P.B.21.14
Vitamin D and MS: relationship with response to treatment and with patient inflammatory profile

R. Alenda[1], *L. Costa-Frossard*[2], *O. Pastor*[3], *E. Rodríguez-Martín*[1], *C. Picón*[1], *J. C. Álvarez-Cermeño*[2], *L. M. Villar*[1];
[1]*Department of Immunology. Hospital Ramón y Cajal, Madrid, Spain,* [2]*Department of Neurology. Hospital Ramón y Cajal, Madrid, Spain,* [3]*Department of Biochemistry. Hospital Ramón y Cajal, Madrid, Spain.*

Objective: Several studies showed an association between vitamin D and the risk of multiple sclerosis (MS).
We aimed to investigate the annual variations of serum vitamin D levels in MS patients and healthy controls (HC), its effect in the response to INF-beta treatment and the possible association between vitamin levels and patient inflammatory profile.
Materials and methods: 96 MS patients and 30 HC were included in the study. Patients initiated treatment with interferon beta and were followed for 2 years. We monitored disability progression, new relapses and new MRI lesions. Samples were obtained before treatment initiation and 6 months after. Serum vitamin D levels were studied by immunoassay. Leukocyte subsets were explored by flow cytometry with a FACS-Canto II.
Results: MS patients showed lower serum vitamin D levels than healthy controls ($p<0.0001$) either in samples obtained in winter ($p=0.001$) or summer months ($p<0.0001$). We found a moderate inverse correlation between vitamin D levels and the percentages of CD_4 T cells showing intracellular IFN-γ production ($r^2 = -0.3495$, $p=0.04$)
No differences were found in serum vitamin D levels between responders and non-responders to IFNB therapy, even after adjusting by the month of extraction.
Conclusions: Our data confirms the association between MS and lower vitamin D levels, it shows that these differences are maintained even in months with high sun exposure and suggest that vitamin D may have immunomodulatory effect on TH_1 cells. On the other hand, vitamin D does not seem to play a role in response to INF-beta therapy.

P.B.21.15
Autologous hematopoietic stem cell transplantation for multiple sclerosis - A Singapore experience

P. Ratnagopal;
Singapore General Hospital, Singapore, Singapore.

Background:Multiple sclerosis (MS) is a disabling disease. Despite immunomodulation therapy, disease progression is common especially among Asian patients. The autologous haematopoietic stem cell transplantation(AHSCT) was introduced in the treatment of refractory forms of multiple sclerosis and clinical outcomes were evaluated for patients with relapsing-remittingRR) MS.
Methods:16 patients with RR MS were treated with AHSCT. Peripheral blood stem cells were obtained by leukapheresis after mobilization with granulocyte colony stimulating factor. Fludarabine and cyclophosphamide were administered as conditioning regimen. Outcomes were evaluated by the expanded disability status scale(EDSS) and progression free survival. No maintenance treatment was administered during a median follow-up of 48 months (range, 2 to 144 months)
Results:No transplant related mortality(TRM) was reported following the treatment. Twelve patients remained relapse free post transplantation up to 48 months. Eleven of these patients had improvement by EDSS, with one having recurrence of active symptoms after 52 months. Another succumbed to septicemia from urosepsis after 65 months. Four patients have just completed the transplant procedure and all showed improvement shortly after completion.
Conclusions: Several studies have shown improvement for AHSCT and local experience shows similar outcomes. Timing of the transplant is crucial for improvement. The primary goal is to eradicate disease altogether and occurs even in the late RR stage, but remarkable improvement occurs in earlier stages. As more studies become available, ABMT looks promising as a possible 2ndor 3rd line contender for treatment of MS.

P.B.21.16
Autophagy and SQSTM1/p62 expression in multiple sclerosis

V. Paunovic[1], *K. Janjetovic*[1], *M. Milenkovic*[1], *Z. Stanojevic*[2], *I. Dujmovic*[3], *J. Drulovic*[3], *V. Pravica*[1], *V. Trajkovic*[1];
[1]*Institute for Microbiology and Immunology, School of Medicine, University of Belgrade, Belgrade, Serbia,* [2]*Institute for Medicinal and Clinical Biochemistry, School of Medicine, University of Belgrade, Belgrade, Serbia,* [3]*Clinic of Neurology, Clinical Centre Serbia, Belgrade, Serbia.*

Introduction: Multiple sclerosis (MS) is an inflammatory disorder in which autoreactive T cells directed against myelin proteins mediate neuropathology and allow disease progression. Failure of myelin-reactive immune cells to undergo apoptosis is implicated in the MS development and maintenance. However, the role of autophagy, a process of controlled cellular self-digestion, has not been thoroughly investigated in MS.
Results: We examined the expression of sequestosome 1 (SQSTM1/p62), an autophagic cargo receptor and selective autophagy target, in MS and its animal mode, autoimmune encephalomyelitis (EAE). We found that the protein levels of SQSTM1/p62 are elevated in popliteal lymph nodes from EAE rats. Quantitative real-time PCR analysis revealed that the expression of SQSTM1/p62, but not other autophagy-related genes, was increased in peripheral blood mononuclear cells (PBMCs) of MS patients compared to healthy controls. Accordingly, protein levels of SQSTM1/p62 were also significantly up-regulated in MS PBMCs. On the other hand, the conversion of cytoplasmic LC3-I to autophagosome-associated LC3-II and the expression of the protein and mRNA levels of the proautophagic Beclin-1 were unchanged. PBMCs from patients and healthy controls responded differently to the PMA/Ionomycin stimulation. In both, the levels of membrane-bound LC3-II were enhanced, while the SQSTM1/p62 levels were increased in control cells and remained unchanged in patient cells.
Conclusions: These results indicate that SQSTM1/p62 expression in PBMCs from MS patients is increased and regulated independently of autophagy, thus implying the existence of other pathways involved in regulation of this multifunctional adaptor protein. The possible role of SQSTM1/p62 in MS warrants further investigation.

P.B.21.17
Increased caspase-1 and EBV load in active phase of Multiple Sclerosis disease

M. Izad[1,2], *M. Behesht*[i1], *Z. Salehi*[1], *B. Nomanpour*[3], *H. Shirzad*[4], *R. Abolfazli*[5], *M. Naseri*[6];
[1]*Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [2]*MS Research Center, Neuroscience institute, Tehran University of Medical Sciences,, Tehran, Iran, Islamic Republic of,* [3]*Department of Microbiology,Kermanshah University of Medical Sciences, Kermanshah, Iran, Islamic Republic of,* [4]*Cellular and Molecular research center, Shahr-e-kord University of Medical Sciences,, Shahr-e- kord, Iran, Islamic Republic of,* [5]*Department of Neurology, Amiralam General Hospital Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [6]*Department of Virology, Faculty of public health, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of.*

Multiple sclerosis (MS) is a T cell-mediated autoimmune disease, characterized by demyelination of nerve fibers in central nervous system. The cause of the disease appears to be a combination of genetic and environmental factors. Among the various proposed environmental causes, viruses such as Epstein - Barr virus (EBV) and Human herpesvirus 6 (HHV-6) have recently considered as potential causes of MS onset and progression. Caspase-1 is a cysteine protease which in response to viral components activates and cleaves precursors of inflammatory cytokines, IL-18 and IL-1β. Caspase-1 is upregulated in oligodendrocytes of Multiple Sclerosis and EAE, and initiates pyroptosis in these cells. In this study we measured serum and cellular caspase-1 and serum levels of IL-18 and IL-1β in Relapsing-Remitting multiple sclerosis patients, in active phase (n=23) and healthy age-and gender-matched controls (n=19) using ELISA assay. Quantitative detection of EBV and HHV-6 DNA in serum of subjects were also performed using Realtime PCR. We observed that caspase-1 level was increased in serum from patients compared to controls ($p<0.05$). There was no significant difference in serum levels of IL-18 and IL-1β between groups. We found 34.8% of patients and 10.5% of controls EBV positive while, 21.7% of patients and 42.1% of controls were positive for HHV-6. There were positive correlation between IL-1B and EBV ($p=0.01$) and between IL-18 and serum caspase-1($p=0.01$). There was a negative correlation between serum and cellular caspase. ($P= 0.05$). Our results suggest that caspase-1 elevates in serum following inflammation that could be because of increasing of EBV load.

P.B.21.18
Differential frequency of CD8+ T cell subsets in multiple sclerosis patients with various clinical patterns

Z. Salehi[1], *M. Izad*[1,2], *M. A. Sahraian*[2], *E. Janzamin*[3], *P. Khosravani*[3];
[1]*Immunology Department, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [2]*MS Research Center, Neuroscience institute, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [3]*Flow Cytometry laboratory, Department of Stem Cell and Developmental Biology, Royan Institute, Tehran, Iran, Islamic Republic of.*

Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system. CD_4+ T helper cells have been the main focus of research in MS for several decades. However, recent evidence points to a pathogenic role for CD8+ cytotoxic T (Tc) cells. Similar to Th cells, Tc cells can be divided into different subsets based on their cytokine profile. In this study we analyzed the frequency of CD8+ T cell subsets, including IFN-γ (Tc_1), IL-4 (Tc_2), IL-10 (Tc_{10}), IL-17 (Tc_{17}), IL-21 (Tc_{21}), IL-22 (Tc_{22}) and TNF-α producing cells, in peripheral blood mononuclear cells of Relapsing-Remitting (n=28), Secondary-Progressive (n=10) and Primary-Progressive MS (n=4) patients in comparison to sex and age-matched healthy controls (n=15) using multicolor flowcytometry. In addition, the level of TGF-β, IL-6 and IL-23 were measured in sera of patients and controls by ELISA.
Compared with controls, elevated levels of Tc_1 and Tc_{17} cells were seen in SPMS ($p =0.04$) and relapse phase of RRMS patients ($p=0.05$) respectively. interestingly, the percentage of TNF-α producing CD8+ T cells in relapse and remission phase of RRMS and SPMS patients were higher than controls ($p=0.01$, $p=0.004$, $p=0.01$ respectively) and Tc_{21} increased in remission phase of RRMS compared to SPMS ($p=0.03$). TGF- β elevated in sera of SPMS and remission phase of RRMS compared with those of controls ($p=0.05$ and $p=0.03$, respectively). There was no correlation of cytokine serum level with Tc_{17} cells frequency.
These data demonstrate the contribution of cytotoxic T cells and neuro-protective and pro-inflammatory role of TGF-β in Multiple sclerosis.