of milk protein and other food allergies .

P6.16.21
Timing of blood sampling for CD4 T-cell count influences HAART decisions

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BACKGROUND: Variability in CD4 T-cell counts has been described for both healthy and HIV-infected persons. It may influence decisions with respect to initiation and monitoring of antiretroviral treatment.
OBJECTIVE: to measure the effect of timing of blood sampling for blood cell count measurement. METHOD: The study population consisted of 71 Ethiopian patients in an observational cohort, either being monitored prior to HAART (n = 40) or receiving HAART (n = 31) at an ART Clinic in Addis Ababa.
RESULT: The median CD4 count demonstrated significantly increasing trends from the morning (8 am) to the afternoon (4 pm), both for patients on HAART (increase of 137 CD4 cell/microl; p = 0.003) and for patients initiating HAART (increase of 56 CD4 cells/microl; p = 0.038). This trend was also observed for CD8+ and CD3+ T-lymphocytes, (initiating HAART p = 0.002 and p = 0.001; patients on HAART p = 0.015 and p = 0.004, respectively).
CONCLUSION: The implications of these findings are for the decision to start HAART or the decision to start prevention of opportunistic infections in Ethiopian patients on HAART.

P6.16.22
***ACTC1, CCR5, TNF/LTA, CCL2, IL12B, IL10, MAL/TIRAP* genes are associated to the development of Chronic Chagas disease cardiomyopathy**

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Chagas disease, due to *Trypanosoma cruzi*, occurs mainly in the Americas. An estimated 300,000 new cases and 50,000 fatalities occur per year. Chronic Chagas cardiomyopathy(CCC) is an inflammatory cardiomyopathy that affects approximately 30% of infected individuals. Approximately 1/3 of patients developing CCC present a dilated cardiomyopathy, with short survival. Familial aggregation of CCC suggests that might be a genetic component to disease susceptibility.
We conduct a genetic study on a large Brazilian population.This study enrolled 315 CCC cases and 118 asymptomatic (controls) subjects. Significant associations were detected: *CCR5*(rs3176763:p=0.006 OR=1.79; rs11575815:p=0.030 OR=1.41), *TNF/LTA* (rs2844484:p=0.007OR=1.51; rs909253:p=0.035; OR=1.50), *CCL2* (rs909253:p=0.037 OR=1.56; rs2530797: p=0.028 OR=1.28), *IL12B* (rs1003199:p=0.008 OR=1.49; rs919766: p=0.007 OR=1.45), *IL10* (rs3024496: p=0.020 OR=1.47), *MAL/TIRAP* (rs8177376:p=0.004 OR=1.42). These associations are consistent with the fact patients who develop CCC display an exacerbated Th1 response and a reduced numbers of IL-10-producing and CD4+CD25+FOXP3$^+$ regulatory T cells.
Cardiac muscle alpha Actin 1 is a protein encoded by the *ACTC1* gene in humans. Proteomic analysis (by 2-D DIGE) on heart biopsies from cases and controls has shown ACTC1 proteins are underrepresented into patient heart biopsies compared to control heart biopsies (confirmed by westernblot).Significant associations were detected with 1 polymorphism located into the promoter (rs640249: p=0.006 OR=1.59). Based on linkage disequilibrium map, a promoter haplotype of susceptibility was identified (rs640249-rs641563). Functional studies done in human cardiomyocytes and an imputation analysis are ongoing to identify functional variants. We hypothesize that *ACTC1* mutations could affect sarcomere contraction and force transmission from the sarcomere to the syncytium leading to cardiomyopathy.

P6.16.23
Serological proteome analysis (SERPA) as a tool for the identification of tumor antigens capable of eliciting immune responses in chronic lymphocytic leukemia (CLL)

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In this study, serological proteome analysis (SERPA) was applied for the first time to identify tumor-associated antigens (Ag) capable of eliciting humoral immune responses in 35 untreated patients (pts) with chronic lymphocytic leukemia (CLL).
Proteins from CLL cells were separated to obtain proteomic maps which were blotted with autologous sera to reveal antibody (Ab)-based reactivity. To verify the CLL-specificity of recognition, 7 pts maps were also probed with sera of 7 healthy donors (HD). Ag spots were identified by Mass Spectrometry (MS). Effector responses were evaluated by IFNγ-ELISPOT upon stimulation of T cells from 6 pts and 4 HD with autologous ENOA-pulsed and control dendritic cells (DC).
Twenty-nine out of 35 CLL sera were immunoreactive and produced 145 Ag spots. By contrast, HD sera were significantly less reactive with 3 spots (p=.007). MS identified 50 different proteins recurrently recognized by autoAb. Sera from 54% of pts exhibited reactivity toward α-Enolase (ENOA). Whereas none of the HD sera was ENOA-reactive. ENOA-pulsed DC stimulated autologous T cells to secrete IFNγ in 4 out of 6 pts. This response was ENOA-specific because it was not induced by unpulsed DC and CLL-specific because IFNγ release was not induced in controls. ENOA Ab-reactivity correlates with parameters of progressive disease, higher lymphocytosis and lower platelet counts.
In conclusion ENOA is a shared Ag eliciting humoral and cellular immune responses in most CLL pts. Our results suggest that the immunoreactivity against self-Ag detected in pts with progressive disease may be the expression of a dysfunctional immune system.

P6.16.24
Impact of excipients and pH on the *in vitro* hemolysis by IgG solutions

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Background & Objectives: In the recent years, an increased number of severe hemolytic reactions associated with the use of intravenous administered IgG solutions (IVIG) have been reported. The aim of this study was to develop an *in vitro* assay to investigate in particular the impact of excipients and the pH value on the IVIG-induced hemolysis. In addition, commercially available IVIG products were compared in this assay.
Methods: The following excipients were used: glycine and L-proline (amino acids), maltose (sugar/disaccharide) and D-sorbitol (sugar alcohol). The excipients were added to the 10% IgG solution at a final concentration of 230-290 mmol/l. In addition, eight commercially available IVIG products were tested. *In vitro* hemolysis was performed using red blood cells (RBCs) prepared from blood samples of healthy donors of different blood groups, including blood group O, Rh- donors.
Results: For all excipients tested, a significant impact of the pH value on the hemolysis was observed. Four of the IVIG products showed significant lysis of RBCs of all blood groups. IgG solutions with

maltose showed lower hemolysis than IgG solutions containing amino acids.
Conclusions: IVIG products vary in pH and concentration of different excipients used in the final formulation. Our studies showed that the kind of excipients and pH have a significant impact on RBC lysis *in vitro*, independent from (or in addition to) hemolysis caused by high haemagglutinin titers. An impact on the *in vivo* hemolysis induced by the IVIG profile cannot be excluded.

P6.16.25
Exhausted CD8 T cells in peripheral blood of breast cancer patients

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Breast cancer (BC) is the most common malignant neoplasm worldwide in women. CD8 T cells have particular importance in mediating elimination of malignant cells, but can become functionally inept. These exhausted CD8 T cells has been identified in several neoplasm but its presence in BC has been only identified in the tumor. Has not been studied whether these cells are increased in peripheral blood respect to healthy women.
Aim: Evaluate and compare the exhausted CD8 T cells in peripheral blood of BC patients and healthy women.
Methods: 48 women were included, 32 BC, 9 benign tumor and 8 healthy. After informed consent, 15 ml of peripheral blood and guided biopsy was obtained. Patients were classified in clinic stage (CS) I, II, III and IV according to clinical criteria and estrogen receptor in biopsy. Mononuclear cells were stained with MoAb against CD3, CD8, CD45RA, PD1 and TIM3. 50,000 cells were analyzed by flow cytometry. Mann-Whitney U and Spearman were used.
Results: Patients were classified in CS-I (n=6), II (n=9), III (n=10) and IV (n=7). Significant differences were found in CD3+CD8+CD45RA-PD1+ percentage between CS-I and healthy women (8.51%±1.37 vs 5.28%±1.84; p=0.035) and CS-I and III (8.51%±1.37 vs 5.87%±1.55; p=0.042). Spearman analysis revealed correlation between CD3+CD8+CD45RA-PD1+ cells and estrogen receptors (Rho=0.411, p=0.024).
Conclusions: Subpopulation CD3+CD8+CD45RA-PD1+ cells are increased in CS-I patients and have positive correlation with expression level of estrogen receptor in biopsy. This subpopulation could be used as a non-invasive tool to predict the tumor aggressiveness in breast cancer. Supported by CONACyT134341

P6.16.26
The impact of polymorphisms in the host immune response genes on the severity of chronic hepatitis C

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Introduction: Polymorphisms in the host immune response genes have been shown to be associated with HCV infection outcome and treatment response. Recently, genetic variations near the IL28B gene have been identified to be associated with spontaneous and treatment-induced clearance of HCV. Therefore, in this study, we investigate the impact of polymorphisms in IL28B, IFN γ and IL10 gene with the severity of chronic hepatitis C.
Materials and methods: 54 patients were included and divided into two subgroups according to the presence or absence of hepatocellular carcinoma. For the IL28B polymorphism, we employed an *in-house* PCR-RFLP method. For the IFN γ and IL10 polymorphisms, we used method published by Dolores L.M. et al. Statistical analysis was done with SPSS 13.0 and $p < 0.05$ is significant.
Results and discussion: Distribution of IL28B, IFN γ and IL10 genotype frequency: There was a significant difference between two groups about CT, AG and TT genotype frequency of IL28B, IL10 and IFN γ gene (65.38%; 75% and 35.71% versus 28.57%; 30.76% and 3.84%) respectively, ($p < 0.05$). Using logistic regression analysis indicated that the severity of liver disease was higher in patients bearing TT genotype of both IL28B and IFN γ ($p < 0.05$). There was no significant correlation observed among IL10 genotypes ($p > 0.05$).
In conclusion, the study indicated that polymorphisms in IL28B and IFN γ gene strongly correlate with the severity of liver disease and this will be able to reach closer step to personalized medicine for treatment of hepatitis C.

P6.16.27
Peptide Microarrays with Near Zero background enable high quality and massive data production from seroscreening of autoimmune diseases

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For the ability of simultaneous analysis of thousands of substances, protein/peptide microarray technology has emerged as an important tool in many fields including disease diagnosis,protemics, and drug discovery. But the high background intensity due to nonspecific protein adsorption has made the data extraction and analysis of protein/peptide microarray more complicated, and then hindered application of the technology in high throughput screening. Here we show the peptide microarray based on iPDMS, an advanced biomaterial which has the intrinsic nonfouling property, can maintain near zero background, which enable high quality and massive data production from seroscreening.
In our previous study, we found the substrate iPDMS showed excellent nonfouling property in sandwich ELISA for tumor marker detection. Here in the seroscreening by peptide microarray based on iPDMS, a maximal [IgG] of 0.5 mg mL-1 was determined to achieve near zero background without any blocking procedure. Since typical [IgG] in human serum is 10-14 mg mL-1, a minimal of 20 fold serum dilution is required. With this near zero background performance, peptide microarray has been successfully applied in epitope identification of autoimmune diseases, such as type 1 diabetes mellitus, systemic lupus erythematosus, and autoimmune thyroid disease. And we believe that peptide microarray based on iPDMS has enormous potentiality in epitope identification and clinical diagnosis of autoimmune diseases.

P6.16.28
Chronic myeloid leukemia patients in prolonged remission after interferon monotherapy have constantly active NK-cells

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Chronic myeloid leukemia (CML) is caused by a BCR-ABL oncokinase. Without any treatment, the disease transforms to fatal acute leukemia in a few years. Before tyrosine kinase inhibitors, CML patients were treated with IFN-α, and a small proportion of patients responded well. Interestingly, about half of the responding patients were able to discontinue the treatment without disease relapse and be cured.
In this study, we aimed to analyze the function of immune cells in well-responded IFN-α treated CML patients in order to understand the putative anti-leukemic effects (IFN-ON (n=5), patients still using IFN-α; IFN-OFF (n=8), patients were off from any treatment and in remission).
Compared to healthy controls, IFN-patients seemed to have a higher amount of NK-cells (median 19.6% vs. 13.2%, p=0.11), and especially in IFN-OFF group the proportion of NK-cells was increased (23.5%, p=0.0031). The phenotype of NK-cells was

CD56DIMCD57+CD62Lneg in IFN-OFF group, whereas IFN-ON patients had more immature CD56DIMCD57-CD62L- NK-cells.
Interestingly, in IFN-α treated patients the baseline degranulation of NK-cells was higher than in healthy volunteers, but due to small number of patients, the difference was not statistically significant (p=0.13). However, especially IFN-ON patients seem to have active, degranulating NK-cells (19.4% vs. 2.8% in healthy, p=0.05). When stimulated with K562 cells, the in vitro cytotoxicity of NK-cells was poor which is in line with the excessive in vivo baseline activation. No differences were observed in the cytokine secretion. To conclude, IFN-α treated patients in remission have increased number of functionally active NK-cells, which may contribute to good response.

P6.16.29
Neutrophil gelatinase-associated lipocalin in chronic heart failure

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Introduction: Chronic heart failure (CHF) is a progressive and complicated clinical syndrome, it is mostly accompanied by chronic renal disease. Glomerular filtration rate (GFR) is decreased in 30 to 50 % of patients with CHF. NGAL, neutrophil gelatinase-associated lipocalin, which is expressed mainly from neutrophils as a response to bacterial infections in innate immunity, is a new marker for renal function. The aim of this study is to evaluate the renal functions of the patients with CHF and determining the clinic importance of NGAL parameter as a biomarker in CHF.
Material and Method: Hundred patients (mean age: 67 ± 12 years) from cardiology outpatient clinic diagnosed as CHF enrolled to this study. Creatinine, CRP, BUN and NGAL levels were measured from venous blood samples in 2 hours after collection. According to CRP results, a possible bacterial infection is excluded. All the cases received written inform on the study including Ethics Committee Approval before the study was initiated. Spearman Correlation Analysis is performed.
Results: Patients with high CRP levels were excluded and NGAL levels did not correlate with normal CRP results(r=0.093, p=0.364). A weak correlation but a statistically significance was observed between NGAL and BUN levels (r=0.293, p=0.03). Also a correlation and a highly statistically significance was found between NGAL and creatinine (r=0.520, p<0.001).
Conclusion: In this study NGAL performance was found significantly better than BUN. A good correlation between NGAL and creatinine indicates that NGAL may be a candidate biomarker for renal functions in patients with CHF.

P6.16.30
Post-traumatic Immunosuppression and its Reversal

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Major trauma suppresses immune defences, and increases vulnerability to nosocomial infections by unknown mechanisms. Post-traumatic immunosuppression (PTI) is further compounded by haemorrhage, surgery, blood transfusions, sepsis and age1. Sterile surgical trauma offered an opportunity to investigate mechanisms underlying PTI. Furthermore, we observed that autologous blood salvaged from the wound site (ASB) and re-infused post-operatively reversed PTI, suggesting novel therapeutic avenues.
Two cohorts of knee arthroplasty patients were recruited; one (n=18) received no ASB whereas another (n=25) received unwashed ASB collected from the wound site within six hours after surgery. Venous bloods were taken pre-operatively and within 3-7 days post-operatively. ASB was sampled at one and six hours after surgery. Fold-changes in cytokine, chemokine and DAMP levels were analysed.
PTI was evidenced in the non-ASB cohort by suppressed pro-inflammatory (IL-1β/2/17A, IFN-γ, TNF-α) and elevated anti-inflammatory (IL-5) cytokine levels. However, PTI was reversed in the ASB cohort as evidenced by increased pro-inflammatory (IL-1β/2/17A, IFN-γ, TNF-α) and decreased anti-inflammatory (IL-4/5/9/10/13) cytokines. This phenomenon was attributed to ingredients in wound blood plasma that included DAMPS (HMGB-1, HSP-27/60/70, S-100A8/A9, α-Defensin, Annexin-A2), chemokines (IL-8, MCP-1, MIP-1α), and pro-inflammatory cytokines (IL-1β/2/6/12p70/17A, IFN-γ, TNF-α).
Thus pro-inflammatory cascades triggered within traumatised tissue involved release of DAMPs which activated inflammasomes (e.g. NALP3) and triggered cytokine and chemokine synthesis. A simultaneous anti-inflammatory process evident systemically accounted for PTI. The fact that PTI was reversed by re-infusion of ASB led us to speculate that ingredients within 'wound-cocktails' may have future application in prophylaxis against infection after major trauma.
1.Bradley et-al.Lancet.2004.363:1025-1030

P6.16.31
Monocyte surface antigen expression in severe sepsis

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Background:
Leukocyte immunophenotyping could improve sepsis diagnostics. Our hypothesis was that monocyte CD14, CD40, CD80 and HLA-DR antigen expressions differ between severe sepsis, non-inflammatory ICU patients and non-septic inflammation (off-pump coronary artery bypass; OPCAB).
Materials and Methods:
Monocyte CD14, CD40, CD80 and HLA-DR expressions were analyzed from 27 patients with severe sepsis, from 7 OPCAB patients and from 8 ICU patients who did not fulfill any SIRS criteria. Blood samples were collected within 48 hours from the beginning of severe sepsis or in non-SIRS patients from ICU admission and on two consecutive days (D0, D1, D2). From OPCAB patients, the first samples were taken on the day of surgery before the skin incision and on two consecutive days (D0, D1, D2). In addition 10 healthy individuals served as controls. Samples were collected, processed and analyzed by using flow cytometry as previously described [1].
Results:
The peak expression of monocyte CD40 was highest in severe sepsis patients compared to the other groups (p<0.05). In severe sepsis, CD40 expression decreased over time (p<0.05). In OPCAB group, CD40 expression slightly increased after surgery (p<0.05).
Conclusions:
Based on this study, monocyte CD40 could be helpful in distinguishing severe sepsis from non-septic inflammation and healthy controls.
References:
1. Jamsa J, Huotari V, Savolainen E-R, Syrjala H, Ala-Kokko T: Analysis of the temperature affects on leukocyte surface antigen expression. J Clin Lab An 2011, 25:118-125

P6.16.32
Change in immune cells population following exposure to the venom from *Hemiscorpius lepturus* scorpion venom

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Introduction: A previous study on the serum of human showed a marked increase in TNFα level in patients envenomed by *H lepturus*

scorpion. The aim of the present *in vitro* study was to assess the changes in change immune cells counts.
Method: Peripheral blood mononuclear cells (PBMC) and heparinized blood samples were exposed to 1 µg/ml of *H. lepturus* venom for 24 hr. Control samples of both preparations were treated similarly in the absence of the venom. Based on specific surface markers, identification and quantization of TC, NK, Th, activated and regulatory T cells and GITR cells was carried by flow cytometry method (BD, USA). The collected data were analyzed by WINMDI software.
Results: Following exposure to *H lepturus* venom the cytometric results for PBMC showed the following changes: a reduction of CD4/CD8 ratio (from 3.14 to 2.08), reduction in CTL cells (from 1.46 % to 089 %); regulatory cells were also reduced from 1.06 % to 0.89%. While a significant increase in Th-activated cells (from 0.21 to 0.65 %) and GITR cells (from 1.94% to 3.03%). No significant changes were observed for NK and B cells. Similar pattern of changes were observed with whole blood.
Conclusion: Besides its cytotoxic properties; the venom from this scorpion has direct immune suppressive and possible indirect immune stimulating actions which may be utilized in development of novel agents for treatment of illnesses that require immune boosting or suppressing interventions

P6.16.33
Clinical utility of interferon-γ releases assay (IGRAs) in new and retreated cases of Pott's disease at a tertiary care hospital

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Objective:Interferon-γ release assay (IGRAs) promised a sensitive and specific diagnosis of latent and active infection of *Mycobacterium tuberculosis(M.tb)*. Therefore, the intent of this study was to appraise the clinical usefulness of Quantiferon-Gold in Tube (QFT-GIT) for diagnosis of *M.tb* infection of Pott's disease.
Method: We prospectively observed 55 clinical-radiological suspected cases of Pott's disease. They were undergone to the detection of tubercular infection and Acid fast bacilli, on QFT-GIT followed by microscopy, culture and histopathological methods. The significance was observed between new and retreated cases. The sensitivity and specificity of QFT-GIT was calculated against the combined results of all performed tests were carried out.
Results:Patients were classified into 2 groups. Group A: 24 (43.6%) were new cases and Group B: 31 (56.4%) were retreated cases. The diagnostic efficiency among group A and B were; 21(87.5%) and 19(61.3%) on QFT-GIT, 10(42%) and 10(32.2%) on microscopy, 14(58.3%) and 13(54%) on culture, 21(87.5%) and 18(58%) on histopathology were analyzed. The significant differences (pvalue <0.05) among group A and B were found by QFT-G IT and histopathology results. Moreover, we found 45 cases as a definite TB and 10 cases were negative by performed tests; on microscopy, culture and histopathology observations. Thus, the overall sensitivity was 84% and 80% specificity calculated for QFT-GIT respectively.
Conclusion:QFT-GIT revealed higher sensitivity in new cases with significant differences of retreated cases. Despite, QFT-GIT showed notable sensitivity and specificity against combined results of laboratory proven methods. Hence, we can use in routine clinical diagnosis of Pott's disease.

P6.16.34
Collagen Triple Helix Repeat Containing 1 (CTHRC1) Acts via ERK-Dependent Induction of MMP9 to Promote Invasion of Colorectal Cancer Cells

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Collagen triple helix repeat-containing 1 (CTHRC1) is known to be aberrantly upregulated in most human solid tumors, although the functional roles of CTHRC1 in colorectal cancer remain unclear. In this study, we investigated the occurrence of CTHRC1 upregulation and its role *in vivo* and *in vitro*. The expression profile and clinical importance of CTHRC1 were examined by reverse transcription-polymerase chain reaction and immunohistochemical analyses in normal and tumor patient samples. CTHRC1 was not detectable in normal tissues, but was highly expressed in tumor specimens. CTHRC1 upregulation was significantly associated with demethylation of the *CTHRC1* promoter in colon cancer cell lines and tumor tissues. Clinicopathologic analyses showed that nodal status and expression of CTHRC1 (95% CI 0.999-3.984, p=0.05) were significant prognostic factors for disease-free survival. Promoter CpG methylation and hypermethylation status were measured by bisulfite sequencing and pyrosequencing analysis. Furthermore, we showed that overexpression of CTHRC1 in the SW480 and HT-29 cell lines increased invasiveness, an effect mediated by extracellular signal-regulated kinase (ERK)-dependent upregulation of matrix metalloproteinase 9 (MMP9). Consistent with this, we found that knockdown of CTHRC1 attenuated ERK activation and cancer cell invasivity. These results demonstrate that CTHRC1 expression is elevated in human colon cancer cell lines and clinical specimens, and promotes cancer cell invasivity through ERK-dependent induction of MMP9 expression. Our results further suggest that high levels of CTHRC1 expression are associated with poor clinical outcomes.

P6.16.35
High expression of prolactin receptor is associated with cell survival of cervical cancer cells

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Prolactin (PRL) regulates many physiological functions such as proliferation, differentiation, cell survival and act as an immunomodulatory. Altered expression of PRL and its receptor (PRLR) has been implicated in carcinogenesis as breast, prostate, laryngeal and colorectal. There are few studies focused in the analysis of the PRL/PRLR in cervical cancer, where the variation of the hormonal status influences in its development. The objective of this work was evaluating the expression of PRL/PRLR and cell survival effect in cervical cancer, as well as to analyze cellular signaling pathways involved. PRL/PRLR expression were evaluated by Western blot, immunofluorecence and Real time PCR in biopsies, cell lines (HeLa, SiHa, and C-33A) derivate of cervical cancer and immortalized human keratinocytes (HaCaT). Proliferation and apoptosis were measured by MTT and TUNEL assays, respectively. The activation of STAT3, Akt and ERK were evaluated by western blot. High PRLR expression was observed in cervical cancer in compare with premalignant lesions and non-tumorigenic keratinocytes. These cells also express variants of PRL of 60-80kDa. Treatment with PRL not induces significant proliferation, but reduce the apoptosis in all cervical cancer cell lines. PRL stimulated phosphorylation of STAT3 in HeLa and C-33A, also increase the phosphorylation of ERK in SiHa. In conclusion, the PRLR over-expressed may acts as important survival factor through the inhibition of apoptosis by signaling pathway STAT3 or ERK in the cervical cancer. PRL autocrine non-induce proliferation or apoptosis in cervical cancer cells maybe exerts a function on immune cells of tumor microenvironment.

P6.16.36
A standardized long-term culture assay to measure antigen-specific memory T cells

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Background: Control of viral infections correlates with the availability of memory T cells with high proliferative capacity and not of activated effector T cells. However, while effector T cells are reliably measurable, measuring memory T cells requires long-term culture

assays that are difficult to standardize. We addressed the unmet need to design a long-term assay that is precise, repeatable and reproducible.
Methods: We quantified antigen-specific T cell precursors with high proliferative capacity (PHPC) using a peptide-based long-term cultured IFN-gamma ELISPOT assay (PHPC assay), shown to identify expandable memory T cells correlating with control of HIV-1 (Calarota S, J Immunol 2008). PBMC from several untreated HIV-1-infected individuals were evaluated in response to HIV-1 Gag p17, p24, and p15 peptide pools. Inter-operator and inter-day variability were assessed with the Intraclass Correlation Coefficient (ICC) with 95% Confidence Interval, intra-sample variability was assessed with the Coefficient of Variation (CV).
Results: Intra-sample repeatability range drastically improved from 5-36%, 8-26% and 50-104% to 0-12%, 8-26% and 1-25% for p17, p24 and p15 CV, respectively. Inter-operator reproducibility also improved from 0.86, 0.68 and 0.42 to 0.94, 0.94 and 0.70 for p17, p24 and p15 ICC, respectively. Inter-day precision remained good with ICC from 0.78, 0.96 and 0.54 to 0.71, 0.92 and 0.63 for p17, p24 and p15, respectively.
Conclusions: We succeeded to make the PHPC test precise, repeatable and reproducible. A standardized test to measure antigen-specific memory T cells will be invaluable to predict and monitor the success of vaccination and immunotherapies.

P6.16.37
Development of the MeDALL allergy-chip, an allergen micro-array comprising 176 components for sensitive detection of allergen-specific antibodies

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In the EU-funded project “MeDALL”, 14 birth cohorts from different European countries provide several thousand serum samples for measurement of different biological parameters to unravel factors and the time course of the development of IgE-reactivities in childhood. The measurement of allergen-specific IgE in these samples using a highly sensitive technology that consumes only 35µl serum will allow to determine the period of allergic sensitization with accuracy.
The MeDALL allergy-chip, a customized version of the ISAC allergen micro-array (Phadia Multiplexing, Vienna, Austria), was developed comprising 176 allergen molecules. Modifications of the protocol and of reagents for antibody detection further increased sensitivity and reduced background of the test. Sensitivity and variation for IgE- and IgG-detection were determined using dilution-series of monoclonal IgE- and IgG_1-antibodies binding to Bet v 1 or Phl p 2, respectively, in direct comparison to ImmunoCAP (for IgE-measurement). To study competition on the chip between antibodies of different isotypes that combine with the same epitope, Bet v 1-specific monoclonal IgE was mixed with a monoclonal IgG-antibody that binds exactly the same epitope, and IgE-levels were measured by micro-array and ImmunoCAP.
The MeDALL allergy-chip is a highly sensitive tool for the reliable measurement of even very low concentrations of allergen-specific IgE and IgG and covers almost all clinically relevant allergen-components. In contrast to ImmunoCAP, it detects competition between IgE and blocking antibodies and is therefore closer to *in vivo* conditions. The MeDALL allergy-chip therefore is a suitable research- and diagnostic tool for the early detection of IgE-reactivities in children.

P6.16.38
Association between reduction of plasma adiponectin levels and risk of bacterial infection after gastric cancer surgery

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Background Infections are important causes of postoperative morbidity after gastric surgery; currently, no factors have been identified that can predict postoperative infection. Adiponectin (ADN) mediates energy metabolism and functions as an immunomodulator. Perioperative ADN levels and perioperative immune functioning could be mutually related. Here we evaluated a potential biological marker to reliably predict the incidence of postoperative infections to prevent such comorbidities.
Methods We analyzed 150 consecutive patients who underwent elective gastric cancer surgery. The patient characteristics and surgery-related factors between two groups (with and without infection) were compared by the paired *t*-test and χ^2 test, including preoperative ADN levels, postoperative day 1ADN levels, and ADN ratio (postoperative AND /preoperative ADN) as baseline factors. Logistic regression analysis was performed to access the independent association between ADN ratio and postoperative infection. Finally, ROCs were constructed to examine its clinical utility.
Results Sixty patients (40%) experienced postoperative infections. The baseline values of age, physical status, operating time, blood loss, surgical procedure, CRP, preoperative ADN, and ADN ratio were significantly different between groups. Logistic regression analysis indicated that T2DM and ADN ratio were significantly independent variables ($p < 0.05$, $p < 0.01$, respectively). ROC analysis revealed that the useful cutoff values (sensitivity/specificity) for preoperative ADN levels, ADN ratio, blood loss, operating time, and CRP levels were 8.81(0.567/0.568), 0.76 (0.767/0.761), 405 g (0.717/0.693), 342 min (0.617/0.614), and 8.94 mg/dl (0.583/0.591), respectively.
Conclusion T2DM and ADN ratio were independent predictors of postoperative infection and ADN ratio was the most useful predictor for postoperative infection.

P6.16.39
Mitral valve proteomic analysis: novel insights into rheumatic heart disease mechanisms

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Introduction: Rheumatic heart disease (RHD) is a life-threatening complication of untreated *Streptococcus pyogenes* infection in susceptible children, leading to severe heart valve damage. Cross-reactivity, T-cell mimicry and epitope spreading are the main mechanisms connecting valve lesions and the infection. The valve proteins which are target of autoimmune reactions are poorly known.
Objectives: To identify protein expression alterations between normal and RHD valve-tissue by a proteomic approach, and to suggest mechanistic involvement in the disease.
Materials and Methods: Mitral valve fragments of RHD patients with regurgitation (n=8) or stenosis (n=6) were compared to control ones (n=7). Protein separation and differential expression analysis was performed by two-dimensional differential gel electrophoresis (2D-DIGE). The differentially expressed spots were identified by mass spectrometry (LC-ESI-MS/MS). Quantification and *in situ* analyses were performed by western blotting and immunoperoxidase staining, respectively. We also performed confocal microscopy for co-locatization studies. Computational metabolic studies were carried out with the Ingenuity Pathway Analysis (IPA) software.
Results: We found 19 differentially expressed proteins out of 451 identified spots. Vimentin, collagen-VI, lumican and vitronectin presented important expression alterations. Collagen-VI and lumican had an increased co-localization pattern in RHD valves.

Computational pathway analysis suggested an involvement of humoral response, antigen presentation and inflammation with the progression of end-stage valve lesions.
Conclusions: Our results demonstrated a differential pattern of valve proteins in RHD, and a network of interactions which are likely attributed to the etiology of the disease.
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P6.16.40
Transient alteration in cytokine patterns following influenza vaccination detected by Ki-67 expression and automated multiparameter analysis of flow cytometry

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We previously showed that seasonal Trivalent Inactivated Influenza Vaccine (TIV) induces a strong, transient CD4 T cell response in healthy adults, measured at days 4-6 after vaccination as influenza-specific CD4 T cells expressing Ki-67. As elderly subjects have blunted immune responses to some vaccinations, we tested whether elderly subjects also induced the transient Ki-67+ population in response to inactivated influenza vaccine. Blood samples were taken before and after vaccination, and analyzed by intracellular cytokine staining and immunophenotyping by flow cytometry. Datasets were analyzed with SWIFT, our new, high resolution flow cytometry algorithm, that uses model-based clustering to objectively detect very small altered populations in large, high-dimensional datafiles. Assigning samples to cluster templates allows rigorous comparison between samples. A strong Ki-67+ influenza-specific CD4 T cell response was detected in subjects of all ages. Using a training/test set strategy, we identified multiple cell populations that were significantly altered in elderly subjects, as evaluated by each of four phenotyping panels. The high resolution provided by SWIFT, operating simultaneously in all dimensions, allowed the identification and quantification of large numbers of clusters, thus facilitating the agnostic discovery of clusters that were consistently altered in elderly subjects. Thus elderly subjects respond to influenza vaccination by transient up-regulation of Ki-67+ CD4 T cells, but have multiple immune cell alterations revealed by automated multiparameter analysis of flow cytometry data.

P6.16.41
Recurrent ulcerative stomatitis and its correlation with psychological factors

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Introduction: recurrent ulcerative stomatitis is one of the most common and painful diseases of mouth epithelium. Effect of depression and anxiety as psychological factors on pathology of this disease is controversial
Methods: In a correlative study at faculty of dentistry of Tabriz University of Medical Sciences in 2011, 75 patients were divided into 3 groups: patients with recurrent ulcerative stomatitis, positive control group (patients with painful mouth syndrome, unusual facial pain and pain due to facial muscles dysfunction) and negative control group (healthy people). These groups were investigated with Spielberger anxiety test and beck depression test.
Results: patients in recurrent ulcerative stomatitis and positive control groups had higher levels of anxiety and depression in comparison to negative control group. Although difference in anxiety level of patients with recurrent ulcerative stomatitis and positive control group was not statistically significant, but difference in depression level between these two groups was statistically significant.
Conclusion: According to these findings, depression and anxiety have a significant effect on pathology of recurrent ulcerative stomatitis and they act as important initiation factors for recurrent ulcerative stomatitis.

P6.16.42
Naturally occurring sepsis in the dog induces changes in transcript levels of genes associated with human apoptosis, autophagy and necrosis

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A recent retrospective analysis found transcriptomic responses in murine models of disease are not representative of human inflammation gene profiles. These data support the growing recognition that induced animal models of disease result in an oversimplification of disease pathogenesis and thus provide limited utility in therapeutic evaluation. We hypothesize the dog may be an alternative model for inflammation, particularly for sepsis, because of similarities in disease phenotype and the fact that canine veterinary patients are treated in a manner analogous to humans. To determine if naturally occurring sepsis is similar across species at the molecular level, we compared inflammation and apoptosis gene profiles in dogs with sepsis and systemic inflammatory response syndrome to that of healthy dogs, using canine orthopedic patients as a model for sterile inflammation. We found that gene expression in septic dogs was significantly enhanced for those genes defining apoptosis, autophagy and necrosis, with significant repression of genes responsible for cell survival. Transcriptomic responses across all disease categories were generally pro-apoptotic. Sterile inflammation was differentiated from sepsis and SIRS by enhanced expression of genes associated with cellular proliferation and repression of genes associated with autophagy. These data strongly suggest that naturally occurring disease in the dog is analogous to human sepsis at the molecular level and is therefore an important resource in therapeutic discovery.

P6.16.43
Serum hepcidin levels in patients with Brucellosis

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Both $CD4^+$and $CD8^+$ T lymphocytes play crucial roles in immunity to *Brucella*, in part because they secrete interferon (IFN)-γ and activate the bactericidal functions in macrophages. Hepcidin is an antimicrobial and iron regulatory peptide produced by the liver in response to inflammation and elevated systemic iron. Recent studies suggest that circulating monocytes and resident liver macrophages - Küpffer cells - may influence both basal and inflammatory expression of hepcidin, and cross-talk between macrophages and hepatocytes and that during inflammation these two cell types act in concert to regulate hepcidin production. Here, we aimed to investigate the association of hepcidin levels with Brucellosis. Serum hepcidin levels in 49 Brucellosis patients were compared with 52 healthy control subjects. Serum hepcidin levels were significantly higher in Brucellosis patients compared those of with healthy controls ($p < 0.001$). There was no statistically significant difference in serum levels of levels among acute, subacute and chronic cases with Brucellosis. However, hepcidin levels seem to be higher in chronic cases in comparison to acute and subacute cases. According to our first results, it may be suggested that the levels of Hepcidin may be a diagnostic marker for Brucellosis, but future studies are needed to evaluate whether hepcidin levels may be used for discriminating three different forms of Brucellosis (acute, subacute, chronic).

P6.16.44
A unique endotoxin tolerance profile strongly predominates during clinical sepsis

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Bacterial products like lipopolysaccharide (LPS), can be potent inducers of inflammation, promoting a systemic production of pro-inflammatory mediators and recruiting/activating immune cells to eliminate the pathogen. However an excessive inflammatory reaction may lead to septic shock, characterized by organ failure and eventually death. In contrast, LPS can also generate an interesting effect known as endotoxin tolerance or cell reprogramming, defined as the reduced capacity of the immune cell to respond to LPS or other bacterial products following a first exposure to this stimulus. A septic patient is normally characterized by undergoing an excessive inflammatory response. To confirm this, we did an extensive bioinformatic meta-analysis using our previously published data characterizing unique LPS and endotoxin tolerance gene signatures in human blood mononuclear cells. We compared these signatures with transcriptional changes observed in human sepsis cohorts performed in our lab and the literature. We also analyzed signaling pathways and cytokine expression. Very interestingly, we found that septic patients strongly present an immunological profile associated with an endotoxin tolerance gene signature, rather than a dominant pro-inflammatory response as believed. In conclusion, these findings challenges the dogma that sepsis is a hyper-inflammatory (cytokine storm) disease, suggesting instead that endotoxin tolerance (an immuno-suppressive state) might occur much earlier in sepsis than previously suspected. This unique endotoxin tolerance gene signature can be used as possible biomarkers, helping us to characterize the critical immunological status of the septic patient, enabling appropriate immunological and supportive therapies that could improve the survival rate during this deadly syndrome.

P6.16.45
Changes in IgA and IgM in saliva and serum of newborns during the first 30 days of life

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IgM and IgA may be used as serum biomarkers of some congenital disorders in newborns because they do not cross the placenta. Saliva may be a good sample for diagnosis, because it is not invasive. Altered IgM and IgA -total or specific- levels have been demonstrated in saliva as biomarkers of some disease conditions in adults, but not in newborns. Changes in basal IgA and IgM levels in saliva and serum of newborns from birth to 30 days of age are unknown, as well as whether there is correlation between these samples. The concentration of IgA and IgM was determined in paired serum and saliva samples from 15 female and 16 male newborns by ELISA. All samples were taken not before three hours of lactation. IgA serum levels clearly increased with age, while in saliva two groups were identified, one characterized by increasing levels along age, and the other without change. This could be due to differences in genetic factors, exposure to microbes or feeding habits. Increase of seric IgM along age was more erratic. This Ig was absent in saliva except for two cases, who were positive at low levels. Two cases with abnormally high IgA concentration in serum, i.e. above 130µg/mL, were identified and four presented abnormally high levels of IgM (>870 µg/mL). One case with congenital toxoplasmosis presented both IgM and IgA in serum and saliva. The results suggest that one or both immunoglobulins may be useful biomarkers in saliva of newborns.

P6.16.46
Lipid profile analysis in spinal trauma patients shows severe distortion of AA/DHA after injury

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Studies have shown fatty acid abnormalities in various neurological disorders with increased arachidonic acid (AA), an omega-6 pro-inflammatory fatty acid, and decreased levels of docosahexaenoic acid (DHA), an omega-3 fatty acid which reduces inflammation. Using a mouse model of spinal contusion injury, we previously showed rapid increases in AA/DHA ratio after spinal injuries. We found that treatment with the drug fenretinide corrected the fatty acid defects and improved recovery in mice. In this study, we hypothesized that AA and DHA may also be impaired in patients following traumas to the spine.
Here we report preliminary results from our study including 23 spinal contusion injury patients, 11 spinal fracture patients. Plasma samples were taken at various times post injury: 1, 2, 3, 8-16, 25-49 days and 1-2 years. We assessed the levels of AA, DHA and malondialdehyde (MDA), a marker of lipid peroxidation.
The greatest changes in fatty acids occurred immediately after spinal trauma with increased AA and decreased DHA in both types of injuries. Thus the AA/DHA ratio was distorted towards a more pro-inflammatory status. Additionally, MDA increased after spinal injury which indicated a higher levels of peroxidation. At later time points, the fatty acid levels returned to normal and peroxidation decreased as well.
Our results provided confirmation for our hypothesis that AA/DHA ratio is distorted immediately after spinal injuries and may be a factor in causing further neuronal damage. Our data suggest that immediate application of fenretinide after a spinal injury might decrease secondary damage and improve neurological outcomes.

P6.16.47
Kinetics of inflammatory markers following traumatic injuries to the spinal cord

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Inflammation following traumatic spinal cord injuries precedes the wound healing process, however, excessive inflammation may lead to irreversible neuronal damage. In this study we assessed whether there are differences in elevation of inflammatory markers in patients with spinal cord injuries (SCI) as compared to those with spinal fractures (SF) without CNS damage.
Patients with SCI and SF were recruited to the study. Blood samples were collected at 1, 2, 3, 8-16, 25-49 days and 1-2 years post injury. We analyzed inflammatory markers in blood plasma (IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IFNα2, IFNγ, and TNFα) and assessed the differences in their levels between patients with SCI and SF injuries. Here we report preliminary data on 7 SCI and 10 SF patients.
IL-6 levels were higher in SCI than SF patients from days 1 to 3, whereas at 8-16 days post injury, IL-6 became significantly lower in SCI patients than SF ($p = 0.0428$). At day 2, IL-10 levels were significantly higher in SCI patients compared to SF ($p = 0.0341$). IFNγ levels were higher in SCI and SF patients at day 3 ($p = 0.0216$). At 8-16 days post injury , IL-17A and IL-8 were higher in SCI than SF patients ($p = 0.0079$ and $p = 0.0033$, respectively).
SCI patients had significantly higher levels of inflammatory markers than SF patients from the first day of injury suggesting that SCI patients may benefit from therapies reducing systemic inflammation immediately following SCI injury.

P6.16.48
Circulating IL-12 and response to therapy in HCV-infected patients: Potential biomarker for a non-favorable outcome?

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Objective: Cytokines play an important role in the regulation of immune responses. In hepatitis C virus infection, the production of abnormal cytokine levels appears to contribute in the progression of the disease, viral persistence, and affects response to therapy. The aim of this study was to explore a possible association between the pattern of serum cytokines with response to therapy in HCV-infected patients.
Methods: The cohort was composed of eighty-three SVR (sustained virological responders) and seventy-seven NR (non-responders). All patients were administered complete pegylated interferon-α and ribavirin combination therapy followed for twenty-four weeks after the cessation of treatment and divided into different groups according to outcomes of treatment based on HCV-RNA tests. Cytokine-bead-array (CBA) was performed by flow cytometry analysis to determine the pro- and anti-inflammatory cytokines plasma levels (IL-1β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IFN-γ and TNF-α).
Results: Increased plasma levels of IL-12 was observed in the group of NR when compared to SVR patients (p<0.0001). Interestingly, IL-12 plasma levels were increased in NR patients when compared to SVR patients independently of viral genotype 1 (p=0,0016) and 2-3 (p=0.0073). In addition, when NR and SVR patients were stratified by progression of liver fibrosis according to METAVIR score, no statistically significant differences were observed in IL-12 plasma levels. None of the remaining cytokines plasma levels were associated with response to HCV-therapy.
Conclusion: These results suggested that IL-12 levels may be used as a predictive biomarker of therapy response in chronic HCV-infected patients.

P6.16.49
Association of IL28B (rs12979860) and IL-12B (rs3212227) polymorphisms with response to HCV-therapy in patients with a mixed genetic background

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Objective: Hepatitis C virus is a major cause of chronic liver disease. Although the standard therapy, Peg-interferon-α and ribavirin, it is well known that many patients will not be cured. In addition, genetic variations among ethnic groups have an important role in determining response.
Methods: Our aim was to study association between genetic polymorphisms (IL-1 rs16944, IL-2 rs2069762, IL-4 rs2243250, IL-5 rs2069812 , IL-6 rs1800795, IL-8 rs4073, IL-10 rs1800871; rs1800872; rs1800896, IL-12B rs3212227, IL22RA2 rs2064501, IL22RA2 rs6570136, IL-28B rs12979860, CTGF rs3037970, CTGF rs6918698, CTGF rs9402373, IFN rs2430561, TNF rs1800629, MMP-1 rs17886084, MMP-9 rs17576, TIMP-2 rs9900972) and antiviral therapy efficiency for HCV-infected patients among a Brazilian cohort. All patients, 92 sustained virological responders and 83 non-responders, were administered Peg-Interferon-α and ribavirin, followed for 24 weeks after the cessation of treatment, and divided according to outcomes. Polymorphisms were genotyped using primers and probes designed for a Taqman Allelic Discrimination Assay.
Results: According to univariate analysis, TT IL-4 rs2243250 (p=0,037;OR=0.325;95%CI=0.119-0.886), CC IL-10 rs1800871 and CC IL-10 rs1800872 (p=0,047;OR=2.062;95%CI=1.052-4.045), AA IL-12B rs3212227 (p=0.013;OR=0.446;95%CI=0.232-0.857), and CC IL-28B rs12979860 (p=1,21x10-5;OR=4.153;95%CI=1.853-9.308) polymorphisms were significantly associated with response to therapy. By multivariate model, AA IL-12B rs3212227 (p=0.00178;OR=0.353;95%CI=0.167-0.744), CC IL-28B rs12979860 (p=0.0009;OR=3.765;95%CI=1.567-9.044) polymorphisms were independent predictors for NR and SVR, respectively.
Conclusion: These findings suggest that IL-28B in combination with IL-12B polymorphism could be used as biomarkers to predict the efficacy of anti-viral HCV therapy.

P6.16.50
HLA-G expression is an independent predictor for survival in high grade ovarian carcinomas

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Background: Expression of classical and non-classical HLA is respectively associated with favourable and unfavourable survival in cancers. However, controversy exists concerning non-classical HLA-G expression and prognosis. The aim of this study was to evaluate the prognostic value of classical and non-classical HLA expression levels in high grade epithelial ovarian cancer.
Methods: Tissue microarrays of formalin fixed paraffin embedded tumour tissue of 167 primary high grade epithelial ovarian cancer patients were constructed. These were immunohistochemically stained for classical and non-classical HLA. Expression was compared with clinicopathological factors and progression free and overall survival using chi-square and log rank test with additional Kaplan-Meier curves. For multivariate analysis Cox Proportional Hazard regression model was used.
Results: Patient with high grade epithelial tumours expressing HLA-G had a better survival (p= 0.014). Median disease specific survival was 26 months shorter for cases without expression of HLA-G. Furthermore, HLA-G expression was associated with longer progression free survival (p=0.036) and chemosensitivity (p=0.016). Also, in multivariate analyses HLA-G expression was found to be an independent predictor for better survival (p=0.011) and for optimal surgery result (p=0.010). Expression or loss of expression of HLA-A, -B, -C and -E was not associated with survival or clinicopathological factors.
Conclusion: Expression of HLA-G is an independent prognostic factor for improved survival in high grade epithelial ovarian cancer. The survival advantage is related to better chemosensitivity and surgery results, suggesting a new role for HLA-G as a prognostic indicator in high grade epithelial ovarian cancer.

P6.16.51
Phenotypic signature of B cell subsets as a tool for the investigation of immunogenicity

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It has recently become clear that different B cell subsets contribute to both the pathogenesis of immune disorders, as well as the maintenance of tolerance, and are therefore implicated in the development of autoimmunity. Patients with rheumatoid arthritis (RA) and systemic lupus erythematosus are characterised by an increase in pathogenic B cells (e.g. memory), and a decrease or functional impairment of regulatory B cells (Bregs), which promote tolerance via the production of interleukin-10. In humans, we and others have shown that B cells with suppressive capacity are enriched in the $CD19^{+}CD24^{hi}CD38^{hi}$ compartment. These cells can interrupt T cell-B cell positive feedback, suppress effector T cell proliferation, and induce regulatory T cell differentiation. Yet conclusive markers for the identification of a pure Breg population, distinct from those with pathogenic capacity, have not been found. Here we describe a new immunophenotyping platform, with which we can screen the expression of 242 cell-surface markers in parallel by flow cytometry. This has enabled us to generate a surface marker “signature” for different B cell subsets, and provided us with a phenotypic counterpart to gene array data. A major goal in the development of biopharmaceutical drugs is to predict which patients are likely to

respond with anti-drug antibodies (ADAs) - one of the principal drawbacks of biological therapies. We will implement our method to compare B cell subset signatures in a longitudinal cohort of RA patients treated with anti-TNFa in order to identify predictive markers for those patients who will become ADA positive.

P6.16.52
Role of the immunity in the aggressive forms of breast cancer seen in Western Kenya

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Breast cancer in African women has a poor prognosis and a high rate of recurrence. There is an urgent need to define the role of the host immune response to tumor in the seemingly aggressive forms of breast cancer in African women. Studies have indicated that the immune system has anti-tumor function and can also facilitate tumor escape and progression. Tumor infiltrated with cells such as CD4, CD8 and macrophages have shown both positive and negative association with tumor relapse and metastasis. Tumor associated M1 and M2 subtypes have a role in suppressing the tumor and enhancing tumor growth respectively. Little has been done on the prognostic value of the immune system in breast cancer. Identifying the role played by the host response to tumor in aggressive breast cancer forms could give an insight in the tumor biology of the unique forms of tumors seen in African women. This study will determine the significance of inter-relationship between tumors and host immune response in the aggressive forms of breast cancer seen in Kenyan young women.

P6.16.53
Gastrointestinal autoimmunity to tryptophan hydroxylase in Autoimmune Polyendocrine Syndrome type 1 (APS-1)

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Background: Autoantibodies to tryptophan hydroxylase (TPHAbs) directed against serotonin-producing enterochromaffin cells (EC) have been reported in APS-1 patients with gastrointestinal dysfunction (GID). Serotonin plays a critical role in enteric function and its serum levels reflect serotonin release from the gastrointestinal tract.
Hypothesis: TPHAbs could mark a distinct autoimmune component of APS-1 characterized by an autoimmune attack toward EC, with subsequently GID.
Methods: TPHAbs were measured in 64 APS-1 patients. Gastric and duodenal biopsy was carried in 16 TPHAbs+ patients (8 with and 8 without GID) and in 2 TPHAbs- patients (without GID); in these patients serotonin serum levels were measured. Immunohistochemistry was performed using antibodies to serotonin, chromogranin-A, CD3, CD4, CD8, and CD20.
Results: 37 of 64 patients were TPHAbs+ (11/12 with GID and 26/52 without GID;P<0.001). Biopsies in 8 TPHAb+ patients with GID showed lymphocytic infiltration (with increased CD3+CD8+ intraepithelial lymphocytes) and absence of EC; mean serotonin serum levels were below the normal range in TPHAb+ patients with GID (P<0.01). In 8 TPHAb+ patients without GID biopsies showed different grades of inflammatory infiltration and reduced number of EC. Mean serotonin serum levels were near the lower limit of the normal range. In all TPHAbs+ patients, biopsies showed a reduced number of chromogranin-A positive cells consistent with enteroendocrine cells depletion. TPHAbs- patients without GID showed no histogical and biochemical abnormalities.
Conclusions: TPHAbs appear to be markers of a distinct autoimmune component of APS-1. Progressive involvement of the gastrointestinal EC leads to the transition from preclinical to clinical disease.

P6.16.54
Blood pulmonary tuberculosis biomarkers on diabetic individuals

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SETTING. Tuberculosis (TB) associated to chronic health conditions such as non-insulin-dependent diabetes mellitus (NIDDM) could represent nowadays a worldwide Public Health threat. The relative odds of developing tuberculosis in diabetic patients ranges from 3 to 8 compared with non-diabetic patients. No molecular diagnostic or prognostic test to distinguish latent (LTB) from active TB (ATB) exists, neither biomarkers to follow TB pharmacology interventions. Given exacerbated inflammation by TB leads to pulmonary damage; key molecules on the inflammation process could be useful as diagnosis biomarkers for TB states, even in individuals with NIDDM.
METHODS. Previous microarrays analysis at our laboratory identified a group of overexpressed genes on mice lungs infected with a Mycobacterium tuberculosis virulent strain that were not induced by a persistent strain that induces no inflammation. Based on the hypothesis that PBMCs from individuals with different TB infectious status could exhibit distinct transcription profiles, the present study evaluated the gene expression capability of 7 mice overexpressed genes on human whole blood, to discriminate subjects with pulmonary TB (PTB) (with or without NIDDM) from non infected or LTB subjects.
RESULTS. In the no NIDDM group, CXCR5 presented lower expression on PTB compared to LTB, while PSTP1 expression was higher. On the other hand, NIDDM-PTB individuals showed higher expression for GBP2, ARHGAP30, NCF1, and FPR2 compared to the LTB individuals, while CXCR5 expression was lower.
CONCLUSIONS. Gene expression profile of four overexpressed genes on TBA were found with potential utility to discriminate on NIDDM, LTB persons from those with activated pulmonary TB.

P6.16.55
Anti-carbamylated protein antibodies (Anti-CarP) precede the onset of Rheumatoid Arthritis

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Objective
Anti-citrullinated protein antibodies (ACPA) and IgM-Rheumatoid Factor (IgM-RF) are auto-antibodies that can be detected many years before the clinical diagnosis of Rheumatoid Arthritis (RA). Since ACPA and IgM-RF are known as predictive and prognostic markers of RA, it is conceivable that these antibodies play a pathogenic role in RA. Recently we discovered anti-carbamylated protein (anti-CarP) antibodies as a prognostic marker in RA and are associated with a conversion to RA in patients suffering from arthralgia. In this study we analyzed whether anti-CarP antibodies can also be detected prior to the onset of the symptoms of RA and which auto-antibody appears first over time.
Methods
Sera of 79 RA patients prior to the onset of symptoms and 141 age and sex matched controls that were regular blood donors were tested for the presence of anti-carbamylated fetal calf serum (Ca-FCS) antibodies, anti-carbamylated fibrinogen (Ca-Fib) antibodies, anti-cyclic citrullinated peptide 2 (CCP2) antibodies and RF-IgM.
Results
Anti-CarP antibodies were present in 39% of the serum samples that were drawn just prior to the diagnosis of RA compared to 4% of the

matched control samples. Anti-CarP antibodies were present in both ACPA positive and ACPA negative patients.
Analysis of the longitudinal samples revealed that anti-CarP antibodies could be detected many years before the onset of symptoms (median 8 years). Both ACPA and anti-CarP antibodies appear (similarly) earlier in time as compared to IgM-RF.
Conclusions
Next to ACPA and IgM-RF also anti-CarP antibodies appear many years before the onset of clinical symptoms of RA.

P6.16.56
An innovative method to identify autoantigens expressed on the cell surface: SARF

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Background: Although autoantibodies against integral membrane proteins are generally considered to be pathogenic, identification of such molecules was difficult by conventional methods, including proteomics. Anti-endothelial cell antibodies (AECAs) are important but unsolved autoantibodies that target cell-surface autoantigens and may play roles in vascular lesions in collagen diseases. To specifically identify cell-surface autoantigens, we constructed a Serological identification system for Autoantigens using a Retroviral vector and Flow cytometry (SARF).
Methods: A cDNA library of human umbilical vein endothelial cells (HUVEC) was retrovirally transfected into a rat myeloma cell line, from which AECA-binding clones were sorted by flow cytometry. Integrated cDNA of the cells was analyzed to identify autoantigens, and the functional significance of the autoantibodies was analyzed.
Results: Distinct AECA-positive clones were isolated using different serum IgG from patients with AECA-positive collagen diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). By DNA sequencing and microarray, we identified three cDNA inserted to each clone, which were fibronectin leucine-rich transmembrane protein 2 (FLRT2) and Pk (Gb3/CD77) from SLE, and intercellular adhesion molecule 1 (ICAM-1) from RA. HUVEC expressed these proteins and the prototype AECA IgGs bound specifically to these molecules-transfected cells. Anti-FLRT2 antibody induced complement-dependent cytotoxicity against FLRT2-expressing cells.
Conclusion: Using SARF, we successfully identified three membrane proteins as targets for AECAs. SARF is useful for specific identification of autoantigens expressed on the cell surface. By thorough identification of cell-surface autoantigens, it may be possible to achieve a comprehensive understanding and the development of more specific intervention strategies of autoantibody-mediated injury.

P6.16.57
Significant cancer-associated autoantibody signatures for early diagnosis and prognosis of gastric cancer

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Gastric cancer (GC) is one of the most common reasons of cancer-related deaths due to asymptomatic early stages and poor treatment efficiency for late stage patients. Cancer autoantibodies (AAb) are attractive biomarkers for early diagnosis as they are specific and stable in blood and form early in disease progression. However no AAb-based test has been developed so far as each individual tumour differs in its antigen repertoire and the frequency of AAbs against each individual antigen is too low.
We have identified >1000 B cell antigens and used them for generation of antigen microarrays. GC-associated antigens were identified by screening sera from 100 GC patients and 100 cancer-free controls (HD). 86 most reactive antigens were selected for production of a validation array screened with an independent set of sera from 235 GC, 154 peptic ulcer and gastritis patients and 213 HD. ROC curve analysis showed that 45 AAb signature could discriminate GC and HD in the validation set with a 90% specificity and 59% sensitivity (AUC 0.79, p=2e-31), thereto early GC was detected with the same precision as advanced GC. Also two sets of seven AAbs were determined as the most significant independent prognostic factors correlating to longer (>1 year) (HzR=0.21, p=0.048) or shorter (<1 year) (HzR=7.7, p=0.002) survival after surgery of late stage patients by the multivariate Cox regression analysis.
We show here that cancer AAbs can be used for early diagnosis and prognosis of GC, and further work is in progress to increase the sensitivity of the test.

P6.16.58
Rates of Persistent Positivity for Anti-Cardiolipin and Anti-Beta-2-Glycoprotein I Antibodies in Routine Clinical Practice

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Purpose: Assessment of anti-phospholipid antibodies is an established practice in the evaluation of patients with suspected anti-phospholipid syndrome (APS). Positivity of anti-cardiolipin (aCL) or anti-beta-2-glycoprotein I (aB2) antibodies (IgG or IgM) on 2 occasions separated by ≥12 weeks fulfills current laboratory classification criteria for APS. The purpose of this study was to assess the frequency of positive serial test results in routine clinical practice.
Methods: aCL and aB2 IgG and IgM antibody results were collected from samples submitted for routine testing between April 1, 2008 and April 30, 2010. Testing was performed by enzyme immunoassay (Phadia). Antibody titers ≥40U/mL were considered positive.
Results: A total of 6,064 patients were identified for whom repeat aCL and/or aB2 IgG and/or IgM testing was performed. Of the positive samples, 63 had repeat testing performed 2-6 weeks following the initial result, 71.4% of which remained positive. For samples in which repeat testing was performed 6-12 weeks (n=89) and ≥12 weeks (n=296), 74.2% and 76.4%, respectively, remained positive. When repeat testing was performed ≥12 weeks following the initial result, 76.4% of samples positive for aCL IgG (n=191) and 72.8% of samples positive for aCL IgM (n=114) remained positive. For aB2 IgG (n=17) and aB2 IgM (n=4), 63.2% and 75.0%, respectively, remained positive.
Conclusions: The frequency of positive aCL and aB2 follow up tests was consistent regardless of the time frame during which the testing was performed. Whether serial positive test results over any specific time period improves the diagnostic specificity for APS remains unclear.

P6.16.59
The effect of dipeptidyl peptidase-IV inhibitor sitagliptin on the immune functions in patients with type 2 diabetes

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Gliptins (e.g. sitagliptin, vildagliptin) are novel anti-diabetic drugs that improve insulin secretion by a GLP-1 mediated mechanism by inhibiting the protease dipeptidyl peptidase-IV (DPP-IV) with a consequent increase in the bioavailability of incretins. DPP-IV is a multifunctional membrane bound serine protease identical with the marker of activated lymphocytes CD26 and its soluble form is found in blood plasma. In addition to incretins, DPP-IV is responsible for the breakdown of several other biologically active peptides such as chemokines and neuropeptides. Given that DPP-IV has diverse effects on the modulation of cell growth and immune functions, its long-term inhibition could lead to unfavorable effects including immune dysregulation.

The aim of this study is to assess the possible differences in lymphocyte differentiation and cytokine production in patients with type 2 diabetes mellitus treated with sitagliptin in comparison to patients treated with other hypoglycemic medications (control group). Patients from both groups are examined before and 4 weeks after the enrolment in the study. The DPP-IV enzymatic activity of plasma, mononuclear cells and immunophenotyping of lymphocytes (Treg, NK, Th1, Th2 and Th17 cells) are performed in heparinized peripheral blood. Our preliminary data show that short term (4 week) sitagliptin treatment leads to changed proportion of lymphocyte populations in peripheral blood in patients with type 2 diabetes. A further follow-up examination is planned at 1 year. These data should improve our understanding of the possible immunological implications of the DPP-IV enzymatic activity inhibition.
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P6.16.60
Tonsils of patients with IgA nephropathy contain cells producing aberrantly glycosylated IgA1 and anti-glycan antibodies: implications for tonsillectomy

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Background: IgA1 in circulating immune complexes and in the glomerular deposits of patients with IgA nephropathy (IgAN) is aberrantly glycosylated, galactose-deficient in *O*-glycans (Gd-IgA1) and is bound by antiglycan IgG/IgA1 autoantibodies. However, the origin of cells producing Gd-IgA1 and the autoantibodies is unknown. Upper-respiratory tract infections and/or tonsillitis are frequently associated with clinical presentation and exacerbation of IgAN, suggesting a link with the disease pathogenesis. Moreover, there is a need to assess the mechanisms and effectiveness of tonsillectomy combined with glucocorticoids on the progression of IgAN.
Methods: In this study, we studied 27 patients with IgAN who underwent tonsillectomy and glucocorticoid therapy (TSP). Tonsillar cells were immortalized with Epstein-Barr-virus and Gd-IgA1 and anti-glycan IgG secreted by these cells were measured. Urinary protein and hematuria, and serum levels of Gd-IgA1, anti-glycan IgG, and anti-glycan IgA were measured before and after the TSP therapy.
Results: Sixteen of twenty-seven patients showed less 0.3 g/gCr proteinuria and 5 red-blood cells/HPF after TSP (Remission group). A rate of decrease in serum levels of Gd-IgA1, anti-glycan IgG, and anti-glycan IgA were greater in the Remission group than in non-Remission group (P<0.01). Furthermore, tonsillar B cells from Remission group secreted greater amounts of Gd-IgA1 and anti-glycan IgG than those from non-Remission group (P<0.01 for both).
Conclusion: In summary, tonsillar immunoglobulin-producing cells from patients with IgAN secreted Gd-IgA1 and anti-glycan antibodies. Moreover, IgA1 and anti-glycan antibodies may be useful serum biomarkers to assess therapeutical responses in IgAN patients.

P6.16.61
Distribution of prostate specific antigen (PSA) among senegalese men with immunoenzymatic sandwich method

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Objective :To explore distribution of total prostate specific antigen (tPSA) values among senegalese men only aged 40 years and upper. Six hundred and eighteen sera were tested. Mean age was 58.67 years. An immunoenzymatic sandwich assay with final detection in fluorescence was used for tPSA measurements. The median tPSA level was 1.32 ng/ml. Approximately 82% presented normal values of tPSA (< 4 ng/ml) and high PSA was found in 18.4% of cases. In healthy subjects, according to the age categories, median tPSA level was respectively 0.92, 1, 1.3, 1.45, 1.45 and 3.61 ng/ml for age strata of 40-49, 50-59, 60-69, 70-79, 80-89, 90 years and above. Among patients suspected of prostate cancer (n = 114), median tPSA level was 8.98 ng/ml. Prostate cancer was confirmed by biopsy in 94 patients. Mean age of diagnosis of prostate cancer was 65.5 years and mean tPSA among the prostate cancer group was 125.7 ng/ml.

P6.16.62
A Peptide based approach for the serological diagnosis of Chikungunya fever

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Chikungunya is a mosquito borne alpha virus, affected large population in Asia and Africa. In general, Chikungunya virus (CHIKV) and Dengue virus (DENV) share common clinicopathological symptoms. In most of the cases, diagnosis of CHIKV is symptomatic but RT-PCR, virus Isolation and immunological approaches are the existing methods of diagnosis of the disease. Some of these methods showed cross reactivity with other related alpha virus family. Confirmatory diagnosis of Chikungunya before treatment initiation is essential to avoid non target effect of drugs. Thus, there is an urgent need to develop diagnostic reagents to diagnose Chikungunya without cross reactivity with other related alpha viruses.
The present study highlights the peptide based detection of IgM antibodies specific for Chikungunya as an efficient and reliable approach for the diagnosis of the disease. To achieve the aim we have selected 7 peptides from E1, 17 peptides from E2 and 1 peptide from E3 by Bcelpred and DNAstar software and surface localization was done by PYMOL software. To evaluate the assay, peptide based ELISA for IgM detection in 83 CHIKV positive, 35 negative control and 33 DENV positive sera was done. Prism Ver: 5 software was used to analyze the data. Of 17 E2 peptides chosen, 10 showed significant seropositivity with all the positive sera similar to virus lysate/E2 protein. The sensitivity and specificity of these peptides were found between 95-97% and 100%, respectively. Importantly, none of the peptides showed serological cross-reactivity with Dengue sera.

P6.16.63
Kinetics of expression of CD62L and CXCR3 on CD8+ T cells during treatment of hepatocellular carcinoma with TACE

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Aim To investigate the effect of treatment with transcatheter arterial chemoembolization (TACE) on the expression of chemokine receptors (CR) on memory T cells in HCC patients. Methods Blood samples from hepatic artery and peripheral vein were collected from 100 patients with HCC before and 4w post treatment with TACE, respectively. PBMCs were isolated to examine the expression of CRs: CD62L and CXCR3 on CD8+T cells in HCC patients. Results Both frequency and quantitation of $CD62L^{low}$CXCR3+ proinflammatory effector T cells in HCC patients post treatment increased significantly than pretreatment with 61.92±8.69% vs 24.45±7.36%, P<0.05. and 18.98±2.33e7/L vs 6.10±1.21e7/L, P<0.001, respectively. No significant difference in their frequency between in artery and peripheral vein was displayed. Furthermore, activated CD69+T cells in HCC patients rose from 2.53±0.51% in artery and 2.38±0.49% in vein respectively to 3.80±0.62% and 4.48±0.75% post treatment, both P<0.05. Conclusion Treatment with TACE may lead to an increase of $CD62L^{low}$ CXCR3+ proinflammatory effector T cells in HCC patients.
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P6.16.64
Cord blood graft composition and its impact on clinical outcome after allogeneic stem cell transplantation

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Today allogeneic hematopoietic stem cell transplantation (ASCT) is used for treatment of hematological malignancies, immunodeficiencies and other non-malignant diseases such as genetic and metabolic disorders. Umbilical cord blood (UCB) is a suitable source of hematopoietic stem cells when no HLA-matched related or unrelated donor is found.
We analyzed material from 29 cord blood units used for allogeneic hematopoietic stem cell transplantation with multi-color flow cytometry. Phenotypical data were correlated with different clinical outcome parameters e.g. survival, graft versus host disease (GVHD), relapse, rejection, viral reactivation, bacteremia etc. The aim was to find cell surface markers that can be used as tools to predict clinical outcome post transplantation.
We found that above-median frequency of CD69+ T cells was associated to patient survival (p=0.025). Additionally, above-median levels of naïve CD8+ T cells (p=0.028) correlated with survival, and an association between patient survival and above-median levels of CD127+ cells B cells (p=0.013) in the graft was found. Moreover, there was a statistically significant correlation between higher levels of CD94+ total T cells (p=0.0031) as well as CD8+ T cells (p=0.0024) and HSV+VZV reactivation post transplantation. This correlation was also seen for the frequency of CD95+ total T cells (p=0.013), as well as CD4+ (p=0.0067) and CD8+ (p=0.033) T cell subsets. Finally, a higher frequency of naïve CD8+ T cells seemed to correlate with aGVHD (p=0.017).
This study highlights the importance of further characterization of cellular graft composition and its impact on clinical outcome after cord blood transplantation

P6.16.65
Characterising the effect of remote ischaemic preconditioning on the immune response in healthy volunteers

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Remote ischaemic preconditioning (RIPC) describes the phenomenon where brief intermittent periods of limb ischaemia are used to protect the heart and other organs from subsequent prolonged ischaemic insults. The precise mechanism through which RIPC exerts its protective effect is unknown but appears to involve inflammatory pathways. This study aims to determine the effect of RIPC on the immune response in healthy human volunteers. Preliminary results indicate that RIPC does not alter the circulating levels of neutrophils or monocytes, but significantly reduces expression of the activation marker, CD11b, in these populations after *in vitro* stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin. In addition, RIPC does not have a direct effect on the response to polyclonal stimuli, or recall T cell responses to the tetanus toxoid; however, PMA and ionomycin stimulated T cells show a greater level of activation and production of the inflammatory cytokine, interleukin 6, following RIPC. These early findings indicate that RIPC has subtle but direct effects on both the innate and acquired immune responses. In light of the challenges translating the success of RIPC from animal studies into human trials, the findings from this study may be useful to help define the clinical indication and limitations of RIPC.

P6.16.66
Validation of in vitro human PBMC assay to determine Lymphocytes activation of new Biotherapeutics

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The development of biopharmaceuticals which includes recombinant proteins and monoclonal antibodies has been on the increase due to the commercial success of many biological products. Regulatory agencies have become interested in potential *in vitro* assays using human cells that may predict immunotoxic and immunstimulatory events in humans especially after the Tegenero incident in London. After the development of *in vitro* assay to characterize and potentially predict the activation of human PBMC lymphocytes induced by different biological compounds, this method were formally validated to the principals of Good Laboratory Practice and based on various white papers and regulatory guideline. Multiple cytokines involved in innate and adaptive immunity were analysed in medium by quantitative immunoassays and in parallel, the expression of lymphocytes activation markers, CD69, CD25, HLA-DR were also assessed by flow cytometry. For validation purposes, human PBMC were isolated from at least six different donors on multiple days and cultured in the presence of vehicle and positive controls. The positive control PHA was found to be the most appropriate control for both Flow Cytometry and Immunoassays and this compound was used to induce an inflammatory response. The aim of the formal validation was to assess reproducibility and robustness of the analytical method but also to assess the biological variability associated with Human PBMC collection from different subjects. Formal validation included evaluation of intra and inter-day precision, subject variability, dilution linearity, stability. All methods were proven to be reliable and to be fit for their intended purpose.

P7.01 Imaging of the immune system

P7.01.01
Malnutrition and thymic hypoplasia in the infancy

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Background and objectives: The thymus is an organ primary linfoide; decisive for the thymopoiesis and development of system inmunitario in the early childood, the nutritional lacks, result in thymus structural and functional changes and in the appearing of infectious complications.
Methods: In this study longitudinal prospective were studied 125 patients undernourished in during 6 moths with thymic hypoplasia and recurrent sepsis among 1-7 years old. The patients stratified in working groups according to the measurement of thymic area for ultrasound themselves: Critical (less than 500mm^2), moderated (501-999 mm^2) and normal (100-1500 mm^2).We realized correlation test between groups.
Results: We found a positive correlation among the malnutrition and the thymic hypoplasia, being more significant in the critical respect to the ones that having the normal thymic area and had a positive correlation also among the malnutrition and the appearing sepsis complicated in the infants, with significant changes in the critical and moderated in relation to them with normal thymic area.
Conclusions: Our results show that the malnutrition result in altered thymic activity in early life, therefore, its monitoring in the infants is very important in the development and functioning of thymus, for the opportune intervention in the fight against the infectious illnesses.

P7.01.02
TIM-1 Protein Trafficking Toward Cell-Cell Contacts

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The T-cell/Transmembrane, Immunoglobulin (Ig) and Mucin domain (TIM) proteins are involved in the regulation of immune responses by modulating effector T cell functions. The TIM gene family is located in a genomic locus linked to autoimmune disease and asthma both in mouse and humans, and they have been associated with immune-related diseases, cancer and viral infections. The TIM proteins are a family of cell surface phosphatidylserine (PtdSer) receptors that can mediate elimination of apoptotic cells. We determined that the Ig variable (IgV) domain of the TIM proteins binds to PtdSer through a conserved pocket termed Metal Ion-dependent Ligand Binding Site (MILIBS). TIM-1 has mostly an intracellular distribution and accumulates in cell-cell adhesive contacts. Currently, we are using

Confocal Microscopy, fluorescent protein and several cell lines to uncover TIM-1 intracellular trafficking and its accumulation at cell-cell junctions. We found that TIM-1 accumulates in endocytic vesicles rather than on the cell surface. This TIM-1 phenotype and its migration to cell-cell contacts is dependent of the integrity of the TIM-1 extracellular portion, including IgV-MILIBS and mucin domain. Currently, we are studying translocation of TIM-1 to the immunological synapse. The accumulation of TIM-1 on cell-cell contacts might trigger costimulatory signals leading to cell activation.

P7.01.03
Cell death detection using antibody-based microarrays and digital holography microscopy on lymphocytic cell lines

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We have combined antibody-based microarray technology with digital holographic microscopy enabling us to capture cells by binding of surface antigens to specific antibodies. We perform holographic measurements for analysis of cancer cell death in terms of loss in cell volume. Development for fast and accurate evaluation tools for cancer treatments will be of great value for clinicians in deciding the most appropriate treatment for patients. We have studied two different leukemia/lymphoma cell lines subjected to high concentrations of cell-death inducing etoposide. T-cell acute lymphoblastic leukemia (T-ALL) cells or diffuse large B cell lymphoma (DLBCL) U2932 cells adhered to antibody-based microarrays and subsequently the mean cell area, cell thickness and cell volume were measured using digital holography. Results showed that the cell number was stable over time. Changes in cell volume of treated cells could be analyzed in experiments performed for up to 980 minutes, whereas untreated cells showed no such decrease in volume. Results support the idea of using the combination of digital holography and antibody-based microarray technology as an evaluation tool for cancer therapies.

P7.01.04
Imaging studies of immunological synapse formation and signaling events using the FlowSight and ImageStream imaging flow cytometers

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Interaction between antigen-specific T cells and antigen presenting cells (APC) cognate ligand involve reorganization of the cytoskeleton and recruitment of adhesive and signaling molecules to the site of intercellular contact. Sustained adhesion of T cells to APCs and formation of the immunological synapse (IS) after T cell receptor stimulation are required for the antigen-specific response. One way to measure IS is by fluorescently labeling the molecules being recruited to the synapse and imaging by fluorescence microscopy. However, these events are rare and therefore difficult to analyze objectively and statistically by traditional microscopy methods. To overcome these problems, we employed the Amnis imaging flow cytometers to objectively collect imagery of large numbers of cells. We report the percentage of T cells involved in an organized immunological synapse, the recruitment of adhesion molecule LFA-1 and signaling molecule Lck to the synaptic complex and translocation of NFkB from the cytoplasm to the nucleus in the T cell. In this study, T cell-APC conjugates are formed with Raji B cells loaded with Staphylococcal enterotoxin B and human T cells. Results from the FlowSight and the ImageStream imaging flow cytometers are compared. Using the FlowSight imaging flow cytometer we demonstrate image-based parameters used to assess the frequency of conjugates with an organized immunological synapse. Employing the ImageStream imaging flow cytometer we further evaluate the specific location of the adhesion and signaling molecules LFA-1 and Lck within the immunological synapse complex in T cells and measure the nuclear localization of NFkB in the T cell.

P7.01.05
Monitoring immunological dynamic processes using large-area high-speed multicolor nonlinear optical microscopy

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Studies of immunological dynamic processes in native physiological environment, call for live imaging methods that have not only high spatial resolution but also the capability of large area, high-speed and multicolor imaging. High-resolution large-area imaging can avoid cell escaping out of the observation volume during long-term monitoring, and allows the visualization of cell-cell, and cell-molecule interactions within proper context of surrounding tissue environment; while, high-speed imaging is essential to provide an accurate readout of instantaneous velocities from multidimensional (x, y, z, time) information; moreover, multicolor imaging allows simultaneous observation of differently labeled cell types, molecules and surrounding environment like vessels, connective tissue, etc. We present a large-area high-speed, multicolor nonlinear optical imaging system that is designed specifically for live animal immunological imaging. The scanning platform of the system is based on the combination of a polygonal mirror and a galvanometer scanner. Thus, an imaging speed beyond 30 frames/s is acquired. In addition, a three-dimensional robotic stage is integrated into the system for sample and objective translation. As a result, large-area and depth-scanning imaging speed is 10 frames/s and 6 sections/s respectively. Furthermore, the realization of multicolor imaging is based on four detection channels covering most of the visual spectrum normally used for fluorescence imaging. This study has the potential to provide new insights into immune system and immune response based on the investigation of cell trafficking.

P7.01.06
Optimization of single- and double-staining indirect immunofluorescence for the detection of anti-pituitary (APA) and anti-GH-producing cells

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Anti-pituitary antibodies (APAs) have been reported in patients affected by autoimmune endocrine disorders, hypopituitarism and pituitary tumors since 1960s, using ELISA, radioligand assay, immunoblotting and indirect immunofluorescence (IIF). A few pituitary cytosolic proteins, factors and hormones, have been suggested as possible immune targets, being GH one of the most attractive especially in the field of pituitary tumors, although the specific autoantigens recognized by anti-pituitary antibodies and their clinical significance remains to be determined. IIF is considered the gold standard method in this field. Major limitations to the detection of APA using traditional methods on commercially available kit/home-made pituitary tissue's slides are the high tissue autofluorescence (due to specific and non-specific Ag-Ab bindings) and the absence of markers for cellular localization (nuclear vs cytoplasm) which highly impair diagnosis and are responsible for false (especially falsely positive) results. We describe the method we optimized for the detection of APA and anti-GH secreting cells using single and double staining IFI working on monkey pituitary tissue from a commercially available kit, and provide images demonstrating the improvements in terms of sensitivity and specificity, leading to an overall significant increase of the method's reliability and results' reproducibility.

P7.01.07
Development of a novel system to visualize basophil degranulation

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Basophils play pivotal roles in allergic reactions, and protective immunity against parasitic infections. They circulate in peripheral blood under steady-state conditions, and are recruited to and accumulated in affected tissues during allergic reactions and parasitic infections. In terms of their functions, it is important to know whether those basophils are indeed activated after infiltrating tissues. It is well known that antigen/IgE-induced cross-linking of the high-affinity IgE receptors on basophils triggers their degranulation and release of inflammatory mediators including lipid mediators and proteases. In this study, we sought to visualize basophil degranulation in order to detect basophil activation in affected tissues. We constructed a novel fluorescent protein-based indicator for monitoring basophil degranulation and introduced them into basophils. The fluorescence intensity in basophils was remarkably increased after antigen/IgE stimulation, whereas it was hardly detected before stimulation. Furthermore, the increase of fluorescence corresponded to the up-regulation of CD63, known as a degranulation marker. Thus, this system appears to be useful for the visualization of basophil degranulation. We are currently applying this technique to the *in vivo* imaging of basophil degranulation.

P7.01.08
Bull's-eyes immunological synapses(ISs),but not multifocal ISs is formed between dendritic cells and T cells

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Immunological synapses (ISs) are multimolecular assembly place of receptors and adhesion molecules, formed at the T cell-antigen-presenting cell (APC) interface during antigen recognition.They play a central role in T cells activation. Usually, multifocal ISs are formed between dendritic cells (DCs) and naive CD4+T or CD8+T cells as well as activated CD4+T cells. On the contrary, classical bull's-eye ISs are formed between B cells, supported planar bilayers and naïve or activated T cells. In our experiments, we found interestingly that bull's-eye ISs, but not multifocal ISs are formed at the murine T cell-DCs interface induced by staphylococcal Enterotoxin B (SEB) stimulation. Similar to the bull's-eye ISs of T-B cells, bull's-eye ISs of T -DCs have mature classical shape and size, and lead to the long time stable contact between T cells and DCs. Compared with the multifocal synapse, bull's-eye ISs lead to different calcium flux in T cells. A dynamic imaging analysis revealed that the mature DCs form more bull's-eye ISs and long-lasting interactions conjugates with naive T cells than immature DCs. Further analysis of the molecular mechanism of bull's-eyes ISs, we found some costimulatory molecules, such as ICAM-1, acting as an important molecular associated with cell cytoskeletal, dynamically participate in formation of bull's-eye ISs. Our results provide an alternative synapse formation between DC-T cells, which will potentially explore the new signal pathway in T cells activation

P7.01.09
Dynamic intravital analysis of T cell activation in the tumor parenchyma and stroma

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Local activation of cancer-associated T cells crucially shapes the tumor immune response; nonetheless, the efficiency of T cell activation in distinct tumor areas in vivo, and following direct- versus cross-presentation, is unknown. We set up a platform allowing the dynamic visualization of the nuclear translocation of the NFAT-GFP reporter in T cells, a hallmark of activation. We recently reported that NFAT is characterized by long deactivation kinetics (~20min) in T cells in vivo, and that during sequential interactions of antigen-specific CD8+ T cytotoxic T cells (CTLs) with cancer cells in the tumor parenchyma, it allowed the integration of intermittent TCR triggering into a continuous transcriptional response.
To study CTL activation upon interaction with hematopoietic elements of the tumor stroma cross-presenting tumor associated antigens, we used mice bearing genetically labeled myeloid cells as recipients of hemagglutinin (HA)-expressing CT26 tumors and HA-specific NFAT-GFP expressing CTLs. Upon MP-IVM, we observed that the myeloid stroma is organized as a rim surrounding the cancer, which CTLs cross on their way to the tumor parenchyma. In this myeloid-rich area, T cells stopped and activated NFAT, with similar kinetics but lower frequency as compared to the tumor nest. Therefore, before tumor invasion, CTLs can get activated during productive interactions with HA-cross-presenting myeloid cells, which strategically surround the cancer parenchyma and likely constitute an important area of immune instruction. We are currently investigating the motility and activation of other lymphocyte types within the myeloid rim, and which other signaling pathways are triggered there.

P7.01.10
Quantitative analysis of single cell distribution inside entire lymph nodes using laser sheet microscopy

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Peripheral lymph nodes (PLNs) and other lymphoid organs perform central functions as a structural interface between the innate and adaptive immune systems. The precise three-dimensional (3D) localization of antigen (Ag)-presenting cells and rare specific lymphocytes (approx. 1-10 cells/PLN) is central for the modeling of relevant molecular and cellular interactions during adaptive immune responses. Thus far, the overall internal organization of lymphoid organ microenvironments and cellular interactions have been mainly determined by two-dimensional tissue sectioning, whereby 3D information is lost, while rare events are difficult to detect.
Laser sheet microscopy, such as selective plane illumination microscopy (SPIM), is a fluorescence imaging technique suited for optical sectioning of whole biological samples at single cell resolution, yet it has not been applied in immunological research thus far. Here, we have used a customized SPIM setup to visualize low numbers of adoptively transferred immune cells and their spatial relation to vascular network structures in entire murine PLNs. Our findings show that SPIM has the resolution to reliably and reproducibly identify fluorescently labeled single cells within an entire, fixed PLN. Anatomical landmarks such as high endothelial venules can be easily stained with the use of fluorescently coupled monoclonal antibodies. Visual information about 3D localization of the cells, such as the distance between cells and anatomical landmarks, can be quantified with appropriate software. In summary, SPIM, by enabling tracking of very small amount of cells, opens the door to a detailed "optical footprinting" of initial events of adaptive immune response with physiologically low Ag-specific cell numbers.

P7.01.11
Imaging T-cell signal transduction by integrative and correlative nanoscopy

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Recent advances in microscopy overcome the optical diffraction limit. Super resolution (SR) optical microscopy or nanoscopy refers to stochastic and deterministic approaches of fluorescent object microimaging with resolution from 200 to 20nm. Stochastic techniques- photoactivated localization microscopy (PALM) or stochastic optical reconstruction microscopy (STORM)- allow to see single fluorescent molecules blinking randomly, leading to resolutions of 20-30 nm in heavily reconstructed images. Deterministic techniques group stimulated emission depletion (STED), ground state depletion (GSD), spatially structured illumination (SSIM) and conical diffraction (SRCD) microscopy; they allow to select few stable fluorescent probes with space resolution from 100 to 35nm by depleting the fluorescence of most others. Fast events can be imaged (sec/frame, msec/line, microsec/pixel) without much computation for the reconstruction of images and allow particle tracking (PT) or raster-image correlated spectroscopy (RICS). We assay molecule numbers, complex stochiometry and dissociation constant in living T-cells by fluorescence correlated spectroscopy (FCS) using labelled antibodies targeting proteins at the cell surface or reporter-fused proteins expressed by transfected T-cells.
We compare the performances and the specificities of these techniques to image cytokine-induced cytoskeleton dynamics and delineate molecular mechanism of signal transduction in fixed and living human primary T-cells. We explore their compatibility with TEM and FIB-SEM electronic microscopy techniques to correlate optical and electronic images in 2D and 3D-cell reconstructions and build up animated models to reconstruct and simulate the signalling machinery in the full T-cell view.

P7.01.12
Use of Nuclear Imaging in tracking of adoptive Treg therapy in transplantation: a pre-clinical model

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Regulatory T cells (Tregs) are key in controlling autoimmune diseases and limiting immune responses to foreign antigens. Tregs are being studied for adoptive transfer immunotherapy for various diseases such preventing transplant rejection. However, key questions such as where therapeutic Tregs go and how long they stay viable in patients remains unsolved. Here we are trying to answer these questions in a pre-clinical set up with the help of nuclear medicine imaging technology.
Imaging of the human sodium/iodide symporter (NIS) via Single Photon Emission Computed Tomography (SPECT) has been used to image various cell types in vivo. This study addresses whether SPECT/CT imaging can be used to visualise the migratory pattern of Tregs in vivo. Murine Tregs were transduced with a construct encoding for NIS.
NIS expressing Tregs were specifically radiolabelled in vitro with Technetium-99m pertechnetate (99mTcO4-) and exposure of these cells to radioactivity did not affect cell viability, phenotype or function. Moreover, we have demonstrated that this method of imaging can be utilised to image migration of Tregs with direct and indirect allo-specificity in a skin transplant model. Interestingly, Tregs that prolonged the transplant survival demonstrated a different pattern of migration compared to Tregs that were unable to prolong the transplant survival.
The data presented here suggests that SPECT/CT can be utilised in preclinical imaging studies of adoptively transferred Tregs without affecting Treg function and viability thereby allowing longitudinal studies within disease models. Moreover, this technology has also the potential to be applied to human Treg studies in future.

P7.01.13
Imaging innate immune response in non-human primates after DNA vaccination against HIV

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Innate immunity is widely considered to be critical in shaping the immune response and its manipulation may improve the design of vaccine strategies. We know that antigen presenting cells (APC) form the bridge between innate and adaptive immunity and they have important immunostimulatory and migratory activities. Thus, improving our understanding of APC behavior at the injection site during the first hours after vaccination is needed.
Here we used a model of DNA vaccination against HIV in non-human primates (NHP) to investigate the spatio-temporal dynamics of skin APC after vaccination with or without electroporation (EP) by *in vivo* fluorescence imaging. Using non-invasive *in vivo* fibered confocal microscopy, we demonstrated the epidermal APC mobilization at 48h after vaccination and their density reduction over time. Moreover, skin APCs were monitored continuously during 24h by confocal fast laser scanning microscopy on skin explants after DNA vaccination. We observed the epidermal APC departure and the analysis of cell velocity and displacement showed the higher activation state of skin APC after DNA vaccination with EP than without EP.
In vivo fluorescence imaging approaches allowed us to track skin APC on the vaccination site in their native environment in NHP. The knowledge about immune cell activation is essential for the optimization of DNA vaccines efficacy.

P7.01.14
Contrasting quiescent G0 phase with mitotic cell cycling in the mouse immune system

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Most lymphocytes are assumed to withdraw from and reenter the cell cycle repeatedly throughout their lifetime. We studied dynamic transition between quiescence and proliferation of lymphocytes using double-Tg mice (#639/#474) generated by crossing FucciG1-#639 and FucciS/G2/M-#474 mice, which expressed mKO2-hCdt1(30/120) and mAG-hGem(1/110) in the hematopoietic cells, respectively. Most cells in LNs, spleen and PP exhibited strong mKO2 signal but no mAG signal and were Ki-67-, which should represent the mKO2++/mAG- G0 cells. After stimulation of mKO2++/mAG- T and B cell, as mKO2++/mAG- cells decreased, both mKO+/mAG+ and mKO-/mAG+ cells increased transiently. Then, mKO2+/mAG- and mKO2++/mAG- cells increased again, indicating that mKO2++/mAG- and mKO2+/mAG- cells correspond to populations of cells in G0 and G1, and mKO2-/mAG-, mKO2+/mAG-, mKO2+/mAG+ and mKO2-/mAG+ cells correspond to cells in the early G1, G1, G1/S, S/G2/M cells, respectively. By intravital microscopy, intact LNs were mostly filled with cells with bright red nuclei. However, a considerable number of migrating cells with green and red nuclei were observed in the draining LN after immunization.
Cells in BM and thymus showed substantital heterogeneity of Fucci-signals. In both differentiating T cells in thymus and B cells in BM, the most immature cells had much more mKO2-/mAG- and mKO2-/mAG+ cells, however as maturation, these cells decreased and intensity of mKO2 increased close to its mature cell level (mKO2++), indicating Fucci-Tg mice visualize cell-cycle progression during differentiation.
In conclusion, we propose that FucciG1-#639 and/or FucciS/G2/M-#474-Tg mice can provide reliable readouts of the cell-cycle regulation of lymphocytes, both in vitro and in vivo.

P7.01.15
High Concentration Of Serum Soluble Fas In Patients With Head And Neck Carcinoma: A Comparative Study Before And After Surgical Removal Of Tumor

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Introduction and Aims: Alternative splicing of Fas transcript can produce a natural secreted isoform of this molecule. Some cancer cells also can make soluble Fas (sFas) which may have suppressive effects on anti-tumor response of the immune system. Elevated concentration of sFas has been detected in serum of patients with different malignancies. To correlate the sFas concentration in sera of head and neck cancer patients with disease indices.
Materials and Methods: Concentration of sFas in sera of patients with head and neck carcinoma, HNC (n=98) and healthy individuals (n=30) were measured by Sandwich ELISA (Bendermed Systems, Austria) and compared to values obtained 6 months after surgical removal of tumor (n=48). Data were correlated with different clinical findings of the patients.
Results: Serum sFas in sera of HNC patients were found to be significantly higher in patients with different tumor stages. Concentration of sFas was not correlated with age or tumor invasiveness however more sFas was found in sera of patients with higher grades of tumor. Surgical removal of tumor in patients resulted in substantial decrease in sFas concentration.
Conclusion: Initial raise in sFas concentration in sera of HNC patients and its consequent decrease could be regarded as a sign of suppressive mechanisms by tumors. Additional studies are needed to fully elucidate this mechanism however these finding may show the prospective use of such biomarkers of the immune system for prognosis of the disease and even immunotherapeutic applications.

P7.02 Systems and theoretical immunology

P7.02.01
Emergence of tissue-level NF-kappaB dynamics during inflammation

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Tissue level dynamics is a function of complex and nonlinear interactions between individual cells that may exhibit emergent properties. During inflammation, these interactions arise via a set of a cellular and molecular cytokine networks including cells of the immune system and key transcription factors such as Nuclear Factor kappa B (NF-kappaB). Pro- (including Tumor Necrosis Factor alpha) and anti-inflammatory cytokines may propagate and resolve inflammatory signals, but many of these cues are inherently noisy and dynamic. Using live-cell imaging and mathematical modeling we previously showed that in response to cytokine stimulation the NF-kappaB system undergoes nuclear-to-cytoplasmic oscillations, which constitutes a key positive cytokine feedback regulation (Science, 324:242, 2009, PNAS 107:11644, 2010).
Here, using systems biology approaches we develop a new quantitative understanding of how tissue-level dynamical phenotype emerges from individual cells during inflammation. We investigate the role of positive cytokine feedback in the control of the NF-kappaB response in single cells as well as multi-scale tissue mathematical models. These models are informed with time-lapse confocal microscopy measurements using RAW264.7 macrophages and mice embryonic fibroblast co-cultures. We hypothesize, that only appropriate control of cellular coupling and molecular noise allows avoiding out-of-control tissue-level inflammatory responses, characteristic to many diseases of the immune system.

P7.02.02
Cytokines prophyle in Gaucher disease type I patients with bone metabolic changes.

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Gaucher disease (GD) is caused by an inherited deficiency of glucocerebrosidase, a lysosomal enzyme responsible for the cleavage of glucosylceramide, a substrate accumulating mainly in macrophages. In addition to organomegaly and haematological abnormalities, type 1 (non-neuronopathic) disease is also characterised by insidiously progressive and often severe skeletal involvement. We hypothesized that cellular alteration in Gaucher disease produces a proinflammatory environement that might be connected to osteoclast activity.
Serum biochemical bone turnover markers and bone mineral density (BMD) were measured in 16 type 1 GD patients and in 50 age-matched controls in order to evaluate bone balance. Cytokines were determined in serum of same GD patients and controls.Our results indicate a marked decrease of bone markers in GD patients, compared to the unaffected controls. These low values were observed in all patients, irrespective of their age, indicating a failure of osteoblastic and osteoclastic function in GD. Additionally we found correlations between bone resorption markers and inflammatory citokines, especialy with IL-6, IL-1β, TNF_{α}. The findings suggest that cytokines play a role in osteoclast formation in Gaucher disease. In conclusion, our data suggests that along with concomitant glucosylceramide accumulation, generates a state of osteoclastogenesis mediated in part by pro-resorptive cytokines.

P7.02.03
Clostridium difficile infected human signaling pathways: An integrated model

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A theoretical model can explore a system of host-pathogen interactions and thus clinical treatment can be optimized to lower the microbial toxin burden in the host. The present method generates data on reaction fluxes in an integrated pathway of the host-pathogen interactions at quasi steady state. The objective function for minimizing toxin expression, with respect to some parameters, viz., concentration of signaling molecules, was formulated and a set of constraints was incorporated. We integrated the toxin expression regulatory pathway in Clostridium difficile, and apoptosis and mitogen-activated protein kinase (MAPK) pathways in an infected host (Homo sapiens). We applied the proposed method in three following cases: 1) integrated toxin expression regulatory pathway of C. difficile and apoptosis pathway of H. sapiens; 2) integrated toxin expression regulatory pathway of C. difficile and MAPK pathway of H. sapiens; and 3) integrated toxin expression regulatory pathway of C. difficile, and apoptosis and MAPK signaling pathways of H. sapiens. On minimization of the toxin expression, we observed that the signal flow values for most of the survival genes were at the higher side, whereas it was the reverse for most of the proapoptotic genes. We also observed increased signal flow values of the molecules in the host ERK pathway as compared to that of the molecules present in the host c-JNK/p38 pathway. In light of these observations, we hypothesize that lower toxin level in a pathogen leads to higher chance of host survival. We validated the results extensively using existing experimental observations.

P7.02.04
Systems Biology Approaches for Predicting and Controlling the Reactivity of Tumor Infiltrating Lymphocytes

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Tumor infiltrating lymphocytes (TIL) is a heterogeneous population with potential anti-tumor reactivity. TIL recognition and reactivity with autologous tumor is associated with increased secretion of IFN-γ. Despite the encouraging results obtained from the use of adoptive cell transfer, in stage IV metastatic melanoma patients, and despite extensive research efforts, little is known about the cellular interactions that determine TIL reactivity. In previous work, we demonstrated that by extensive phenotypic analysis of Selected-TILs combined with computational tools we generated a decision tree algorithm that accurately predicts the degree of TIL reactivity in terms of its subpopulation constituents. Our current studies are focused on understanding the molecular basis that enables the control and prediction of TILs. Analyzing pre-REP young-TILs, we found significant correlation between reactivity and non-reactivity, and subpopulation signatures. These signatures are distinctive. Importantly, we revealed that pre-REP Young and Selected TILs share similar signatures for reactivity vs. non-reactivity. Further analysis of Pre-REP vs. post-REP Young-TILs revealed the unexpected finding that they differ in the signatures for reactivity. Expansion of TILs induces alternations in subpopulation composition and thus, changes TIL reactivity and subpopulation signatures. We aim to determine the biological processes that govern TIL reactivity during the expansion and the relationships between the TIL subpopulations and functions using systems biology approaches. Our studies may shed light on the molecular mechanism that control TIL reactivity combined with controlling the long-term reactivity, and improve the clinical efficacy of TILs. It will also set the foundations to study other types of heterogeneous cell populations.

P7.02.05
Utilizing next-generation sequencing of the humoral repertoire for monoclonal antibody discovery

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Historically, monoclonal antibody (mAb) discovery has relied on high-throughput screening of hybridomas or recombinant surface display libraries. While these techniques have been incredibly successful they require extensive labor, resources, and time for discovery; furthermore, potentially valuable candidates may go undiscovered due to the constraints of the screening method chosen. Here, we present mAb discovery methods that rely on analysis of the in vivo humoral repertoire of antibodies in order to isolate clones of interest. These novel methods utilize next-generation DNA sequencing (NGS, Illumina miSeq system) and bioinformatic analysis of antibody variable region repertoires obtained from B cell populations of protein antigen immunized animals. Specific candidate antibody genes are identified based on bioinformatic and statistical analysis of repertoires, primarily by clonal frequencies and ranking. These antibodies are then recombinantly expressed for characterization by biochemical and biophysical binding assays (e.g., ELISA). While our methods bypass extensive screening steps, repertoire analysis by NGS can also be combined with surface display screening technologies to further optimize mAb discovery. Finally, by performing a deep analysis of the antibody repertoire following immunization, we are able to gain quantitative insight on the diversity of B cell responses.

P7.02.06
Therapeutic Potential of Inhibition of Complement Activity for Alleviating Degenerative Eye Diseases

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Vaccinia virus complement control protein (VCP) has structural similarity to complement control proteins (Kotwal and Moss Nature 335(6186):176-8,1988). VCP exerts control over the complement (C) system (Kotwal et al. Science 250(4982):827-30, 1990). During the past decades, VCP has been extensively used in preclinical inflammatory disease models to demonstrate that controlling C mediated inflammation has improved outcomes and potential for therapeutic treatment. Published reports suggest controlling inflammation using VCP has therapeutic potential in CNS diseases, eg. AD, as well as CVS diseases eg. atherosclerosis and autoimmune diseases, eg. rheumatoid arthritis. Age-related macular degeneration (AMD) is the leading cause of vision loss amongst the aging population in the western world. A defect in C regulation in the eye has been associated with AMD. Recently, the role of C regulation in degenerative diseases of the eye in 2 separate models was investigated. In one model, mice are anesthetized and their pupils dilated. Krypton red laser photocoagulation was used to generate multiple laser spots in each eye. VCP was injected in one eye and the other served as control. Choroidal neovascularization (CNV) was measured by analysis of confocal microscopy of the tissue and VCP was shown to be effective in preventing and/or treating CNV, a hallmark of wet AMD. In light-induced retinal degeneration in albino rats, a model for dry AMD, VCP showed protective potential as judged by the quality of cell layer integrity, the reduction of photoreceptor loss and by inhibiting C activity as measured by C3d deposition in the tissue.

P7.02.07
Hgshs

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Neurodegenerative diseases, such as Alzheimer's, are characterized by a progressive loss of neural function. With symptoms including dementia, movement difficulties and speech problems, these diseases are devastating and sometimes even fatal. Despite this, and the large economic burden that they represent, there is no known cure. Recently, however, research on neural progenitor cells (NPCs) has offered hope for treatment of these diseases.
NPCs are potent immunomodulatory agents, capable of promoting tissue repair in neurodegenerative disease. Conversely, microglia, the primary immune cells of the brain, are a major instigator of cell death in disease states. Interestingly, it has been shown that mesenchymal stem cells modulate the action of microglia, encouraging a therapeutic phenotype. Here, we investigate the interactions between NPCs and microglia, by observing the dynamics of a major neuroinflammatory mediator, the gene transcription factor Nuclear Factor kappaB (NF-κB).
NF-κB regulates the expression of a variety of vital inflammatory genes, in a cell-type and stimulus-specific manner. Its complex pattern of shuttling between the nucleus and cytoplasm determines gene expression profile and, therefore, contributes to cell fate during neuroinflammation.

P7.02.08
A new model for estimation of cell proliferation dynamics using Carboxyfluorescein succinimidyl ester (CFSE) data

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Lymphocyte proliferation, which follows antigenic stimulus resulting from an invading microorganism, is a hallmark of the adaptive

immune system. Studying the quantitative dynamics of lymphocyte proliferation is essential for a better understanding of immune responses. Since it was first described, serial dilution of the fluorescent dye carboxyfluorescein succinimidyl ester (CFSE) has become the almost universal method for the determination of cellular division histories. To obtain dynamic information from CFSE-labelled proliferating cell populations, the fluorescence intensity profiles need to be analysed. Individual peaks can be fit with predefined shapes, usually normal or log-normal distributions, so that cell numbers per generation can be estimated. Therefore, the ability to reliably estimate cell numbers per fluorescence peak depends on clear peak separations that is often not attained in experiments using blood-derived human lymphocytes.
Here, we have developed a mathematical model that predicts the population structure evolution of human CD4 and CD8 T cells from histograms of time-series measurements of CFSE-labelled, phytohaemagglutinin-stimulated peripheral blood mononuclear cells. It combines (i) the direct analysis of flow cytometry-derived CFSE histograms with (ii) a statistical evaluation of the variability in the histogram data and (iii) a cyton model-based description of cell fate decisions. This model enables to derive biologically meaningful proliferation parameters and to reconstruct the population generation structure for viable cells. As the model is not dependent on clear CFSE peak separations, it is potentially of broad utility for the detailed characterization of the immune cell dynamics from human PBMCs in studies of human infections or vaccinations.

P7.02.09
High-throughput antibody repertoire analysis in S. pneumoniae immunized mice

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Despite currently existing conjugate vaccines, Streptococcus pneumoniae remains a considerable threat for infants and immune-compromised individuals1. This is mainly due to the small coverage and the rapid evolution of bacterial serotypes, which evade current vaccines and antibiotic treatment. Here, we present a systems immunology approach to gain a quantitative molecular description of the humoral immune responses that develops against S. pneumonia.
We have previously shown that next-generation DNA sequencing and bioinformatics analysis of plasma cell antibody repertoires can be used to rapidly generate recombinant monoclonal antibodies against protein antigens2. Here, we apply a similar approach to gain a comprehensive picture of the antibody repertoires that develop in mice following exposure to the complex pathogen S. pneumoniae. Specifically, we isolate plasma cells from spleen and bone marrow, which is followed by RNA extraction and antibody variable region VL (light chain) and VH (heavy chain) library generation by PCR. Libraries are sequenced using the Illumina miSeq platform, which enables full-length reads of VL and VH regions. Bioinformatic analysis of variable regions will include ranking according to transcript frequencies, revealing which B cells have expanded the most after immunization. Next, we combine recombinant antibody expression with high-throughput screening to determine whether high-frequency antibodies are pathogen-specific.
In summary we are demonstrating a novel systems immunology approach to quantitatively profile humoral immune responses that develop following immunization with the complex pathogen S. pneumonia. This platform might develop into a valuable tool for rapid monoclonal antibody discovery and selection of new vaccine candidates.

P7.02.10
Development of a bioinformatic framework for the study of high-throughput antibody repertoire data

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High-throughput antibody repertoire analysis (HTRA), which combines next-generation DNA sequencing (NGS), bioinformatics, and statistical analysis, has become a powerful way to quantitatively address fundamental questions in immunology related to antibody repertoire diversity and evolution and as a biotechnology tool for antibody engineering. On both a genetic and protein structural level, the complementarity determining regions (especially CDR3) define antibody clonality and antigen specificity.
Therefore, we developed a modular and extensible program that identifies the various CDR and framework regions within antibody variable gene NGS data using species-specific germline data (openly available on the Immunogenetics Information System, IMGT). This program is compatible with paired-end Illumina miSeq results and processes 10^6 reads in <5 minutes. Using NGS data sets from a variety of B-cell populations and species, we show that this program is comparable or better than IMGT analysis (HighV-quest) in terms of quality control, allowed input size and speed. We have used the characteristic identifiers of antibody sequences to expand bioinformatic methods to study antibody evolution using phylogenetic trees. Additionally, taking advantage of the distributional depth of NGS data, we characterized the diversity of antibody repertoires using statistical measures which take into account both clonal diversity and abundance.
Our approach to HTRA implements an improved processing and a better understanding of antibody NGS data by providing repertoire-characterizing statistical measures both of which can be capitalized upon to gain greater insight into adaptive immune responses following vaccination and infection.

P7.02.11
Heterogenous differentiation patterns in individual CD8+ T lymphocytes

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Upon infection antigen-specific CD8+ T cell responses display a highly reproducible pattern of expansion and contraction that is generally assumed to reflect a uniform behavior of individual naïve T cells. To investigate this assumption we have tracked the progeny of individual CD8+ T cells by in vivo lineage tracing and demonstrate that individual T cells follow highly heterogeneous differentiation patterns. First, even for T cells bearing identical T cell receptors, clonal expansion and differentiation vary considerably. Second, T cell diversification upon primary antigen encounter leads to stable clonal dominance during recall infections. The observation that the fate of individual naïve T cells is highly discordant suggests a division of labor between the progeny of individual naïve T cells and demonstrates that reproducibility of CD8+ T cell responses is only achieved at the population level. These findings may have important implications for the rational design of vaccines to infectious diseases and cancer.

P7.02.12
Thorough characterization of TCR-pMHC binding free energy estimated by string model and Miyazawa-Jernigan matrix

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Estimation of TCR-pMHC binding free energy based on its structural information is an important issue to elucidate the mechanism of immunological self-image. Some strict methods to evaluate binding free energies have been reported, however, their computational costs were quite expensive. Chacraborty's group has proposed an estimating method of the binding energy based on string model and Miyazawa-Jernigan (M-J) matrix, and applied to explain the presence of super-control of AIDS (Košmrlj A. et al., PNAS 105:16671-16676, 2008, Nature 465:350-354). We generated 106 of random peptides as T cell receptor CDR3s, educated them with self-peptides selected from FANTOM library of RIKEN using position specific scoring matrix from MEME-suite, examined the reactivity against IEDB-registered epitopes, and found that no registered epitopes was recognized by

selected receptors. This estimating method predicts a larger educational epitope pool results in the increased number of hot spots for selected TCRs. However, we found that this apparent increase of recognition-specificity involved the convergence of selected TCRs to some common sequences. This paradoxical behavior of selection process derived from the physicochemical character of M-J matrix. Chakraborty's group has further proceeded their analysis and treated the thymic selection process as an extreme-value problem based on this evaluation method (Košmrlj A et al., Phys Rev Lett 103:68103, 2009, J Stat Phys 1-17, 2011). We also carried on our analysis and further characterized this evaluation method. (This work is a collaboration with Dr. Takuya Takahashi at Ritsumeikan Univ.)

P7.03 Structural biology in immunology

P7.03.01
Polyclonal T-cell Receptors recognise an unusually long tumour antigenic peptide complexed to HLA-B7

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We recently reported a novel immunodominant 13 mer epitope derived from cancer testis antigen NY-ESO-1. This peptide is restricted to HLA-B*0702 and is naturally presented by melanoma cells. Although it adopts an extensive bulge in the centre when complexed to HLA-B7, unlike other reported long peptides, the $CD8^+$ T cell response to this 13 mer is not only immunodominant but also involves broad TCR repertoire, at least 10 different Vβ families. We hypothesise that the polyclonal recognition of NY-ESO-1_{60-72}/HLA-B7 is probably a result of flexible arrangement of the bulged peptide upon being recognised by different TCRs. To date, four NY-ESO-1_{60-72}-specific $CD8^+$ T cell clones with different TCRs have been generated and functionally characterised in various *in vitro* assays. All individual full-length TCR alpha and beta chain sequences have been identified and cloned. Sequence analysis revealed that the TCRs bear different complementarity-determining region (CDR3) sequences. These TCRs have been shown to form functional surface receptor in TCR-negative human thymoma cell line, SKW-3 after retroviral transduction. High purity soluble recombinant TCRs and NY-ESO-1_{60-72}/HLA-B7 complex have been successfully produced. Gel filtration assay and Surface Plasmon Resonance analysis confirmed the interaction between purified recombinant TCRs and NY-ESO-1_{60-72}/HLA-B7 complex. Recombinant protein crystals have been successfully grown in CrystalMation screenings. High resolution X-ray diffraction dataset of 2.0 Å, 2.8 Å and 3.2 Å have been collected for three TCR-NY-ESO-1_{60-72}/HLA-B7 crystals. The knowledge acquired from this study will aid to the current understanding of the TCR-pMHC I interaction and may have important implications for future vaccine design.

P7.03.02
Use of The Alcoholic Extract of Alfalfa Root Instead of SLO for ASO Diagnostic Test

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INTRODUCTION: Alfalfa (Medicago sativa) is a flowering plant in the pea family Fabaceace. Alfalfa has been used as an herbal medicine for over years. Streptolysin O (SLO) is one of the several toxic immunogenic exoenzyme produced by group A β-hemolytic Streptococci. Anti-streptolysin O (ASO) is a routine test for the diagnosis and management of acute rheumatic fever and acute glomerulonephritis. Currently, recombinant Streptolysin O antigens are used for the ASO test. This study explored the application of this extract instead of SLO.
METHODS: Roots of extraction of Alfalfa was prepared using the maceration method. At frist we determained hemolysis effect of the extract on Human Red Blood Cell (HRBC). This extract was used instead of SLO antigen in neutralization test (Macro) .The effect of extract was evaluated on reaction between SLO latex and ASO in slide method (Agglutination). Nonetheless, the antigenic properties were determined by SRID test.
RESULT: The Alfalfa Extract exhibited considerable hemolysis activity on hRBC. The ASO inhibited hemolysis activity of the extract in a specific concentration. The extract inhibit ASO, agglutination test and showed precipitation by ASO on agarous gel.
CONCLUSION :There is significant similarity of antigenic property in both Alfalfa extract and SLO. Thus, purified extract, instead of SLO, can by used for the ASO test.

P7.03.03
Mapping the N- and O-glycome of mouse Peyer's patches

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The interaction between L-selectin on lymphocytes and carbohydrate, especially 6-sulfo sialyl Lewis X, cover on the surface of high endothelial venules mediates lymphocyte homing. However, this glycan epitope has no significant influence on lymphocytes homing to Peyer's patches. Thus, characterization of other sulfated glycan structures on Peyer's patches is critical to understand the contribution of carbohydrate to lymphocyte homing. Nevertheless, lack of antibodies could detect sulfated glycans. Therefore, total glycan are released from Peyer's patches tissue section, chemically derivatized by permethylatiton, and liquid chromatography mass spectrometric(LC-MS)-based glycomics methodologies are utilized to analyze the N- and O- linked glycan of mouse Peyer's patches. Carbohydrate carrying one or two sulfated group are identified on N- and O- linked glycan, and sulfated group are located at either galactose or N-acetylglucosamine. Moreover, the sulfated Sda like antigen is the major glycan of O-glycans on mouse Peyer's patches. Utilizing this method, we identify the glycan structure of lymphatic tissue which might interact with glycan-binding protein to cause lymphocytes homing to Peyer's patches, and might thus have critical function for innate immunity.

P7.03.04
Disintegrin-like molecule isolated from snake venom induces endothelial cell migration and inhibits adhesion on extracellular matrix components

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Jararhagin-C (jar-C), a disintegrin-like molecule which contains an ECD sequence (Glu; Cys; Asp), was isolated from Bothrops jararaca venom. The ECD sequence can competitively binds to cell membrane integrins to disrupt platelet-collagen interaction. As endothelial cells express different integrins receptors, they represent a potential target for interaction with ECD-disintegrins. These molecules are able to induce angiogenesis, melanoma cell adhesion and wound healing. This study aimed to investigate the effects of jar-C in the adhesion and migration of human vascular endothelial cells (HUVECs) on different extracellular matrix substrates. The cell migration assay was performed on a monolayer line scraped HUVECs grown on collagen I; IV and fibronectin. After 24 hours of jar-C treatment cells were stained and analyzed by optical microscope. The inhibition of cell adhesion was evaluated incubating HUVECs with jar-C and seeding them on well plates coated with collagen I, IV or fibronectin. HUVECs were washed post-seeding and the adherent cells were quantified by MTT method. Our results show that jar-C is associated with HUVEC migration on collagen I substrate rather than collagen IV or fibronectin. By the other side, the inhibition of cell adhesion was effective on collagen IV. Both the migration process and the adhesion are modulated by the expression of integrins by binding the matrix components. There are a number of integrins that bind to collagen and serve to anchor the cells to the basement membrane and

interstitial matrix. The jararhagin-C ability to bind to α2β1is probably the responsible by the effects observed here.
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P7.03.05
Pathological Characteristic of Experimental Colorectal Cancer Model in Three Rat Strains

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OBJECTIVE: In the present study, we found that the development of rectum-associated lymph nodules (RALN) in the experimental ulcerative colitis model induced by dextran sulfate sodium (DSS) is different in various kind of strain in rats. In order to investigate the mechanism, we examined the relationship between ulcerative colitis and rectum-associated lymph nodules in three kinds of strain [Wistar ($RT1^u$), DA ($RT1^a$) and Lewis ($RT1^l$)].
METHODS: (1) Animals: Specific pathogen-free (SPF) male rats of inbred strains Wistar ($RT1^u$), DA ($RT1^a$) and Lewis ($RT1^l$) were given drinking water containing 3 % dextran sulfate sodium (DSS). The local ethical committee approved the animal studies. (2) Tissue preparation. (3) Antibodies: Mar1 (anti macrophage specific), Mar3, ED1 (anti macrophage differention antigen specific), and ED2 (anti macrophage subset). (4) *Immunohistochemical staining. (5)* Isolation of rectum macrophages. (6) Flow cytometric analysis
RESULTS: The weak degree of ulcerative colitis was observed at 3 days in Wistar rats; at 11 days in DA and Lewis rats ($p<0.05$) after drinking water containing 3% DSS. The immuno-histochemical and Flow cytometric analysis found that the dome, lymph follicule formation and cellular components of RALN in DA and Lewis rats were different from Wistar rats during the early postnatal period and DSS administration.
CONCLUSIONS: These results suggest that there is close relationship between the pathological changes of ulcerative colitis and the development of RALN in the three strains of rats.
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P7.03.06
Ubiquitin ligase MARCH I involves peptide discrimination for MHC II complex

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We examined whether ubiquitin ligase MARCH I distinguish the structural fluctuation of MHC/peptide complexes. Ubiquitination of MHC II is necessary for intracellular transport from cell membrane to acidic endosomes and vice versa. However, functional properties of ubiquitination are still in question. We used dendritic cell from MARCH I KO mice to examine antigen presentation pathway and resulting MHC/peptide complex formation with peptides that bind to I-A^k with high and low affinities. In biophysical study, we observed various range of structural fluctuation of MHC/peptide complex; the degree of structural fluctuation was proportional to the sensitivity to SDS that break complex to α-chain and β-chain in acrylamide gel. We hypothesized that MARCH I detect the motion of MHC II. DC derived from wild mice showed clear elimination of low affinity peptide for binding to I-A^k, on the other hands, DC derived from MARCH I KO mice did not show such discrimination; comparable amount of MHC/peptide complex formed with both high and low affinity peptides. We next examined decay of the formed complexes; since low affinity peptide complex has shorter half-life than high affinity one with an intracellular mechanism. To our surprise, the half-life of MHC/peptide complex was almost same in MARCH I KO mice as wild type. Thus, accelerated decay of MHC/low affinity peptide should be mediated by other mechanism beside ubiqutination, or physiologically unknown ubiquitin ligases such as MARCH VIII or other families. We will discuss on MHC II sorting and DM catalysis as the function of ubiquitination.

P7.03.07
Structural analysis of the class II HLA molecule, DP5, in complex with the Cry j 1 antigen of Japanese cedar pollen

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Japanese cedar pollinosis is a type I allergy caused by Cryptomeria japonica (Cry j, Japanese cedar). Cry j 1, one of the major Japanese cedar pollen allergens, reacts with IgE antibodies in Japanese cedar pollinosis patients. We previously analyzed the association between Japanese cedar pollinosis and class II HLA alleles, and found that the frequency of HLA-DP5 is significantly higher in the patients. Furthermore, we identified an immunodominant peptide that induces Th2 restricted by HLA-DP5, among overlapping peptides spanning the entire length of Cry j 1. However, the specific mechanism of Cry j 1 recognition by HLA-DP5 remained unknown, due to the lack of structural information.
In the present study, we determined the crystal structures of HLA-DP5 (DPA*02022 and DPB1*0501) in complex with a Cry j 1-derived nine-residue peptide (KVTVAFNQF), at 2.4 angstrom resolution. The antigen-binding groove formed by the DP5α and DP5β chains captures the nine-residue epitope. The lysine at P1 is an important anchor residue that binds in the negatively charged pocket, and we confirmed that HLA-DP5 retains the Cry j 1 binding-activity in vitro by a structure-based mutant analysis. Furthermore, we identified the characteristic residues of HLA-DP5 that are responsible for the distinct properties of the groove, by comparing the structure of HLA-DP5 and the previously reported structures of HLA-DP2 (DPA1*0103 and DPB1*0201) in complex with pDRA. The comparison revealed that the HLA-DP5/Cry j 1 complex forms several hydrogen-bond/salt bridge networks between the receptor and the antigen that were not observed in the HLA-DP2/pDRA complex.

P7.03.08
Structures of the Pore-Forming Toxin Monalysin solved by X-ray crystallography and electron microscopy

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Pore Forming Toxins (PFT) represent the largest class of bacterial protein toxins and are often important virulence factors of a pathogen. These proteins are initially produced as soluble molecules and association with a target membrane, generally via specific receptors, induces their oligomerization in a ring-like structure. Subsequent structural rearrangements of each protomer lead to the formation of a water-filed pore. Depending on the PFT, pore formation is aimed at permeabilizing the target membrane or can serve as a translocation channel to introduce other virulence factors into the target cell. The structure of several PFT has been determined, but for only one the structures of both the soluble and the pore form are available. Nevertheless, the molecular mechanisms involved in the soluble to transmenbrane form transition of PFT are still unknown.
Monalysin was recently identified as a new virulence factor secreted by the Drosophila pathogen Pseudomonas entomophila. The studies we carried out highlighted that Monalysin is a PFT involved in the ability of the pathogen to induce intestinal cell damages into the host gut. Here we present the structures of pro-Monalysin, cleaved form and inactive mutant that have been solved by X-ray crystallography or electron microscopy. On the basis of these structures a possible mechanism of action for Monalysin is presented.

P7.03.09
Homology modeling of the extracellular region of the B cell protein CD19

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CD19 is a member of the immunoglobulin superfamily of proteins that is found on B cells from the earliest stages in the bone marrow until it is lost upon terminal differentiation to plasma cells. CD19 is known to have a role in signal transduction as part of a co-receptor complex containing CD21 (CR2) and CD81 (TAPA-1) that, together with the B cell antigen receptor (BCR), is involved in various pathways related to B cell development and activation. CD77, also known as Burkitt's lymphoma-associated antigen and globotriaosyl ceramide (Gb3), is a marker for germinal center stage B cells and a cellular receptor for Shiga toxin. Several studies have linked CD19 and CD77 functions and expression. Previously we identified a potential CD77-binding region on CD19 with amino acid sequence similarities to the CD77-binding Shiga toxin B-subunits. CD77 remains the only ligand for the extracellular region of CD19 described to date. In the current study, we present a structural model of the CD19 extracellular region based on homology modeling using SSEARCH, MODELLER, PROCHECK and ProSA. Shiga toxin B-subunit models were used as templates to construct the model of the proposed CD77 binding region, and immunoglobulin superfamily members and other proteins were used for additional areas of the CD19 extracellular region. The proposed CD77-binding site of this CD19 model is most similar to CD77-binding Site 1 of published Shiga toxin B-subunit structural models and contains many amino acids of the previously proposed binding region based solely on amino acid sequence similarity.

P7.03.10
Assessing changes expression and presentation of NKG2D under the influence of serum factors in breast cancer patients

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Introduction: Breast cancer is the most common cancer in women worldwide. Nk cells play an important role in tumor cells killing by NKG2D binding to the MICA proteins on tumor cells surface. accumulated soluble MICA from tumor cell surface in serum may lead to the down regulation of NKG2D expression that has been proposed to be a novel mechanism used by cancer cells to evade the immune system. In this study we assessed influence of sMICA on changes expression and presentation of NKG2D on NK cells from breast cancer patients.
Method:
In heparinised PB samples from 49 healthy and 49 breast cancer patients before surgery and chemotherapy, NKG2D expression and presentation were assessed by qRT-PCR and flow cytometry methods respectively and to determine the serum sMICA we used ELISA. The results of flow cytometry were analyzed with WinMDI software. Statistical analysis was performed with SPSS software.
Results:
In contrast to healthy volunteers, significant amounts of sMICA was detected in sera from majority of patients. The expression and presentation of NKG2D in patients was significantly lower compared to healthy individuals, with an inverse correlation to sMICA and direct correlation to tumor stage.
Conclusion:
Our study reveals that sMICA might act as an effective factor in diminishing NKG2D expression in breast cancer patients compared to healthy individuals, and sMICA can be candidate as target for elevate expression and presentation of NKG2D that directly contributing in tumor cell killing. More work is needed to determine other confounding factors in cancer patients.

P7.03.11
How T cells engage an EBV-associated cancer epitope

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Epstein-Barr virus (EBV) is a flourishing microparasite presently infecting over 90% of the human population. The virus is also a global disease burden and associated with 18 different malignancies. The latent EBV protein LMP2 is a key target of the cellular immune system since it encodes a number HLA-A*0201 (A2) restricted epitopes including FLY (FLYALALLL). FLY is a promising therapeutic and prophylactic target since it is expressed on the surface of most EBV^+ malignancies and exhibits complete sequence conservation across all known virus strains. How T cells engage this important epitope has been unknown. Here, we have solved the structure of the FLY-A2 molecule on its own and in complex with the SB7 T cell receptor (TCR) and have identified key areas of T cell contact. Understanding the molecular basis of this engagement may aid in optimizing T cell recognition as well as the design of intelligent FLY mimic compounds and super-agonists for use in the clinic.

P7.03.12
Human milk immunoglobulins contain various combinations of antigen-binding sites resulting in binding polyspecificity and catalytic polyreactivity

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In the classic paradigm, antibodies are considered as monospecific molecules that have stable structures and two or more identical antigen-binding sites. We have shown that IgG and sIgA of human milk eluted from several specific affinity sorbents under the conditions destroying strong immune complexes demonstrate high catalytic activities in hydrolysis of ATP, DNA, oligosaccharides and phosphorylation of proteins, lipids and oligosaccharides.
In preparations of human milk IgG and sIgA we have found chimeric κλ-IgGs and κλ-sIgAs, which contain both types of immunoglobulin light chains simultaneously. Chimeric κλ-IgGs were presented by all four subclasses (IgG1-IgG4). Moreover, adding of reduced glutathione and milk plasma containing no immunoglobulin to two IgG or sIgA fractions with different affinity for DNA-cellulose leads to antibodies transition from one fraction to the other. We explain this phenomenon by the possibility of HL-fragments exchange between different IgG, IgA and sIgA molecules, but not the exchange of only light or heavy chains.
Some chimeric molecules of sIgA may contain from two up to four HL-fragments to various antigens high affinity interacting with different sorbents and catalyzing various chemical reactions. Our data essentially expand the ideas explaining the phenomenon of polyspecificity and cross-reactivity of human milk antibodies. Further investigations will be focused on allocation of human milk factor that provides exchange of immunoglobulin structural components. This could lead to a method of producing bispecific antibodies.

P7.03.13
Synergistic inhibition of Her2 overexpressing tumor cells by a combination of anti Her2 monoclonal antibodies

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Her2 proto-*oncogene* is critical in the biology of breast cancer and an important therapeutic target by monoclonal antibodies (MAbs). Trastuzumab (Herceptin) is the first approved anti Her2 therapeutic MAb. We prepared a panel of mouse MAbs specific for different epitopes of Her2. The anti proliferative effect of these MAbs was investigated on Her2 *overexpressing* human breast cancer cell line BT474, using radioactive thymidine incorporation assay. Our results demonstrated that while two of the MAbs (1T0&2A8) inhibited cell proliferation dose dependently, similar to Trastuzumab, four MAbs

(1F2, 1B5, 1H9 and 4C7) augmented cell proliferation. Treatment of BT474 cells with different combinations of two MAbs induced either synergistic inhibitory or stimulatory effects. Combinations of these MAbs with significant synergistic inhibitory function may improve the *therapeutic* efficacy of Her2 specific MAbs.

P7.03.14
Nicotinic receptors in nervous and immune systems:identification and functional roles

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Nicotinic acetylcholine receptors (nAChR) are present in neuro-muscular junctions, in the brain and other tissues, including skin and the immune system cells. They are involved in normal functions ranging from muscle contraction and regulation of immune processes to cognition. nAChR malfunctioning , due to mutations, disturbances in the expression levels or to wrong interactions are associated with such autoimmune diseases as myasthenia gravis, as well as with different channelopathies, psychiatric and neurodegenerative diseases. Since nAChRs are composed of 5 identical or different subunits, the important task is identification of the individual receptor subtypes in norm and at pathologies. Due to high homology of nAChR subunits, antibodies cannot give an undoubted answer. More reliable are specific peptide and protein neurotoxins. Using Alexa-alpha-bungarotoxin, we developed a method for identification of alpha7 nAChR present both in the nervous and immune systems (J. Neurochem. 109, 1087 (2009)). We isolated or designed novel protein (J.Biol.Chem. 287, 2709(2012)) and peptide (Mar. Drugs, 9, 1698, (2011)) neurotoxins for these purposes. Snake toxins proved useful to identify neuronal-type nAChRs in immune cells (Int.J.Biochem.Cell Biol.43, 516(2011)) and to probe the involvement of alpha7 nAChRs in the Alzheimer's diseases (J. Alzheimers Dis., 25,747 (2011)) . Combination of radioactive and fluorescent derivatives of snake alpha-neurotoxins, together with mRNA analysis and application of appropriate knock-out animals, made possible identification of functionally active alpha7 nAChRs in outer membrane of mitochondria (PloSOne, 7, e31361(2012)) which might be of importance for clarifying the role of the latter in apoptosis.

P7.03.15
Rules that govern high TCR-CD3 cell surface expression

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TCR gene transfer is an attractive strategy, which enables rapid engineering of large numbers of T-cells with defined antigen-specificity. TCRs introduced via gene transfer have to compete for cell surface expression with the endogenous TCR, as well as with mixed TCR dimers. Our work has uncovered that introduced TCRs differ in their ability to compete for expression at the cell surface. Although the expression of several TCRs could be improved by codon optimization and cysteine modification, their cell surface expression was still disadvantaged in the presence of endogenous strong competitor TCRs. Therefore, TCR gene therapy might benefit from new insights into additional TCR-characteristics that govern efficient cell surface expression.
In this study, we isolated several virus-specific T-cell populations from different healthy individuals. Previously, we have shown that to some extent it can be predicted based on specificity whether virus-specific T-cells exhibit a weak or strong competitor phenotype. We hypothesized that virus-specific T-cell populations dominated by T-cells with a public TCR would be mainly of strong competitor TCR phenotype, since efficient TCR cell surface expression might benefit T-cell selection. Indeed, our results demonstrate that presence of a public TCR in some virus-specific populations coincides with a strong competitor phenotype. However, also some virus-specific T-cells with a weak competitor phenotype exhibited a dominant public TCR. These different public TCRs will be used to study the detailed interactions between the TCRα and -ß-chains as well as their capacity to efficiently capture CD3 subunits using both protein and cellular assays.

P7.05 Evolution of the immune system and comparative immunology

P7.05.01
Innate immunity of the Mytilus galloprovincialis mussel and environmental pollution: Study of some parameters and interactions

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The immune system of bivalves is based on innate defenses developing cell-mediated responses and on other humoral immune systems being able to resist in a polluted aquatic environment. The aim of this work is to evaluate the innate immunity at molecular level in mussels exposed to Cadmium and Chrysene (PAH). The study of the gene expression of some biomarkers such as Catalase and Superoxide Dismutase (SOD), which occur during phagocytosis in cell-mediated response, and MgBD3 (AMP) and Lysozyme (in humoral-mediated response), is of considerable interest to understand the phenomena of the ecotoxicological effects of the environmental chemical variations of these parameters. Mussels with an average length of 7 to 8 cm are exposed to different concentrations (25, 50 and 100 µg/L) of Cadmium and Chrysene in vivo for 24 hours and 7 days to quantify the gene expression levels with RT-PCR after removal of hemolymph and isolate total RNA from hemocytes. The results show that Cadmium and Chrysene modulate the expression of the studied genes in different ways depending on the type of response and contamination, and on the dose and duration of exposure. Indeed, in the case of MgBD3 and Lysozyme (humoral-mediated response) the response to Cadmium is expressed almost in the same way for both exposure durations with same contaminant, while in the case of exposure to Chrysene, Catalase and SOD (cell-mediated response) the effect differs with the exposure time for the same contaminant.

P7.05.02
The role of bidirectional activity of NWC promoter in the evolution of RAG transposon

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NWC is a third gene in RAG (recombination activating genes 1 and 2) locus. In contrast to RAG genes which entered the genome at some point of the deuterostome evolution as a result of a horizontal transfer of a mobile genetic element, NWC gene appears to be more conserved, as its orthologs can be found in the genomes of different groups of eumetazoan and parazoan animals. It was recently shown that many transposon-derived genes that are still functional in their host genomes are localized in the immediate vicinity of the 5' end of host genes that are controlled by bidirectional promoters. Such an arrangement enables integration of the transposon, ensures its expression and protects against host-mediated epigenetic gene silencing. We have shown that the murine NWC promoter exhibits bidirectional activity which results in the transcription of RAG-2 coding exon in non-lymphoid cells and that the promoter is activated by ZFP-143 transcription factor which binds to two sites within the promoter. Similar structure and localization of the putative NWC promoters was found in other vertebrates and ongoing investigation is aimed to verify the hypothesis that the bidirectional activity of NWC promoter is an evolutionarily conserved feature that enabled integration and survival of RAG transposon in the genome of the common ancestor of jawed vertebrates.

P7.05.03
Amyloidogenesis as protective response in invertebrates and vertebrates

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Foreign molecule contacts from bacteria (LPS), fungi (PMA), parasites, and chemicals provoke stress stimuli inducing protective responses from acquired and/or innate immunity. In the case of innate immunity any stress event sets off a mix of responses from immune and neuroimmune systems. Animals generally show fundamental biological principles revealing conserved regulation of the involved processes. This is also true for cellular stress responses, a complex and dynamic process of restoring cellular homeostasis characterized by the same specific stages. We have previously demonstrate that cellular stress conditions are able to promote in different animal models (invertebrates and vertebrates) the same massive morphological and physiological modifications Any kind of insult mimicking a stress condition (sundry chemical, immune, neuroendocrine and inflammatory) provokes, always and in any type of cell/tissue, detectable series of events that start with ROS over expression, ACTH/α-MSH activation, and cytokine such as IL-18 production. These general overexpressions sustain a massive amyloid fibril synthesis that provides a resistant scaffold in turn driving melanin deposition. But the amyloid production is not only linked to precursor melanin activation and synthesis. In fact it is basic to guaranty a state of cellular redox equilibrium due to regulation of ROS presence, *i.e.,* amyloid fibrils production could be considered as a basic cellular compensatory response endeavouring to attenuate oxidative stress in different cell types. Moreover, the relationship between amyloidogenesis and stressors allows to surmize a new background of information on the effects of stress.

P7.05.04
Innate immunity and evolution of antioxidant defenses: the methionine sulfoxide reductase genes in the ciliated protozoan Tetrahymena thermophila

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Accumulative post-translational modification to proteins, mediated by the action of reactive oxygen species (ROS), is thought to be one of the major causes of aging and age-related diseases. Mechanisms have been evolved to prevent or reverse these protein modifications. While most protein damage by ROS is irreversible, methionine oxidation to proteins can be reversed by the methionine sulfoxide reductase (Msr) system, which includes MsrA (that repairs methioninesulfoxide S-enantiomer) and MsrB (that repairs the methionine-sulfoxide R-enantiomer). The action of the Msr system contribute to cellular antioxidant resistance, resulting in life span extension of the organism. Moreover, many work demonstrated that overexpression of Msrs enhances the defenses against various diseases. In particular, methionine oxidations in both inhibitor of kappa B-alpha and Ca^{2+}/calmodulin-regulated phosphatase calcineurin may alter their functions and consequently affect transcription levels mediated by NFAT and NFκB, especially in T-lynphocytes of the immune system. With the aim to explore this problem we projected some experiments using the ciliated protozoan *Tetrahymena*, as model organism, characterizing the genes codifying for Msrs. Total RNA has been purified from *T. thermophila* cells cultured in PPYG medium and the cells were harvested after three days during exponential growth. Three genes potentially codifying for MsrA were cloned and the nucleotide and aminoacid sequences of all genes were compared with orthologous of other organisms belonging from Alveolata group and used for phylogenetic analyses. Time-course of gene expression was analyzed by RT-PCR in *T. thermophila* cells grown in normal condition and after pro-oxidant exposure. (M.I.U.R. grant)

P7.05.05
Evolution of the complement system: ancient molecules and new evidences from tunicates

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The complement system is a key player in innate immunity but, recently, it is becoming even more evident that complement plays also important roles in adaptive immunity.
Components of the human complement system possess unique domain structures and are classified in protein families: C3, factor B (Bf), mannan-binding protein-associated serine protease (MASP), C6 and factor I (If) family.
These complement families probably derive from exon shuffling, which created the unique domain structures of each family, and gene duplication and subsequent functional divergence, which increased the number of members in each family. Accumulating information on the complement system of vertebrates indicates that these gene duplications, which played a pivotal role in establishing the classical and the lytic pathway, occurred in jawed vertebrates. In contrast, information on complement genes of invertebrate chordates is limited and, so far, only the ascidians, such as Ciona intestinalis and Halocinthia roretzi, and the cephalochordate Branchiostoma floridae have an almost complete set of the complement gene families: C3-, Bf-, MASP-, and C6-like genes.
In the present work we demonstrate the presence of C6-, C3-, MASP-, MBL- and Bf-like genes in the colonial ascidian Botryllus schlosseri and the enhanced transcription after zymosan infection, which indicates their involvement in the ascidian immunity. The observation of a transcribed C6 gene suggest that the presence of the lytic pathway predates the appearance of the vertebrates. We are now carrying out new investigation to demonstrate a complement-related lytic activity in B. schlosseri.
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P7.05.06
The determination of antibodies against human growth hormone by use electrochemical methods combined with surface plasmon resonance and the application for the analysis in human serum

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Optical and electrochemical methods registering antigen-antibody binding's events are widely used in clinical examinations, biochemical analyses, in other areas such as environmental pollutants and food quality control. Direct immunoassay of human growth hormone (hGH) using simple to use, rapid and cost-effective electrochemical techniques have become an important topic in bio- and immuno-analysis.
This work reports the attempt for the application of optical and electrochemical techniques on the same chip detecting interactions of the specific anti-hGH antibodies with hGH immobilized on the surface plasmon resonance (SPR)-chip surface. A novel SPR immunoassay with atomic force microscopy and electrochemical detection for the determination of hGH has been developed.
Pulsed amperometric (PA), cyclic voltammetric (CV), square wave voltammetric (SWV) and differential pulse voltammetric (DPV) were used for the detection of anti-hGH. Higher sensitivity of created immunosensor was observed using PA, CV: analytical signal registered using PA method was 2.50 times higher if compare with CV, 16.3 times higher - with SWV and 24.5 times higher - with DPV methods. Similar reproducibility of the analytical signal (0.13) was characterised for SPR, PA and CV detection. In the case of PA method the limit of detection was 75 nM, in the case of CV method - 108 nM. The electrochemical signal dependences at wide analyte concentrations (230 - 834 nM). This work demonstrates successful simultaneous exploitation of several techniques to detect the anti-hGH antibodies using direct immunoassay format on the same area of SPR-chip. Sensitive PA was chosen for the determination of anti-hGH in human serum.

P7.05.07
Characterization of the IL-17 and IL-17 receptor families in lampreys

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Interleukin-17 family members have been identified in lampreys. These jawless vertebrates have an alternative adaptive immune system wherein variable lymphocyte receptors (VLRs) composed of leucine-rich-repeats are used for antigen recognition. Nevertheless, jawless vertebrates have T- and B-like lymphocytes that express VLRA and VLRB respectively. Prior studies in our laboratory suggested that *IL-17* transcripts (*IL-17D* orthologue) are expressed preferentially by $VLRA^+$ lymphocytes and at higher levels after activation. Conversely, $VLRB^+$ lymphocytes express *IL-17 receptor* transcripts (*IL-17RA* orthologue named *IL-17RA1*). Because lampreys represent an early phylogenetic juncture between innate and adaptive immunity, we sought to characterize the IL-17 family members and their cognate receptors in order to examine their potential roles in coordinating $VLRA^+$ and $VLRB^+$ cellular interactions. Nested PCR was used to obtain full-length cDNAs of the lamprey *IL-17D* (591-bp open reading frame) and three *IL-17R* family members with *IL-17RA* homology (*IL-17RA1*, *IL-17RA2* and *IL-17RA3*). Quantitative RT-PCR analysis indicated that the lamprey *IL-17D* orthologue is expressed highly in skin, gill and $VLRA^+$ lymphocytes, while lamprey *IL-17RA1* is expressed highly in hematopoietic tissue typhlosole, $VLRB^+$ lymphocytes and monocytes. A recombinant IL-17D-Fc fusion protein was produced and shown to bind to both monocytes and $VLRB^+$ lymphocytes, and not to neutrophils or $VLRA^+$ lymphocytes. The lamprey IL-17RA1 recombinant protein is directly associated with the IL-17D-Fc protein. These findings are consistent with the hypothesis that IL-17D produced by $VLRA^+$ lymphocytes may interact with a cognate IL-17R on $VLRB^+$ lymphocytes and monocytes to facilitate immune responses in lampreys.

P7.05.08
Genomic analysis in inbred mice provides insights into the evolutionary history of NKG2D ligand genes

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NKG2D ligands (NKG2DLs), represented by MIC and ULBP in humans and RAET1, H60 and MULT1 in mice, are MHC class I-like molecules, the expression of which is induced by cellular stress such as transformation and infections. Engagement of NKG2DLs with NKG2D, a major activating receptor expressed on NK cells, enables NK cells to kill transformed cells and infected cells. We previously showed that the ancestors of the MIC, ULBP/RAET1/H60/MULT1, and MILL families emerged by tandem duplication within the MHC region in a common ancestor of marsupial and placental mammals (*Immunogenetics*, 2010). In the present study, we compared the genomic organization of the NKG2DL-encoding region and its adjacent region in inbred mice. This comparison showed that laboratory strains fall into two major groups with disparate repertoires of NKG2DL genes. Mouse strains with inactivated H60a ligand generally have two NKG2DL genes of the *Raet1* family, *Raet1d* and *Raet1e*, whereas those with functional H60a ligand have three *Raet1* genes, *Raet1a*, *Raet1b*, and *Raet1g.* These results suggest that some *Raet1* genes are likely allelic. Our present work revealed the complex evolutionary history of the genomic region encoding NKG2DLs.

P7.05.09
Cranial base (CB)/brain and immune system of the fetus and neonate in concert in evolutionary development

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Introduction: Human CB/brain growth follows same developmental pattern as immune response maturation, 1st postnatal year; these systems' rapid extensive intra/extra uterine growth abounds despite somatic growth slowing, 4.4 months gestation. Methods: This paper explores this intertwined relationship of CB/brain and immune systems for evolutionary and ontological significance.
Results/Discussion: Human CB studies show fetal 2nd trimester anterior CB begins faster elongation rate, 1.6mm/week vs 0.9mm/week, $p<0.001$, matching midline-extension brain enlargement. A significant phylogenetic identity shift, early fusion of intrinsically controlled mid sphenoid synchondrosis, an elongation enhancement, includes crista galli/olfactory receptors. Unexpected link between immune/olfactory systems by major histocompatibility complex (MHC) transmits information about MHC genotypes/influences behavior/reinforces *new* human identity. 1st year postnatal life, human immune response maturation and CB/brain growth follow same developmental pattern. *Yet*, conserved genome plasticity of immune response is 500 million years old, when basic antigen presentation strategy by MHC polymorphism was established. Conserved gene plasticity for neo-cortex development is 57 years young; genes *RFPL1,2,3* duplication/cluster arrangement began neural expression for size/organizational changes. Life History Theory favors trade-offs for privileged CB/brain/immune systems, investment associated with increased society longevity, ongoing 125K years. Embryonic gene assembly confers vulnerability/responsiveness to environment the immature brain relies on, establishing/guiding pathways/connections. Fetal-capacity immune responsiveness begins development 8 weeks gestation; driven by environmental pathogens, infancy growth investment is then maximized by rapid development of individual specific immune repertoire, offering phylogenetically the *most* antigen binding specificities. Conclusion: Evolutionary features as central design provide insight for understanding preeminent relationships of ontological significance for CB/brain and immune systems.

P7.05.10
A Research Coordination Network in Ecological Immunology

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Ecological immunology, or eco-immunology, is one of the most rapidly growing fields of evolutionary biology. In 2010, the US National Science Foundation funded a Research Coordination Network to foster its conceptual and technical development. Since funding, the RCNE has flourished including: the spawning of several collaborations; the convening of two topical workshops in the USA (Florida and Michigan) and two in Europe (Scotland and Germany); the establishment of an email list-serv; extensive support of trainee research exchanges; and the production of a website. The group's efforts have also been influenced the Society of Integrative and Comparative Biology (SICB) to vote soon to found a new Division (Ecoimmunology and Disease Ecology). We present this poster to increase the visibility and membership of the RCNE and thus broaden and deepen immunology, from molecular to landscape scales.

P7.05.11

Functional analyses of lymphocytes and granulocytes isolated from the thymus, spiral valve intestine, spleen, and kidney of juvenile Australian lungfish, Neoceratodus forsteri

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The Australian lungfish Neoceratodus forsteri is an evolutionary important species, data suggesting that it's the closest living relative to the tetrapods. Our current understanding of the lungfish immune system is limited. This study characterizes the immune cells separated from primary and secondary immune organs of the Australian lungfish, N. forsteri. Flow cytometry was used to conduct functional studies on immune cells extracted from the thymus, spiral valve intestine, spleen, and kidney. The different characteristics of lymphocytes and granulocytes were analyzed by utilization of viability, phagocytosis, oxidative burst, and apoptosis assays. The profile of N. forsteri thymocytes resembles those from the thymus of high vertebrates. Notably, most of the nonviable intestinal immune cells were lymphocytes. Depending on the organ, 6-25% of the total population, predominantly granulocytes from organs other than the intestine, underwent phagocytosis where the splenic cells were the most and intestinal cells the least phagocytic cells. Cells responded positively but differently to stimulation with phorbol myristate acetate (PMA) to produce radical oxygen species, an indication of their oxidative burst activity, which was mainly associated with granulocytes. Although cells were induced by dexamethasone to undergo apoptosis, such an induction did not follow a consistent pattern of dose of dexamethasone or incubation time between the different organs. In the absence of antibodies directed against lungfish immune cells, these functional flow cytometric analyses aid our understanding on the functionality of immune cells.

P7.05.12

The introduction of monoclonal antibody Institute: the region's largest student center

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The monoclonal antibody research center was first established by Tabriz University of Medical Sciences in 2011 to provide service and research. This research center is meant to concentrate and coordinate research and production of antibodies against human and animal cells. Production of synthetic and semisynthetic monoclonal antibodies needed for research centers and companies, production of laboratorial kits for diagnostic and therapeutic purposes have played an important role in approaching Iran's national research goals.
In a short period of time, this center has produced many antibodies such as antibody against sperm surface antigen, anti-ferritin, PSA, BSA, Inhibin, different cancerous peptides and more than 68 polyclonal, enzyme-conjugated and fluorescent-conjugated products. In this research center polyclonal and monoclonal antibodies can be produced and purified. One of the other purposes of this research center is educating Iranian researchers, sharing technologies with other centers and holding research workshops. This research center is a member of European biotechnology network, so in order to cooperate with this network, besides many productive research projects, this center has held practical and theoretical workshops about monoclonal and polyclonal antibodies.

P7.05.13

The cattle germline IGHV repertoire is very limited

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Ruminants are thought to have a small germline immunoglobulin gene repertoire, but the IGHV genes have been poorly known. We characterized the bovine IGHV repertoire based on the genome assemblies, raw sequencing data and targeted sequencing of several animals.
In the genomic data, 36 IGHV genes were identified and assigned to 3 subgroups. Fetal IGHV cDNA sequences, containing less somatic mutations than in adults, aligned to these genes with 99.6% average identity. Only 10 of the genes appear functional, all in subgroup IGHV1, with identical FR and CDR lengths and canonical CDR structures. Targeted sequencing yielded 17-20 different sequences homologous to the functional genes per animal (including allelic variants), indicating that the bovine genome contains 10-20 paralogous IGHV loci. Thus, the germline IGHV repertoire is very small.
The evolution of the IGHV repertoire appears highly dynamic within Cetartiodactyla. The bovine IGHV genes belong to clans I and II; no clan III genes were found even by clan-specific PCR. Of related species, sheep and elk were negative for clan III, but deer, pig and dolphin were positive. The functional subgroup in cattle was likely generated by recent duplication events. It is homologous to the murine IGHV2 (Q52), but lacks a clear human homolog.
The complex IGH locus is incorrectly assembled in current genome versions. Most IGHV genes were found in UMD_3.1 but missing from Btau_4.2. In UMD_3.1, they were distributed in BTA21 and BTA7. FISH analysis confirmed the locus on BTA21 but suggests that the BTA7 locus is an assembly error.

P7.05.14

Risk Factors Associated with the Acquisition of HIV/STIs in Female Sex Workers (FSWs) in Lagos Nigeria

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This study assessed the risk factors that influence the acquisition of STIs/HIV infections in Nigerian FSWs. ***Methods*****:** 450 FSWs aged (15 – 35 years) were randomly selected in 3 local government areas in Lagos, Nigeria. A pre- and post-test counselling was conducted after a written informed consent was obtained. Participants provided demographic data, information on sexual behaviour, contraceptives use, number of new sexual partners within 3 months and vaginal douching. Cervical and high vaginal swabs were screened for the presence of STIs using standard microbiological methods. Quick view Chlamydia test kit was used for detection of Chlamydia antigen on cervical swabs. Blood samples were screened for HIV antibodies using ELIZA kits and Western blot tests, syphilis antibodies using RPR and TPHA kits. ***Results*****:** HIV infection (36.0%) and (31.7%) was seen in those involved in anal and oral sexual practices respectively. HIV infection in those who douched was high (32.3%) and was low (10.0%) in those who do not. HIV (21.8%) was prevalent in FSWs who had 20-30 new sex partners within 3 months. ***Conclusion:*** Anal, oral, douching, increased number of new sex partners and improper use of condom have been identified as risk factors to HIV/STIs acquisition in FSWs**.** Information on the need to access health programmes for early treatment of STIs/HIV, counseling on sexual practices, vaginal douching/proper use of condoms in order to maximize benefits should be made available, regular surveys on these risk factors would help in developing new strategies for control/ prevention of STIs/HIV diseases in FSWs.

P7.05.15
Boosting heterosubtypic neutralization antibodies in recipients of the 2009 pandemic H1N1 influenza vaccine

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BACKGROUND: A mass vaccination has been implemented to prevent the spread of 2009 pandemic influenza virus in China. Highly limited information is available on whether this vaccine induces cross-reactive neutralization antibodies against other subtypes of influenza viruses.
METHODS: We employed pseudovirus-based assays to analyze heterosubtypic neutralization responses in serum samples of 23 recipients of 2009 pandemic influenza vaccine.
RESULTS: One dose of pandemic vaccine not only stimulated effective neutralizing antibodies against cognate influenza virus 2009 influenza A (H1N1), but also raised broad cross-reactive neutralizing activities against seasonal H3N2 and highly pathogenic avian influenza virus H5N1 with lesser activity to H2N2. The cross-reactive neutralizing activities were completely abolished by IgG removal. In contrast, H1N1 vaccination alone in influenza-naive mice elicited only vigorous homologous but not cross-reactive neutralizing activities.
CONCLUSIONS: Our data suggest that the cross-reactive neutralizing epitopes do exist in this vaccine and could elicit significant cross-reactive neutralizing IgG antibodies in the presence of preexisting responses. The exposure to H1N1 vaccine is likely to modify the hierarchical order of preexisting immune responses to influenza viruses. These findings provide insights into the evolution of human immunity to influenza viruses after experiencing multiple influenza virus infections and vaccinations.

P7.05.16
R4 regulators of G-protein signaling share a common ancestry with the MHC paralogons

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Regulators of G Protein Signaling (RGS) modulate a multitude of biological processes by inhibiting GPCR-mediated signaling pathways. The smallest RGS proteins, members of the R4 subfamily, are responsible for the regulation of immunological processes such as chemotaxis. In many vertebrates, genes encoding the R4 RGS proteins such as RGS1 and RGS16 are located clustered together in the same genomic regions. In this study we show that the genomic context of these genes constitutes a synteny group well conserved across tetrapods. Our findings indicate that the RGS1/RGS16 synteny group can be linked to the MHC paralogons and has been likely coevolving with them. Indeed, RGS1/RGS16 region genes can often be found on the same scaffolds as the hallmark MHC markers in the cephalochordate Branchiostoma floridae, a species with well-conserved ancestral configuration. Moreover, we show that genes of this region have immune function not only in mammals, but also in an evolutionarily distant animal - the amphibian Xenopus tropicalis.

P7.05.17
Comparative study on primary structure and function of rhesus monkey and human granulocyte-macrophage colony stimulating factor

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Recent studies revealed the pivotal roles of granulocyte-macrophage colony stimulating factor (GMCSF) in inflammation and arthritis, suggesting GMCSF-neutralizing agents might be developed as novel therapeutic approaches for these immune disorders. Since the rhesus monkeys *Macaca mullata* are usually used as animal models for pathogenesis and drug discovery for human disease, it is needed to compare the human GMCSF (hGMCSF) and *M. mulatta* GMCSF (mmGMCSF). Here, a 432 bp transcript encoding mmGMCSF was amplified by RT-PCR. The predicted mmGMCSF contains 144 amino acid residues with approximately 96% identity to hGMCSF. The mature mmGMCSF differs from hGMCSF at 6 amino acid residues. MmGMCSF was expressed in freshly isolated PBMCs of monkey at low level. In contrast, Con A- and allogenic antigen-stimulation significantly increased the accumulation of mmGMCSF in the media of PBMCs. Moreover, the amount of secreted mmGMCSF was consistent with the proliferation of stimulated PBMCs. These results suggested that mmGMCSF was predominantly produced by activated T cells. The recombinant mmGMCSF produced by *E. coli* is comparable to hGMCSF in supporting TF-1 cell growth. In the presence of human interleukin 4, mmGMCSF induced the progenitor cells in monkey PBMCs to differentiate into DCs. The hGMCSF receptor protein and the neutralizing anti-hGMCSF antibody reduced the activity of mmGMCSF in supporting TF-1 cells survival. These results suggest that the recombinant mmGMCSF might be a useful tool for *in vitro* evaluation of novel hGMCSF-neutralizing agents prior to *in vivo* evaluation in the rhesus monkey model.

P7.05.18
The Drosophila immune response to Invertebrate Iridescent Virus 6

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Although insects lack an adaptive immune response, they mount a robust innate response against many pathogens. The *Drosophila* immune response against bacteria and fungi is largely NF-κB mediated and RNA viruses initiate a strong small interfering (si)RNA response. However, very little is known regarding the response to DNA viruses. To determine which pathways respond to DNA virus infection in *Drosophila*, we have utilized Invertebrate Iridescent Virus 6 (IIV-6), to identify pathways required to protect against infection with this DNA virus. IIV-6 is an large, dsDNA virus with a nucleo-cytoplasmic replication cycle. Infected wild-type flies present an iridescent blue color and abdominal swelling in later stages of infection, before succumbing approximately thirty days post-infection. We have revealed roles for several different signaling pathways, notably the Piwi-interacting RNA (piRNA) pathway, in protection from this virus.
Canonically, the piRNA pathway functions in the germline to protect the genome from transposons, mobile genetic elements that can cause mutations and double strand breaks if uncontrolled. piRNAs are transcribed from specific genomic loci, processed, and loaded onto Piwi proteins to form an RNA-induced Silencing Complex, targeting complimentary transcripts. Additionally, these piRNAs can be amplified through a secondary pathway: the ping-pong amplification loop. We have evidence that the secondary piRNA pathway, consisting of Aubergine and AGO3, is strongly protective against IIV-6. Given the similarities between transposons and viruses, it is intriguing that this pathway is operating in an anti-viral manner against a DNA virus.

P7.07 Veterinary immunology

P7.07.01
Innate and acquired immunity and apoptosis of lymphocytes and granulocytes in rabbits experimentally infected with RHDV

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RHDV is a factor causing viral haemorrhagic disease, being a appropriate model for hemorrhagic fevers and hepatitis in humans. This paper presents an immunological picture in 30rabbits (10infected with RHDV strain-237/04, 10 infected with an antigenic variant

RHDVa-72-V/2003 and 10control) infected with RHDV. The blood was sampled from the marginal ear vein at 0 ,8, 12, 24h p.i.
The innate immunological picture was determined by the ability of adherence, absorption (absorption index, % absorbing cells), NBT test (spontaneous, stimulated, spectrophotometric, stimulation index), myeloperoxidase activity in PMN cells, the concentration and activity of lysozyme in the serum and the percentage of T-cells with receptor CD8+, while acquired immunity was determined by percentage of monostained T-cells with receptor CD5+, CD4+ and CD25+, and of B-cells with receptor CD19+. The apoptosis of lymphocytes and granulocytes was determined by the total caspase 1-9 activity.
Studies have shown that the strain 237/04 of RHDV in comparison to strain 72-V/2003-an antigenic variant (RHDVa), provides more than 40% changes in studied parameters. Those changes with the RHDV strain were recorded as increases and decreases in the 8, 12, 24h of study, and changes with RHDVa, were registered mostly as decreases in 12, 24h and rarely in 8h (CD8+). The apoptosis of lymphocytes and granulocytes in case of RHDV incorporated at 8, 12, 24h, and in case of RHDVa at 12, 24h.

P7.07.02
Tumoral necrosis factor alpha (TNF-alpha) and interleukine 1 alpha (IL-1 alpha) expression in intestinal mucosa of young alpacas (vicugna pacos) with histological diagnostic of necrotic enteritis

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The aim of this study was to determine mRNA levels of pro-inflammatory cytokines TNF-α and IL-1α by real time RT-PCR in intestinal mucosa of neonatal alpacas with histological diagnosis of necrotic enteritis (n = 27). Relative quantification was performed with the 2 Delta Delta Ct method ($2^{-\Delta\Delta Ct}$), using 5 young healthy alpacas as control samples and 3 newborn alpacas as the calibrator sample. Total mRNA was extracted from each sample and subsequently real time RT-PCR was performed. It was determined that sick animals express more TNF α (13.58 times) than healthy (12.2 times) when compared with the calibrator; likewise IL-1α expression was higher in animals with necrotic enteritis (21.83) than in healthy animals (17.5 times) when compared with the calibrator. The expression levels of both cytokines in the intestinal mucosa suggest constant confrontation to proinflammatory agents in young alpacas, there being a slight increase in proinflammatory cytokines expression in animals with necrotic enteritis.

P7.07.03
Dynamics of T and B cells in lymphoid organs during the experimental infection of specific-pathogen free (SPF) layers with Fowl adenovirus (FAdV) serotype 4

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Fowl adenovirus (FAdV)-serotype 4 is involved in the etiology of inclusion body hepatitis, a disease that causes significant economic losses for the poultry industry. The aim of this study was to evaluate the dynamic changes that occur in the subsets of $CD3^+$, $CD4^+$, $CD8^+$ and B-lymphocytes among lymphoid organs after experimental infection with FadV-4.
Thirty-five SPF layers of 12-week-old were split into two groups. Group 1 (n=19) was infected with $6.25x10^9$ copies of FadV-4 while group 2 (n=16) remained uninfected (control). The percentages of $CD3^+$, $CD4^+$, $CD8^+$ and B-lymphocytes in spleen, thymus, bursa and peripheral blood were evaluated by flow cytometry at 2, 4 and 7 days post-infection (dpi). At 4 dpi, a significant decrease of $CD8^+$ T cells (median=20.18%) in spleen, B cells (median=68.39%) in bursa, and $CD8^+$ T cells (median=12.48%) and B cells (median=2.54%) in peripheral blood was observed in the infected group compared to $CD8^+$ T cells (median=27.44%, $p<0.05$) in spleen, B cells (median=96.96%, $p<0.05$) in bursa and $CD8^+$ T cells (median=18.91%, $p<0.05$) and B cells (median=10.64%, $p<0.05$) in peripheral blood from the control group. In addition, $CD45^{hi}$ population in thymus was significantly higher in the infected group (median=89.64%) than in the control group (median=66.58%, $p<0.05$). By 7 dpi, no differences in the T-cell subsets and B cells were observed between the different groups.
FadV-4 causes a rapid decay of $CD8^+$ T and B cells among lymphoid organs. Our results suggest that the thymus can be related to the T cell repopulation and immune reconstitution after disease.

P7.07.04
Immunological assessment of Aleuria Aurantia Lectin

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Background: We have previously shown, that Aleuria Aurantia Lectin (AAL) microparticles target murine peyer's patches in the gastrointestinal tract and can modulate an ongoing Th2-dominated response towards Th1. In this study, we further wanted to assess stability and binding of AAL to canine tonsils and intestinal sites. Moreover, the immunological properties of AAL were investigated.
Methods: AAL was incubated with Pepsin or Trypsin and stopped after different time-points, subsequently stability was assessed by SDS-PAGE following Coomassie or Silver staining. NfkB/AP1 activation upon AAL-addition was determined in human THP1 monocytic cell line, transfected with an NfkB/AP1 alkaline phosphatase reporter and quantification with Quanti-Blue. AAL-binding of paraffin-embedded canine tonsils and small intestine was analysed immunhistochemically.
Results: AAL alone was sensitive to Pepsin-digestion, when incubated longer than 15 minutes, whereas trypsin was not able to digest AAL at all. Furthermore, a modest, but concentration-depent increase in NfkB-activation was observed by adding AAL to THP1-cells. In dogs AAL-binding was predominantly observed on the apical side of epithelial cells lining canine tonsils and intestine.
Conclusion: AAL is considerably resistant to gastric digestion, is able to activate NfKb and binds to epithelial cells of tonsils, peyer's patches and intestinal villis, hence, making it suitable as an oral delivery system in canine patients.

P7.07.05
Immunodiagnosis of porcine cysticercosis: Identification of accurate antigens through immunoproteomics

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Background: Taenia solium cysticercosis is an infection mostly endemic of developing countries in Latin America, Africa and South East Asia that affect humans and pigs. Prevalence of infection in pigs, the intermediate host for T. solium, has been used as a monitor of disease transmission in endemic areas, however, accurate and specific diagnostic tools for porcine cysticercosis remain a major goal. Aim: To identify specific antigens that can diagnose with high efficiency and specificity porcine cysticercosis. Methods: Sera from well characterized pigs, including infected and non-infected animals and infected with other common pig infections, were used to select antigens by immunoproteomics that were exclusively recognized by antibodies present in the sera from cysticercotic pigs. Results: Seven antigens well characterized using the Taenia solium genome database were identified as specific for porcine cysticercosis: None of these proteins were cross-reactive when tested with sera from pigs

infected with Ascaris spp., Cysticercus tenuicollis and hydatid cysts of Echinococcus spp or with a serum from a Taenia saginata infected cow. Comparison among orthologues indicated that the amino acid sequences of two antigens possessed highly specific regions, what makes them adequate candidates for the development of a specific diagnosis for porcine cysticercosis. Perspectives: Further studies will be conducted to evaluate the potential of the 7 selected antigens (individually or in combination) using a larger number of sera from naturally infected pigs, as well as with sera and cerebral spinal fluids from neurocysticercotic patients. Project supported by DGAPA IT201613.

P7.07.06
Monocyte chemoattractant protein-1 polymorphisms influence T cell proliferation and T subsets

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Monocyte chemoattractant protein-1 (MCP-1), a widely known pro-inflammatory cytokine, belongs to a family of secreted proteins involved in immune-regulatory and inflammatory processes. MCP-1 is produced from a wide variety of stimulated normal cells, such as mononuclear leucocytes, fibroblasts, endothelial cells, smooth muscle cells, and epithelial cells. Single nucleotide polymorphisms (SNPs) are thought to be ideally suited as genetic markers for establishing genetic linkage and as indicators of genetic diseases. MCP-1 gene variants have been known to be associated with blood pressure in ischemic heart disease, type 1 diabetes, differential susceptibility to pulmonary tuberculosis and coronary artery disease. In the present study, we characterized the MCP-1 SNPs and investigate the associations between MCP-1 SNPs and phenotypes of cell proliferation and cell surface markers on porcine peripheral blood mononuclear cells (PBMC). We firstly identified two SNPs on the MCP-1 intron 1, SNP 336 and SNP 383. Significantly lesser CD8+ and reduced cell proliferation was found in PBMC with MCP-1 SNP 336 C/C than those with 336 C/A and A/A types. In addition, lesser CD4+ cells was found in those with MCP-1 SNP 383 G/G than those with 383 G/A and A/A types while cell proliferation of PBMC with MCP-1 SNP 383 A/A was significantly lower than other two genotypes. Thus, MCP-1 SNPs may affect disease-resistance by influencing T cell proliferation and cell surface antigen expressions of PBMC.

P7.07.07
Soluble FliC fusion protein cloned from *Salmonella Enteritidis* stimulates TLR7 and TLR8 expressions

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Flagellin, a TLR5 ligand, is the major protein component of bacterial flagella, which provide motility and allow adhesion to host mucosal tissues. Numerous studies have demonstrated that flagellin contributes to the efficacy of various novel vaccines for Yersinia pestis, West Nile virus, Plasmodium falciparum, Tetanus toxoid, and influenza. Flagellin activates TLR5-positive dendritic cells, neutrophils and epithelial cells. Mucosal administration of flagellin has been shown to induce primarily the Th2-type adaptive immune response, while also enhancing levels of mucosal and systemic IgA. Thus, flagellin may trigger the innate immunity that in turn is important for subsequent adaptive immune responses. In the present study, the full-length of flagellin was cloned from *Salmonella Enteritidis* and expressed as a soluble fusion protein (FliC). The biological activities of FliC were examined in the peripheral blood mononuclear cells (PBMC) of specific-pathogen free (SPF) pigs. Results showed that the FliC significantly stimulated gene expressions of toll-like receptor 7 (TLR7) and TLR8 and secretions of IL-4 and IL-10 in the porcine PBMC. Thus, the FliC soluble fusion protein with improving activities of porcine immune cells can be used as a potential porcine vaccine adjuvant candidate.

P7.07.08
Gentamicin-attenuated Leishmania infantum vaccine: protection of dogs against canine visceral leehmaniasis in endemic areas of southeast of Iran

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An attenuated line of Leishmania infantum (L. infantum H-line) has been established by culturing promastigotes in vitro under gentamicin pressure. The safety and the ability of protection of the attenuated vaccine were conducted on dogs in the high endemic areas in the rural district of Baf in the southeast of Iran. One hundred and three dogs from non-endemic area were randomly divided 2 groups (38 dogs in vaccinated group and 65 dogs control group). The dogs were vaccinated subcoutanously with L. infantum H-line, and the prospective incidence of natural infection by this vaccine was assessed during a two-year follow-up from July 1020. The safety and protection of the vaccine in the endemic area was assayed by monitoring the clinical signs and measuring the serum specific anti-Leishmania IgG. No clinical signs of disease including anorexia, apathy, local swelling on injection site occurred and no vomit and diarrhea were developed after vaccine injection. The results show only 4% of vaccinated dogs developed CVL while 45.6% of controls developed clinical signs of disease. The gentamicin attenuated line L. infantum vaccine induced a significant and strong protective effect against canine in the field.

P7.07.09
Bm86 and Hd86 anti-*Hyalomma scupense* tick vaccine trials in cattle, efficacy and limits

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The *Rhipicephalus microplus* recombinant Bm86-based tick vaccines have shown their efficacy for the control of several *Hyalomma* cattle ticks genera. However, *H. scupense* species, the most important tick in North Africa has never been studied. Vaccination trials using recombinant Bm86 and Hd86-based vaccines were conducted in cattle against immature and adult *H. scupense* ticks. The results showed a 59.19% reduction in the number of *scupense* nymphs engorging on Hd86 vaccinated cattle. However, cattle vaccination with Hd86 did not have an effect on *H. scupense* adult ticks infestations. In order to explain these *data*, Hd86 mRNA expression levels between these two tick developmental stages were measured. The results showed a significant reduction in the number of transcripts during feeding and, particularly, moulting to adults. The most interesting result was noted after moulting of engorged nymphs in unfed adults where the expression levels decreased significantly by 12.78 (10.77-17.39) and 9.25 (5.77-15.72) fold in unfed males and unfed females, respectively. Comparing unfed nymphs to unfed adult ticks, the *Hd*86 expression levels decreased by 13.82 (5.39-24.45) and 9.93 (2.87-22.08) fold in males and females respectively. Moreover, Bm86 vaccine didn't show any efficacy against the both stages of the *H. scupense* ticks. Amino-acids sequence variations between Bm86 and Hd86 could explain these results.

P7.07.10
Evaluation of vaccines against foot and mouth disease in the mouse model, correlation with efficacy in cattle

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The use of mice as a laboratory animal model for assessing vaccine potency provides an attractive alternative to the use of cattle and overcomes the disadvantages of high costs and facility requirements. There is international consensus to reduce, replace, refine animal experiments.
Protection against podal generalization (PGP) and quantification of post vaccination antibodies by liquid phase blocking ELISA (lpELISA) are the official control tests in South America for assessing potency of foot-and-mouth disease (FMD) vaccines. In this report, FMD oil adjuvant vaccines were inoculated in cattle and BALB/c mice. Specific antibody titres were measured in cattle and in mice at 60 and 21 days post vaccination (dpv) respectively by lpELISA. The cut-off points for lpELISA at 60 dpv in cattle have been officially determined and are correlated with the PGP test. A statistically significant association between antibody levels in cattle and mice was shown (concordance between both methods was 92%) as supported by Fisher exact test and by Kappa index of 0.68 (good concordance). At 21 dpv, we found that IgG2b/IgG1>1 and high avidity indexes (>50%) of mice antibodies against FMDV correlated with efficacy of commercial vaccines.
In addition IFN-γ secretion and IgA mucosal level were studied in vaccinated mice although still no relationship with protection against challenge were found.
This report describes the correlation established between immune response in cattle and mice following vaccination against FMD. This alternative method is a refinement of a current test and addresses animal welfare concerns by reducing the number of animal experiments performed.

P7.07.11
Producing Anti-Somatostatin-14 in Eggs of Immunized Chickens and Evaluating Antibody by ELISA

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Egg yolk is an important source of antibodies. Hens were immunized with Somatostatin-14 Human globulin conjugate, The biggest obstacle for isolation of chicken antibodies (IgY) is the removal of lipids, which are present in abundance in egg yolk. We have used a two-phase system to separate egg yolk. The use of an aqueous two - phase system with phosphate and PEG 6000, made separation of lipids and water -soluble proteins possible.
An indirect ELISA system was developed to measure anti - Somatostatin antibodies titer after their extraction. The sensitivity of ELISA was also affected by the coupling method used in the conjugation of Somatostatin and carrier protein. The results of total protein content of eggs during the immunization period showed that the protein amount of ss-14 injected group is higher than control group and the maximum level of protein content was attained at day 40, after first injection and then the amount of protein content decreased. Total protein content examined in egg yolk as indicator of total IgY amount of immunized an non immunized chicken that immunized group showed higher level of IgY content than control group after conjugated somatostatin-14 (SS-HSG) injection.

P7.07.12
Development of monoclonal antibodies against (PPR) virus

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Peste des petits ruminants (PPR) is an acute, febrile, viral, disease of small ruminants with great economic importance. PPR and rinderpest (RP) viruses are antigenically related and need to be differentiated serologically. The use of monoclonal antibodies (Mab) in ELISA for specific diagnostic and separation PPR and RPV will be important. For this purpose six BALB/c mice were immunized with inactivated antigen from the Nijeria strain. Fusion cloning was performed 3 months later by directly using cloning plates, and by selecting the hybridoma colonies at an early stage with an inverted microscope and transferring them into 96-wells plates with a micropipette. From 300 wells nearly 56 hybridoma were found which after testing in ELISA, only 11 clones with higher titer were selected. Among them only two clones were placed for limiting dilution (1H1, 6A12). There was only one clone (6A12L1F12) that had no cross-reactivity with RPV and reacted with the N protein and was of IgG2 isotype.

P7.07.13
Participation of Treg cells in equine Recurrent Airway Obstruction

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Recurrent Airway Obstruction (RAO) is a common allergic inflammatory disease that affects stabled adult horses. As etiological factor, environmental air borne antigens are implicated, and airway hypersensitivity types I and III, are well recognized factors of the disease. A deeper study of the disease, clearly show that other immunologic components are involved. In this work we explored the participation of the T regulatory cells (Tregs) in equine RAO.
For this purpose, a RAO susceptible herd of nine horses were exposed to *Aspergillus fumigatus* and the clinical condition monitored before and after the allergen exposure, obtaining between other samples bronchioalveolar lavage fluid (BALF) in order to evaluate the cell content the airways, and in particular the presence of Treg cells.
The results show that the Treg CD-4+, CD-25+, FoxP3+ increases in BALF obtained from healthy animals from 0.56% to 1.59% in RAO horses, if we consider as Treg population the CD-4+, FoxP3+, CD25- cells, we find that the content of this sub-population increases from 1.80% to 3.54% in normal and RAO positives animals. This increment represent represents the largest contribution to the increase of total FoxP3 cells in airways and has a direct correlation with number of neutrophils in BALF as well as with the clinical and the mucus score shown by each animal.
The role of Treg in the resolution of the disease as well as the significance of the great number of FoxP3 positives Tregs that do not show the CD-25 molecule in the membrane are discussed.

P7.07.14
Emetic potential of newly identified staphylococcal superantigens

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Staphylococcal enterotoxins (SEs) are exotoxins that cause staphylococcal food poisoning in humans. Classical types, SEA, SEB, SEC, SED and SEE have been characterized. Recently SEG, SEH, SEI, SER, SES and SET were identified as potential agents of food poisoning. In addition, new proteins (SElJ, SElK, SElL, SElM, SElN, SElO, SElP, SElQ, SElU, SElV and SElX) with similar amino acid sequences as the above SEs have been identified. SEs are also known as members of the superantigens. During the last few decades, a lot of studies have been conducted on the nature of SEs and their superantigenic activities. However, the emetic activity of those toxins has not been studied. To understand the etiologic nature of staphylococcal food poisoning extensively, emetic potential of SEls should be evaluated.
We demonstrated the emetic activity of SElK, SElL, SElM, SElN, SElO, SElP, and SElQ using primate model (cynomolgus monkeys) in which the number of vomiting, the time to the first vomit (latency period), and behavioral changes were recorded. We compared their emetic activities between classical and new SEs, and among three large groups on the basis of similarity of amino acid sequences.

Furthermore, we studied their mitogenic activities, and will discuss the relationship between emetic and superantigenic acitvities.
Additionally, we demonstrated their emetic activity in a recently established system using house musk shrews. The use of monkeys investigating SEs is severely restricted by high cost, the availability of the animals, and ethical considerations. We will discuss the usefulness of the system using house musk shrews.

P7.07.15
The technique for large-scale production of CpG DNA adjuvant and applications in avian vaccines

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Research of animal vaccines is one of the most influential developing industries in the demand for the national policy regarding agricultural biotechnology industrialization. Development of adjuvants is one of the keys to development of vaccine technologies. Use of an adjuvant has proven to yield advantages improving the deficits of traditional vaccines by increasing safety and enhancing vaccine effectiveness. DNA adjuvants containing unmethylated CpG motifs have been confirmed to promote human, mammal, and even avian vaccine efficacy. Our lab has developed one species-specific, structurally stable CpG DNA adjuvant with promoting humoral and cellular immune responses in chickens. The objective of this research is to produce the CpG DNA adjuvants in a large-scale and apply the CpG DNA adjuvants in the chicken vaccines. The highest chemically purified CpG DNA plasmid contents with the 64 copies of CpG motif was achieved at the 6-10 hr under the typical fermentative conditions and the chemically purified CpG DNA plasmid with the 64 copies of CpG motif significantly stimulated gene expressions of IFN-gamma, TLR7 and TLR8 of the chicken immune cells. These technologies can be also applied for vaccine/adjuvant design, production and implementation for other animals, including mammals and even pets, and thus have the potential to drastically facilitate the development of the animal vaccine industry.

P7.07.16
Development of an ELISA for determination of potency in snake anti venoms

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The venom of Naja Naja Oxiana, Vipera Lebetina, Vipera Albicornouta, Echis Carinatus, Pseadocerastus Persicus and Agkistrodon Halys snakes are used to determine the potency of the polyvalent sera in neutralizing the venom. LD50 value per mouse was determined 6.5 µg. Neutralization methods for obtaining potency of anti venom are competitive ELISA (Enzyme linked immunosorbent assay) and in vivo assay (ED50). Both of these tests were performed to estimate the serum potency, in 15 samples of hyper-immune equines. Results of competitive ELISA showed that at 1/12000 dilution of serum can inhibits 50 percent of antigens. Five fold of LD50 as challenge dose of venom for lethality neutralization test was applied for all serums. Competitive ELISA was compared with current biological assay ED50. Significant correlations between ELISA titers and values of ED50 at level of P≤ 0.01 and r=0.95 was observed, that indicates competitive ELISA can estimate antibody neutralizing capacity of the serum as well as the in vivo assay. The results of present study shows C-ELISA that measures Ag-Ab complex can used as a suitable replacement method for lethal neutralizing in vivo method.

P7.07.17
Determination of lymphocytes subpopulations in peripheral blood cells of dogs with atopic dermatitis

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Canine atopic dermatitis (AD) is a genetically-predisposed inflammatory and pruritic allergic skin disease, with clinical features commonly associated with IgE-antibodies to environmental allergens. Th1 and Treg lymphocytes play an important role in AD, regulating immune response i.e. by inhibiting the development of allergic responses of Th2 cells. During early phases of AD Th2 response predominates and is followed by Th1 domination in chronic AD. The aim of this study was to determine the profile of lymphocyte subpopulations in peripheral blood mononuclear cells (PBMC) in dogs with AD. Twenty dogs with AD (patients of Small Animal Clinic) were included in this study. Diagnosis was made based on Willemse's and Prélaud's criteria, confirmed by serological allergy tests and intradermal skin tests. Three healthy dogs were included as controls. PBMC were isolated from blood and analyzed using flow cytometry.
The most commonly encountered positive results were against house-dust mites (over 70%), and less frequently against various pollens: grass weed, tree. Percentage of Th1 cells was higher in atopic dogs (12%) compared with controls (6.7%). The Th1/Th2 ratio equaled 3 in AD dogs, 1.3 in controls. The number of Treg lymphocytes was two times higher in atopic dogs. Tc cells population was also elevated in AD dogs vs. controls (19.5% vs. 16.8%). B cells percentage was lower in AD dogs. To summarize, dogs with AD had increased Th1 and Tc subpopulations, and decreased number of Th2 and B cells, indicating that a transition from early humoral to cell-mediated immunity occurred in the examined patients.

P7.07.18
Human anti-langerin (CD207) polyclonal antibodies detect epidermal Langerhans-like cells in the sea turtle (Chelonia mydas)

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Dendritic cells form the link between the innate and adaptative immune response, particularly on mucosal and epidermal surfaces. Langerhans cells play a key role in the skin immune response, however, no molecular marker for these cells has been identified in endangered marine reptile green turtles, thereby complicating the understanding of the pathogenesis of diseases such as fibropapillomatosis which induces internal and skin tumors in this species worldwide. Langerhans cells present different molecular markers, among them, HLA-DR, MHC-II, CD1A, and specially CD207. We demonstrate that the polyclonal anti-human langerin (CD207) antibodies strongly stained Langerhans-like cells in the suprabasal layer of epidermis in cryosections and cytospin preparation from migration assay. The morphology of these cells was round to amoeboid in normal skin and cytospin preparation, but in skin with ulcerative dermatitis, langerin+ cells aggregated around ulcers and adopted a more pleomorphic morphology. To our knowledge, this is the first identification of Langerhans-like cells with a molecular marker in a reptile species.

P7.07.19
Isolation and characterization of recombinant single chain fragment variable antibodies against Toxoplasma gondii surface antigen

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Toxoplasma gondii an obligate, intracellular parasite, which is widely spread in the world. The parasite is able to infect all warm-blooded hosts including human and farm animals. The infection in humans often occurs after the ingestion ofraw or undercooked meat containing tissue cysts, although the ingestion of food or water contaminated with oocysts excreted by infected cats is another major transmission route. Several methods have been applied to detect this parasite in contaminated foods. The object of present study was to isolate recombinant monoclonal antibodies against this important parasite that could be used in future in diagnostic tests. The purified Toxoplasma gondii surface antigen p30 was coated to immunotubes and used as a target for selection of antibodies from the Tomlinson I and J phage display libraries of single chain (scFv) antibodies. Clones able to recognize antigen were isolated by three rounds of binding, elution and amplification. scFv antibodies chosen from the resulting panel, their specificity were confirmed by ELISA, dot blotting and western blotting.
The study showed that several recombinant antibodies were able to bind specifically to antigen with high affinity. Therefore these isolated soluble single chain antibodies are good candidates to apply as monoclonal recombinant antibodies in diagnostic kits for detection Toxoplasma gondii in contaminated samples.

P7.07.20
Failures of immunization in animals with reference to the West Africa sub-region

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In West Africa, outbreaks among livestock vaccinates of vaccine-preventable anthrax, brucellosis and clostridial diseases, have been common occurrences suggesting that no vaccine could be 100% protective. Longitudinal studies conducted over the last five years to determine active immunization against these diseases have shown in general that the antibody titres found in members of vaccinated populations were distributed on a statistically “normal” curve; a few animals having very high titres and another small group failing to respond at all, however many times the animals were re-vaccinated. This varied response is probably due to individual genetic make-up. In general we also observed that a supposedly live vaccine may be dead due to poor storage especially during transportation, or the animal may recently have been treated with an antibiotic, residues of which may be sufficient to inactivate a live bacterial vaccine. If for example more than one antigen is given at one time, the balance of antigens may be such that the response to one or more of them is overwhelmed by a greater stimulation of antibody production effected by the others. In very young animals passive immunity obtained from colostrums or through the placenta may be the cause. The implication of these failures is that where an enzootic disease such as anthrax, brucellosis or tetanus is concerned, many deaths will occur in the unprotected animals because individuals are exposed to the chance of infection over long periods of their lives.

P7.07.21
Polyspecific antigen binding by bovine immunoglobulin heavy- and light-chain variable domains

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The complementarity regions (CDR) in the variable heavy and light chain domains of immunoglobulin determine the affinity and specificity of the antigen-binding site.
Generation of an exceptionally long CDR3H (up to 61 amino acids) in the bovine heavy chain variable region (VH) provides an additional mechanism to generate antibody diversity, not found in other species to date. These long CDR3H loops, present in polyspecific IgM, originate from VDJ recombination encoded by a specific VH gene, unusually long single DH-gene and insertion of 15-18 base long conserved short nucleotide sequences at VH-DH junction. In cattle, it is thought that antigen binding is mainly a function of the variable-region VH where light chain provides only structural support. To test this hypothesis, single chain variable fragment (scFv) and single domains (Fd) from polyspecific IgM were constructed. Both purified scFv and FdVH showed polyspecific binding to structurally dissimilar antigens in an ELISA. Whether scFv and FdVH bind to multiple epitopes on an antigen or if they recognize the same epitope can now be determined. The structural-functional complexities of these antibody fragments and the role of the heavy- and light-chains in antigen binding will be discussed. [Supported by NSERC Canada]

P7.07.22
Pathogenesis of pancytopenia in canine visceral leishmaniasis: bone marrow morphological alterations and IGF-I and IL-7 mRNA expressions

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Pancytopenia is an important alteration in active visceral leishmaniasis (VL) but the pathogenesis is poorly known. We examined 13 naturally *L. (L.) infantum*-infected dogs and 10 control dogs. Dogs presenting *Erlichia canis* coinfection were discarded. We performed hematological exam, myelogram and IGF-I and IL-7 mRNA expressions by Real Time PCR. All VL dogs exhibited normocitic normochromic anemia, leucopenia and/or thrombocytopenia. From 13 VL dogs 5 presented pancytopenia and 8 bicytopenia. In the myelogram we observed dysgranulopoiesis (100%), dyserythropoiesis (100%) and dysmegakaryocytosis (53.8%) and an increase in the myeloid:erythroid ratio. The precursor erythroid cell: mature erythroid cell ratio was higher in pancytopenic than in bicytopenic dogs. We also observed an increase in the mature myeloid and a decrease in the mature erythroid cells when compared with control dogs. In the differential count, we observed an increase in the number of lymphocytes in VL dogs than in controls. We observed a significant decrease of the plasma cells in VL dogs compared to control dogs. The IGF-I mRNA expression was lower but the IL-7 mRNA expression higher in VL dogs when compared with control dogs. In canine VL, the morphological analysis suggests the occurrence of dishematopoiesis with gradual disturbance in the maturation process. Furthermore, a decreased IGF-I mRNA expression may be implicated in the hematological changes mainly of erythroid series and IL-7 mRNA expression in the lymphocyte proliferation. Supported by FAPESP, CAPES, CNPq, LIM-38 (HC-FMUSP).

P7.07.23
Booster BCG vaccination modulates response against Mycobacterium bovis-PPD in dairy herds with tuberculosis

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Vaccination with *Mycobacterium bovis* BCG and booster with protein subunit vaccine induces a significant degree of protection against bovine tuberculosis (bTB). We performed studies to determine the profile of immune response on commercial dairy herds with high bTB prevalence (30.6±9.0%) after vaccinated with BCG (5 x 10^6 CFU) and/or boosting with a subunit vaccine. We assessed gamma interferon (IFN-γ) and also (purified protein derivative [PPD])-specific Immunoglobulin G (IgG) antibody levels in serum. IFN- γ response showed a sustained increase along the time (from 1000 to 2000-4000 pg/ml) with higher peaks one month after initial vaccination (5000 pg/ml) and after the first (4000 pg/ml) and second booster (7000 pg/ml) in single tuberculin skin test reactors (TST^+). When comparing the IFN- γ in non-reactors cows, the levels were lesser, but also increased at mo 3th (2000 pg/ml; after the 1st booster) and at mo 13th (over 3000 pg/ml; after the 2nd booster). The increased response in both groups of animals, suggest the induction of a Th1 memory response in the immunized animals. We found that the IgG antibody response to this antigen was inversely correlated to the cellular immune response measured by IFN- γ production. In TST^+ the mean IgG antibody levels increased at the first month (p<0.05), although

decreased (p<0.05) along time post-vaccination (i.e., at mo 2th and 7th). We explored different cut off values for classification of test results using receiver operating characteristics curve analysis, which could enhance the power of discrimination between vaccinated and bTB infected and non-infected cattle.

P7.07.24
Improvement in bovine haemorrhagic septicaemia vaccine potency and efficacy by aluminium nanoparticles and keyhole limpet hemocyanin, and an approach to develop a DIVA strategy

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Haemorrhagic septicaemia (HS) caused by *Pasteurella multocida* B:2 serotype is a major cause of morbidity and mortality in cattle and buffaloes in India and other Asian countries. Alum adjuvanted and oil emulsion commercial vaccines for HS control suffer from poor potency, efficacy, safety and other problems. Moreover, these vaccines are unable to differentiate infected from vaccinated animals (DIVA strategy) and the HS control programme cannot be monitored effectively. The present study aimed to improve HS vaccine qualities by using aluminium nanoparticles and keyhole limpet hemocyanin (KLH) as adjuvants, and to develop a DIVA strategy by incorporating KLH. Aluminium nanoparticles and KLH were mixed with formalin-inactivated *Pasteurella multocida* B:2 P52 strain. Various controls were also used. Swiss albino mice (n=8/group) was inoculated twice at three-week interval. Two weeks after booster, mice were challenged with 50xlethal dose$_{50}$ of virulent bacteria. All the mice (n=7) immunized with nanoparticle+KLH+bacterin (group I) survived the challenge. While aluminium nanoparticles (group II), KLH (group III) and alum-precipitated bacterin (group IV) groups (n=7/group) had six, four and one survivors, respectively. Anti-bacterial antibody levels in the group I-III were significantly higher than those of group IV at every week post-immunization. Anti-KLH antibodies were produced in high titres in all KLH groups and persisted as long as those against bacterial antigens had. Anti-KLH antibodies were absent in sera samples of cattle and buffaloes. In conclusion, efficacy and potency of HS bacterin was improved by aluminium nanoparticles and KLH. KLH inclusion was also suitable for DIVA strategy for HS control programme.

P7.07.25
TIR8/SIGIRR is expressed in tissues of adult and embryo chicken

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The orphan receptor TIR8/SIGIRR, belongs to the TIR superfamily. TIR8 does not activate NF-kB and IRF3 and negatively modulates the inflammatory responses. It acts as antagonist for the TIR family members and triggers a negative pathway of the TIR receptor system, crucial for dampening inflammation. TIR8 was well characterized in mouse, humans and in other mammals, but it is poorly known in birds.
We investigated TIR8 distribution in adult and embryo chicken samples. The pattern of expression of chicken TIR8 in adult was ubiquitary and similar to mammals, but it resulted unique in pancreas, female reproductive tract, heterophils. Different TIR8 isoforms were detected, suggesting the occurrence of post-translational modifications or alternative splicing. Immunohistochemistry revealed TIR8 immunolabeling in the intestine, thymus and oesophageal ganglia. These results demonstrate that TIR8, although evolutionarily conserved, show species-specific peculiarities. We analyzed TIR8 expression in embryos of layer- and broiler-chicken at different incubation time-points. TIR8 was detected since the first stages of development, but it reached a remarkable expression level at day 10. TIR8 was ubiquitously expressed, but the highest expression were found in liver and kidney. This pattern was comparable to those observed in adult chickens and in mammals examined to date. No differences were observed between the two different chicken breeds despite their immunological discrepancies. These are the first findings concerning TIR8 expression in developmental stages and they contribute to better understand the reproductive physiology and transovarian pathogen transmission, together with the recent findings of TLR expression in ovary and embryos of different species.

P7.07.26
Infection and immunity during leptospirosis disease in Golden Syrian Hamster: approaching for vaccine biological models

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Leptospirosis is considered by WHO the zoonotic disease most disregarded in last decades. One of the major obstacles in developing prophylactic vaccines is the absence of cross-protection among unrelated serovars and the lack of knowledge about the immune response against Leptospira spp. The main objectives of the study were to assess the existence of cross-protection among vaccine strains of different serovars and to characterize the immune response during leptospirosis in the most used animal vaccine model. The infection model reproduces the test of different doses of DL50 of several virulent leptospiras serovars. After inoculation the antibody response , cellular response, biochemical and haematological parameters and survival rates were measured. The carrier state on main organs and histopathology studies were determined after death. The results demonstrated that a cross-protection among unrelated Leptospira serovars strain is possible. Canicola strain is able to induce protection against homologous, Ballum and Copenhagen strains. Instead, Mozdok strain induced only protection against homologous challenge. The other strains showed a moderate cross-protection against heterologous challenge. It was demonstrated that elimination of the carrier state is correlated with high IgM and IgG1 antibody response. The cellular response is absent or not detected in ex vivo cultures. These results indicate that protection conferred by LPs is serovar specific in contrast to the response against external membrane proteins which is related to cross-protection among unrelated serovars. Also the humoral response is predominant during the immune response but it is not correlated to protection.

P7.07.27
Ethoneuroimmunoendocrinology of fear-related behavior disorders in dogs and cats

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Ethoneuroinmunendocrinology is a veterinary specialty that studies relationships between body control systems, which are communicated through common signaling molecules (neurotransmitters, cytokines, hormones); and views diseases as a result of the rupture of this systems´ balance.
Behavior disorders of dogs and cats can be analyzed from an integrative perspective, addressing the clinical presentation, diagnosis and treatment from the body-control systems´ interactions point of view. Fear-related disorders (phobias and anxiety) often occur along with immune-related chronic physical pathologies in dogs and cats, such as skin disorders (atopic dermatitis and other allergies), endocrinopathies (hypothyroidism), gastrointestinal diseases (IBD, chronic diarrea or vomiting , gastritis, gastric dilatation/ volvulus syndrome), urinary disorders (idiopathic interstitial cistitis, lower urinary tract disease), respiratory diseases (feline bronchial asthma, canine tracheal collapse), neurological diseases (hydrocephalus, epilepsy, strokes). It is not easy to distinguish which is the primary pathology (if any), or if they are comorbid pathologies.
There are several hypotheses about the joint occurrence of physical and emotional conditions: 1) Fortuit association. 2) Genetic association. 3) Gene interactions. 4) Emotional disturbances as causal factors of physical illness. 5) Physical illness as causal factor of emotional disturbance. 6) Physical illness and emotional disturbance coexist because they share molecular mediators and signaling pathways. Likely, there is a combination of all the above, which would be difficult to be studied separately.
Evidence suggests that the state of chronic stress form living with excessive fear or anxiety can have deleterious effects on health, welfare and lifespan of pets.

P7.07.28
Ocular infectious bronchitis virus vaccination induces different immune responses in the mucosal and systemic immune compartment

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Infectious bronchitis virus (IBV) is an economic problem in the poultry industry despite extensive vaccination. Live attenuated IBV vaccines are delivered via spray or drinking water to induce T and B cell responses in head-associated lymphoid tissues (HALT), i.e., Harderian glands and conjunctiva-associated lymphoid tissue, and spleen. HALT lymphocytes undergo an expansion phase from day 7 through 10 during the IBV-induced immune response, after which they undergo a contraction phase, which coincides with the effector phase of the T and B cell response after IBV vaccination. The IFN-gamma response to IBV is biphasic, consisting of an earlier response at the beginning of the expansion phase and a second response coinciding with the effector phase. The secondary IBV IFN-gamma response is restricted to the spleen, indicating that IBV memory cells are located in the systemic immune compartment rather than mucosa-associated lymphoid tissues. A distinct, earlier IBV-specific IgA spot-forming cell (SFC) response is observed in the spleen compared to HALT, while both display a significant SFC increase 10 days after vaccination. IBV vaccination increases IBV-specific IgA levels in plasma and tears 3-4 days earlier than IgG levels. To address whether antibody responses are affected by frequent mutations in the amino terminal portion of the IBV spike protein (S1), the target for neutralizing antibodies, an overlapping peptide array is used. Distinct B cell epitopes are recognized which are altered in variant IBV strains enabling immune escape. These data indicate distinct roles of the mucosal and systemic immune compartments in the IBV response.

P7.07.29
Expression of TNF-alpha mRNA in canine mammary tumors as a potential marker for tumor evolution

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TNF-alfa is a pleiotropic cytokine, which promotes variable effects on a large number of cells. The findings on the effects of TNF-alfa on tumors are diverse and controversy, because this cytokine exerts both anti and pro-cancer activities. Thus, TNF-alfa damages tumor vasculature and has necrotic effects in tumor cells, or, it can inhibit DNA repair, promotes angiogenesis and acting as growth factor for tumor cells. Our purpose was to establish an association between a histological classification of mammary gland tumors (MGT) in dogs with TNF-alfa gene expression. Twenty nine female dogs with MGT and 5 normal dogs were selected for biopsy of mammary gland. The histopathology examination classified 3 adenomas and 26 simple carcinomas (11 tubular; 9 papillar; and 6 solid). The gene expression for TNF-alfa mRNA was measured by quantitative real-time RT-PCR. The statistical analysis was performed by Student-Newman-Keuls Test. All groups of dogs with MGT showed increased levels of TNF-alfa mRNA expression, which are significant different ($p < 0.05$) from the average level measured for the control group (mean 0.53; SD 0.48). The mean of carcinoma group (mean 575.88; SD 720.08) was higher than that found for adenoma group (mean 25.704; SD 34.15). Thus the TNF-alfa mRNA expression in MGT can be positively associated with tumor development and/or malignancy and the overexpression of this cytokine could be associated with the progression of MGT, hypothesizing that it is acting by different modulating effects in tumor microenvironment, which may lead to different tumor evolution pathways. Financial support and fellowship FAPESP (2012/09385-0; 2010/12946-8).

P7.07.30
Novel dendrimeric peptide: Immune response and protection against Foot and Mouth Disease Virus (FMDV) induced by synthetic peptides in cattle

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Previous work has shown that a synthetic dendrimeric peptide of FMDV containing a T epitope [3A(21-35)], and four copies of a B epitope [site A; VP1(136-154)] of serotype C protected 100% of vaccinated pigs.
The aim of the study was to evaluate the immunogenicity and protective response induced by B2T and B4T (B epitope of serotype O) peptide as FMD vaccine in cattle.
Hereford steers were inoculated twice with B2T (n=4) or B4T (n=4) by intramuscular injection with 2 mg of peptide emulsified with commercial oil adjuvant. One animal was left as unvaccinated control.
At 38 dpv, the α-FMDV IgG titers in the B4T-group were 3.5±1.0, whereas in the B2T-group were 4.1±1.0 and the predominant isotype induced in both groups was IgG1. Likewise, α-B4T antibody titers were 4.9±0.4 whereas in the B2T-group were 5.0±0.4.
At 32 dpv neutralization indexes were ≥1.8 for 3 animals in the B2T -group and for 4 animals in the B4T-group, However neutralizing antibody titers were lower than 2.
We observed positive proliferative response of PBMC of vaccinated animals, when stimulated with both peptides. IFN-γ was secreted when cells were stimulated either with virus or peptides.
At 42 dpv cattle were challenged with FMDV by nasal instillation. Two animals in both experimental groups delayed the onset of symptoms while only one animal of each group presented no foot lesions. Cattle receiving the commercial vaccine were protected against challenge. The higher level of protection was observed in animals vaccinated with peptide that presented higher cellular and humoral responses.

P7.07.31
Establishment and characterization of monoclonal antibodies against tumor-associated antigen(s)in Mc29 virus-transformed chicken hepatoma cells

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The tumors induced by the myelocytomatosis retrovirus Mc29 are recognized as suitable model systems in experimental oncology. The aim of our study was to develop monoclonal antibodies (Mabs) recognizing tumor-associated antigen(s) in Mc29-virus-transformed chicken hepatoma cells (cell line LSCC-SF-Mc29).
Ninety six hybridoma clones were obtained after fusion between mouse myeloma P3U1 cells and splenocytes harvested from two BALB/c mice immunized with LSCC-SF-Mc29 cells and with extract in 3M KCl from the same cells. Eight clones were found to produce antibodies that gave a positive reaction with chicken hepatoma cells (or extract in 3M KCl from these cells) in the indirect immunofluorescence test, dot-immunobinding assay and ELISA. No cytotoxic effect was found in the complement-dependent cytotoxicity test.
The intraperitoneal injection of hybridoma cells in BALB/c mice pretreated with Pristane resulted in the development of solid tumors in mesentherium which did not produce ascetic fluid.
Using SDS-polyacrylamide gel electrophoresis and Western blotting, one of the MAbs (1A6 - proved to be of subclass IgG1) was shown to visualize tumor-associated antigen with molecular weight of ~25 kDa that was expressed intracellularly as well as on cell membrane. The examined antibody did not recognize any antigen in cultured cells of various tumors in chicken, mouse, rat and human. No cross-reactivity with chicken embryonal cells was detected.
Investigations are in progress to further characterize the developed monoclonal antibodies and their corresponding antigen(s) as well as the role of these antigens in tumor growth.

P7.07.32

Expression of the novel antigen receptor (IgNAR) in the bamboo shark and isolation of antigen-specific IgNAR domains from shark antibody repertoires

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Fish represent the oldest and most diverse group of vertebrates with over 29,000 species. Within this group, the cartilaginous fish represent the oldest extant jawed vertebrates, having evolved for more than the past 400 million years. This evolutionary ancient class already contains an adaptive immune system. Despite the fact that fundamental molecules of the immune system are similar in fish and mammals, chondrichthyans comprise a unique antibody-like molecule, named Novel Antigen Receptor (IgNAR), which is a homodimer of heavy chains devoid of light chains. Hence, the antigen-binding site is composed of only one variable domain, vNAR. Like camelid VHHs, vNAR domains have several structural and biophysical features that render them interesting molecules for biomolecular applications: vNARs are extraordinary stable proteins and have remarkable folding properties. Furthermore, due to the loss of a conventional CDR2, the vNAR domain is the smallest naturally occurring antigen binding domain which leads to greater tissue mobility and to the possibility to target more cryptic and recessed epitopes otherwise accessible only to small molecules. [1, 2]
We analyzed the vNAR repertoire and the tissue-specific expression of this antibody-like molecule of the bamboo shark (*Chiloscyllium plagiosum*). Furthermore we utilized this repertoire to generate antibody-based binding molecules against several disease-related antigens, including CD 326 (EpCAM) and EGFR *via* yeast surface display. These molecules potentially can function as tools for diagnostic applications *in vitro* and *in vivo*.

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AUTHOR INDEX

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