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274 Association Between Body Mass Index (BMI) and Fraction Of Exhaled Nitric Oxide (FeNO) Levels In The National Health and Nutrition Examination Survey (NHANES) 2007-2010

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RATIONALE: Obesity is characterized by chronic, low-grade systemic inflammation. Elevated FeNO levels reflect airway inflammation in various lung diseases including asthma.

METHODS: This is a cross-sectional analysis of data.Participants younger than 20 years old with history of cough/cold symptoms in the past 7 days, smoking, exercise in the previous hour, consumption of nitric oxide rich meats/vegetables, or use of inhaled corticosteroids during the previous 2 days were excluded. BMI (in kg/m2) was divided in to 4 categories: underweight (UW) (0-18.5), Normal (N) (>=18.5 to < 25), Overweight (OW) (>=25 and <30) and Obese (O) >=30. Complex sample multiple regression analysis was used to determine the association between BMI and FeNO levels.

RESULTS: There were a total of 149,629,652 weighted participants: (UW (22,235,218), N (45,021,536), OW (5,1670,522) and O (50,199,974); 50.36% were men and 49.63% were women. Mean FeNO levels (in ppb) increased with increasing BMI category: UW (12.52±1.05) N (16.25±0.64), OW (16.62±0.34), and O (16.78±0.39) [p=0.0035]. There is a weak yet statistically significant correlation between FeNO levels and both age [r=0.11, p<0.001] and BMI [0.028,p=0.019], respectively. Our multivariate analysis predicting FeNO level based on BMI category, adjusting for age and gender, found age less than 60 years (vs. 20-40 and 40-60 years, [p<0.0001]), male gender (vs. female, [p<0.0001]), and UW category (vs. obese, [p=0.0056]) were associated with statistically significantly lower FeNO levels.

CONCLUSIONS: Age, female gender, and BMI have weak positive associations with FeNO levels. Controlling for age, gender, and race, obese individuals have a statistically significantly higher FENO level than underweight individuals.

275 Daily Global Stress Is Associated With Nocturnal Awakenings Due To Asthma In School-Age Children

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RATIONALE: Since day-time stress is associated with nocturnal awakening in adults, we sought to determine the associations of child daily stress with nocturnal asthma in school-age children.

METHODS: 46 children ages 6-11 years with persistent asthma for ≥1 year and nocturnal symptoms in the past 6 months participated in a 12 week daily diary card study (median 12 weeks, range 1-12) recording stress measures and asthma symptoms. Daily global stress was indicated by response to the question "How was your day today?" with very bad, bad, good or very good. Children chose pictorial representations of not at all, a little or a lot for "Circle the face that represents your emotion today". Diary responses were analyzed for frequency and factors related to nocturnal awakenings.

RESULTS: 59% of children experienced ≥1 nocturnal awakening from asthma (median 2, range 1-32). Children whose global daily stress was very bad or bad versus very good were more likely to awaken from asthma that night (OR 3.3, 95%CI 1.8-5.9), even when controlling for albuterol use that day (OR 2.1, 95%CI 1.1-3.9). There was no association between child daily global stress and awakening for another reason besides asthma that night (p=0.69). Children were more likely to rate daily global stress as very bad when they experienced emotions a lot that day (p<0.0001).

CONCLUSIONS: Child-reported greater daily global stress was associated with increased emotions that day and increased likelihood of awakening from asthma that night. Developing interventions to address daily global stress may decrease nocturnal asthma symptoms and their associated morbidity.

276 A Seven Minute High Intensity Workout Is Well Tolerated In Adults With Asthma and Results In Decreased Salivary Leukotrienes

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RATIONALE: Cysteinyl-leukotrienes (Cys-LT) are important mediators in the pathophysiology of asthma. Salivary Cys-LT are elevated in asthmatic individuals. Aerobic exercise has been shown to decrease urinary LTE4 in healthy individuals. We hypothesized that the 7 minute workout (7MW), which does not require expensive equipment or extensive time investment, will result in decreased salivary total Cys-LT in individuals with asthma.

METHODS: Thirteen individuals with asthma performed the 7MW, which includes 12 exercises (jumping jacks, wall sit, push-up, abdominal crunch, step-up, squat, triceps dip, plank, high knees, lunge, push-up with rotation, side plank) each performed for 30 seconds with 10 seconds of rest in between. Heart rate (HR), oxygen saturation, and saliva were obtained before and immediately after exercise. Total Cys-LT levels were assayed by ELISA.

RESULTS: All 13 individuals completed the workout, which was well tolerated. One individual complained of chest tightness after completing the workout, which was relieved with albuterol HFA. HR increased from 78.8+/-2.5 (mean+/-SEM) to 132.6+/-5.0 (p<0.0001, paired t-test) after exercise. Mean % maximal HR was [72.6+/-2.7] after exercise. In the 10 individuals with evaluable salivary total Cyst-LT, mean levels decreased after exercise from 431+/-132 pg/mL to 207+/-57 pg/mL (p=0.05).

CONCLUSIONS: In this pilot study of individuals with asthma, a single episode of high intensity exercise with the 7MW was well tolerated and decreased salivary total Cyst-LT. The individuals reached a level of moderate exercise as indicated by their % maximal HR. The 7MW may prove to be an inexpensive and time efficient therapeutic modality in the management of asthma.

277 Obesity and Depression Affecting Asthma Morbidity: An Analysis Of Montefiore Asthma Center's Poorly Controlled Asthmatics

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RATIONALE: Previous studies have demonstrated an increased prevalence of obesity and depression in asthma patients. The purpose of our study is to investigate this association with asthma morbidity in a high asthma prevalence area, the New York City borough of the Bronx.

METHODS: We reviewed 100 charts of poorly controlled asthma patients at Montefiore's Asthma Center at their initial evaluation. Patient criteria included at least one asthma-related emergency department visit or asthma-related hospitalization within the last 12 months or receiving 3 or more steroid courses within the last 12 months for the treatment of asthma exacerbation. For each patient, we reviewed the demographic, body mass index (BMI), Patient health questionnaire for Depression PHQ-4, Asthma Control Test (ACT) and spirometry data at their initial Asthma Center visit.

RESULTS: Our analysis revealed that 82% of the patients were considered to have uncontrolled asthma with an ACT score less than 19. The majority of these patients lived in the Bronx (89%) and was a minority group (55% Latino and 36% African-American.) Almost half (48%) of the patients screened positive for depression on their PHQ-4 (score ≥ 3.) The majority of patients were considered overweight (21%, BMI 25 – 29.9) or obese (63%, BMI ≥ 30.)

CONCLUSIONS: There appears to be an association between obesity and depression in uncontrolled asthma patients in the Bronx. This relationship requires further investigation as it may have therapeutic value in future management and prevention of asthma exacerbations.

278 Psycho Social Stresses and Asthma Morbidity In Children

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RATIONALE: The purpose of this review is to investigate the influence of psycho-social stress on asthma morbidity in children.

METHODS: Data on 56 asthmatic children aged 5-18 years were collected. Subjects were examined for the influence of psychological stresses (AHDH, Anxiety, Depression) and social factors (parental divorce, poor school performance, chronic illness in the family and any other stress in the family). The study subjects were divided into two groups. Group A (N= 30) comprised of children without personal or family history of psychosocial stress. Subjects in Group B (N= 26) had 2 or more background psychosocial stressors.

RESULTS: Group A: Average asthma related hospital admission rate for group A was < 1 /year/subject. 7 subjects in this group had >2 ER/Urgent care visits/year. 10 subjects had >2 courses of systemic steroids/year. Average asthma related missed school days/parental job time in the group was 4 days/year. Non compliance with asthma medications/care plan was found in 11 subjects Group B: Average hospital admission rate for group B was > 1.5 /year/subject.16 Subjects had >2 ER/Urgent care visits/year. 17 subjects had >2 courses of systemic steroids /year. Average missed school /parental job time in group was 9 days/ year. 16 subjects in Group were found to be non-compliant with medication/asthma care plan.

CONCLUSIONS: These data suggest that psychosocial stressors may act as risk factors for asthma morbidity. Further research is needed to characterize the relationship with stress at multiple levels to more fully understand and address asthma morbidity in children.

279 Does The Sinusitis Affect Bronchial Hyperresponsiveness In Asthmatic Children ?

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RATIONALE: Severe asthmatics are thought to have severe rhinitis. How the sinusitis affects bronchial hyperresponsiveness (BHR) in the asthmatic children has not been fully investigated. The aim of this study was to investigate whether sinusitis affects BHR in asthmatic children.

METHODS: The BHR in 69 patients (range 4-9 year-old) with asthma was evaluated during a stable period. A challenge test was performed with acetylcholine chloride (Ach). The patient inhaled Ach dilution until more than 20% decrease of FEV_1. We obtained the provocation concentration of Ach producing a 20% decrease in FEV_1 (PC_{20}). The FeNO was examined by the recommended on-line method. FeNO and lung function were performed before Ach challenge test and spirometry were performed. The sinusitis was evaluated by Waters X-ray. Forty-six patients were treated with inhaled corticosteroids (ICS).

RESULTS: Subjects with a sinusitis (n=26) exhibited an earlier onset of asthma ($p<0.05$) than ones without sinusitis (n=43) (median age 2 vs. 3). There are no differentiations between the two groups in PC_{20}, FeNO, pulmonary functional parameters, serum IgE and treatment with ICS.

CONCLUSIONS: Asthmatic children with sinusitis presented symptoms of asthma earlier than ones without sinusitis. The sinusitis does not affect severity of asthma during stable period.

280 Allergic Bronchopulmonary Mycosis. Are We Overdiagnosing In Cape Town, South Africa?

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RATIONALE: Allergic bronchopulmonary mycosis (ABM) is rare in paediatric patients with asthma. Asthmatics have sensitisation to moulds, but the incidence of ABM is unknown in children. The reference ranges for IgG to aspergillus in South African childhood asthmatics is not known, nor are intradermal tests to aspergillus done at our hospital.

METHODS: Over the past 22 months we have assessed 46 patients with difficult asthma for ABM and 30% have been treated for ABM. The median age of presentation was 117 months (IQR 114-128), and all had cystic fibrosis excluded. All patients were on high dose inhaled corticosteroids (ICS), and 12/14 (86%) were on long acting beta agonist and ICS combination therapy. Leukotriene agonists were used as add on therapy in 9/14 (64%) patients. The mean FEV1 was 76.5% predicted pre treatment.

RESULTS: The median total IgE was 3994kU/l (IQR 1611-1872), and the median specific IgE aspergillus was 13.8kU/l (IQR 1.55-21.3). The median specific IgG aspergillus was 14.94mg/ml (IQR 7.52-14.5). All patients were treated with oral corticosteroids. In four (28%) patients, steroid tapering was unsuccessful and azoles were added to wean steroids. One patient remains on alternate day low dose steroids.

CONCLUSIONS: The South African paediatric allergy experience with ABM is limited. Currently the treated patients are controlled and their asthma severity downgraded. All FEV1 are above 90% predicted, and have not relapsed clinically. Possibilities for our practice would be the introduction of intradermal skin tests, to establish our own reference range for IgG values to aspergillus and then treat according to a standardised protocol.

281 Risk Factors For Postoperative Pulmonary Complications After Noncardiothoracic Surgery In Adult Asthma Patients

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RATIONALE: Postoperative pulmonary complications (PPC) are common problems after noncardiothoracic surgery in the patients with chronic obstructive airway disease. Despite variable risk factors and preoperative management have been described for PPC, there are only a few studies that focused on asthma patients. The objective of this study is to investigate the incidence and risk factors of PPC after noncardiothoracic surgery with adult asthma patients.

METHODS: The electronic medical records at the Pusan National University Yangsan Hospital (Jan. 2009 ~ Dec. 2012) were retrospectively reviewed to identify asthma patients who had evaluated preoperative risk associated with asthma by respiratory physician before noncardiothoracic surgery. Clinical characteristics, history of previous asthma management and the results of preoperative examination were analyzed for determine their association with the occurrence of PPC.

RESULTS: Total of one hundred and twenty-seven patients was included and thirty-seven (29.1%) experienced PPC. Twelve patients (32.4%) had pneumonia, 9 (24.3%) bronchospasm, 7 (18.9%) atelectasis, 6 (16.2%) prolonged mechanical ventilation and 3 (8.1%) acute respiratory insufficiency. Risk factors for PPC were age, presence of respiratory symptoms and low FEV1. No significant difference between patients with or without PPC was found for gender, abnormal chest radiologic imaging.The development of PPC was associated with prolonged hospital stay ($p<0.05$).

CONCLUSIONS: The incidence of PPC after noncardiothoracic surgery with adult asthma patients was 29.1% and the most common PPC was pneumonia. Risk factors were age, presence of respiratory symptoms and low FEV1.

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282 Rates Of Comorbidities Are Related To Level Of Asthma Control

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RATIONALE: Co-morbidities have been postulated to contribute to poor asthma control. We hypothesized that patients with either very poorly controlled or not well controlled asthma would have a different incidence of co-morbidities than those with well controlled asthma.

METHODS: We compared the presence and absence of co-morbidities in well controlled vs. very poorly controlled or not well controlled asthma patients (N=2884) entered Into the Asthma IQ Program from either specialists (n=2614) or primary care physicians (n=270), including second hand smoke exposure from 1531 patients under age 18.

RESULTS: Obesity was significantly ($p<0.05$) more common among patients with either very poorly(18.5%) or not well controlled asthma(13.2%) vs. well controlled asthma(7.6%). Depression was also significantly more common in patients with very poorly controlled (4.6%) vs. not well(1.8%) or well controlled (1.1%) patients. GERD was less common in well controlled (10.5%) vs. not well (15.0%) or poorly controlled (18.3%) asthma. Rhinosinusitis was significantly more common in not well controlled (52.6%) vs. very poorly controlled (46.6%) or well controlled (46.4%) patients. Among children, a significantly higher percentage of those with not well controlled asthma (11.1 %) had second hand smoke exposure vs. well controlled asthma (6.9%).

CONCLUSIONS: Data from the Asthma IQ Program support the postulate that asthma patients whose asthma is not well controlled or very poorly controlled are more likely to have co-morbidities than those with well controlled asthma. A stronger emphasis on managing co-morbidities could help improve overall asthma outcomes.

283 Rates Of Co-Morbidities Are Related To Asthma Severity

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RATIONALE: Co-morbidities have been postulated to contribute to asthma severity. We hypothesized that patients with more severe asthma would have a different prevalence of co-morbidities than those with less severe asthma.

METHODS: We compared the presence and absence of co-morbidities in intermediate, mild persistent, moderate persistent, and severe persistent asthma patients (n=2884) entered into the Asthma IQ Program from either specialists (n=2614) or primary care physicians (n=270) including second hand smoke exposure from 559 patients under age 18.

RESULTS: Obesity was significantly ($p < 0.05$) more common among patients with severe persistent asthma (10.7%) than among those with intermittent disease (4.5%). GERD was significantly more common in moderate persistent (11.5%) vs. intermittent asthma (6.5%). Rhinosinusitis was significantly more common in severe persistent (63.1%) vs. moderate persistent (52.5%), mild persistent (47.0%), or intermittent disease (42.5%). Furthermore, patients with moderate persistent disease were significantly more likely to have rhinosinusitis than those with intermittent disease. Among children, those with mild persistent (12.6%), moderate persistent (12.4%) or severe persistent (11.8%) disease were significantly more likely to be exposed to second hand smoke than those with intermittent disease (5.0%). Depression was not significantly related to asthma severity.

CONCLUSIONS: Data from the Asthma IQ Program support the hypothesis that patients with more severe asthma are more likely to have comorbidities than those with less severe asthma. Awareness of these relationships can help physicians in managing more severe asthmatic patients.

284 Exercise-Induced Bronchospasm In Adolescents: Characteristics Of Lung Function and Accuracy Of Symptoms

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RATIONALE: Exercise-induced bronchoconstriction (EIB) describes acute airway narrowing that occurs after exercises; although evidence-based documents exist to guide health care providers with regard to its diagnosis, it is often in medical practice considering only the symptoms, without a confirmatory test, to determine BIE. The aim of this study is to estimate the prevalence of EIB among adolescent students with and without reported asthma and to evaluate the accuracy of symptoms in detection of EIB.

METHODS: Cross-sectional study with 220 adolescents students from 12 to 14 years. A bronchial provocation exercise-induced test was performed and symptoms questionnaire for asthma and EIB was applied. Besides to estimate the prevalence of EIB and accuracy of symptoms to detect BIE, we compared the fall in FEV1 between asthmatics and non-asthmatics. The significance level was 95%.

RESULTS: The prevalence of EIB was 19.1% (n = 42) (95% CI 14.0 to 24.0) and asthma was 18.6% (n= 41). Asthmatics with EIB showed significant decrease in FEV1 ($p = 0.000$) at 5 minutes post-exercise, showing increased earliness and severity of EIB when associated with asthma. Diagnosis of EIB by symptoms allocated 62 students. However, after bronchial provocation test, only 14 (22.6%) had a decrease > 10% in FEV1 (sensitivity 33%, specificity 73%, PPV: 22%, and NPV: 82%).

CONCLUSIONS: There was synergism between EIB and asthma in reducing FEV1 post-test. Suggestive symptoms of EIB have low accuracy, with unacceptable false positives and negatives values for diagnosis. The clinical relevance is to discourage doctors in diagnosing EIB based only by symptoms.

285 Variation Of Bronchial Hyperresponsiveness According To Age and Gender In Pediatric Population

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RATIONALE: Bronchial hyperresponsiveness (BHR) is a key feature of asthma that varies according to personal factors such as age and gender. We evaluated the prevalence of BHR according to age and gender in pediatric population and analyzed the risk factors for gender differences.

METHODS: Methacholine bronchial provocation test was performed in 2,030 children above 6 years old from daycare center to middle school in metropolitan area. The prevalence of BHR defined as provocative concentration of 20% decrease of FEV_1 (PC_{20}) below 8 mg/mL or 16 mg/mL was evaluated with age and each gender. The sexual difference of BHR prevalence at each age was calculated by Chi square test. In females, logistic regression analysis was performed to evaluate the risk factors for BHR.

RESULTS: The prevalence of BHR (PC_{20}≤8mg/mL or PC_{20}≤16mg/mL) among each gender was decreased with age (P value for trend < 0.001 in both gender). BHR prevalence (PC_{20}≤8mg/mL) showed increased tendency after 11 years old in females compared to that of males (males=1, females=13, P=0.019 at age 13). Also higher BHR prevalence (PC_{20}≤16mg/mL) was showed at age 12 (males=6, females=17, P=0.045) and age 13 (males=2, females=20, P=0.002). In logistic regression analysis, menarche (P=0.019, 95% CI 1.234-10.026) and allergic sensitization (P=0.005, 95% CI 1.393-6.561) had significant risk for BHR (PC_{20}≤8mg/mL) in females.

CONCLUSIONS: BHR was decreased with age in pediatric population. In females, menarche and atopy can explain the increased prevalence of BHR after puberty.

286 Nasal Provocation Test In The Diagnosis Of Mite Allergic Rhinitis : Standard Or Rapid?

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RATIONALE: Diagnosis of mite allergic rhinitis is difficult. Limited studies of Standard Nasal Provocation Test (SNPT) against mites have shown that about 20% of rhinitics sensitized to mite had negative NPT. SNPT is time consuming and impractical in routine. We develop a "rapid" NPT (RNTP). Objective: Determine the sensitivity and specificity of SNTP and compare the RNTP to SNTP.

METHODS: 91 patients (52 sensitized to mites and 39 controls) were included (mean age 27,7 years). Patients achieved the 2 tests separated for 4 weeks with Stallergènes Dpt extracts (Derp1 content:7.9 mg/ml for 100 IR). SNTP was positive if the nasal resistance increased more than 100% and/or if the clinical score was more than 5 points (score of Lebel-Bousquet). RNTP was positive only with a score greater than 5 points.

RESULTS: 27 men and 61 women were included. Skin prick tests to Dpt was 6.9 mm (+/-2.6). Sensitivity and specificity of SNTP were 94% and 100% . RNTP sensitivity was lower (79%) and specificity similar (98%). A significant correlation between the 2 tests was found (kappa 0.794 {0.668-0.920}). RNTP presented a significant correlation with the initiale dose of Dpt and serum specific IgE (r=0.31). Cumulative doses by nostrils were higher for the SNTP. No serious side effects occurred with the 2 tests.

CONCLUSIONS: We have validated SNRP in a young population susceptible to be candidate for immunotherapy. RNTP appears to be a good tool and could replace STNP to confirm the diagnosis of mite allergic rhinitis. Study supported by Stallergènes Laboratories ®.

287 Nocturnal Asthma In Latino Children Is Associated With Severe Disease and Allergenic Triggers (GALA II Study)

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RATIONALE: Nocturnal asthma in Latino asthmatics is not well-characterized.

METHODS: We utilized data from a large study in asthmatic Latinos(GALA II Study) conducted in 5 cities in the United States and Puerto Rico from 2008-2011. Data included questionnaire, allergy skin test, serum IgE and lung function. We used logistic regression to determine factors associated with nocturnal asthma.

RESULTS: Among 1992 Latino asthmatic subjects aged 8-21 years, 1099(55%) reported trouble sleeping because of asthma over the previous year. Comparison of baseline characteristics between those with and those without nocturnal asthma were similar with respect to age(mean+/-SEM: 12.4+/-0.1 vs.12.7+/-0.1), gender(54% vs.56% males) and BMI-percentile(73%ile+/-1 vs.74%ile+/-1). Compared with asthmatics without sleep trouble, those with nocturnal asthma had slightly lower FEV1%-predicted(89.3%+/-0.5 vs.92.8%+/-0.5, p<0.0001), higher plasma IgE(612+/-30 vs 435+/-27 kU/L, p<0.0001), higher frequency of severe asthma(55% vs. 29%, p<0.001), were taking >1 controller medications(19% vs. 12%, p<0.001), missed more school days in the previous 12 months(74% vs. 29%, p<0.001), and were of Puerto Rican ethnicity(56% vs. 35%, p<0.001). Logistic regression adjusted for demographics, plasma IgE, BMI, smoking, ethnicity, and education level revealed modifiable risk factors associated with nocturnal asthma such as presence of indoor cat(OR=1.62[95%CI=1.14-2.32], p=0.007), dog(OR=1.3[95%CI=1.02-1.62], p=0.03), and to the following environmental triggers of asthma symptoms: pollen(OR=1.59 [95%CI=1.22-2.05], p<0.001), house dust(OR=1.81[95%CI=1.40-2.32], p<0.001), pets(OR=1.87[95%CI=1.38-2.52], p<0.001), and mold(OR=2.30[95%CI=1.76-3.00], p<0.001). Nocturnal asthma was not associated with positive skin testing to aeroallergens.

CONCLUSIONS: Nocturnal asthma in Latino youth is associated with more severe disease, Puerto Rican ethnicity, plasma IgE, and allergenic triggers. Avoidance of triggers may improve nocturnal symptoms and disease severity.

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288 Nasal Challenge With 50mgs Of L-Aspirin For Diagnosis Of ASA Exacerbated Respiratory Disease

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RATIONALE: The ASA exacerbated respiratory disease is an aggressive and continuous inflammatory disease of the airways. The diagnosis can be established with provocation test using increasing doses of ASA, the oral test has the higher sensitivity.

METHODS: We assigned three groups of 20 patients. Group 1 were patients with AERD, group 2 were patients with asthma and nasal polyps, and group 3 were healthy volunteers. We administrated 50 mgs of L-aspirin in each middle tourbinate and 30 minutes after we performed another spirometry and rhinomanometry, it was considered positive if the FEV1 felt down 20% or more or the nasal flow decreased 40% or more. At the same time we recorded the clinical changes in the patients

RESULTS: With the clinical changes we had a sensibility of 38.2% and a specificity of 96.2%. If the FEV1 felt down at least 20% or more, it had a sensibility of 50% and a specificity of 96%, , and if the nasal flow felt down at least 40%, it had a sensitivity of 73.5% and a specificity of 96%, and if we had at least 1 positive result of the three parameters we had a sensitivity of 85% and a specificity of 96%.

CONCLUSIONS: The intranasal challenge is a safe and practical study for the diagnosis of AERD, and has a sensitivity and specificity similar to the oral challenge without the hospitalizations and the risk of severe reactions

289 Clinical Usefulness Of Bronchial Mannitol Provocation Test In Children With Asthma Symptoms

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RATIONALE: Airway hyperresponsiveness (AHR) to inhaled mannitol are related to allergic inflammation characterized by eosinophil infiltration and a clinical response to treatment with anti-inflammatory agents in subjects with asthma. The aim of this study was to examine the clinical usefulness of mannitol provocation test in a pediatric population presenting with asthma symptoms.

METHODS: Children aged 5-17 years, referred for possible reactive airway disease to the Pediatric Allergy and asthma Department of Severance Children's Hospital from March in 2010 to February in 2013. Bronchial provocation test with mannitol dry powder (MDP) (AridolH, Pharmaxis, Australia) and methacholine, exhaled NO measurement and sputum induction were performed. Data of patients with airway hyperresponsiveness to mathacholine was only positive and to mannitol was only positive and both test positive were compared.

RESULTS: One hundred and thirty five children completed mannitol provocation test (mean age 9.6 years). Sixty-six (48.9%) patients had positive result both mannitol and methacholine provocation test. (PC_{20}=5.1 mg/mL, PD15=195.1mg) Twenty-eight(20.7%) patients had only positive results to mannitol. In 24 patients, response to mannitol was negative but was positive to methacholine. There was no difference in sex, age, blood eosinophil, serum total IgE and ECP level between three groups. The geometric mean of PD15 mannitol values are low in patients with elevated exhaled nitric oxide (FeNO) level. ($p<0.05$) But, There is no association with the geometric mean of PC20 methacholine values and FeNO level.

CONCLUSIONS: Mannitol provocation test demonstrate AHR and may be a clinically useful marker for assessing the airway inflammation of asthma at once.

290 Salivary Alpha Amylase Activity Is a Potential Surrogate Biomarker For Inhaled Beta-2 Agonist Responsiveness

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RATIONALE: Salivary alpha-amylase activity (sAA) is an indirect method of measuring beta-receptor activation. Increases in sAA correlate with the activation of beta-receptors in isoproterenol -infused mice and in humans under physical or mental stress. Thus we hypothesize that sAA levels will parallel albuterol-induced increases in airway beta-2 receptor activity.

METHODS: Seven healthy controls and seven asthmatics were enrolled. Spirometry, sAA and vital signs were collected at baseline and 15, 30, 60, and 120 minutes after inhaled albuterol.

RESULTS: Baseline FEV1 (% predicted) in controls was 95+/-13.3 (% predicted, mean+/-SEM) and 87.9+/- 2.9 in asthmatics. Fifteen minutes after albuterol inhalation, FEV1 improved to 93.1+/-2.8 through 120 minutes ($p<0.01$ compared to baseline) in asthmatics. As expected, FEV1 did not change from baseline for controls. Albuterol inhalation induced increases in sAA from baseline in both groups at all time points measured, with a peak at 15 minutes. For controls, baseline sAA(U/mL) was 39.6+/-12.2, with a peak of 217.9+/-40 ($p<0.001$ compared to baseline). For asthmatics, sAA increased from a baseline of 82.4+/-14.6 to a peak of 221.3U/mL+/-68 ($p<0.05$). The increase in sAA paralleled the increase in FEV1 in asthmatics. No statistical difference in blood pressure or pulse from baseline was found after albuterol inhalation.

CONCLUSIONS: This is the first study to demonstrate that inhaled albuterol increases sAA. Parallel increases in FEV1 and sAA in asthmatics suggest that sAA is a surrogate marker for beta-2 receptor activation upon albuterol inhalation. sAA may be useful for assessing the mechanisms of tachyphlaxis or unresponsiveness to albuterol.

291 Feno Decreases Significantly During Improved Symptom Control In Pediatric Patients After Acute Asthma Exacerbations Necessitating Emergency Department Care

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RATIONALE: Fractional exhaled nitric oxide (FeNO) is elevated in pediatric patients during acute asthma exacerbations. We sought to determine whether patients with high FeNO values during acute exacerbations have meaningful decreases during improved symptom control.

METHODS: Participants were selected from a cohort of children aged 5 – 17 years who had FeNO measurement previously performed in our pediatric emergency department (ED) during acute asthma exacerbations. We recruited participants who had FeNO values above the median value for this cohort. Exclusion criteria included a febrile illness, systemic corticosteroid treatment, or an acute exacerbation necessitating ED care within the preceding 4 weeks. FeNO was measured and each participant completed a questionnaire to ascertain asthma severity and current asthma medications. We performed a multivariable linear regression model accounting for the repeated measures to calculate the adjusted mean difference of FeNO at follow-up compared to the value during the acute exacerbation.

RESULTS: Between April 11 and September 4, 2013, we enrolled 42 participants with median [IQR] age 12 [11, 15], male gender 22 (52%), and African-American race 13 (31%). Median FeNO at the time of prior exacerbation was 57 ppb [44, 77] and at the follow-up visit was 40 ppb [24, 68]. Using multivariable regression analysis, there was an adjusted 36% (95% CI: 12%, 53%; p<0.01) decrease in FeNO level at follow-up compared to values obtained during exacerbations.

CONCLUSIONS: Pediatric patients with elevated FeNO during acute asthma exacerbation have lower values of eNO at a time of improved symptom control.

292 Risk Factors Of High Feno Levels & Cut-Off Value Of Feno In Elementary School Children With Asthma

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RATIONALE: Fractional exhaled nitric oxide (FeNO) may serve as a non-invasive marker of airway inflammation in asthma. However, the relationship between FeNO levels and variable factors has not been evaluated in a general population of children. The aim of this study was to provide the risk factors influencing FeNO levels and cut off values of FeNO.

METHODS: A total sample of 1,080 school children was divided into two groups; a group of children (6-13 years of age) with physician–diagnosed asthma and a control group. Bronchial hyperresponsiveness (BHR), blood eosinophil counts, serum total IgE levels, skin prick tests, lung function tests, and FeNO levels were evaluated in all children.

RESULTS: BHR, blood eosinophil counts, serum total IgE levels, and positive reactions on skin prick tests were significantly associated with FeNO levels. However, results of lung function tests were not associated with FeNO levels in the total population. Risk factors for high FeNO levels were atopy, blood eosinophil counts, serum total IgE levels, and BHR (aOR: 5.15, 4.19, 3.76, 1.95, respectively, $P<0.05$). Cut–off value of FeNO in asthmatic children with BHR (PC20 < 16 or <8) was 13.5 ppb (PC20<16: sensitivity 87.5%, specificity 65.8%, $P=0.005$, AUC [area under curve] 0.744; PC20<8: sensitivity 90.9%, specificity 59.5%, $P=0.009$, AUC 0.758).

CONCLUSIONS: This study suggests that FeNO levels can be a useful biomarker to predict the BHR, serum eosinophil counts, total IgE levels, and positive skin prick tests.

293 Metabolomic Profiles Of Exhaled Breath Condensate In Asthmatics

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RATIONALE: Exhaled Breath Condensate (EBC) contains volatile and non-volatile components of airway lining fluid. Characterizing components of EBC may be helpful in establishing biomarkers useful in the evaluation and management of asthma. We hypothesize that distinct metabolite profiles exist in the EBC of asthmatic adults compared to healthy controls.

METHODS: Adults with asthma and healthy controls were recruited through the University of Texas Medical Branch and enrolled into this non-randomized controlled cross-sectional study. Spirometry, EBC, basic medical history and demographic information were collected from subjects. EBC was analyzed using nuclear magnetic resonance (^{1}H-NMR) imaging and mass spectrophotometry (MS).

RESULTS: We observed significant differences in the metabolomic profiles of EBC from asthmatics compared to healthy controls at 500-MHz ^{1}H-NMR spectral regions 0.77-0.83 ppm, 1.09-1.39 ppm, 1.94-2.18 ppm and 3.3-3.6 ppm.

CONCLUSIONS: Characteristic metabolomic profiles exist in the EBC of asthmatics compared to healthy controls. EBC may be a useful tool in distinguishing asthma phenotypes.

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294 Extended Nitric Oxide Analysis and Bronchial Hyperresponsiveness In Children With Asthma According To Atopy

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RATIONALE: Extended nitric oxide analysis such as bronchial NO and alveolar NO has been studied ardently because it is not affected by exhaled flow rate. The aim of this study is to evaluate whether extended nitric oxide analysis parameters is correlated with BHR.

METHODS: A total 117 children with a typical asthmatic symptom was evaluated. All children performed exhaled nitric oxide test and methacholine challenge test for BHR ($PC_{20} \leq 25$ mg/ml) on the same day. We measured exhaled nitric oxides at 30, 50, 100, 200 mL/s and defined $FeNO_{50}$ at 50 ml/s exhaled flow rate. Atopy was defined as a positive skin test result or a more than 0.7 KUa/L specific IgE or a more than 150 IU/ml total IgE.

RESULTS: Among 117 children, atopic subjects were 75. In atopic subjects, J'awNO (total NO flux from the airways) (r=-0.388, p=0.001) and $FeNO_{50}$ (r=-0.353, p=0.002) were correlated with PC_{20} significantly. On the receiver operating characteristics (ROC) curve for deciding the BHR, area under the curve (AUC) of J'awNO and $FeNO_{50}$ were 0.716 (p=0.002) and 0.703 (p=0.003), respectively. In non-atopic subjects, only CawNO (airway wall NO) showed the significant correlation with PC_{20} (r=-0.331, p=0.032) and the AUC of the ROC curve of CawNO for deciding the BHR is 0.741 (p=0.023).

CONCLUSIONS: The usefulness of $FeNO_{50}$ and extended nitric oxide analysis parameters for predicting of BHR was different according to atopy. The J'awNO in the atopy and the CawNO in the non-atopy were able to be suggested as a useful tool to predict BHR in children with asthma.

295 Reference Values and Determinants Of Fractional Concentration Of Exhaled Nitric Oxide (FeNO) In Healthy Children

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RATIONALE: Measurement of the fractional concentration of exhaled nitric oxide (FeNO) is a quantitative, noninvasive, simple and safe method of assessing airway inflammation. FeNO measurement has been standardized, but reference values for children in the general population are not yet in Korea.

METHODS: FeNO was measured according to American Thoracic Society guidelines with a chemiluminescence analyzer (NIOX Exhaled Nitric Oxide Monitoring System, Aerocrine, Sweden). FeNO was measured in 295 preschool children and 808 elementary school children defined as healthy controls.

RESULTS: Geometric mean of FeNO were 9.66 (95% confidence interval [CI] 6.71-12.59) ppb, 15.25 (95% CI 11.81-18.69) ppb and 11.22 (95% CI 7.95-14.48) ppb in non-atopic, atopic, and all healthy controls in children, respectively. The FeNO reference equations that determined by multiple linear regression analysis, taking into account the variables of age, height, weight, total IgE, eosinophil percent, and bronchial hyper-responsiveness (methacholine PC_{20}) were as follows: FeNO = 0.914 + 0.003 × total IgE + 0.363 × eosinophil percent, coefficient of determination (R^2) = 0.096 in the healthy non-atopic controls; FeNO = -14.564 + 1.388 × eosinophil percent – 2.003 × log methacholine PC_{20}, R^2 = 0.213 in the atopic healthy controls; FeNO = -9.036 + 0.132 × Height + 0.005 × total IgE + 1.007 × eosinophil percent, R^2= 0.210 in the all healthy controls.

CONCLUSIONS: This study provides reference values for FeNO that can be used to evaluate airway inflammation in healthy children. This study assesses affecting factors to FeNO values in preschool children and elementary school children.

296 Improvement Of FENO In Youth With Asthma After Attending An Asthma Summer Camp

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RATIONALE: Measurement of Fractional exhaled Nitric Oxide (FENO) has been proposed as a measure of compliance with asthma therapy. We hypothesized that measurements of FENO would be significantly lower after 1 week of asthma camp as a result of daily education sessions and supervised asthma therapy administration.

METHODS: FENO measurements were obtained from 59 children with asthma at the beginning and end of a five day asthma education camp. FENO values were compared for the entire cohort and also dichotomized by initial FENO measurement. A FENO measurement greater than one standard deviation above the mean was considered significantly elevated. Significance was determined by Wilcoxon signed-rank test, pre and post camp value correlation was determined using Pearson coefficient.

RESULTS: There were no significant differences between pre (24ppb S.D. 22) and post (21ppb S.D. 20) camp FENO measurements for the entire cohort of 59 subjects. FENO values before and after camp correlated well (r=0.78). Of the 59 participants 5 subjects initially had elevated FENO levels with an average of 79 ppb that significantly improved to 56 ppb by the end of camp (p=0.05).

CONCLUSIONS: The good correlation between pre and post camp FENO values indicates consistency of measurement in this group. While the population as a whole did not show significant change in FENO due to supervised medication administration at asthma camp, the significant decline in FENO among the group entering camp with significantly elevated FENO suggests a benefit of supervised therapy and possibly a lack of compliance prior to asthma camp.

297 Exhaled Nitric Oxide (FeNO) and T-Helper 2 Cell Biomarkers: Can They Predict Treatment Response To Dupilumab, An IL-4Rα Antibody, In An Eosinophilic Asthma Population?

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RATIONALE: Dupilumab (DPL), a fully human monoclonal antibody against IL-4Rα, inhibits both IL-4 and IL-13 signaling, drivers of Th2 inflammation. Biomarkers may guide therapeutics by documenting the time course of effect or predicting response.

METHODS: Adults with moderate to severe uncontrolled eosinophilic asthma (blood eosinophils [EOS] ≥300 cells /μL or sputum EOS ≥3%) were treated with DPL 300 mg (n=52) or placebo (PBO; n=52) subcutaneously once weekly for 12 weeks. ICS and LABA were withdrawn between Week 4 (W4) and W8. Clinical response assessments included incidence of asthma exacerbations and changes in FEV1, FeNO and Th2-associated biomarkers in blood.

RESULTS: DPL caused a decrease (mean % change from baseline) in FeNO (W4: -40 DPL vs. -5 PBO; W12: -29 DPL vs. +35 PBO), TARC (W4: -29 DPL vs. +7 PBO; W12: -26 DPL vs. +8 PBO), eotaxin 3 (W4: -37 DPL vs. +3 PBO; W12: -46 DPL vs. +5 PBO) and IgE (W4: -10 DPL vs. +14 PBO; W12: -37 DPL vs. +6 PBO) irrespective of ICS/LABA treatment. YKL-40 was not affected. Periostin increased (W4: +5 DPL vs. +7 PBO; W12: +24 DPL vs +26 PBO). In some patients, EOS increased on DPL. FEV1 changes correlated inversely with changes in FeNO at W8 and W12. A consistent treatment effect of DPL was observed irrespective of EOS counts and biomarker levels at baseline.

CONCLUSIONS: In a population preselected for eosinophilia, several biomarkers associated with the Th2 phenotype decreased during treatment, but none further defined a subpopulation more responsive to treatment.

298 Asthma Management Costs With Feno In Addition To Standard Guidelines

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RATIONALE: Published data have shown that healthcare practitioners choosing to incorporate Fractional Exhaled Nitric Oxide (FeNO) assessment into their asthma management practice may experience improved patient outcomes, including reductions in the number of asthma exacerbations experienced, as well as in the severity of the exacerbations. Data are presented to evaluate asthma management costs with FeNO in addition to standard guidelines.

METHODS: A single-center, observational study evaluated the impact of FeNO assessments on asthma management decisions in 50 subjects aged 7 to 60 years with asthma. Standard asthma assessments, including spirometry and ACT questionnaire were performed. Healthcare practitioners (HCPs) normally assessed patients and made treatment decisions. FeNO was subsequently measured and HCPs had an opportunity to review the results and adjust treatment based on FeNO.

RESULTS: Overall, 18/50 (36%) of subjects had their prescriptions altered, most commonly by the addition (increases) or deletion (decreases) in ICS therapy. Based on published cost estimates, use of FeNO in addition to standard guidelines has the potential to save approximately $600/year in asthma management costs per patient. If this cost analysis is applied to the 18 patients who had their prescriptions altered, continued use of FeNO in addition to the standard guidelines may save nearly $11K in the first year and potentially save as much as $54K in asthma management costs over a 5-year timeframe.

CONCLUSIONS: Knowledge of FeNO augments routine clinical assessment of airway inflammation and affects medication treatment decisions; both have important cost effectiveness implications.

299 Developmental Assessment Of Serum Periostin As An Asthma Biomarker In Children

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RATIONALE: Increased periostin levels in peripheral blood have been identified as a biomarker of type 2 airway inflammation in adult patients with asthma, which may have important clinical implications for individualized therapy. In children, *in vitro* studies have demonstrated that airway epithelial cells from children with allergic asthma express greater periostin in comparison to children without asthma. We aimed to explore serum periostin as a potential systemic biomarker of asthma in children.

METHODS: A total of 288 children from the Childhood Origins of ASThma (COAST) study were followed prospectively from birth. Serum samples were collected at ages 2, 4, 6, and 11 years, and periostin was measured using a proprietary immunoassay developed by Genentech. Relationships among age, asthma, and periostin were assessed.

RESULTS: Serum periostin levels were approximately 2-3 fold higher in children than those previously observed in adults. Levels were highest at 2 years of age ($p<0.0001$), and did not change significantly between ages 4 years and 11 years. Children who developed asthma by age 6 years had increased serum periostin at ages 2 years ($p=0.02$), 4 years ($p=0.002$), 6 years ($p=0.12$), and 11 years ($p=0.01$) compared to children who did not develop asthma by age 6 years.

CONCLUSIONS: Serum periostin levels are significantly higher in children compared to published adult values and change developmentally, which may be due to bone turnover and growth. Despite this, serum periostin appears to be a predictor of childhood asthma development and warrants further study as a promising biomarker in preschool children.

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300 T-Cell Profiles In Bronchoalveolar Lavage (BAL) Of Wheezing Children With and Without A History Of Respiratory Syncytial Virus (RSV) Lower Respiratory Tract Infection

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RATIONALE: No data on airway T cell subsets in "wheezing" children (WC) is available. CD25, found on activated T cells and CD45RO "memory"cells, associated with T cell development, T cell activation and IgE synthesis, were analyzed in BAL from WC and normals.

METHODS: We evaluated 18 WC with BAL. WC were further subdivided into children with history of RSV (WC+RSV)(n=10, median age 17.0 months) or without RSV (WC-RSV)(n=8, median age 9.3 months). Comparisons were made with normal controls (Control)(n=5, median age 19.5 mos). BAL cell counts and differentials were determined. Cell blocks were cut and immunohistochemically stained for the following T cell surface markers: CD3, CD4, CD8, CD25, and CD45RO. Co-expression of CD25 or CD45RO on CD3, CD4, and CD8 T cells was evaluated by an NIH image analysis program on sequential sections.

RESULTS: The total number of CD3 cells was significantly elevated in the WC+RSV compared with the Control (p=0.008). WC+RSV had significant (p=0.003) elevations in # of CD45RO cells/1000 cells compared to Control and the total number of CD45RO was significantly elevated in the WC+RSV compared with the WC-RSV (p=0.033) and the Control (p=0.001). Although the % of CD4 T cells expressing CD45RO was greater in WC+RSV than WC-RSV, the elevation was only statistically significant compared to Control (p=0.005).

CONCLUSIONS: These findings suggest that specific T cell profiles exist in WC+RSV which differ from WC-RSV and Control. Furthermore CD45RO cells may play a role in the immune processes of WC+RSV.

301 Increased Blood Th2-Like Invariant Natural Killer T Cells In Patients With Asthma

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RATIONALE: Invariant natural killer T (iNKT) cells might play an important role in the pathogenesis of asthma in humans. Our previous study has shown no difference in the number of blood iNKT cells between asthmatics and controls. However, few studies have reported about the function of blood iNKT cells in human asthma.

METHODS: Twenty asthmatics and eight controls were included. Blood iNKT cells were identified with double staining with both anti-Vα24 and anti-Vβ11mAbs or both 6B11 and anti-Vβ11mAbs. Intracellular IL-4, IL-10 and IFN-γ cytokines were stained in blood iNKT cells with the respective mAbs and isotypes, and their relationships with clinical parameters were analyzed.

RESULTS: The number of Vα24+Vβ11+ iNKT cells or 6B11+Vβ11+ iNKT cells did not differ between asthmatics and controls. However, among Vα24+Vβ11+ iNKT cells, the proportion of IL-4+ cells of Vα24+Vβ11+ iNKT cells was increased in 20 asthmatics (7.0 ± 3.0%) compared with 8 controls (0.5 ± 0.4%, $p < 0.05$). There were no differences in the proportion of IL-10+ or IFN-γ+ cells of iNKT cells between asthmatics and controls. Proportion of IL-4+ cells of 6B11+Vβ11+ iNKT cells was inversely correlated with FEV_1 expressed as % predicted value in asthmatics ($Rs = -0.64$, $p < 0.05$, $n = 19$).

CONCLUSIONS: Blood iNKT cells are thought to be Th2-like type in human asthma, and IL-4-producing iNKT cells may be associated with lung function, indicating that a new drug regulating Th2-like iNKT cells may control refractory asthma in humans.

302 Establishment and Treatment Of a Steroid Resistant Asthma Model By Adoptive Transfer Of Helper T Cell Clones

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RATIONALE: To investigate the role of helper T (Th) cells in steroid resistant (SR) asthma, steroid sensitive (SS) and resistant (SR) Th clones were selected *in vitro*, and then adoptively transferred into unprimed mice. Effect of CTLA4-Ig was analyzed both *in vitro* and *in vivo*.

METHODS: For *in vitro* evaluation, ovalbumin (OVA) reactive Th clones were cultured with antigen presenting cells and OVA in the presence of various concentrations of dexamethasone (DEX). Proliferative responses of Th clones were measured by ^{3}H-thymidine incorporation. For *in vivo*assessments, unprimed BALB/c mice were transferred with Th clones, challenged with OVA, and administered with DEX subcutaneously. Bronchoalveolar lavage fluid (BALF) was obtained 48 hr after challenge, and the number of infiltrating cells was differentially counted. CTLA4-Ig was administered through nasal inhalation or venous injection.

RESULTS: SS and SR clones were selected based on the effect of DEX on the proliferative responses of antigen-stimulated Th clones. Airway infiltration of eosinophils and lymphocytes of mice transferred with SS clones were effectively inhibited by the administration of DEX. In contrast, those of mice transferred with SR clones were not significantly inhibited by DEX. Administration of CTLA4-Ig significantly suppressed the proliferation of DEX-treated SR clones *in vitro*, and the eosinophil infiltration of SR asthma model transferred with SR clones *in vivo*.

CONCLUSIONS: Steroid sensitivity of Th clones assessed *in vitro* was consistent with that of adoptively transferred asthma model assessed *in vivo*. Costimulatory signal mediated through CD28 is crucial for the induction of steroid resistance both *in vitro* and *in vivo*.

303 CD4+ T Cells From Nasal Polyp Explants Contain Abundant Th2 Cells Expressing Functional Interleukin-25 Receptors Together With Th17 Cells

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RATIONALE: Chronic rhinosinusitis with nasal polyposis (CRSwNP) in western countries is characterized by eosinophilia and IgE production. Surprisingly the local T cell response in polyposis remains poorly characterized *in vitro*. We previously showed that T cell phenotypes compartmentalize in the healthy nasal mucosa, emphasizing the importance of studying local responses. We sought to test our hypothesis that the local T cell response in CRSwNP is associated with Th2 and non-Th2 pathogenic effector populations that may represent therapeutic targets.

METHODS: Polyp tissue and blood were obtained from patients undergoing nasal polypectomy. Healthy nasal biopsies were obtained as controls. Tissue was cultured in an explant model and surface phenotype/intracellular cytokines assessed by flow cytometry and/or immunostaining.

RESULTS: Polyp-derived cells contained a discrete population of IL-25R$^+$CD4$^+$ T cells, which were not present in peripheral T cells or controls (polyp mean = 18.8% vs. blood 1.9% (n=12; p=0.001), vs. healthy biopsy 2.4% (n=7; p=0.002). IL-25R$^+$CD4$^+$ cells expressed IL-5 and IL-13 that was increased by IL-25 addition. Immunohistochemistry suggested the predominant source of IL-25 in polyps to be epithelial cells. Polyp- and biopsy-derived but not peripheral cells contained significant CD4$^+$CCR6$^+$IL-17$^+$ T cells, consistent with Th17 cells.

CONCLUSIONS: This is the first demonstration of human explant-derived IL-25R$^+$CD4$^+$ Th2 cells obtained directly from tissue with eosinophilic inflammation. IL-25R is functional since these cells respond to IL-25 *in vitro* with augmented Th2 cytokine production. The presence of putative Th17 cells in polyp and healthy nasal mucosa suggests that these cells may represent the default T effector phenotype in normal nasal mucosal immunity.

304 Successful Desensitization To Paclitaxel For Stage 4 Ovarian Cancer

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RATIONALE: Paclitaxel is a taxane chemotherapeutic agent that has a rare side effect of hypersensitivity reactions. We describe our experience with an effective desensitization protocol following an anaphylactic reaction in a 66 year-old female patient with stage 4 ovarian cancer.

METHODS: A patient was unable to complete chemotherapy after developing anaphylaxis on 2 prior cycles of paclitaxel, and was referred to an allergist consultant. Premedication prior to desensitization included dexamethasone, diphenhydramine, and famotidine were administered 13 hours, 7 hours, and 1 hour prior to the infusion of 250 mg of Paclitaxel to our patient who was monitored in the intensive care unit. Divided doses of Paclitaxel were given in 1:100000, 1:10000, 1:1000, 1:100, and 1:10 graded dilutions and infused over 3 hours. Post-medication was provided with dexamethasone and an oral prednisone taper to prevent biphasic sequellae

RESULTS: The patient tolerated the desensitization protocol well with the premedication of dexamethasone + diphenhydramine + famotidine. The graded dilutional desensitization approach infused over 3 hours allowed the patient to achieve tolerance to the drug without any significant adverse events and resume her cycles with paclitaxel.

CONCLUSIONS: This protocol of graded desensitization in a closely monitored setting permits the continuation of life sustaining antineoplastic treatment protocols in patients that have varying degrees of hypersensitivity to paclitaxel.

305 IL-17 Plays a Major Role In Driving The Recruitment Of B Cells Into Bronchial Tissue Of Asthmatic Patients

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RATIONALE: B cells play an important role in asthma development mostly via the production of IgE. In this proposal, we hypothesized that IgE is increased in lung tissue of asthmatic patients due to increased infiltration of B cells to this tissue. We also investigated the possible involvement of IL-17 in the migration of B cells to the mucosal surface of the airways.

METHODS: We determined the number and pattern of infiltrated B cells into bronchial biopsies from asthmatic versus healthy subjects by staining for B cells marker (CD20) using Immunohistochemistry. Migration of B cells towards Th-17 cytokines were examined using Boyden Chamber migration assay. Mechanism of IL-17 induced B cell migration were tested using MAP kinase inhibitors to determine IL-17 induced MAP kinase pathways involved in this process.

RESULTS: The number of CD20 positive cells in asthmatic biopsies was significantly higher than those in healthy subjects. Interestingly, we have also observed an increase in B cells lymph follicle numbers in asthmatic airways compared to healthy subjects. Although B cells were shown to migrate in vitro towards both IL-17A and IL-17F, lower concentrations of IL-17F, compared to IL-17A, were sufficient to induce migration. Blocking IL-17 signaling using either anti-IL-17R antibodies or p38 MAP kinase inhibitors prevented in vitro migration of B cell towards IL-17.

CONCLUSIONS: These results indicated that IL-17 might drive the migration of B cells in the lung tissues of asthmatic patients. Activation of p38 MAP kinase seems to be required for IL-17 activity on B cells.

306 A Comparison Of Asthma Prevalence and Severity Among Urban and Rural African American Teenage Youth

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RATIONALE: The high prevalence of asthma in African American (AA) populations residing in urban areas of the United States has attracted major attention and research, yet the prevalence of asthma in rural areas is poorly documented.

METHODS: The prevalence of asthma was compared in 7550 youth attending six public high schools in urban, Detroit, MI and in 2523 youth attending four public high schools in rural Georgia (GA) counties. Using the Lung Health Survey, diagnosed asthma was defined as a physician diagnosis and symptoms in the past 12 months while undiagnosed asthma was defined as respiratory symptoms in the past 12 months with no physician diagnosis. Chi-square tests were used to compare prevalence by location.

RESULTS: In Detroit, 7235 (95.8%) youth were AA compared to 1514 (60.0%) in GA. Average population density in high school ZIP codes was 5628 people/mi^2 in Detroit compared to 45.1 people/mi^2 in GA. Prevalence of diagnosed asthma was 20.2% in Detroit and 19.4% in GA. Undiagnosed asthma prevalence was 7.7% in Detroit and 7.5% in GA. Detroit youth with a diagnosis experienced more symptom-days, nights awakened, days needing medication, changed plans, and school missed in the prior 30 days compared to GA counterparts ($p<0.005$). However, the prevalence of symptoms was similar among the undiagnosed youth.

CONCLUSIONS: Among AA youth, asthma prevalence is remarkably similar in urban Detroit and rural GA. For those with diagnosed asthma, symptoms were more commonly reported in the Detroit population, but this pattern was not observed among those with undiagnosed asthma.

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307 Interleukin-4 and Transforming Growth Factor-Beta Single Nucleotide Genes Polymorphisms Confer Susceptibility To Atopic Dermatitis

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RATIONALE: Atopic dermatitis (AD) is a chronic inflammation of the skin, seems to have a strong genetic background. Interleukin-4 (IL-4) is one of the cytokines implicated in promotion of allergic diseases and transforming growth factor-beta (TGF-β) is an anti-inflammatory cytokine promoting counter-regulatory responses to IL-4. This study aimed to investigate association of polymorphisms at genes encoding IL-4 or TGF-β with susceptibility to AD.

METHODS: This case-control study comprised 89 children with established diagnosis of AD and 139 healthy controls. Single nucleotide polymorphisms at *IL-4* -1098T>G (rs243248), -590C>T (rs2243250), -33C>T (rs2070874), and *TGF*-β codon10C>T (rs1982073) and codon25G>C (rs1800471) were typed. Frequencies of alleles, genotypes and haplotypes were estimated and compared between patients and controls.

RESULTS: Patients with AD had significantly higher percentage of T allele at *IL-4*-1098T>G (84.4%) and C allele at *IL-4*-590C>T(85.0%) and *IL-4* -33C>T (84.4%, all p<0.001). TT genotype at *IL-4*-1098T>G [OR 3.65, 95%CI (1.99-6.71)] and CC genotype at both *IL-4*-590C>T [OR 31.75, 95%CI (13.61-76.07)] and *IL-4*-33C>T [OR 3.52, 95%CI (1.92-6.52)] were overrepresented in AD patients (p<0.001). Consistently, TCC haplotype was significantly more frequent in AD patients [(OR 5.15, 95%CI (3.28-8.08), p<0.001]. Considering *TGF*-β gene in AD patients, C allele was significantly higher at both loci and CC at codon10C>T [OR 15.34, 95%CI (7.55-31.55)] and CG at codon25G>C (100%) were overrepresented (p<0.001). CC haplotype of *TGF*-β was overrepresented in patients [(OR 6.86, 95%CI (3.88-12.21), p<0.001)].

CONCLUSIONS: Considering higher frequencies of specific genotypes of both *IL-4* and *TGF*-β in patients with AD, the gene polymorphisms of these cytokines could confer susceptibility to AD.

308 A Comparison Of Regulatory T-Cell Receptor Vβ (3,5) Expression In Patients With Food Allergy and Atopic Dermatitis

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RATIONALE: Regulatory T (Treg) cells play an important role in the maintenance of self-tolerance, and patients who lack these cells have an increased incidence of atopic dermatitis (AD) and food allergies (FA). Staphylococcus aureus superantigenic toxins have been shown to be important in AD, but their role in FA and their potential influence on Treg cells is not well understood. To explore the interplay between Staphylococcus superantigens and Treg cells in atopy, we characterized Treg cell phenotypes and T-cell receptor (TCR) Vβ (3,5) chain superantigen specificity in patients with FA and AD.

METHODS: Peripheral blood mononuclear cells were isolated from 21 patients recruited at the Ann & Robert H. Lurie Children's Hospital of Chicago and analyzed using flow cytometry as part of an ongoing study. Populations of Treg cells, defined as $CD4^+CD25^+CD127^-Foxp3^+$, were isolated and percentages of TCR Vβ (3,5) expression were compared using the Mann-Whitney-Wilcoxon test between the following 4 patient groups: +FA and +AD (4), -FA and +AD (0), +FA and -AD (7), and +FA and +AD (10).

RESULTS: Percentages of TCRs expressing Vβ (3,5) were significantly higher (p<0.01) in $Foxp3^+$ T cells of patients with FA who did not have AD compared to patients with FA and AD. There was a trend toward significance for Treg cells of patients without FA and AD to have the highest percentages of Vβ (3,5) expression.

CONCLUSIONS: There appears to be differences in TCR Vβ expression of Treg cells in patients with FA and AD.

309 Correlation Between Fractional Exhaled Nitric Oxide and Asthma Exacerbation

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RATIONALE: Fractional exhaled nitric oxide (FeNO) levels have been used as the marker of airway inflammation. FeNO levels ≥49 ppb has been proposed to predict asthma exacerbation. This study aim to determine the correlation between FeNO levels and asthma exacerbation

METHODS: A prospective study was performed in patients with atopic asthma aged ≥7 years. The participants were assessed every 2 months for 1-2 years. FeNO levels and spirometry were measured every 6 months.

RESULTS: At 6-month-follow up, 56 patients (38 boys) with median age of 12.1 years (7.03-28.0 years) were evaluated. Most of them (66%) were treated with inhaled corticosteroids. Sixteen percent of the cases had FeNO levels ≥49 ppb with the median of 94.8 ppb. In the cases with FeNO levels <49 ppb, the median was 20.9 ppb. Asthma exacerbation occurred more often in those with FeNO levels ≥49 ppb than in those with the levels <49 ppb (22.2% vs 8.5%, P = 0.223). Successful step-down treatments were more commonly achieved in the patients with FeNO levels <49 ppb than in those with the levels ≥49 ppb (57.4% vs 11.1%, P <0.05). FeNO levels ≥49 ppb was significantly correlated with maternal atopy (P = 0.021) and the longer duration of asthma (P=0.045). There was a significant correlation between the FeNO levels and the percent improvement of FEV1 after bronchodilator (r = +0.300, P = 0.026).

CONCLUSIONS: Asthma exacerbation occurred more often among those with FeNO levels ≥49 ppb. Step-down treatments were more successful in those with FeNO levels <49 ppb

310 IL17RB+ Granulocytes In Asthma Patients

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RATIONALE: IL-25 and its receptor IL-17RB have been shown to promote Th2 type allergic responses. Numerous IL-17RB+ cells that produce Th2 cytokines have been identified. A granulocytic cell population that expresses IL-17RB as well as IL-4 and IL-13 was identified in patients with asthma and termed Type 2 Myeloid (T2M) cells. This study aims to confirm T2M elevation in asthmatic patients and its potential correlation with severity of asthma.

METHODS: 1) Flow cytometry. Peripheral blood from subjects with physician diagnosis of asthma (N=17) and healthy controls (N=8) were collected. Isolated granulocytes were incubated with cell-specific fluorescent antibodies and the percentage of T2M (CD11B+CD16+CD177+IL-17RB+) and IL17RB+ granulocytes were determined. 2) Clinical assessment. Through spirometry testing and patient interview, severity of asthma was assessed (number of exacerbations, FEV1, FEV1/FVC, and asthma control test).

RESULTS: Compared to healthy controls, asthma subjects have significantly higher percentage of both IL17RB+ granulocytes (P=0.013) and T2M (P=0.037). Results identified that the T2M cells were a distinct population of granulocytes separate from eosinophils. Among asthmatics, the percentage of IL17RB+ granulocytes or T2M did not show significant correlation with number of exacerbations, FEV1, FEV1/FVC or ACT scores.

CONCLUSIONS: A higher percentage of granulocytes in asthmatic patients expressed the IL-25 receptor, IL17RB. Asthmatics also have significantly more T2M than controls. The presence of either the IL-17RB+ or T2M cell population did not correlate with severity of asthma in patients during their clinic visit. Future studies will examine patients during active asthma exacerbations to determine correlations of T2M cells with disease severity.

311 Pollen From Genetically Modified Bt Maize Does Not Promote Allergic Responses In Mice

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RATIONALE: The genome of the genetically modified (GM) maize event MON810 contains a truncated version of a transgene, *cry1ab*, from the soil bacterium *Bacillus thuringiensis* (Bt). Hence, the GM plants express a modified Cry1Ab protein, which is toxic for some yield-reducing maize pests. The Cry1Ab protein shares 87% sequence homology with the recognized mucosal adjuvant Cry1Ac, and accordingly possesses potential adjuvant properties. We investigated whether pollen from the Cry1Ab-expressing maize affects airway responses or acts as an adjuvant on antibody production against ovalbumin (OVA) in an airway allergy mouse model.

METHODS: On days 0, 1, 2, 21, 22 and 23 mice were intranasally exposed to OVA with either 0.5 mg of recently shed (<24 hrs) pollen from MON810 or the near isogenic non-GM maize, or the purified Bt-derived protoxin Cry1Ab. OVA with cholera toxin (CT) was used as positive control for adjuvant effect. Blood and bronchoalveolar lavage fluid (BALf) were collected on day 26, and anti-OVA specific IgE, IgG1, IgG2, anti-Cry1Ab specific IgG1, and Th1/Th2/Th17/Treg cytokines, respectively, were determined.

RESULTS: While this airway allergy model detected adjuvant effects of a known potent adjuvant (CT), anti-OVA specific IgE, IgG1 and IgG2a were not elevated in mice receiving MON810 pollen or the Cry1Ab protoxin. Exposure to protoxin Cry1Ab, but not MON810 pollen, induced an anti-Cry1Ab specific IgG1 response. Cytokine levels in BALf were low in all groups.

CONCLUSIONS: In the mouse model employed, exposure to pollen from the genetically modified Bt maize event MON810 does not trigger enhanced allergic sensitization, general immunogenicity or airway responses.

312 Effects Of rs3744262 On DNA Methylation and Symptoms In Participants With Allergic Rhinitis During Grass Pollen Exposure In The Environmental Exposure Unit (EEU)

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RATIONALE: Genotype can affect epigenetics, as SNPs can alter CpG sites. Surrounding CpG sites may also be affected, but genetic-epigenetic interactions are not well understood. rs3744262 is a CpG-SNP in ephrin-B3. Ephrin-B3 is a transmembrane ligand for receptor tyrosine kinases, involved in bidirectional signaling. Ephrin-B3 is crucial in epithelia and enhances the T cell response. However, the effects of this CpG-SNP on nearby methylation sites and effects on symptoms during allergen challenge in an Environmental Exposure Unit (EEU) have not been examined.

METHODS: 38 participants with allergic rhinitis were exposed to grass pollen for 3 hours on two consecutive days. DNA was isolated from blood drawn at baseline, 3H and 27H post-exposure. All participants recorded their total symptom scores (TSS) and peak nasal inspiratory flow (PNIF) at baseline and 30 minute intervals during exposure. All participants were genotyped at rs3744262 and pyrosequencing was performed on 16 participants at the site of interest plus three surrounding CpG sites. Kruskal-Wallis tests and Spearman correlations were used.

RESULTS: rs3744262 was associated with DNA methylation at that site ($p<0.0001$). Genotype was not associated with TSS, PNIF or methylation of surrounding sites. However, one of the CpG sites surrounding rs3744262 was negatively correlated with baseline TSS ($p=0.03$). Another nearby CpG showed a trend towards positive correlation with baseline PNIF ($p=0.08$).

CONCLUSIONS: Epigenetic modifications in the region of the EFNB3 gene are associated with allergic rhinitis symptoms. Further studies are needed to examine the effects of genetics and epigenetics on allergic rhinitis.

313 Chronic Spontaneous Urticaria – An Evaluation Of An Indirect Immunofluorescence Method For Detecting Anti-Mast Cell IgG Antibodies

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RATIONALE: An autoimmune basis is believed to be responsible for about half of all cases of chronic spontaneous urticaria (CSU) with specific IgG auto-antibodies directed at mast cells. The autologous serum skin test (ASST), basophil histamine release assay and basophil activation test have been used to diagnose autoimmune form of CSU. These tests are limited because they are indirect form of mast cell/basophil degranulation.

METHODS: Sections were cut from paraffin embedded blocks from skin biopsied infant with bullous mastocytosis. Serum from 69 patients with CSU was used, and fluorescein conjugated human IgG was used to label fixed antibody. An ASST was performed on 66 of the patients with severe urticaria and was found to be positive in 45.45%. 27 of these patients were receiving intravenous immunoglobulin (IVIG) or received it in the past.

RESULTS: A positive indirect immunofluorescence was found in half the patients. It was positive in 22.73% of the patients with a positive ASST, but was also positive in 25.76% with a negative ASST. The sensitivity and specificity of ASST were calculated to be 46.88% and 52.94%, respectively. When IVIG treated patients were omitted from the calculations the sensitivity and specificity was 34.62% and 77.27%, respectively

CONCLUSIONS: Positive indirect immunofluorescence was found in half the patients with CSU. When IVIG treated patient were excluded the ASST was associated with is a high specificity but with low sensitivity. Indirect immunofluorescence should be considered as better indicator of the autoimmune form of urticaria.

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314 Protease Activity Of Per a 10 Causes CD 40 Cleavage On Dendritic Cells and Th2 Polarization

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RATIONALE: Per a 10, a serine protease is a major allergen of *Periplaneta americana*. The aim of present study is to elucidate the effect of protease activity on dendritic cell activity and subsequent T cell polarization.

METHODS: Mice were sensitized and challenged with cockroach extract (CE), heat inactivated cockroach extract (ΔCE) and PBS. Bone marrow was cultured in 10%RPMI media containing rmGMCSF and rmIL-4. Immature DCs were stimulated with native (n) Per a 10, heat-inactivated (Δ) Per a 10, LPS or media alone for 48hrs. Cell surface markers and cytokines (in supernatant) were analyzed by flow cytometry and ELISA respectively. DCs and murine naïve CD4+ CD62L+ T cells were co-cultured at ratio of 1:5 and supernatant analyzed by ELISA.

RESULTS: Eighty five percent of cultured bone marrow cells acquired DC phenotype (CD11c+, I-A/I-E+) at 8th day of culture. DCs stimulated with n Per a 10 caused increased CD86 expression and significantly reduced CD40 expression as compared to Δ Per a 10. IL-12 level was also reduced in supernatant of DCs stimulated with n Per a 10, indicating CD40 cleavage by proteolytic activity. Protease activity of n Per a 10 caused higher secretion of proinflammatory cytokines TNFα, IL-6. Naïve T cells co-cultured with n Per a 10 stimulated DCs caused higher secretion of IL-4, IL-13 and lower secretion of IFNγ due to lower levels of IL-12 in microenvironment, favoring Th2 polarization.

CONCLUSIONS: Proteolytic activity of Per a 10 causes cleavage of CD40 and lowered secretion of IL-12 by DCs and thus favors Th2 polarization.

315 Type 2 Immunity Can Have a Protective Role In Host Defense Against Venoms In Mice

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RATIONALE: Besides their beneficial role in defense against certain helminths, type 2 immune responses are best known in the context of allergies, where they can result in deleterious and sometimes fatal responses against a wide range of allergens, including components of venom. However, it has been hypothesized that "allergic responses" also can represent defense mechanisms against toxins (M. Profet. *Quart. Rev. Biol.* 66:23-62, 1991; N. W. Palm *et al. Nature*. 484:465-472, 2012). Therefore, we assessed whether and to what extent type 2 immunity induced by honeybee venom (BV) or Russell's viper venom (RVV) influenced the resistance of mice challenged with potentially lethal amounts of the venoms.

METHODS: Wild-type C57BL/6 and BALB/c mice mock-immunized with PBS (as a control) or immunized with a sub-lethal dose of BV or RVV were assessed for the development of venom-specific T-cell and antibody responses and, three weeks after injection with venom or PBS, were challenged with potentially lethal amounts of that venom.

RESULTS: BV or RVV induced type 2 immune responses that conferred increased resistance of mice (assessed by body temperature and survival) to challenge with a potentially lethal amounts of BV or RVV, respectively. Furthermore, such acquired resistance to BV or RVV could be passively transferred by serum, suggesting that soluble factors in the serum (most likely venom-specific type 2 antibodies) are responsible for this protective effect.

CONCLUSIONS: Type 2 immunity can enhance resistance to potentially lethal amounts of BV or RVV, revealing a novel role for type 2 immunity in host defense.

316 CD4+ and CD8+ T Cells Bearing Naïve and Memory Markers In Blood Of Immigrants To Brooklyn Who Develop Asthma and Allergic Disease

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RATIONALE: Brooklyn has ongoing immigration from regions of low allergy/asthma prevalence (40% of Brooklyn residents are foreign born); these immigrants develop seasonal allergies and asthma after 8 years. We determined whether there is a difference in blood CD4+ and CD8+ T cell subsets bearing markers associated with naïve (CD45RO-$CD62^{Hi}CD11a^{Lo}$) and memory (CD28-CD45RO+) T cells in immigrants who develop allergy and asthma compared to those without allergy or asthma.

METHODS: Blood was obtained from immigrants to Brooklyn (n=112 Chinese and Hispanic; n=57 with self-reported allergy or asthma [allergy/asthma group]; n=55 without allergy or asthma [non-allergy/asthma group]). Distributions of CD4+ and CD8+ T cells with naïve (CD45RO-$CD62^{Hi}CD11a^{Lo}$) or memory (CD28-CD45RO+) markers were determined (flow cytometry). Total serum IgE levels also were determined (fluoroimmunoenzymeassay). T-test was used for analysis.

RESULTS: The allergy/asthma group had significantly higher IgE levels compared with the non-allergy/asthma group (p=0.016). Nevertheless, the allergy/asthma group had significantly more CD4+ and CD8+ CD45RO-$CD62^{Hi}CD11a^{Lo}$+ T cells compared with the non-allergy/asthma group (p=0.01, p=0.03, respectively). Although the allergy/asthma and non-allergy/asthma groups had similar numbers of CD4+ CD28-CD45RO+ T cells (p=0.2), the non-allergy/asthma group had significantly more CD8+ CD28-CD45RO+ T cells than the allergy/asthma group (p=0.03).

CONCLUSIONS: Decreased CD8+ memory T cells may be associated with development of allergy/asthma.

317 17β-Estradiol Increases IL-17A Protein Expression From Mouse CD4+ Th17 Differentiated Cells

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RATIONALE: Th17 cells have increased IL-23/IL-23 receptor (R) signaling leading to increased IL-17A and IL-17F protein expression. Th17 cells are associated with severe asthma as well as multiple sclerosis and lupus. After puberty, women have a higher prevalence of these diseases, suggesting a role for sex hormones, but the mechanisms by which sex hormones affect Th17 cell cytokine production remain unknown. We hypothesized that *in vivo* 17β-estradiol (E2) increases IL-23R, IL-17A, and IL-17F expression in *ex vivo* differentiated Th17 cells from mice.

METHODS: Naïve CD4+ T cells from female, male, and ovariectomized mice ages 3 weeks (pre-pubescent) or 8-10 weeks (adult) were differentiated into Th17 cells. Placebo, 17β-E2, progesterone, or 5α-dihydrotestosterone (DHT) pellets were implanted into ovariectomized mice 21 days prior to naïve T cells isolation. IL-23R mRNA expression was measured by qPCR, and IL-17A and IL-17F protein expression were measured by ELISA.

RESULTS: In pre-pubescent mice, IL-23R mRNA expression and IL-17A and IL-17F production were decreased by approximately 50 fold in Th17 cells from female compared to male mice. In adult mice, IL-23R mRNA expression and IL-17A and IL-17F production were increased by about 5 fold in Th17 cells from females compared to male and ovariectomized mice. *In vivo* administration of 17β-E2, but not progesterone or 5α-DHT, significantly increased IL-17A and IL-17F production by Th17 cells by 2.5 fold compared to ovariectomized mice receiving placebo pellets.

CONCLUSIONS: *In vivo* 17β-E2 increases Th17 cell differentiation and provides a biological mechanism to explain the switch in prevalence of Th17-mediated diseases at puberty.

318 17-β Estradiol Positively Correlates With IL-17A+ CCR6+ Memory CD4+ T Cells In Patients With Severe Asthma

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RATIONALE: Severe asthma prevalence is slightly increased in boys compared to girls. However, after puberty and until menopause the prevalence of severe asthma is increased in women compared to men. This change in asthma prevalence strongly suggests a role for ovarian hormones. IL-17A is secreted from Th17 cells and has been positively correlated with asthma severity. We hypothesize that ovarian hormones positively correlate to IL-17A secretion from memory Th17 cells.

METHODS: CD4+ memory T cells were isolated from the blood of women (n=16) and men (n=14) with severe asthma ages 18-45. Cells were cultured overnight with anti-CD3 and anti-CD28. Cells were then restimulated with PMA, ionomycin, and Golgi-stop for 4 hours. Memory CD3+ CD4+ CCR6+ T cells were gated and IL-17A intracellular staining was assessed by flow cytometry. CCR6 is a surface marker for Th17 cells. Plasma was also isolated from and assessed for 17β-estradiol (17β-E2) and progesterone by radioimmunoassay.

RESULTS: CCR6+ memory CD3+ CD4+ T cells were similar in women and men, but women with severe asthma had increased CCR6+ IL-17A+ memory T compared to men ($p<0.05$). 17β-E2, but not progesterone, concentrations positively correlated with IL-17A+ CCR6+ cells ($p<0.05$).

CONCLUSIONS: Our results provide a potential explanation for the increased prevalence of severe asthma in women compared to men. Further, the correlation between 17β-E2 and IL-17A protein expression provides an explanation for the switch of severe asthma prevalence at puberty and menopause. Identifying the immune mechanisms underlying severe asthma is essential to optimizing therapeutic regimens.

319 Allergic Sensitization and Determination Of Serum Eosinophil Cationic Protein and Triptase In Preschool Population In Hermosillo, Sonora, Mexico

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RATIONALE: One of the most reliable in vitro method to diagnose allergic sensitization is the qualitative determination Phadiatop®. We detected allergic sensitivity against a panel of common allergens present in the northwestern region of Mexico, in a preschool popullation, and also quantified tryptase and eosinophil cationic protein (ECP).

METHODS: Approved by the Ethics Committee. A total of sixty four children (ages 3-6 years) serum samples were analyzed by Phadiatop ® for determined specific IgE, and tryptasa and ECP by Phadia ImmunoCAP® 100. The group 1 included children sensitized (n = 10), and group 2, with non-sensitized children (n=54).

RESULTS: All children studied were sensitive to pollens, specifically Artemis vulgaris, Fraxinus americana and Phleum pratense. Only 9 children were found specific IgE antibodies against Betula verrucosa. It was observed that seven children were sensitive to the cockroach (Periplaneta americana) and house dust. Additionally four children were sensitized to dog dander and three to house dust mites (Dermatophagoides pteronyssinus). Finally, in three children the analysis found specific IgE antibodies against cat dander and one positive to Alternaria alternata. In the determination of markers of inflammation, no significant differences were observed between the two groups, ECP values ($p=0.062$) and tryptase ($p=0.518$).

CONCLUSIONS: This study is important because it teaches the importance of early detection of allergic sensitization by a positive Phadiatop ® test in still asymptomatic children or children with symptoms but no diagnosis and leads to take steps to avoid the allergic march.

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320 Fungal Cross-Allergenicity In Specific Ige Testing

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RATIONALE: Fungal allergens are often suspected to have cross-reactivity even between organisms considered to be far separated in taxonomic organizations. Sequencing of fungal allergen component proteins has identified several families of proteins that appear in many species of fungi. To examine the observed cross reactivity in fungal specific IgE testing the following studies were conducted.

METHODS: Results from all specific IgE testing performed for a 12 year period (2000 - 2012) at a hospital laboratory using ImmunoCap reagents were retrieved. Results for individuals tested for specific IgE against a combination of four fungi (Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum and Penicillium chrysogenum) were selected for comparison. Tests with values greater than 0.35 kIU/L were considered positive.

RESULTS: Results of 6565 individuals were retrieved. There were 26260 individual tests of which 7058 were positive with 2607 individuals having at least one positive test. The highest number of positive tests was to Alternaria (2156) and the fungus with the fewest positive tests was Cladosporium (1508). Of the persons testing positive to only a single fungus (785) Alternaria was the most frequent (465) followed by Aspergillus (139) Penicillium (138) and Cladosporium (43). Of those individuals testing positive for only two fungi Alternaria and Aspergillus dominated (151) and of those testing positive for only 3 fungi Alternaria, Aspergillus and Cladosporium dominated(143). Most significantly, of the individuals having at least one positive nearly half (1208) tested positive for all four fungi.

CONCLUSIONS: Specific igE testing indicated the possibility of significant cross-reactivity among these four fungi.

321 Monocytes From Peanut-Allergic Patients Express Higher Levels Of RALDH2 In Response To Peanut Protein Than Monocytes From Tolerant Subjects

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RATIONALE: Retinoic acid (RA), the active metabolite of vitamin A, is involved in the differentiation and gut homing of regulatory and effector T cells. We previously observed that human myeloid dendritic cells and monocytes stimulated with peanut protein show increased expression of the RA-producing enzyme RALDH2. In the present study, we aim to compare peanut protein-induced RALDH2 expression between peanut-allergic, non-peanut-allergic atopic, and non-atopic individuals.

METHODS: Peanut allergy and atopy were diagnosed based on clinical history, peanut-specific and total IgE levels. Whole blood was collected from all individuals, and PBMC were isolated. CD14+ monocytes were purified from PBMC by magnetic bead separation, and cultured for 16h with or without plate-bound peanut protein or the TLR2 agonist Pam3Cys. Cells were harvested for total RNA isolation, and expression of RALDH2 and IL6 was measured by RT-qPCR.

RESULTS: Monocytes from peanut-allergic patients showed a stronger increase in RALDH2 expression in response to peanut protein (7.6-fold increase as compared to unstimulated cells) than monocytes from non-peanut-allergic atopic patients (2.8-fold) and non-atopic subjects (2.8-fold). In contrast, monocyte responsiveness to Pam3Cys, as measured by expression of IL6, was not different between the groups.

CONCLUSIONS: Monocytes from peanut-allergic patients are more sensitive to the RALDH2-inducing effect of peanut protein than cells from subjects tolerant to peanut. This is not due to a generally increased responsiveness to innate immune stimuli. Increased production of RA by monocytes and dendritic cells in susceptible individuals exposed to peanut may have consequences for the polarization of the peanut-specific T cell response.

322 Peanut Protein Induces Expression Of RALDH2 In Human Dendritic Cells In a TLR2-Dependent Manner

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RATIONALE: Dendritic cells instruct naïve T cells to differentiate into various effector cells determining immune responses such as allergy or tolerance. Our objective was to determine peanut protein (PP)-induced changes in gene expression in human myeloid dendritic cells (mDC), identify possible signaling receptors, and assess the role of differentially expressed genes in the induction of T cell differentiation.

METHODS: mDC (CD11c+BDCA1+) and naïve Th cells (CD4+CD45RA+) were isolated from blood bank donors. mDC were incubated for 24h with medium alone, PP, or control stimulants (LPS, CT). mRNA was isolated for use in gene expression array and RT-qPCR. To assess T cell differentiation, mDC were cocultured for 6d with autologous naïve T cells and the RALDH2 substrate retinal.

RESULTS: PP induced a unique expression profile in mDC, including the gene that encodes RALDH2, a rate-limiting retinoic acid (RA)-producing enzyme. RT-qPCR confirmed the ~20-fold induction of this gene in PP-treated mDC. PP-treated mDC also demonstrated a 7-fold increase in enzymatic activity of RALDH2. Naïve T cells cocultured with PP-treated mDC showed a 3-fold increase in expression of the RA-sensitive surface markers CD38 and $\alpha4\beta7$-integrin. Blocking antibodies against TLR2/TLR1 reduced expression of RALDH2 in PP-stimulated mDC by 70% (n=9).

CONCLUSIONS: PP induces RALDH2 in mDC by signaling through the TLR2/TLR1 heterodimer. This leads to production of RA, which acts on T cells to induce gut-homing integrin. RA has been implicated in potentiating differentiation of gut-homing regulatory and effector T cells. RALDH2 induction by PP could therefore be an important factor determining allergic or tolerant responses to peanut.

323 IL-4 Receptor Alpha and STAT6 Single Nucleotide Polymorphisms Are Associated With Increased Risk Of Asthma In a Saudi Arabian Population

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RATIONALE: IL-4Rα rs1805010 and rs1801275 SNPs have been found to be significantly associated with asthma susceptibility in different ethnic groups; some STAT6 SNPs, including rs324011 and rs324015, have also been associated with asthma predisposition and/or IgE levels. Risk evaluations of IL-4Rα and STAT6 SNPs in association with asthma have never been evaluated in Saudi Arabian populations. We investigated whether IL-4Rα (rs1805010 and rs1801275) and STAT6 (rs324011 and rs324015) polymorphisms are associated with asthma in a population of asthmatic patients from Saudi Arabia.

METHODS: Saudi Arabian patients with documented history of severe asthma (n=190) and healthy subjects (n=194) were recruited. Allelic and genotype association to asthma was assessed for IL-4Rα and STAT6 polymorphisms using nucleotide sequencing.

RESULTS: Genotype frequencies were analyzed by testing distinct genetic models, either adjusted or not for covariate gender. The IL4Rα rs1801275 SNP A/G-G/G genotypes, but not the A/A genotype, were significantly associated with asthma predisposition (OR=0.47; 95% CI=0.31-0.72; P<0.001*; dominant model); IL4Rα rs1805010 SNP was also significantly associated with asthma (OR=0.62; 95% CI=0.39-0.99; P=0.043*). Similarly, for STAT6 rs324011, odds were significantly higher that homozygous T/T genotype could be associated with asthma; contrariwise, STAT6 rs324015 genotypes were not significantly associated with asthma, according to this analysis (genotype A/A: OR=0.70, 95% CI=0.29-1.70, P-value=0.43; recessive model).

CONCLUSIONS: The minor allele, G, of IL-4Rα rs1805010 and rs1801275 SNPs, and the corresponding A/G and G/G genotypes were significantly associated with asthma predisposition in asthmatic patients from Saudi Arabia; STAT6 rs324011 T/T genotype was also significantly associated with asthma predisposition, whereas rs324015 genotypes were not.

324 Infants With Idiopathic T Cell Lymphopenia Identified On New York State Newborn Screen: A Follow Up Report

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RATIONALE: Quantification of T-cell receptor excision circles (TRECS) through newborn screening has introduced early diagnosis of idiopathic T cell lymphopenia (ITCL). As the TREC assay is relatively new, the clinical characteristics, laboratory monitoring, and outcomes of ITCL are not yet well described.

METHODS: From September 29, 2010 through July 26, 2013, infants with TREC values <150 copies/μl (or < 200 copies/μl if flagged for repeat) were referred to Mount Sinai Immunology for diagnostic evaluation. This report is a retrospective chart review looking at the follow up for patients diagnosed with ITCL.

RESULTS: Fourteen patients had absolute T cells <2000/cubic mm, and 6 were followed in clinic for ITCL. Initial TREC levels averaged 76 copies/μl, and initial absolute T cell counts averaged 934/cubic mm. The number of flow cytometries performed ranged from 2 to 4, and follow up flow was generally done around 4 months, 9 months, and 1 year of age. Vaccination was deferred until T cell counts had an upward trend towards normal, and prophylactic antibiotics were only used in 2 patients. Patients who were not followed longitudinally did not necessarily have a higher absolute T cell count on their final flow, but were clinically asymptomatic over a period of months with levels close to the normal range. All the patients generally did well without significant infections or complications.

CONCLUSIONS: ITCL may be identified more readily as the TREC assay is included in more state-mandated newborn screens. While patients seem to do well clinically, more information is needed on monitoring and outcomes.

325 Frequency Of Cellular and Humoral Immunodeficiencies In Digeorge Syndrome Patients Seen At New York Presbyterian Columbia Between 2006-2012

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RATIONALE: DiGeorge syndrome is most commonly associated with 22q11.2 deletion, one of the most frequent microdeletion syndromes in the US population. Diagnosis is often made following identification of cardiac abnormalities. About 60-70% of patients with DiGeorge syndrome have T-cell deficiencies. B-cell deficiency is also seen but data are limited. We sought to determine the prevalence of T cell as well as B cell immunodeficiency among a cohort of patients with DiGeorge syndrome evaluated at New York Presbyterian Columbia between 1/2006-1/2012.

METHODS: A retrospective chart review was performed on patients with ICD 9 code for DiGeorge syndrome using the electronic medical record (EMR). Descriptive statistics were assessed.

RESULTS: We have reviewed 30/200 patients' clinical and laboratory data to date. All patients had a congenital cardiac diagnosis and Tetralogy of Fallot was the most prevalent cardiac defect (10/30). 22q11.2 deletion was seen in 26/30 patients. One patient had tbx1 gene mutation. Absolute lymphocyte counts (ALC) were checked in 23/30 patients within the first 3 months of life and 83% (19/23) showed low ALC. Lymphocyte subsets were checked in 17 patients and quantitative immunoglobulins were checked in 12 patients in the first 3 months of life. Twelve (71%), 14 (82%), and 13 (76%) patients had low CD3, CD4, and CD8 counts respectively and 8 (67%) had low immunoglobulin levels.

CONCLUSIONS: It is known that cellular immunodeficiencies are common in DiGeorge syndrome. Our preliminary data suggest that B cell immunodeficiency also may be common in the first 3 months life.

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326 A Novel ORAI1 Mutation Resulting In $T^+B^+NK^+$ SCID With Normal Lymphocyte Proliferation

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RATIONALE: *ORAI1* encodes a calcium channel subunit essential for lymphocyte activation. Severely impaired lymphocyte proliferation is a hallmark of human mutations in *ORAI1*, resulting in $T^+B^+NK^+$ SCID with opportunistic infections and hypotonia. We report two patients with a novel frameshift mutation in *ORAI1* that permits normal lymphocyte proliferation, but causes impaired NK and $CD8^+$ T cell cytotoxicity, CMV viremia, and hypotonia.

METHODS: Immunophenotyping was performed on Patient 2 prior to transplant; Patient 1 had been previously transplanted. Whole genome and Sanger sequencing was performed on patients' fibroblast lines, their parents, and a healthy sibling. ORAI1 expression was studied with immunoblotting. Store-operated calcium entry in fibroblast lines was assessed with intracellular fluorescent calcium ion indicators.

RESULTS: Our patient had normal T, B, and NK cell numbers. Lymphocyte proliferation to mitogens was normal and present to antigens. IgG and IgM were normal, IgA was elevated, and he had positive pneumococcal titers. NK cell cytotoxicity was absent and $CD8^+$ T cell cytotoxicity was minimal. Whole genome sequencing revealed a frameshift nonsense mutation in *ORAI1* (p. H164PfsX1) that abolished expression of the protein's last two C-terminal domains. Fibroblasts from both patients demonstrated significantly decreased, but not absent, store-operated calcium flux. This may due to residual ORAI1 expression or compensation by ORAI2/3. Ongoing studies will determine if retroviral reconstitution of ORAI1-deficient fibroblasts with $ORAI^{H164PfsX1}$ permits residual ORAI1 expression and calcium flux.

CONCLUSIONS: This is the first human ORAI1 mutation that permits normal lymphocyte proliferation to mitogens, thus expanding the clinical phenotype associated with this disease.

327 Coronin-1A Oligomerization Is Critical For Host Defense Against Viral Pathogens

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RATIONALE: Coronin-1A is an actin regulator important for T cell homeostasis. Mutations abolishing Coronin-1A expression have been associated with $T^-B^+NK^+$ SCID and EBV-induced lymphoproliferation. Coronin 1A oligomerizes and associates with the cytoskeleton via two C-terminal domains. We present two siblings with disseminated varicella, cutaneous warts, and T cell lymphopenia with a novel frameshift mutation in *CORO1A* that replaces the last two C-terminal domains by a novel sequence, resulting in failure of the expressed mutant protein to oligomerize.

METHODS: The *CORO1A* mutation was identified through whole genome sequencing of the patients and their mother and confirmed by Sanger sequencing. Coronin-1A expression was evaluated by immunoblotting cell lysates. cDNA was sequenced using 3' RACE. Protein complex formation was evaluated by native PAGE.

RESULTS: The two siblings had T cell lymphopenia with intact proliferation to PHA and anti-CD3 stimulation. NK cell degranulation was normal. The patients were homozygous for a single nucleotide insertion in *CORO1A* (1191_1192insC). The mutation would result in the replacement of the last 62 C-terminal a.a. of the protein by a novel 92 a.a. sequence. The Coronin-1A mutant protein was expressed in the patients' cells and migrated ~3 kb higher than wild type protein. Native gel electrophoresis demonstrated impaired oligomerization of the mutant protein.

CONCLUSIONS: We describe a novel mutation in *CORO1A* in two siblings with immunodeficiency. The mutation alters the last two domains of Coronin-1A and impairs the ability of the mutant protein to oligomerize. The data indicate that oligomerization is critical for Coronin-1A function *in vivo* and host defense against viral pathogens.

328 Newborn Screening For Severe Combined Immunodeficiency In Iowa: TREC Assay Results and Characteristics Of SCID and T-Cell Lymphopenic Patients

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RATIONALE: T-cell receptor excision circle (TREC) analysis on dried bloods spots (DBS) is used in newborn screening (NBS) for severe combined immunodeficiency (SCID). We hypothesized that the Iowa TREC assay would correctly identify known SCID and other selected T-cell deficient patients and that levels would correlate with degree of lymphopenia, PHA response, infection severity, diagnosis and age of diagnosis.

METHODS: Stored DBS of ten Iowa children ($\leq$ 6 years) followed in the University's Immunology Clinic with established SCID/other T cell lymphopenia were pulled and TREC assays were run using real time quantitative PCR with RNase P control. Clinical and laboratory data were extracted by retrospective chart review.

RESULTS: Four SCID subjects meeting selection criteria were identified (IL2Rγ, NEMO, RAG1 and RAG 2); all had undetectable TRECs. The mean age of SCID diagnosis was 11 months. Four subjects with 22q11 deletion syndrome had abnormal TRECs with mean age of diagnosis of 3.75 months. Two T-cell lymphopenic subjects (congenital renal disease and hypogammaglobulinemia/cytopenia) had normal TREC assays. Only SCIDs had abnormal PHA responses. Lymphocyte counts, infection severity, and age of diagnosis did not correlate with TREC level.

CONCLUSIONS: The Iowa TREC assay run on stored DBS correctly identified children with known SCID and 22q11 deletion syndrome. Regardless of TREC level, only SCID subjects had abnormal PHA responses. Similar to other states screening for SCID, abnormal TRECs were detected in 22q11 deletion syndrome. The average age of diagnosis in the SCID subjects was 11 months, highlighting the importance of NBS for earlier SCID diagnosis.

329 Thymus Graft Factors Critical For Negative Selection Of Direct Allospecific T Cells

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RATIONALE: Mechanisms underlying thymic negative selection of direct allospecific T cells remain unclear and become even less well-understood when mediated by allogeneic thymus tissues that are cultured for transplantation. Thymus transplantation is used to treat infants with T cell deficiency due to congenital athymia.

METHODS: Cultured FVB/NJ thymus tissues were transplanted into epaxial muscles of C57Bl/6 nude mice. Thymus tissues were cultured for up to 7 days; recipients were given grafts cultured for 3, 4, or 5 days. A second cohort received single, double, or quadruple 4-day cultured allogeneic thymus grafts. Thymus graft activity was monitored noninvasively by *in vivo* bioluminescence assessments. Direct alloreactivity toward thymus graft alloantigens was assessed using mixed lymphocyte cultures.

RESULTS: Marked depletion of viable bone marrow-derived cells was observed during the first 2 days of thymus tissue culture. Recipients of allogeneic thymus grafts cultured for 3 or 4 days demonstrated T cell-mediated direct alloreactivity toward thymus donor alloantigens ($p < 0.05$). This alloreactivity was lost when thymus grafts were cultured for 5 days. Direct alloreactivity toward donor alloantigens in recipients of 4-day cultured thymus allografts was not rescued by higher doses of graft tissue.

CONCLUSIONS: Thymus graft epithelial cells may play an important role in negative selection of direct allospecific T cells after transplantation. These cells may acquire key yet-to-be-determined cell-intrinsic tolerogenic properties over time as they are cultured for transplantation. The total quantity of tissue transplanted appears to be irrelevant. Further research is needed to identify specific mechanisms, principles, and signaling pathways by which acquisition of tolerogenic properties occurs.

330 Successful Hematopoietic Stem Cell Transplant For CD40 Deficiency Manifesting As Hyper-IgM Syndrome With Absent CD40 Expression and Marked Lymphocytosis

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RATIONALE: CD40 deficiency is a rare cause of Hyper-IgM syndrome, with little known about outcomes following hematopoietic stem cell transplant (HSCT). Here we present a patient affected by a mutation resulting in absent CD40 protein expression, treated successfully with HSCT.

METHODS: CD40 gene sequencing was performed by Correlagen Diagnostics and protein expression was determined by FACs.

RESULTS: The patient was born to first-cousin parents of Moroccan origin, and family history was significant for a brother that died at 18 months of recurrent infection and failure to thrive. She presented at 6 months with *Pneumocystis jirovecii* pneumonia requiring intubation. Immune workup was notable for persistent and stable lymphocytosis up to 50,000 cells/uL without evidence of underlying infection, undetectable immunoglobulins except for normal IgM, elevated B and T cell counts with naïve predominance, and decreased T cell proliferations. CD40 gene sequencing revealed a homozygous missense mutation in exon 3 (c.170C>T, resulting in p.Thr57Met). CD40 protein surface expression was absent. The patient received HSCT with a 9/10 mismatched unrelated donor, conditioned with busulfan, cyclophosphamide, and ATG. Transplant course was complicated by RSV and CMV pneumonitis and renal tubular dysfunction. Now nearly one year out from transplant, she is fully engrafted, off GVHD prophylaxis without evidence of GVHD, and doing well, though continues to have marked lymphocytosis of unclear etiology.

CONCLUSIONS: We describe a case of CD40 deficiency treated successfully with HSCT from a 9/10 mismatched unrelated donor transplant. Her clinical disease is unusual for marked lymphocytosis that recurred post-transplant.

331 IL-2 Receptor Gamma-Chain(IL2RG) Defect Can Present With Features Other Than Increased Susceptibility To Infection

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RATIONALE: Mutations in IL2RG are the recognized cause of X-SCID, a disorder that presents in infancy with overwhelming infection and fatal if untreated. Rare phenotypes of IL2RG mutations were identified in patients presenting later in life with history of severe recurrent infections. We report novel IL2RG mutation in young adult presenting with diffuse cutaneous lesions and immune dysfunction in absence of systemic infections.

METHODS: Flow Cytometry, Serum-Immunoglobulin Measurements, Lymphocyte Proliferation studies, Skin Biopsies, Whole-Exome Sequencing

RESULTS: 18-year-old-male presents with five-year history of diffuse red-purple plaques on face/extremities and pink, confluent macules/patches on chest. Laboratory findings were suggestive of combined immunodeficiency: IgG-133mg/dL, IgA-54mg/dL, IgM-10mg/dL, CD4-159cells/ul, CD8-480cells/ul, CD19-148cells/ul, NK-5cells/ul, no lymphocyte proliferative response to mitogens/antigens. He had no significant sinopulmonary or systemic infections. Skin biopsies of lesions: cutaneous granulomas and epidermodysplasia verruciformis(EDV). EDV is associated with cutaneous Human Papillomavirus infection in immunodeficiency syndromes. Intravenous immunoglobulin was started at replacement doses as well as antibiotics(later discontinued when infectious cause for granulomas was ruled out). Granulomatous lesions responded well to systemic steroids. EDV lesions were refractory to topical-5%-imiquimod and remained unchanged. Due to unusual presentation, whole-exome sequencing was performed showing novel mutation of IL2RG (Val152Ala) affecting extracellular-domain of gamma-chain.

CONCLUSIONS: Unforeseen finding of novel IL2RG mutation indicates that spectrum of disorders associated with this type of defect is not limited to severe impairment in host defenses. It is important to consider combined immunodeficiency evaluation and possibility of IL2RG mutation in patients with diffuse cutaneous granulomas and EDV even in absence of history of systemic infectious complications.

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332 Studies On Cohort Of Infants With Di-George Syndrome Detected By New York State Newborn Screening For Severe Combined Immunodeficiency (SCID)

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RATIONALE: To assess immune and clinical status of infants with Di-George Syndrome referred to Mount Sinai from the New York State (NYS) newborn screening program for severe lymphopenia.

METHODS: We collected clinical and laboratory data on infants with Di-George Syndrome referred to Mount Sinai, one of 8 referral centers in New York State. Patients with levels of T-cell receptor excision circles (TRECs) of below 125 were referred. Data collected included TREC values, CD3, CD4, CD8, NK and B cell absolute numbers as well as genotype and phenotype.

RESULTS: In the first 33 months of newborn screening we saw 6 patients at Mount Sinai finally diagnosed with Di-George syndrome. 5 of these patients were female. 4 patients had undetectable TREC levels at birth (ranging from average 0 to 188 (?) and 0 to 889 on 2-4 follow up visits). All but one of those patients also had congenital heart defects of different degrees. T-cell lymphopenia ranged from 0 to 2189/cu mm absolute CD3. At least half of the patients were known to have a 22q11 deletion. One patient with 22q11 deletion had hypocalcemia and lymphopenia but no cardiac involvement. Another patient had complete DiGeorge Syndrome without chromosomal deletion due to maternal diabetes and subsequently successfully underwent thymic transplantation. A third patient with a maternal history of Di-George syndrome and 22q11 deletion was found to have Di-George syndrome with the same chromosomal abnormality.

CONCLUSIONS: Newborn screening for SCID can identify patients with Di-George syndrome with varying degrees of lymphopenia.

333 Immunologic Profile Of Single Ventricle Survivorship Participants: Risk Factors For Clinically Significant Immunologic Dysfunction

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RATIONALE: Congenital cardiac anomalies are associated with both primary and secondary immunodeficiency. Surgical thymectomy, manipulation of the thoracic duct and protein losing enteropathy (PLE), a condition related to stressed Fontan hemodynamics, are thought to result in low peripheral absolute lymphocyte counts (ALC) and quantitative immunoglobulins. We hypothesized that although immunologic perturbations are common in this population, immunologic intervention is rarely required.

METHODS: A retrospective chart review of the immunologic parameters of all patients enrolled in the Single Ventricle Survivorship Program (SVSP) at the Children's Hospital of Philadelphia (CHOP) was performed.

RESULTS: The median age of the 178 SVSP patients was 7.4 years, with ages ranging from 3 to 26 years. Among those with PLE, the median ALC and IgG were lower (672/ul and 200mg/dl) than those without (1610/ul and 868mg/dl). Interestingly, in the non-PLE group, the range of ALC varied from 530-5322/ul, with 17 non-PLE patients maintaining an ALC <1000. Despite lymphopenia in the majority of SVSP patients, few were severely clinically affected: 25% had delayed clearance of cutaneous viral infections, 63% had atopy, and one died of EBV-associated Hodgkins lymphoma. IVIG was clinically indicated for 4 patients, one of whom had CVID. Four patients with normal splenic function were treated with antibiotic prophylaxis.

CONCLUSIONS: Patients with repaired single ventricle physiology often demonstrate T cell lymphopenia and hypogammaglobulinemia, most commonly associated with PLE. A significant portion of patients without PLE also have lymphopenia. In the overall cohort, most patients did not demonstrate clinical immunodeficiency despite these laboratory abnormalities.

334 Chronic Non-Iatrogenic Lymphatic Loss Syndromes Identified Though Abnormal TREC Analysis From The Texas Newborn Screening Program (NBS)

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RATIONALE: Chronic non-iatrogenic lymphatic loss syndromes are associated with lymphopenia, infections and high mortality. We previously presented 6 children with chronic chylous losses in a three-year chart review of 567 cases at Texas Children's Hospital(TCH). Since initiation (12/2012) of Texas SCID Newborn screening (NBS) by measure of T-cell receptor excision circles (TREC), 4 infants with low TREC and chronic lymphatic loss were identified. This represents 3 times expected incidence for TCH suggesting missed diagnoses prior to NBS.

METHODS: Retrospective review of infants born 12/2012-7/2013 (n=60) referred to TCH Immunology by geographic Texas residence for abnormal NBS TREC. Chart review assessed demographics, immune studies, clinical diagnoses, treatment, and outcome.

RESULTS: Sixty infants with abnormal TREC were referred to our center. Four had chronic chylous loss syndromes: hydrops(n=2), omphalocele(n=1), and chylous pleural effusion(n=1). Mean ALC was 1602cells/mm^3(Standard-Deviation[SD] 803, <50% of aged-matched controls[AMC]). Mean CD3 count was 581(SD803, 23% AMC), mean CD4 was 409cells/mm^3(SD 540, 26% AMC), and mean CD8 was 164cells/mm^3(SD249, 29% AMC). CD4+45RA+ were preferentially lost (mean 316cells/mm^3; SD459, 26% AMC) with CD4+45RO+cells spared. Serum Immunoglobulin (IgG) levels were variable in 3 patients (mean 241mg/dL, normal range:254-909mg/dL). All were treated for sepsis (proven or presumed) and 2 with IgG replacement. Three infants died, one remains critically ill.

CONCLUSIONS: NBS identifies chylous loss disorders in neonates prior to their demise. As the United States moves to universal NBS for SCID, chylous lymph loss in neonates will be diagnosed earlier. Immune management of these disorders is controversial; given high mortality, formulation of a systematic approach is needed.

335 An Adult With Disseminated Herpes Zoster Infection Found To Have Rare Combined CD4, CD8 T-Cell and NK-Cell Deficiency

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RATIONALE: Cellular immuodeficiencies other than those associated with human immunodeficiency virus (HIV) are rare, and they usually arise in early childhood (eg, severe combined immune deficiency). Only idiopathic CD4+ lymphopenia has been well-defined to develop in adulthood. Case reports have reported adult-onset T-cell deficiency associated with impaired CD2 expression, and a subtype of common variable immunodeficiency with severe T-cell defect. Here we present an adult with recurrent herpes zoster infection found to have CD4, CD8 T-cell and NK cell deficiency.

METHODS: HIV DNA/RNA PCR and lymphocyte mitogen stimulation were performed by Quest Diagnostics.

RESULTS: This Caucasian male initially presented at age 7 with acute varicella infection. At age 25 the patient presented with pruritic rash involving T10 dermatome, diagnosed with herpes zoster. At age 40, patient presented with diffuse papulovesicular rash, malaise, and headache. Vesicular fluid showed positive varicella zoster virus (VZV) PCR. Immune evaluation was performed at this point. CD3+/CD4+ T cell 248 $x10^6$ cells/L, CD3/CD8 T cell 94 $x10^6$ cells/L, and CD3-/CD56+ NK cell 69 $x10^6$ cells/L, varicella IgG 1.51 g/dL. White blood cell count, quantitative immunoglobulins IgG, IgM, IgA, IgE, B cell count, streptococcal antibody titer, lymphocyte mitogen stimulation, HIV RNA PCR, and tetanus toxoid/tuberculin/candida delayed hypersensitivity skin tests were normal. Patient was given hepatitis B and herpes zoster vaccinations.

CONCLUSIONS: We believe this is a unique, rare case of adult-onset combined CD4, CD8 T-cell and NK cell deficiency. This novel late-onset cellular immunodeficiency is of unknown prevalence, genetic origin, and may be of very significant clinical utility in the future.

336 Clinical and Virological Characteristics Of HIV-Associated Lymphomas In Patients With Perinatally-Acquired HIV In The Era Of Antiretroviral Therapy

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RATIONALE: Since the advent of cART, HIV-associated lymphomas have decreased in incidence and there has been a shift towards Burkitt (BL) and Hodgkin lymphoma (HL). However, in contrast to non-Hodgkin lymphoma, HL has been increasing in incidence. EBV, most common oncogenic virus involved in pathogenesis, is present in approximately 40% of cases. To date, there have been no reports of HIV-associated lymphoma in patients with perinatally-acquired HIV in the US.

METHODS: Clinical, virological, and histologic characteristics of HIV-associated lymphomas in 5 patients with perinatally-acquired HIV were collected restrospectively through chart review.

RESULTS: Median age at diagnosis was 24 years [22, 25]. All patients had moderate to severe immunosuppression (median CD4 220/μL [85.5, 263.0], median viral load 1761 copies/mL [113, $2X10^6$]) at the time of diagnosis. The median CD4 nadir was 350/μL [4, 446.5] and duration at nadir was 48 months [18, 72]. Two patients had diffuse large B-cell lymphoma (DLBCL), one of which had a plasmablastic subtype of DLBCL. Two patients had nodular sclerosing HL, and one had BL. Four patients had serologic and/or immunohistochemical evidence of EBV infection. Four patients had favorable response to chemotherapy, no disease progression, and median disease free survival of 3.4 years [1.2, 7.1] thus far. One patient, with DLBCL, had disease progression including leptomeningeal involvement and is currently receiving salvage chemotherapy.

CONCLUSIONS: In this series, HIV-associated lymphomas are found in patients with perinatally-acquired HIV during the second decade of life. Prognosis remains favorable for those that respond to first line chemotherapy.

337 Study On Air Pollution and Respiratory Health Of Children In Delhi, India

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RATIONALE: Air pollutants may cause respiratory problems in children during adolescence. The study was aimed at assessing the respiratory health of children exposed to pollution in different areas of Delhi.

METHODS: Schools were selected from commercial-Chandni Chowk (CC), industrial- Mayapuri (MP) and residential -Sarojini Nagar (SN) areas. Indoor and outdoor levels of SO_2, NO_x and Particulate matter (PM) were estimated using samplers in classrooms and at monitoring stations. Questionnaire was designed based on internationally valid questionnaires for respiratory illness. Students completed the questionnaire and performed spirometry test.

RESULTS: Indoor and outdoor PM_{10} concentration was highest in CC (815±354.45 & 337±85 $\mu g/m^3$) and lower in MP (694.6±322.9 & 274±78$\mu g/m^3$) and SN (534.3±94.22 & 197±48 $\mu g/m^3$). These were too high & above the permissible limits of 100$\mu g/m^3$. However, levels of SO_2 and NO_xwere below the limit. Students (1814) aged between 12–16 years participated in the survey. Questionnaire data showed that "wheeze, cough and cold" were most prevalent in CC (29.6%) panel of students followed by MP (15.9%). Spirometry tests demonstrated that 19% of CC & 14% of MP subjects suffered mild obstruction whereas 16% of CC & 32% of MP subjects had severe obstruction. Conditional logistic regression analysis of the association between chronic exposure to PM and respiratory symptoms showed a significant positive relationship in these areas.

CONCLUSIONS: Commercial and industrial zones with high traffic movement and human activities contribute more PM which affects the pulmonary health. The study indicates that high pollution levels may lead to respiratory illness in children.

338 Trans-Generational Transmission Of Ozone – Induced Airway Dysfunction

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RATIONALE: Epigenetics is responsible – environmental interaction of asthma development. The role of epigenetics behind ozone - induced airway dysfunction is not known yet.

METHODS: 6 weeks old BALB/C female mice were exposed to filter air or O3 (2ppm, 3hr/day, day1 to day 21). Airway resistance (Pehn), and BAL procedure were performed at weekly interval. For trans-generational transmission study, 6weeks old BALB/C female mice (F0) were exposed to filter air (Air-F0) or O3 (1ppm, Ozone-F0) during pregnancy. Pehn was measured at 5 and 6 weeks of age in F1∼F3 mice grown up in filter air, and cytokines (IL-1a, ß, 4, 5, 8, 9, 10, 12, 17, 22, 23, LxA4, RvE1, PGE2) were measured in BALF using ELISA kit. mRNA expression of DNMTs, MeCP2, MBDs, HDAC3, Chrm1,2,3 and ADRß2 genes were measured in RNA extracted from lung tissue using Real-Time PCR.

RESULTS: After Ozone exposure, Pehn value was significantly increased in a time dependent manner with concomitant BAL neutrophilia. MBDs and HDAC3 increased at hour 3, while DNMTs, MeCP2 started to increase at day 14 exposure. In the trans-generational models, F1∼ F3 mice born from Ozone-F0 showed persistent elevation of Pehn and BAL neutrophilia. IL-5, 8, 9,10, and 12 elevated in F1 of Ozone F0, but returned in F2 and F3 compared to those of Air F0. DNMTs of Ozone-F0 to Ozone-F3 increased, while Chrm2 and ADRß2 were decreased compared to those of AirF0.

CONCLUSIONS: Ozone - induced airway dysfunction may be transmitted to F3 generation with altered expression of the genes related with airway function potentially via epigenetic changes.

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339 Dust Mite Allergen In Bed Dust Predicts Rhinitis Symptom Persistence In Urban Pre-Adolescent Children With Higher Elemental Carbon Particulate Matter Exposure

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RATIONALE: Few studies have examined environmental risks for persistent rhinitis symptoms in urban populations during pre-adolescence. Combustion byproduct particulate matter has been shown to promote the T-helper cell-2 response to allergens. We hypothesized that preadolescents exposed to both combustion byproducts and domestic allergens would have more persistent rhinitis symptoms than those exposed to one or neither.

METHODS: The New York City (NYC) Neighborhood Asthma and Allergy Study recruited 7-8 year-old children with (cases) and without (controls) asthma through a health insurance plan. Bed dust was collected for measurement of indoor allergens. Neighborhood-level annual outdoor elemental carbon (EC) was estimated for each child at baseline based on NYC Community Air Survey data. Questionnaires were administered at age 7-8 and age 9-10. Children reported to have had rhinitis symptoms in the past 12 months were categorized as having "current rhinitis."

RESULTS: Of 102 children who had current rhinitis at age 7-8 and were followed for 2 years, 54 (53%) had current rhinitis symptoms at age 9-10. In multivariable regression analyses, neither dust-borne allergen concentrations nor EC concentration were associated with persistence of rhinitis. However, Der f 1 concentration was associated with rhinitis persistence (relative risk (RR)=1.33, P=0.03) among the children with higher EC (greater than median)[$P_{interaction}$=0.096].

CONCLUSIONS: Children with rhinitis may be more likely to have persistent symptoms if they are also exposed to combustion byproduct particulate matter than if they are not. Exposure to EC may thus contribute to the persistence of rhinitis symptoms in urban communities.

340 Associations Between Outdoor Of PM2.5 With Cough and Wheeze Symptoms In Asthmatic Children In Korea

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RATIONALE: Exposure to traffic-related pollutants poses a serious health threat to residents of major urban around the world. Short-term exposure to air pollution can trigger asthma exacerbation in children, but it is not known which components of air pollution are most important. We monitored their outdoor air pollution and asthma symptoms.

METHODS: Daily 24-hr personal samples of $PM_{2.5}$, including the elemental carbon fraction, were collected for 40 children with asthma during approximately 1 month each. Asthma Control Questionnaire is a survey tool for the measurement of overall asthma control. We conducted a repeated measure panel study to examine weekly associations between ACQ scores and traffic- and non-traffic air pollutants among asthmatic children.

RESULTS: Of the 40 study participants, 24 were males and 16 were females. The average age was 10 years (range 7-14). The average indoor $PM_{2.5}$ was 8.7 μg/m3. The odds ratio for a standard deviation increase in ambient $PM_{2.5}$ was 1.18 (95% CI 0.89-1.58) for cough and 1.07 for wheeze. We found elevated same-day relative risks of wheeze [1.45; 95% confidence interval (CI), 1.03-2.04)], shortness of breath (1.41; 95% CI, 1.01-1.99), and total symptoms (1.30; 95% CI, 1.04-1.62).

CONCLUSIONS: Cough was more prevalent than wheezing in this inner-city panel of asthmatic children. The study suggesting that the $PM_{2.5}$ is also most responsible for pollution-related asthma exacerbations among children. Studies that rely on exposure to PM mass may underestimate PM health impacts.

341 Effect Of Duration Of Residence In Brooklyn On IgE Responses Of Immigrants

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RATIONALE: Inner city areas including Brooklyn, New York suffer disproportionately from atopic disease. Brooklyn has ongoing immigration from regions of low allergy/asthma prevalence. We previously reported that ethnicity and history of hepatitis and other infections significantly affected development of new asthma and seasonal allergies in Brooklyn immigrants. Whether residence in Brooklyn is associated with increasing IgE response has not been determined.

METHODS: Immigrants to Brooklyn (n-298) were interviewed about early life living conditions, previous infections, and total IgE, and HSV1 were determined. A generalized linear regression model was constructed, with dependent variable $\log_{10}$(total IgE). Predictors were history of hepatitis, Herpes simplex 1 IgG, number of children in house, age, sex, rural/town/city and region of origin (4 groups). Tests of utility of polynomial terms in continuous predictors were conducted. Model residuals were examined for skew and for outliers. Model-generated means with standard errors are reported. Analysis of effect included generation of Chi-Square value and degrees of freedom in order to determine significance.

RESULTS: While IgE levels decreased with increasing age, residence in Brooklyn was associated with annual increases in serum IgE (p=0.0003 and 0.016, respectively). Each additional year of age decreases IgE by 2.0% (95% CI 0.5, 3.1). Each additional year in Brooklyn increases IgE by 1.5% (95% CI 0.3, 2.6). No other predictor was significantly associated with IgE (p=ns).

CONCLUSIONS: The decline in IgE levels with age is countermanded by local immune stimuli which stimulate development of IgE responses in previously non-allergic immigrants residing in Brooklyn. These local stimuli may contribute to the ongoing asthma epidemic.

342 Impact Of Energy Expenditure On Ozone-Induced Inflammation

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RATIONALE: Ozone exposure induces an inflammatory airway response as measured by decrements in spirometry and airway neutrophilia. Exercise may also induce airway neutrophilia, but the impact of energy expenditure during exercise itself on ozone-induced inflammation is not known. We sought to determine the effect of energy expenditure on ozone-induced inflammation.

METHODS: 72 individuals were exposed to 0.4ppm ozone while exercising for 1 hour (Group 1) or 0.08ppm ozone while exercising intermittently for 6 hours (Group 2). Induced sputum was collected at baseline and 24 hours after ozone exposure. Linear regression analyses were used to determine the impact of energy expenditure (joules) on changes in sputum percent neutrophil (%PMN) counts after adjusting for age, gender, ethnicity, and BMI.

RESULTS: Both ozone exposure conditions lead to significant increases in sputum %PMN at 24 hours (group 1 $p<0.0001$; group 2 $p=0.0002$). There was no significant difference in the change in sputum %PMN at 24 hours between the two groups. Energy expenditure among volunteers ranged from 0 to 2,051,460 joules. For each 10,000 joule increase in energy expenditure, there was a significant 0.12% increase in the change in sputum %PMNs post-ozone exposure ($p=0.05$). Asthmatics had significantly greater increase in change in sputum %PMN post-ozone exposure for each one joule increase in energy expenditure compared to non-asthmatics ($p<0.0001$).

CONCLUSIONS: Increased energy expenditure leads to greater airway neutrophilic inflammation post-ozone exposure; this response is exaggerated in asthmatics. Additional study is needed to fully explore the impact of differing ozone exposure conditions and physiological parameters on the ozone-induced inflammatory response.

343 Exposure Of Mice To Silica Crystals and Poly I:C Synergistically Enhances Neutrophil Infiltration and Epithelial Damage In The Airway

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RATIONALE: Silica crystals (silica) are the main mineral component of volcanic ash and desert dust. Silica-containing dust is known to exacerbate chronic respiratory diseases such as asthma and COPD. We previously reported that simultaneous exposure of normal human bronchial epithelial cells to silica and poly I:C induced caspase-9-dependent apoptosis, but not caspase-1-dependent inflammasome activation.We investigated the in vivo effects of silica in mice.

METHODS: Silica and poly I:C were intranasally administered to C57BL/6 mice for 3 consecutive days, and airway inflammation and histopathological changes were evaluated on day 4.

RESULTS: The intranasal administration of silica and poly I:C to mice synergistically enhanced neutrophil infiltration into the airway mucosa, without IL-1β release into the BALF. Consistent with the cytokine profile of BAL cells, combined administration of silica and poly I:C synergistically enhanced the levels of such inflammatory cytokines as IL-6, TNF-α, IFN-γ and HMGB-1 in the BALF. However, neither IL-1β nor activated caspase-1 proteins were detected in the lung tissues. Histopathological analysis revealed that silica or poly I:C alone induced marginal airway inflammation, whereas their combined administration significantly enhanced both inflammation and epithelial damage in the airway.

CONCLUSIONS: Our results suggest that inhalation of silica-containing dust may cause inflammasome-independent airway inflammation, possibly by damaging the epithelial barrier, especially at the time of viral infection. These responses may also be involved in acute lung injury induced by inhalation of silica-containing dust.

344 Modest Effects Of Bisphenol A Exposure In Mouse Models Of Respiratory Allergy and Food Allergy

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RATIONALE: The impact of Bisphenol A (BPA) exposure from diet is heavily debated. Epidemiological associations between BPA exposure and diabetes and obesity as well as animal models showing effects of BPA exposure on allergy, behavior and diabetes development, prompted us to further investigate effects of BPA exposure in mouse models of respiratory and food allergy.

METHODS: Balb/cOlaHsd mice were exposed during pregnancy to BPA (10mg/l or 100mg/l) through drinking water and the offspring were sensitized and challenged with ovalbumin (OVA) intranasally. Inflammatory cells in bronchoalveolar lavage fluid (BALF) and serum OVA-specific IgG an IgE were analyzed. In a food allergy model using C3H/HeJ mice and lupin as the allergen, the animals were exposed to BPA through drinking water (1, 10 or 100mg/l) in utero and until adulthood. Anaphylactic score, drop in body temperature, total IgE and specific lupin IgG in serum were analyzed.

RESULTS: Transmaternal BPA exposure (100mg/l) resulted in increased numbers of eosinophils in BALF and a trend of increased IgE levels in serum in the respiratory allergy model. In the food allergy model, long term BPA exposure resulted in decreased MCP-1 serum levels, although overall allergic responses were not affected.

CONCLUSIONS: Our data show that transmaternal BPA exposure through drinking water augments allergic reactions in a mouse model of respiratory allergy but not in a food allergy model. The results suggest that allergy-promoting effects of BPA are modest and possibly give tissue-specific effects.

345 In Vitro Allergy Testing: Relationship Patterns Between Allergen Pairs

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RATIONALE: We noted that during specific IgE testing, patients tend to be negative for all allergens tested or they tend to be positive for multiple allergens. We determined the nature of the relationship between any given pair of allergens.

METHODS: Data from *in vitro* testing using ImmunoCAP® technology were extracted from instrument log files. Approximately 2.5 million test results were examined covering a 4.5 year span. Allergens were paired in all combinations, with all patient samples tested for any given pair being compiled and analyzed to determine the nature of the relationship.

RESULTS: We have meaningful data for about 10,000 pairs of allergens. For one pattern of results, levels of IgE against one allergen closely correlate to levels against the second. For a second pattern of results, levels of IgE against the second allergen are consistently higher than the levels against the first allergen.

CONCLUSIONS: This study correlates results of *in vitro* specific IgE tests among various allergens. Allergen pairs which show highly correlated IgE levels indicate that there is a common component in the two allergens with the amount of specific IgE being measured to be approximately the same, regardless of which allergen is being tested. Allergen pairs in which the level of measured IgE is preferentially higher for one allergen over the other indicate allergens which have components in common combined with an additional allergenic component in one of the two. We have identified allergen pairs for which IgE levels show these two patterns.

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346 Comparison Of Total Protein Profile Of Alternaria Alternata Extract Obtained From Various U.S. Allergenic Extract Manufacturers

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RATIONALE: *Alternaria alternata* sensitization can cause rhinoconjunctivitis and asthma, occasionally fatal. The allergenic extract used for diagnosis and immunotherapy for the condition is non-standardized. Multiple allergens, strain variability, different growth and extraction conditions impart high variability. The purpose of this study is to assess the variability, and to develop a multiplex antibody-based assay for measuring overall potency of *A. alternata* extracts.

METHODS: We prepared *A. alternata* extract as follows: 5 g of dried, defatted powder was mixed with PBS containing protease inhibitors overnight at 4°C. The extract was centrifuged and filtered. In addition, we analyzed six *A. alternata* allergen extracts from six manufacturers. The extracts were analyzed using 2D gel electrophoresis and GelFree fractionation system. Separated proteins were transferred onto PVDF for human IgE immunoblotting. Proteins detected by IgE were excised from Coomassie stained gels and identified using peptide mass fingerprinting.

RESULTS: As expected, *A. alternaria* allergen extracts exhibited extensive compositional differences by SDS-PAGE and 2D fractionation, and a broad range of Alt a 1 levels (0.1-9.0 mcg/mL) (Alt a 1 ELISA kit, Indoor Biotechnologies). On IgE immunoblot of 2D gel electrophoresis, >10 protein spots were detected, including heat shock protein 70/Alt a 3 (356578610), TBP-associated factor 15B (261336148), beta-glucosidase 2 precursor (380007310) and exoglucanase (6179889).

CONCLUSIONS: There are significant composition differences at the protein level in different *A. alternata* extracts. The range of Al t a 1 levels is broad. We have identified novel allergens that need to be further characterized for the development of a multiplex assay.

347 IgE Antibodies To Cat and Cat Components In Relation To Asthma In a Population Study Of 963 18 Year Olds From Six Schools In Northern Sweden

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RATIONALE: In Norbotten, the dominant indoor allergens are cat and dog and there is no significant exposure or sensitization to mite, cockroach, Alternaria or Aspergillus.

METHODS: Sera were assessed by ImmunoCAP for IgE to cat, dog and horse (dander); birch and timothy pollen; mite, cockroach, Alternaria and Aspergillus. The results were compared to asthma, prevalence and severity.

RESULTS: Significant associations with asthma were observed for IgE to animal dander and pollens; only danders remained significant in multivariate analysis: odds ratio 5.7 (3.5-9.4) $p<0.001$, for the association of IgE ab ≥17.5 IU/ml (class 4) and physician diagnosis of asthma. Sera positive for dander were assayed for IgE to Feld1, Feld2 (albumin), Feld4 (lipocalin), and Feld5 w (Cat IgA, alpha-gal): Positive results were seen in 197, 25, 81 and 1 sera respectively. The quantitative results for Feld1, Feld2 and Feld4 correlated with asthma; only Feld1 and Feld4 were significant in multivariate analysis: Odds Ratio 3.0 (1.5 – 5.9) $p<0.01$, and 4.8 (1.0 – 23) $p<0.05$ respectively. Interestingly, while Feld1 results correlated most strongly with IgE to cat Rs 0.86 ($p<0.001$), the results for Feld2 correlated equally, with cat, dog and horse; 0.30, 0.33, 0.26. The results for Feld4 correlated strongly with IgE to horse Rs 0.78, $p<0.001$, which may reflect the dominance of the lipocalin Equc1 in the horse ImmunoCAP extracts.

CONCLUSIONS: The results illustrate the complexity of component analysis for cat and strongly support the importance of both specificity and titer of IgE antibodies.

348 Epitope Mapping Of An Anti-Bla g 1 ScFv Used For Cockroach Allergen Quantitation

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RATIONALE: Bla g 1 is one of two primary allergens used to measure cockroach allergen exposure. A panel of avian scFv antibodies was developed for composition profiling of whole body cockroach extract. Herein, we mapped the epitope of an anti-Bla g 1 scFv in order to better understand the binding efficiency and cross reactivity with other group 1 cockroach allergens.

METHODS: X-ray crystallography was used to determine the structure of the scFv. The scFv epitope on Bla g 1 was assessed by alanine scanning site-directed mutagenesis and ELISA. The allergen-scFv complex was modeled based on the results. The scFv was tested by ELISA for the ability to block the binding of IgE antibodies from cockroach allergic patients to Bla g 1.

RESULTS: Twenty-four rBla g 1-GST alanine mutants were assessed for variations in binding to the scFv compared to wild type. Five mutants showed a significant difference in affinity. These mutations clustered to form a discontinuous epitope comprising four helices of Bla g 1 with high sequence identity to Per a 1. The scFv did not inhibit the interaction of patient IgE antibodies with Bla g 1.

CONCLUSIONS: The anti-Bla g 1 scFv is expected to have good detection and quantitation properties for both German and American cockroach species. As the scFv does not interfere with IgE antibody binding, it should act as a good capture antibody for quantifying anti-Bla g 1 serum IgE levels by ELISA.

349 Antigenic Analysis Of The Major Cockroach Allergen Bla g 5 and Its Dust Mite Homolog Der p 8

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RATIONALE: Bla g 5 induces the highest IgE antibody (ab) titers compared to cockroach allergens from groups 1, 2, 4 and 7. Bla g 5 and dust mite Der p 8 are glutathione-S-transferases (30% and 26% identical in sequence and surface, respectively). Despite reported IgE ab cross-reactivity for GSTs from mite and cockroach, cross-reactivity between both allergens is unknown.

METHODS: Bla g 5 and Der p 8 were expressed in *Escherichia coli* and purified by GST affinity chromatography. Six monoclonal antibodies (mAbs) were raised against Bla g 5. Specific IgE to Der p 1, Der p 2 and Bla g 5 in sera/plasma were measured by ImmunoCAP. Ab binding assays were performed by ELISA.

RESULTS: Six anti-Bla g 5 mAbs showed lack of reactivity to Der p 8, and inhibited IgE ab binding to Bla g 5 up to 78%, indicating overlapping epitopes with IgE ab. Fifty two percent ($n=12/23$) of plasma/sera from dust mite allergic patients, with IgE specific for *Dermatophagoides pteronyssinus* allergens, reacted with Der p 8, and none reacted with Bla g 5. Conversely, 15 sera with Bla g 5 specific IgE did not show significant IgE reactivity to Der p 8. No significant IgE or mAb cross-reactivity was observed between both allergens, in agreement with a low molecular surface homology.

CONCLUSIONS: Patients' IgE binding to Bla g 5 and Der p 8 result from co-sensitization. Immunological analysis of homologous allergens allows evaluation of antigenic cross-reactivity and to assess the relevance of homologous allergens from different sources.

350 Immunomodulatory Effects Of Rye Grass Pollen Allergen Lol p 5 On The Prostaglandin E_2 Pathway and Kallikrein-Kinin System Of Respiratory Epithelial Cells

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RATIONALE: Several important aeroallergens are known to modulate respiratory epithelial (RE) function. The rye grass pollen Group 5 allergen is a significant contributor to pollen allergy but its immunomodulatory effects on RE function are unknown.

METHODS: Rye grass pollen allergen rLol p 5 and its N- and C-terminal domains were expressed in *E. coli*. Physicochemical studies were conducted using SDS-PAGE, native-PAGE, HPLC, circular dichroism and Phyre2 modelling. Allergenicity was determined by ELISA and immunoblot, and ribonuclease activity by enzyme assay. RE IL-8 release was examined using ELISA and A549, 16HBE14sigma- and Detroit562 cell lines. mRNA level expression of enzymes/proteins associated with the prostaglandin E_2 (PGE_2) pathway and kallikrein-kinin system (KKS) by RE cells were determined using RT-PCR.

RESULTS: 3D-modelling showed rLol p 5 to be structurally similar to Timothy grass pollen Group 5 allergen; circular dichroism analysis showed heat stable proteins with a predominance of α-helices and coils, consistent with the 3D structures. HPLC and native PAGE indicated trimerization (mature and N-terminal domain) and dimerization (C-terminal domain). Each protein was enzymatically active, and reacted with IgE from seven rye grass pollen sensitive patients. All three cell lines released IL-8, and A549 cells showed PGE_2 release. RT-PCR with A549 showed up-regulation of COX-2, mPGES-1, mPGES-2 and cPGES in the PGE_2 pathway, and gC1qR, UPAR, UPA, HSP90α and PAI associated with the KKS.

CONCLUSIONS: The ribonuclease Lol p 5 and its component domains were allergenic, existed as oligomers, induced IL-8 and PGE_2 and up-regulated proinflammatory PGE_2 and KKS pathways by, as yet, unknown mechanisms.

351 Development and Characterization Of a Murine Model Of Repeated Dry Exposure To Aerosolized Fungal Conidia

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RATIONALE: Personal fungal exposures are associated with a variety of adverse health outcomes, including invasive disease, allergic sensitization, and asthma. Most murine models of fungal exposure have utilized aspiration or inhalation of uncharacterized extracts or liquid conidial suspensions that do not resemble natural human exposures. These studies were conducted to characterize a novel dry aerosol repeated exposure model to *Aspergillus fumigatus* with a goal towards defining the resulting immune response.

METHODS: In these studies, immunocompetent Balb/c mice were repeatedly exposed to *A. fumigatus* wild-type (WT) or melanin-deficient (Δ*alb1*) conidia via aerosol exposure of dry conidia using an acoustical generator. Flow cytometric and histopathologic analyses were conducted to characterize the immune responses and the associated lung pathology following repeated exposures.

RESULTS: Histological analysis demonstrated *in vivo* germination in mice exposed to *A. fumigatus* conidia. WT exposure led to increased numbers of adaptive immune system cells (B cells and T cells) and innate immune effector cells (eosinophils, neutrophils, and macrophages). Importantly, $CD8^+IL\text{-}17^+$ (Tc17) cells were also elevated in exposed mice, which appeared to closely correlate with the germination of WT *A. fumigatus* conidia.

CONCLUSIONS: The data presented here are among the first to characterize the immune responses to repeated dry fungal exposures in immunocompetent animals. Dry aerosol exposures via the acoustical generator may provide more accurate analyses of immune responses following exposures to other environmentally prevalent fungi.

352 Cross-Reactivity Between Recombinant Tropomyosin From *Chortoglyphus* and Natural Tropomyosin Of Other Extracts

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RATIONALE: Tropomyosin is a pan-allergen with high homology among species, involved in cross-reactivity between mites, crustacean, mollusks and insects. The objectives were to produce the recombinant tropomyosin from *Chortoglyphus arcuatus* and to investigate the cross-reactivity between different species.

METHODS: Recombinant *C. arcuatus* tropomyosin (r-tropomyosin) was cloned, sequenced, expressed in *Escherichia coli* and purified by HPLC (ion exchange and affinity chromatography). Polyclonal anti-tropomyosin antibodies were produced in mice. IgE recognition to r-tropomyosin was checked by immunoblot with a pool of sera from patients sensitized to storage mites from Galicia. The native tropomyosin was identified in the complete extract of mites by immunoblot inhibition after inhibiting the human pool of sera with r-tropomyosin. Tropomyosin was also identified in shrimp, cockroach and *Anisakis* extracts by immunoblot, incubating with anti-tropomyosin antibodies. Cross-reactivity between r-tropomyosin and Der p 10 was studied by immunoblot inhibition.

RESULTS: A 40 kDa protein corresponding to tropomyosin (GeneBank, JN596422), with a purity higher than 95% and a yield of 1.85 mg/l of bacterial culture, was obtained. r-tropomyosin was recognized by a pool of sera from sensitized individuals. *C. arcuatus* tropomyosin was identified in the whole extract. Tropomyosin was also identified in Anisakis, shrimp and cockroach extracts. r-tropomyosin completely inhibited the recognition of Der p 10 by a monoclonal anti-Der p 10 antibody, therefore cross-reactivity with Der p 10 was demonstrated.

CONCLUSIONS:

- Recombinant *C. arcuatus* tropomyosin was purified demonstrating its capacity to recognize IgE by a specific pool of sera
- Cross-reactivity between mite tropomyosins was demonstrated.
- Anti-tropomyosin antibody recognized tropomyosin in other extracts from invertebrates.

353 Identification Of The Cysteine Protease Amb a x As A Novel Major Allergen From Short Ragweed Pollen (Ambrosia artemisiifolia)

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RATIONALE: Allergy to pollen from short Ragweed (*Ambrosia artemisiifolia*) is a serious and ever expanding health problem in the US and in Europe. Herein, we investigated the presence of unrecognized allergens in Ragweed pollen, with the goal to improve diagnostic and treatment modalities.

METHODS: A Ragweed pollen extract was analyzed by 2D gel electrophoresis and IgE immunoblotting using 70 individual sera from Ragweed pollen-allergic donors (from the US and Central Europe). IgE-reactive protein spots were characterized by mass spectrometry.

RESULTS: Four distinct patterns of IgE sensitization were identified among patients, in both American and European patients. High resolution 2D gel electrophoresis allowed to identify a new allergen termed Amb a x*, with IgE reactivity confirmed in more than 60% of patients. Based on partial amino acid sequencing, the gene was PCR cloned, demonstrating the high sequence homology of Amb a x with known cysteine proteases, such as the house dust mite Der p 1 allergen. Amb a x was purified, fully characterized by mass spectrometry and its three-dimensional structure established by homology modeling. IgE reactivity was confirmed on purified natural and recombinant forms of Amb a x. Our results suggest that the allergenic activity of Amb a x was previously unrecognized and likely inappropriately ascribed to Amb a 1.

CONCLUSIONS: We identified Amb a x as a new major allergen belonging to the cysteine protease family. Amb a x should be considered as an essential component for diagnosis and specific immunotherapy of Ragweed pollen allergy. * pending IUIS official nomenclature

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354 Characterization Of The Allergenic Activity Of Tropomyosin From *Aedes Aegypti*

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RATIONALE: Mosquito bites are an important cause of skin lesions in allergic and non-allergic individuals worldwide. The inhalation of mosquito allergens may also be a cause of allergic respiratory diseases. It has been suggested that there may be cross-reactivity among mosquitoes and other arthropods such as mites, Chironomidae and shrimps. We hypothesized that the panallergen tropomyosin may be involved in this process.

METHODS: Tropomyosin was purified from a whole body *A. aegypti* extract by size exclusion and anionic exchange chromatography. Specific IgE binding to the purified tropomyosin was evaluated by immunoblot and ELISA in allergic patients with positive ELISA to *A. aegypti* allergenic extract and control individuals. Cross-reactivity was evaluated by immunoblotting and ELISA inhibitions using tropomyosin derived from the giant tiger prawn *Penaeus monodon* (nPen m 1) and from the whiteleg shrimp *Litopenaeus vannamei* (rLit v 1).

RESULTS: A purified fraction from the allergenic *A. aegypti* extract showed four bands of several molecular weights with reactivity to anti-Pen m 1 rabbit serum, which showed also IgE reactivity to positive sera to mosquito extract. The IgE reactivity to these bands disappears when the sera where adsorbed with shrimps tropomyosin (nPen m 1 and rLit v 1). 25% of the mosquito allergic individuals had detectable specific IgE levels to this fraction. Inhibition of IgE reactivity higher than 90 % was demonstrated in a sera pool adsorded with nPen m 1 and rLit v 1.

CONCLUSIONS: We have demonstrated the presence of tropomyosin in *A. aegypti* extract, which cross-react with shrimp tropomyosins.

355 Allergy To Ferret

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RATIONALE: Allergy to domestic ferret (Mustela Putorius), a popular mammalian pet, has scarcely been reported and its allergens are not well known.

METHODS: Four atopic patients with rhinitis and asthma symptoms related to ferret exposure were studied. Skin prick tests (SPT) with common commercial aeroallergens as well as with an extract prepared from ferret hair samples were carried out. Specific IgE levels were evaluated by ImmunoCAP^c and microarray ISAC^c assays. Proteins from ferret hair extract were separated using SDS-PAGE. Allergenic profiles were analyzed by imunoblotting assays and IgE binding bands were characterized.

RESULTS: SPT were positive to grass pollens, cat, dog epithelium and the hair ferret extract in all patients. Specific IgE determination to ferret epithelium by CAP were positive, varying from 1.11 to 4.21 kU/L. ISAC profiles showed sensitization to rFel d 1, rFel d 4, rCan f 1, rEqu c 1 in most of patients. Pattern reactivity to ferret hair extract was variable, showing multiple IgE binding-bands between 10 and 66 kDa. Two prominent immunoreactive bands at 34 and 14 kDa were found in 100% patients. A 28 kDa IgE-binding band was detected in three patients. Additional allergens included bands at 66, 51, 26-24, 17 and 10 kDa. Immunoblotting with a control patient serum exposed to ferret did not show any band.

CONCLUSIONS: This study confirms that domestic ferret causes rhinitis and asthma, with an IgE mediated response. Immunoblotting studies revealed IgE reactivity to three prominent protein bands, at 34, 28 and 14 kDa, which can be new major ferret allergens.

356 Do Residual Wheal Skin Prick Test Responses To Perennial and Seasonal Allergens Correlate With Their Specific IgE Levels In Allergic Subjects?

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RATIONALE: The correlation between allergen specific skin prick tests (SPT) and specific lgE levels in patients allergic to either perennial (cat) or seasonal (ragweed) allergen is, to our knowledge unknown. Our aim was to determine if SPT wheal size could be predictive of IgE level.

METHODS: Data from 1307 subjects (18-65 years old) allergic to either cat or ragweed allergen was analyzed. All subjects had a minimum of one year history of allergy and positive skin prick test to either cat or ragweed allergen of 3 or 5mm. A Spearman Rank Correlation between the SPT wheal response and the corresponding specific IgE was performed.

RESULTS: The SPT residual wheal responses analyzed ranged from 3 to 60 mm while the IgE levels ranged from 0 to 466 kU/L. A moderate to low correlation between the allergen specific SPT and IgE was observed ($r=0.384$). There was a similar correlation between the perennial cat allergies ($r=0.346$, $n=323$) compared to the seasonal ragweed allergies ($r=0.349$, $n=984$). A SPT response ≥ 5 was highly likely to yield an IgE class of II or greater, with 94% of the IgE levels being >0.7kU/L.

CONCLUSIONS: This study demonstrates that allergenicity assessed by SPT and IgE is consistent between perennial and seasonal allergens. SPT response is a reasonable indicator of expected IgE level.

357 Peanut Epicutaneous Immunotherapy (EPIT) In Peanut-Allergic Children: 18 Months Treatment In The Arachild Study

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RATIONALE: Peanut EPIT using a new pharmaceutical patch containing 100µg peanut proteins (pp) is under investigation in the pilot Arachild study. Efficacy results after 18 months of treatment are shown.

METHODS: A French multicenter study enrolled 54 pediatric patients (5-17 years, median=10.5) with IgE>5KU/L, SPT wheal≥8mm and peanut allergy documented by allergic reaction to a cumulative reactive dose (CRD)<300mg pp at screening DBPCFC. EPIT was carried out with daily active patch application on the skin for 18 months for approximately half of the subjects (25 patients) The other half received placebo 6 months then 12 months of active treatment. Desensitization was monitored by DBPCFCs every 6 months. CRD≥10-fold basal CRD or CRD reaching ≥1000mg pp was the success criterion. Peanut-specific IgE and IgG4 were also monitored.

RESULTS: Eighteen-month EPIT showed a treatment response of 40% overall. Interestingly the subgroup population of 15 children aged 5-11 years showed 67% response rate. CRD increased constantly over time in these 15 children, with the following mean values: baseline: 24.27±29.98 mg pp; 6mo: 122.6±239.2 mg; 12mo: 308.3±673.9 mg; 18mo: 357.7±542.9 mg, $p<0.001$ between serial CRD values (Wilcoxon matched-pairs signed rank test). In this age population, a progressive increase in IgG4 was seen over time, reaching a mean value of 5.13±5.9 mg/L at 18 months, $p<0001$.

CONCLUSIONS: Peanut EPIT with a patch at 100µg pp induced a progressive and significant increase in peanut CRD in children; this increase was highly correlated with the specific IgG4 rise.

358 Predictors For Allergic Symptoms During Build-Up and Maintenance Phases Of Oral Immunotherapy To Peanut

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RATIONALE: Risk factors for adverse reactions during peanut oral immunotherapy (PN OIT) are unknown. We sought to determine if concurrent atopic disease or serum-specific IgE level (PN-ImmunoCAP) predicts systemic reactions during build-up visits (BU) or maintenance dosing (MD).

METHODS: Ninety-seven patients (mean=9.4 yrs± 3.4) were treated with PN OIT. Asthma, other atopic diseases, and PN-ImmunoCAP were evaluated as potential predictors of systemic reaction during BU and at MD.

RESULTS: 86/97 reached the MD of 450 mg of PN protein daily. Fifty-four children had asthma, 44 had allergic rhinitis and 26 had eczema. Thirty-one patients (32%) had symptoms requiring dose adjustments and extra visits during BU. Oropharyngeal itching and mild gastrointestinal reactions were the most common symptoms. During MD, 14 patients (16%) experienced more severe systemic reactions. Co-factors associated with these included exercise, hot showers, viral illness and decreased carbohydrate content in pre-dose meal. Initial PN-ImmunoCAP level>100 kU/L increased the likelihood of reaction during BU requiring dose adjustment ($p=0.001$). The association between baseline PN-ImmunoCAP and severe systemic reactions during MD approached clinical significance ($p = 0.09$). Asthma or other atopic disease did not predict a risk for symptoms during BU or MD immunotherapy.

CONCLUSIONS: Systemic reactions during PN OIT are common. PN-ImmunoCAP levels >100 kU/L are associated with increased risk of reactions during PN OIT necessitating slower up-dosing. Highly elevated anti-PN IgE may be a risk factor for severe systemic reactions at MD. The presence of asthma or other atopic disease did not predict reactions during BU or MD PN OIT.

359 Quality Of Life With Sublingual Immunotherapy For Peanut

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RATIONALE: While sublingual immunotherapy (SLIT) has proven successful in desensitizing peanut-allergic patients, data on quality of life (QoL) after peanut SLIT is lacking. Using a new food allergy QoL questionnaire, we sought to determine the impact SLIT has on QoL.

METHODS: QoL was assessed in 28 peanut-allergic children (1-11 years) from a previously published placebo-controlled SLIT trial (22 peanut, 6 placebo). We used a validated food allergy QoL instrument, consisting of three subscores (emotional impact, food anxiety, and social and dietary limitations) and an additional set of questions. Subjects were assessed at the start of therapy and one year later at maintenance. Double-blind placebo-controlled food challenge was used to assess desensitization at maintenance. Statistical analysis included paired t-tests and Wilcoxon rank-sum tests.

RESULTS: Comparing baseline and maintenance questionnaires, there were no differences in overall QoL score or subscores. Parents' expected improvements in QoL at baseline were higher than their actual improvement at maintenance, in all domains of QoL, including emotional ($p<0.0012$), physical ($p<0.0018$), social ($p<0.0001$), and work ($p<0.0001$). In the overall QoL and subscores, there was no difference between subjects on placebo versus peanut. Of patients on peanut SLIT, subjects who passed their challenge had better emotional impact scores than those who failed ($p<0.012$), with no differences in overall QoL or other subscores.

CONCLUSIONS: Peanut SLIT does not appear to change QoL in the first year of administration. Patient expectations of QoL improvements were higher than their actual improvements during immunotherapy. Further studies with longer follow-up and larger populations are needed.

360 Low Dose Maintenance Peanut Oral Immunotherapy Can Produce Sustained Unresponsiveness

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RATIONALE: Multiple studies have shown that peanut oral immunotherapy (OIT) is effective in inducing desensitization and even sustained unresponsiveness. However, the optimal dose to produce sustained unresponsiveness is uncertain.

METHODS: Informed consent was obtained from 20 peanut-allergic subjects (ages 4-19) to receive OIT with peanut flour. Initial escalation, build-up and maintenance (300 mg) phases were followed by an oral food challenge (OFC) at approximately 1 year to assess desensitization. Subjects were then transitioned to 1-2 peanuts daily (~300-600 mg peanut protein). When serum peanut-specific IgE was <15 IU/mL, sustained unresponsiveness was assessed by a second OFC following 4 weeks of intentional avoidance. IgE levels and laboratory studies were performed at regular intervals.

RESULTS: Seventeen of 20 subjects achieved desensitization passing a cumulative dose OFC of 5000 mg peanut protein. Three subjects withdrew early in the study because of side effects. The initial median peanut sIgE was 285.4 IU/mL (range, 22.1-795.0 IU/mL). Post-desensitization, 10 subjects continued home dosing with 1-2 peanuts daily for a median of 8.7 months (range, 4-17) until peanut sIgE was <15 IU/mL. All 10 subjects passed a second 5000 mg OFC after 4 weeks of intentional peanut avoidance. Seven subjects continue to consume 1-2 peanuts daily pending qualification for intentional avoidance.

CONCLUSIONS: Low dose maintenance peanut OIT of 300-600 mg protein daily can promote sustained unresponsiveness as peanut sIgE falls below 15 IU/mL. Encouraging continued peanut consumption after desensitization is one of the challenges of OIT, and low dose maintenance using actual peanuts was well-tolerated and effective in producing sustained unresponsiveness.

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361 Increases In Peanut-Specific IgA1 and IgA2 During Peanut Immunotherapy Do Not Correlate With Clinical Tolerance

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RATIONALE: The mechanistic role of IgA1 and IgA2 in food allergy is not well characterized. We retrospectively determined peanut-specific IgA1 and IgA2 levels in plasma from peanut-allergic pediatric subjects who completed either Sublingual Immunotherapy (SLIT) or Oral Immunotherapy (OIT) for peanut allergy.

METHODS: Plasma from 12 peanut-allergic subjects who underwent SLIT and 14 peanut-allergic subjects who underwent OIT were available at the following time points: baseline, one year of immunotherapy, and end of immunotherapy. Plasma from eight age-matched peanut-allergic subjects, who received placebo for the first twelve months, were available for controls. An ELISA was developed to measure peanut-specific IgA1 and peanut-specific IgA2 in subjects' plasma samples.

RESULTS: Both peanut-specific IgA1 and IgA2 levels increased significantly following 12 months of peanut SLIT ($P=0.0010$; $P=0.0010$) as well as 12 months of peanut OIT ($P=0.0067$; $P=0.0001$). Peanut-specific IgA2 plasma levels remain significantly elevated at the end of immunotherapy when compared to baseline for both SLIT ($P=0.0210$) and OIT ($P=0.0001$). There was not a significant change seen in peanut-specific IgA1 and IgA2 levels in subjects treated with placebo for one year. There were also no significant differences in peanut-specific IgA1 and IgA2 levels in subjects who passed tolerance challenges versus subjects who did not pass.

CONCLUSIONS: The data reveals a likely role of IgA1 and IgA2 in treating food allergy. However, the observed increases in peanut-specific IgA1 and IgA2 do not correlate with clinical tolerance. Further investigation is needed to determine the roles allergen-specific IgA1 and IgA2 play in food tolerance.

362 Peanut OIT-Induced IgG Suppresses Ex Vivo Activation Of Allergic Donor Basophils Via a Combination Of Antigen Interception and Receptor-Bound Inhibition

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RATIONALE: We previously reported that plasma from subjects on peanut oral immunotherapy (PNOIT) suppresses ex vivo basophil activation in peanut-allergic subjects. We investigated the identity of the suppressive plasma factor and its mechanism of action.

METHODS: Plasma was removed from whole blood from peanut-allergic subjects and replaced with pooled plasma from PNOIT subjects ($n=10$). Plasma from 0 and 12 months on PNOIT was used unaltered, diluted, or diluted and depleted of IgG. After incubation, samples were stimulated with peanut and assessed for %CD63+ basophils. In some samples, 12 month plasma was added, then removed and replaced with the original allergic plasma ("double replacement", $n=5$) to assess the bound versus soluble fraction of IgG responsible for suppression of basophil activation.

RESULTS: The pooled plasma samples from 12 months on PNOIT decreased basophil activation when compared with pooled plasma from 0 months on PNOIT ($p<0.01$). IgG-depleted samples from 0 and 12 months did not show significantly different activation. Depletion of IgG in the 12 month plasma also increased activation compared with undepleted 12 month plasma ($p<0.01$). In addition, samples receiving diluted 12 month plasma had higher activation than their undiluted counterparts ($p<0.05$), a finding not observed for 0 month plasma. Sample size for double replacement limited statistical analyses, but the results followed the same pattern: "double replaced" samples had lower activation than 0 month samples, and activation equal to or higher than 12 month samples.

CONCLUSIONS: PNOIT-induced IgG suppresses ex vivo activation of allergic donor basophils in a combination of antigen interception and cellular receptor binding.

363 Omalizumab Pretreatment Does Not Protect Against Peanut Oral Immunotherapy-Related Adverse Gastrointestinal Events

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RATIONALE: Co-administration of omalizumab may reduce the side effects of oral immunotherapy (OIT) and permit accelerated dosing. However, we question whether the protective effects of omalizumab extend to gastrointestinal symptoms, a prominent side effect limiting participation in OIT.

METHODS: An interim safety analysis was performed, focusing on patients unable to continue a study that assesses tolerance through the use of omalizumab with OIT. Dropouts were compared to those who stayed in the protocol. Ten peanut-allergic patients, aged 12-20 years, with peanut-specific IgE > 5kU/L were recruited. Subjects were treated with omalizumab for 4 months, followed by a modified rush OIT, then a build-up phase consisting of dose escalations to maintenance dosing of 4000 mg. Food challenges were eventually performed to assess desensitization and tolerance.

RESULTS: Of the 10 patients who initiated, 4 dropped out. Three of the 4 had persistent gastrointestinal symptoms that resulted in eventual withdrawal. Median baseline peanut IgE of 35 kU/L (interquartile range: 12, 100), median baseline total IgE of 93 kU/L (45, 497), median baseline peanut skin prick test of 14 mm (4, 16) and withdrew after median 481 days of OIT (117, 593). There were no differences in sex, age, baseline total or peanut IgE levels, baseline skin prick test, prevalence of atopic diseases, days on therapy, or maximum dose of OIT when compared to those who remained in the protocol.

CONCLUSIONS: We report a high incidence of gastrointestinal side effects leading to withdrawal from the study (30%) despite pretreatment with omalizumab used as an adjunct to peanut OIT.

364 Single Practice Five-Year Experience Treating Food Allergy With Oral Immunotherapy: Efficacy and Epinephrine Treated Reactions

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RATIONALE: FOIT has been reported for decades and has been the subject of several studies but is not yet a common practice in the outpatient clinical setting. We report a five-year experience with FOIT in a single A/I practice.

METHODS: Retrospective record review of all patients receiving FOIT approved by the North Texas IRB. Patients received escalating FOIT doses under direct supervision and continued the tolerated dose twice a day at home until reaching maintenance (mg of protein: cashew 2,000, egg 4545, milk 8,000, peanut 2,000, wheat 8,000).

RESULTS: 228 patients with documented history of food allergy (cashew 3, egg 34, milk 60, peanut 128, wheat 3) were treated with escalating doses of the allergenic food until reaching the target (maintenance) dose or discontinuing. 193 patients (cashew 3, egg 32, milk 52, peanut 103, wheat 3) reached the target dose. 112 epinephrine treated reactions (ETRs) (cashew 0, egg 9, milk 37, peanut 66, wheat 0) occurred during 81,044 escalation doses (cashew 920, egg 11,090, milk 25,764, peanut 42,420, wheat 850). There was one ETR/ 724 doses; a median of 312 doses required to reach maintenance.

CONCLUSIONS: 85% of patients reached maintenance (cashew 100%, egg 94%, milk 87%, peanut 81%, wheat 100%). While ETRs occurred in some FOIT patients during escalation, the majority of patients reach their target dose and continued maintenance dosing for at least three years. FOIT may be done in an A/I office with a manageable rate of ETRs and a high success rate.

365 Single Practice, Five-Year Experience Treating Food Allergy With Oral Immunotherapy (FOIT): Successes and Failures

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RATIONALE: FOIT has been reported for decades but the effectiveness of treatment remains uncertain. We report an analysis of 193 FOIT treated patients who reached their target dose and 35 who didn't.

METHODS: Retrospective record review of all patients receiving FOIT approved by the North Texas IRB. Patients received increasing FOIT doses reaching maintenance (mg of protein): cashew 2,000, egg 4545, milk 8,000, peanut 2,000, wheat 8,000).

RESULTS: The rate of patients reaching maintenance (MP) (3/3 cashew, 32/34 egg, 52/60 milk, 103/128 peanut, 3/3 wheat) differed by food. MP and those who discontinued (DP) did not differ in age or sex. Median pre-FOIT asIgE (kU/L) values of MP/DP were egg 5.3/60.3, milk 10.9/12.1, peanut 29.6/28.6. All cashew and wheat treated patients reached maintenance. 50% (97/193) of MP and 71% (25/35) of DP had a history of systemic reactions prior to FOIT. 11% (21/193) of MP and 14% (5/35) of DP had >1 epinephrine treated reaction. 57% (109/193) of MP and 71% (25/35) of DP had reactions that were not treated with epinephrine. Patients discontinued FOIT because of reactions (12), GI problems/EoE (11), lost to f/u (5), anxiety (3), siblings with reaction (2), difficulty taking doses (1), moving away (1).

CONCLUSIONS: Gender, age, pre-treatment asIgE, and >1 ETR were not predictors of FOIT treatment success. Patients with a history of systemic reaction before beginning FOIT may be more likely to discontinue. Additional data is needed to determine if reactions during escalation that are not treated with epinephrine help predict FOIT success.

366 Single Practice Five Year Experience Treating Food Allergy With Oral Immunotherapy (FOIT): Effect On Antigen Specific IgE (asIgE)

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RATIONALE: Food oral immunotherapy (FOIT) is known to alter antigen-specific IgE (asIgE) levels. We report changes in asIgE in 155 FOIT-treated patients who reached the target escalation dose.

METHODS: The data were collected by a retrospective record review approved by the North Texas IRB. Individuals with documented food allergy underwent FOIT for egg (n=13), milk (n=48), peanut (n=92), and wheat (n=2). As part of the FOIT procedure, asIgE was obtained immediately prior to starting, one month after reaching maintenance, and every 1-2 years subsequently. Those patients with asIgE >100 kU were scored as equal to 100.

RESULTS: Among patients who reached maintenance, median asIgE decreased 57.9% from baseline one month after reaching maintenance. 64.5% had at least a ≥50% reduction in asIgE from baseline upon reaching maintenance. For those individuals on maintenance for one to two years, median asIgE decreased 78.9% from baseline. A ≥50% reduction was seen in 82.9% of patients.

For individual foods, median asIgE decreases upon reaching maintenance from baseline were: 78.9% egg, 75% milk, 53% peanut, 67.3% wheat. The majority of individuals had a ≥50% reduction in asIgE: 84.6% egg, 61.5% milk, 60.9% peanut, 50% wheat. At one to two years after reaching maintenance, the median asIgE changes from baseline were 97.7% for egg, 94.4% for milk, 71.5% for peanut, and 94.4% for wheat.

CONCLUSIONS: FOIT results in significant reductions in asIgE that increase over time, suggesting that reactions during maintenance treatment will become less likely and that some patients may become tolerized.

367 Secondary Eosinophilic Reactions During ORAL Immunotherapy

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RATIONALE: Abdominal complaints which are not associated with dose administration, is an adverse reaction during oral immunotherapy (OIT). In many cases, eosinophilia-related responses was presumed to be the etiology. We sought to characterize the prevalence of these reactions and their association with eosinophilia, during OIT.

METHODS: A retrospective analysis of milk, peanut and egg allergic patients enrolled >60 days in an OIT program (n=500) was performed. Patients reported daily on their clinical status. Blood eosinophil counts were obtained monthly. Patients were classified as symptomatic with eosinophilia > 1400 (Group A), symptomatic with eosinophilia 900-1400 (Group B), or asymptomatic with eosinophilia > 1400 (Group C).

RESULTS: Eosinophilia was noted In 68/500 (13.6%) of patients, with the highest frequency during milk (64/432, 14.8%) as compared to peanut (2/42, 4.65%) and egg (2/25, 8%) OIT. In 66% (42/64 patients) of milk-OIT cases, the eosinophilia was associated with a new onset of symptoms (Groups A+B), primarily vomiting. In the four symptomatic patients that underwent upper endoscopy, histologic results demonstrated esophageal eosinophilia (30-65/hpf, range). Treatment modifications such as decreasing or temporarily discontinuing OIT therapy reversed the symptoms and the blood eosinophil count returned to baseline. In only two patients, eosinophilia and symptoms recurred upon the resumption and escalation of therapy.

CONCLUSIONS: Abdominal complaints associated with blood eosinophilia, occur in 8-15% of OIT patients and can associated with esophageal eosinophilia. These reactions appear to be reversible upon modification of treatment. The blood eosinophil count may serve as a good peripheral biomarker for resolution of disease.

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368 Rate Of Anaphylaxis Caused By Oral Immunotherapy In Children With Cow's Milk Allergy

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RATIONALE: The safety represents the major concern for the treatment of IgE mediated food allergy with oral immunotherapy (OIT). The aim of the survey was the detection of the rate of Anaphylaxis during immunotherapy with cow's milk (CM – OIT).

METHODS: Since 2006 we have treated 48 patients with CM – OIT. Children of both sexes aged 4 to 14 years (median 9 yrs) with demonstrated IgE mediated cow's milk allergy (CMA) underwent OIT with a weekly updosing protocol. Reactions severity, during updosing regimen(s) was assessed through the 'Grading of Food Induced Anaphylaxis according to Severity of Clinical Symptoms' which could be summarized from grade 1 to 5. Grade 3 to 5 were considered as severe or life threatening events.

RESULTS: One patient had symptoms of grade 3 and two patients had symptoms of grade 4, none had symptoms of grade 5. Therefore, among children who underwent OIT a rate of 6% of severe or life-threatening side effects was detected. These children had been successfully treated with i.m. Adrenaline, plus other rescue medications, and desensitization was stopped. Of note, the appearance of mild to moderate adverse effects was quite frequent in the remaining children.

CONCLUSIONS: Among 48 children treated with CM-OIT , 3 (6%) had anaphylactic reactions which were well controlled with i.m. Adrenaline. Therefore, CM –OIT could be considered rather safe in the majority of patients. However, in our opinion, immunotherapy with cow's milk or other foods could be performed only in selected medical centers and under strict medical supervision.

369 Long-Term Follow Up In Cow's Milk Anaphylaxis After Successful Rush Oral Immunotherapy

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RATIONALE: Oral food desensitization is a promising therapeutic aproach in patients with persistent cow's milk allergy (CMA). Although it seems that these protocols show a better outcome in those afflicted with "milder" symptoms (i.e. non anaphylactic reactions) there are controversial results in highly sensitised subjects.

METHODS: Patients with persistent CMA and severe uncontrolled anaphylactic symptoms despite a correct restrictive diet. We performed a two-day desensitization procedure at the Pediatric Critical Care Unit in our Institution. Starting from a 1/100 milk dilution, the patients were progressively exposed to incresing doses ut to 4 ml of undiluted milk. The second phase of the current study was weekly scheduled in the Outpatient clinic to reach a final cumulative dose of 250 ml of undiluted milk. Clinical and serological date were collected every six month for a five-year period.

RESULTS: Ten children (2-15 y.o.) were included. All children reached the final dose of 250 ml of undiluted milk in less than ten weeks. Significant clinical and serological changes were obtained not only in the first six months but during the subsequent five years.

CONCLUSIONS: Highly sensitised CMA patients may benefit from rush oral Cow's Milk immunotherapy. Clinical and serological changes have been found both at early and long-term stages of the follow-up.

370 Milk Oral Immunotherapy. Standard Versus Personalized Protocols: Efficiency and Safety

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RATIONALE: To determine the security, efficacy and efficiency of personalized oral immunotherapy (OIT) protocols based on the results of the challenge test in children with cow's milk allergy.

METHODS: A retrospective, observational study was led in our pediatric allergy unit. Patients with persistent milk allergy that had undergone OIT treatment were included. Anaphylactic patients were excluded. Patients were divided into groups, depending on the OIT protocol. Group 1: personalized protocol based on the tolerance threshold demonstrated in challenge test. Group 2 (control): selected patients that had undergone conventional 14 weeks protocol, starting at 0.1 ml. Data stored and analyzed with SPSS 20. Quantitative variables were expressed as median and range, qualitative variables were expressed as frequencies. Associations were determined through Mann-Whitney test and Fisher's exact test.

RESULTS: Group 1: 42 patients (26 boys, 16 girls), age 5 years (1-7), starting dose 15 ml (5-100). Group 2: 58 patients (38 boys, 20 girls), age 4 years (1-10). No significant differences were found for sex, age, athma, AD, other food allergy, sIgE to milk, casein, beta-lactoglobulin, alpha-lactalbumin and severity of symptoms. Duration of treatment was shorter in group 1 (11, 4-35 weeks) than group 2 (14, 6-56 weeks) ($p=0,009$), number of adverse episodes was smaller in group 1 (1, 0-7) than group 2 (3, 1-15) ($p<0,0001$). There were no differences for success rate (group 1: 100%; group 2: 94,8%).

CONCLUSIONS: The determination of tolerance threshold has proven useful to design personalized OIT protocols. Custom milk OIT allowed an increase in efficiency and security, while maintaining efficacy.

371 Cross-Desensitization To Goat and Sheep Milk Protein In Cow's Milk Protein Desensitized Patients

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RATIONALE: Over 90% cow's milk protein (CMP) allergic patients cross react to both goat's milk protein (GMP) and sheep's milk protein (SMP) due to shared kappa-casein protein epitopes. We investigated whether CMP allergic patients who have been successfully fully desensitized in an oral immunotherapy (OIT) program would be able to consume GMP and SMP without an allergic reaction.

METHODS: 62 patients (age 4-29 years) who completed an OIT program and were consuming 240 ml of cow's milk and dairy foods daily were evaluated. Skin puncture testing (SPT) followed by oral challenge of 3000mg GMP and 6000 mg SMP was then performed.

RESULTS: Positive SPT's to GMP and SMP were identified in 34/53 and 33/53 respectively. 2/62 (3.2%) of patients challenged with GMP reacted. One patient had mild GI symptoms which resolved without treatment and the second experienced a systemic reaction which required treatment with epinephrine. 2/60 (3.3%) challenged with SMP reacted with mild conjunctivitis treated with antihistamines. The reactions to GMP and SMP occurred after one hour following the last dose and to relatively large amounts of protein.

CONCLUSIONS: Over 95% of desensitized CMP-allergy patients are also cross-desensitized to GMP and SMP. However, a supervised oral challenge to GMP or SMP should be performed before these patients are allowed to consume these related milk proteins.

372 Long Term Follow Up Of Children Who Incorporated Extensively Heated (baked milk) In The Diet

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RATIONALE: Most milk-allergic children tolerate baked-milk. We sought to determine the long-term outcomes in the baked-milk clinical trial.

METHODS: Following a baked-milk food challenge (OFC), children ingested 1-3 baked-milk servings daily. They returned for periodic follow-up and OFC to unheated milk. Current status of milk allergy was determined with telephone follow-up and record review.

RESULTS: Out of 100 children, 85 were followed for median 83 months, range 63-106; 15 were lost for follow–up. Among 52 initially baked milk-tolerant children, 37 (72%) became unheated milk-tolerant; 10 (19%) continued to ingest baked-milk only, whereas 5 (9%) avoided all forms of milk. Of those, 3 reacted to a pizza-OFC and stopped eating baked-milk, 1 developed ulcerative colitis and 1 reported delayed diarrhea at home following a baked-milk OFC. Among 38 children with unrestricted milk intake, 2 (5%) developed eosinophilic esophagitis (EoE); 2 reported exercise-induced symptoms following milk intake, and 8 reported dislike of liquid milk, including 5 with mild oral symptoms with liquid milk. There were no significant differences in any of the initial immunologic parameters in baked milk-tolerant children in regards to the final outcome of milk tolerance. Among 7 unheated milk-tolerant children at enrollment, 1 developed exercise-induced anaphylaxis and started avoiding all forms of milk. Among 23 baked milk-reactive subjects at enrollment, 18 (78%) avoided all forms of milk, 3 started tolerating baked-milk and 2 became tolerant to unheated milk.

CONCLUSIONS: Baked-milk diet is safe and well tolerated in the vast majority of baked milk-children, in contrast to the milk oral immunotherapy.

373 Baked Milk Oral Immunotherapy For Severe IgE-Mediated Cow's Milk Protein (CMP) Allergic Patients: Interim Results

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RATIONALE: A select group of highly sensitive IgE-mediated CMP allergic patients, failed desensitization during milk oral immunotherapy (milk-OIT) due to the frequency and severity of reactions. The safety and efficacy of baked milk (BM)-OIT for this group of patients has not been well studied.

METHODS: Patients (n=15, ages 4-13 years) were evaluated for their baseline sensitivity to BM and unheated milk (UM) by oral food challenge. BM was administered in a slowly escalating OIT program with a goal to reach 1.92 gram within 6-months, after which reactivity to UM will be assessed. Single individualized doses of low-fat dried milk powder were introduced into a muffin batter and baked at 180^0C for 30 minutes.

RESULTS: Three out of 15 patients (20%) failed the program on the first day, two due to allergic reactions occurring at ≤15 mg of BM and the third secondary to psychological stress. The remaining twelve patients began the BM-OIT program, but one discontinued treatment due to IgE-mediated reactions. Patients were able to tolerate baked milk at higher levels than at which they reacted to unheated milk ($z=2.91$, $p<0.01$, n=11, 1-tailed Wilcoxon Signed-Rank Test). Patients reached an average dose of 160 mg and 252 mg after 30 and 60 days, respectively. Allergic reactions requiring epinephrine occurred in 2/12 (16.6%) of patients during treatment. Patients responded similarly in the basophil activation test to UM and heated milk.

CONCLUSIONS: While IgE-CMA patients with a low threshold reactivity to CMP can progress in BM-OIT, it is not without risk for anaphylaxis to BM.

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374 Progression Towards Increasing Tolerance To Less Extensively Heat-Denatured Milk Products

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RATIONALE: The majority of milk-allergic children tolerate baked-milk products. We sought to determine the predictors of tolerance to increasing doses of baked-milk in children on baked-milk diets.

METHODS: Milk-allergic children were challenged sequentially over 2 days (up to 2 foods per day) to foods baked under different conditions and containing increasing amounts of milk at baseline. Based on the baseline challenges, different baked foods were incorporated into the diet and challenges repeated at 12-months. Antibody concentrations were measured with UniCAP®; basophil activation and T regulatory cells were measured by flow cytometry; skin prick tests were done with commercial extracts, unheated-milk and boiled-milk.

RESULTS: 136 children (70% males) were enrolled (median age: 7 yrs; inter-quartile range, 5-9 yrs). Forty-one (30%) reacted to muffin, 31 (23%) to pizza, 11 (8%) to rice pudding, 43 (32%) to unheated-milk; 10 (7%) tolerated unheated-milk. At 12 months, among 85 milk-allergic children who added baked-milk to the diet, there were no significant differences between those who progressed to tolerating increased doses of less extensively baked-milk (n=52) and those who did not (n=33) in any of the baseline immunologic parameters, including serum specific IgE and IgG4 antibody levels, skin test wheal size, other humoral parameters, and in peripheral blood T-regulatory cells. Among 41 baked-milk-reactive children, 7 became baked-milk tolerant and had lower baseline milk-and casein-IgE levels than children who remained baked milk-reactive.

CONCLUSIONS: Baseline immune parameters in milk-allergic children subsequently placed on diets consisting of baked-milk products do not predict who will progress towards increasing tolerance to less extensively heat-denatured milk products.

375 Cow's Milk Allergen Specific CD4+ T Cell Responses In Patients With Persistent Cow's Milk Allergy

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RATIONALE: Food allergies (FA) are increasing with approximately 4% of children affected. Cow's milk (CM) is a common cause of fatal/ near fatal anaphylactic reactions. Understanding of CM specific CD4+ T cells responses should elucidate the pathologic mechanisms of persistent CMA. Hypothesis: Patients with persistent, IgE mediated CMA may have abnormal T cell responses to casein.

METHODS: Patients 12 months and up were recruited based on their clinical history of systemic symptoms consistent with anaphylaxis to CM. Patients must also have a positive IgE by either skin prick testing or serum IgE class 3 or higher to CM. Whole blood was obtained from each subject and processed per protocol to isolate CD4+ T cells and grown in culture stimulated with CM proteins. Subjects were HLA typed. Tetramer guided epitope mapping was conducted to identify whey and casein specific T cell epitopes and analyzed via flow cytometry. Non-allergic subjects were also recruited and similar mapping experiments were performed.

RESULTS: All allergic subjects have Th2 type CD4+ T cells to whey protein, B lactoglobulin, and as1 casein (6/6). 83.3% (5/6) are reactive to B casein and 50% (3/6) to as2 and Kcasein. These cells are releasing IL-4 and IL-5 by cytokine analysis consistent with Th2 allergy phenotype. T cell responses in non-allergic subjects have Th1 phenotype, and were weaker in magnitude compared to allergic subjects.

CONCLUSIONS: Casein appears to be the fraction conferring lifelong CMA. Knowledge of T cell epitopes to CM consistently responsible for lifelong CMA will be useful in devising strategies to halt/reverse progression of CMA.

376 The Role Of Skin Prick Testing and Specific IgE To Boiled Versus Unheated Cow Milk In Cow Milk Allergic Children

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RATIONALE: Some cow milk(CM) allergic children can tolerate extensively heated milk. There is a commercially available IgE antibody measurement to boiled milk(BM) but the clinical significance is unknown.

METHODS: Children ages 2 to 18 with known CM allergy underwent skin prick testing (SPT) to CM extract and CM extract that was boiled at 95°-100°C for 5, 10, and 20min. IgE antibody to CM, BM for 5min,10min and 20min were measured (values in kU/L). Subjects were then challenged to BM.

RESULTS: 21 subjects were recruited. Mean CMSPT wheal was 10.14mm(range 4-20mm) compared to BMSPT wheals of 8.33mm(range 3-20mm), 8.33mm(range 0-21mm), and 7.6mm(range 0-20mm) at 5,10 and 20min respectively. Mean CM specific IgE was 10.4(range <0.35-43.9). Mean BM specific IgE at 20min was 7.38(range <0.35-50.3). The Pearson correlation coefficient for the CM and BM IgE was 0.840($p<0.0001$). 14 subjects completed BM challenge. 11 subjects passed the BM challenge and their CMSPT mean wheal was 9.45mm and BMSPT wheals at 5,10, and 20min were 7.36mm, 8.18mm, and 7mm. Their specific IgE to CM was 4.61(range <0.35-23.6) and IgE to BM at 5,10 and 20min were 2.9, 3.1 and 1.99 respectively. Three subjects failed the OFC, mean CMSPT wheal was 15mm and BMSPT wheals at 5, 10, and 20min were 15mm, 13mm, and 9mm. The mean specific IgE to CM was 22.2 and for 5min, 10min and 20min BM were 21.63, 24.5 and 22.5.

CONCLUSIONS: The CM and BM specific IgE appear to be highly correlated such that the BM IgE may have questionable clinical utility in predicting BM tolerant children.

377 Withdrawn

378 Comparing The Utility Of Skin Prick Testing Using Commercial Extracts and Fresh Food In Diagnosing Peanut Allergy

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RATIONALE: Skin prick tests (SPT) are commonly used to screen patients with suspected IgE-mediated food reactions. During preparation of commercial extracts (CE) for SPT, labile proteins that are responsible for IgE-mediated sensitivity may be degraded or lose allergenicity. For this reason, fresh food (FF) SPT may be performed.

METHODS: This is a retrospective analysis of children seen at National Jewish Health for evaluation of peanut allergy who underwent SPT using both commercial peanut extract and peanut butter diluted in buffered saline. An open oral food challenge (OFC) to peanut was performed in all subjects, and results were determined by the judgment of a physician. We sought to correlate the results of SPT using CE and FF to OFC outcomes and compare results obtained using each form of SPT.

RESULTS: In 62 subjects, SPT were positive in 47% using CE and 50% using FF. The two methods were in agreement in 48 (77%) subjects. OFCs were positive in 15 (24%) subjects. The positive predictive value of SPT for the OFC was 31% using CE (95% CI[16, 51%]) and 29% using FF (95% CI [15, 48%]). The negative predictive values of SPT using CE and FF were 82% (95% CI[64, 92%]) and 81% (95% CI[62, 92%]), respectively.

CONCLUSIONS: The predictive power of SPT using CE and FF in diagnosing peanut allergy is similar, though slightly better for CE. The use of SPT with FF is not superior to CE, nor is it a useful adjunct to CE in diagnosing peanut allergy.

379 Variability Of Major Allergens In Commercially Available Peanut Extracts For Skin Prick Testing

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RATIONALE: Peanut allergy is the most common cause of anaphylaxis in children. Its prevalence continues to increase over the past two decades. Commercially available crude peanut extracts are routinely used in skin prick testing (SPT) to confirm the clinical diagnosis of peanut allergy in the background of a positive medical history; yet multiple major peanut allergens have been characterized and standardized peanut extracts are not available. In this study, we investigated lot-to-lot variability of major allergens between commercial peanut extract products.

METHODS: Six peanut allergic patients with a positive clinical history of peanut allergy, positive peanut SPT, and peanut-specific IgE (ImmunoCap, Thermo-Fisher) level above class III were included. A total of 8 lots of commercial peanut extracts were obtained from 2 different companies. Those extracts were immunoblotted with patients' sera and an anti-human IgE secondary antibody to detect the relative abundance of various peanut allergens.

RESULTS: Relative densities of Ara h1, Ara h2 and other peanut allergens were measured. Significant lot-to-lot variations of major allergen abundance were observed between the two different companies as well as within lots from the same company.

CONCLUSIONS: Significant variations exist in commercially available peanut extracts used for SPT, making the interpretation of SPT results in clinical setting a challenge. Efforts to standardize peanut allergens, the use of adjunctive assays, such as the ImmunoCap assay for various peanut component allergens, and testing only in the presence of a strong clinical history are essential for proper diagnosis of peanut allergy.

380 Ara h 2 and The Relative Risk Of Other Sensitizations

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RATIONALE: IgE sensitization to the peanut seed storage protein (SSP) Ara h 2 is the most accurate single serological test for the diagnosis of peanut allergy. This study shows the relative risk of sensitization to other components that may influence a diagnostic strategy.

METHODS: In an IRB-approved cross-sectional study, ISAC112 microarray results from 154 subjects with known atopic disease, age 11 months to 70 years, were separated into those Ara h 2 IgE positive (n=62) and those Ara h 2 IgE negative (n=92). For the two groups, the prevalence of sensitization to other components and the relative risk of sensitizations were determined.

RESULTS: Those sensitized to Ara h 2 were 60 times more likely to be sensitized to the peanut SSP Ara h 3 and 11 times more likely to be sensitized to the cashew SSP Ana o 2. Sensitization to the peanut lipid transfer protein (LTP) Ara h 9, PR-10 Ara h 8 and profilin Bet v 2 was equally distributed between the groups. By history, Ara h 2 positive subjects reported local or systemic symptoms. SSP negative subjects who were sensitized to peanut LTP (n=8), PR-10 (n=24) or profilin (n=19) did not report systemic symptoms when peanut was included in their diet.

CONCLUSIONS: Sensitization to Ara h 2 increases the pre-test likelihood of sensitization to other SSPs. Sensitization to Ara h 2 does not affect the likelihood of sensitization to cross-reactive proteins LTP, PR-10 and profilin. Further studies are warranted before extending these observations to a formal predictive strategy using component-resolved diagnostics.

381 Variability Of Repeat Peanut Serum IgE Levels

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RATIONALE: Food specific serum IgE (sIgE) levels correlate with oral food challenge outcomes, however no guidelines exist regarding the interval to repeat testing, though this is common practice. We examined the utility of repeating peanut sIgE levels.

METHODS: This was a chart review of all patients at our teaching institution who had serum peanut sIgE drawn on 3 or more occasions and have had a diagnosis code of food allergy (693.1), personal history of allergy to peanut (V15.01), or anaphylaxis (995), between January 1, 2003, and January 1, 2013.

RESULTS: 966 patients had 3 or more peanut sIgE levels performed. 387 patients had an initial level of 14 kUA/L (95% predictive of clinical reactivity) to <100 kUA/L (median 40.30 kUA/L, median age 4 years). Only 8 patients (2.1%) had any subsequent peanut sIgE level <5 kUA/L (50% predictive of clinical reactivity), with only 2 decreasing below 3 kUA/L. 187 patients had an initial peanut sIgE level >100 kUA/L (median age 5 years). Only 8 patients (4.3%) had any subsequent peanut sIgE <50 kUA/L, and only 2 (1.1%) dropped below 14 kUA/L, though both patients had a repeat peanut sIgE level the same year of >100 kUA/L, indicating possible lab error.

CONCLUSIONS: It is unlikely that repeating peanut sIgE levels yearly in patients whose level is >14 kUA/L is necessary. Further analysis is necessary to determine precisely when to repeat these levels, if it all, and when to repeat levels in patients with more modest peanut sIgE levels.

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382 Does Peanut Allergen Conjunctival Provocation Test Reflect Specific IgE Levels To Peanut?

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RATIONALE: Allergen specific conjunctival provocation test (CPT) is proposed for diagnosing food allergy. We hypothesized that the challenge dose of peanut extract in the CPT was associated with level of specific immunoglobulin E (s-IgE) to peanut including component allergens.

METHODS: A double blind CPT with peanut extract was performed in 102 children (aged 5-17) with peanut sensitisation and/or peanut allergy. One drop of peanut extract diluted with NaCl 0.9 % in steps 0-5 (1:160 (step 0) to 1:1 (step 5)) was placed sequentially in the lower conjunctival pouch, and placebo in the other eye. The test was considered positive if ≥2 symptoms appeared (redness, itching, chemosis and/or lacrimation). S-IgEs were analysed by ImmunoCAP.

RESULTS: A significant association was found between challenge steps causing a conjunctival reaction and levels of s-IgE to peanut (r=-0.2, p=0.05), Ara h 1 (r=-0.23, p=0.02) and Ara h 8 (r=0.21, p=0.04), but not to Ara h 2, Ara h 3 or Ara h 9. Median s-IgE in children with positive CPT step 1 (n = 12) was 160.5 kU/l while 4.1 kU/l in children with positive CPT step 5 (n = 9). Children with a history of anaphylaxis (n=13) to peanut reacted to a lower conjunctival step than those without (2.08 (95%CI 1.56, 2.60) vs 2.74 (95%CI 2.46, 3.03)) (p=0.03). No severe adverse reactions occurred.

CONCLUSIONS: Peanut CPT is easy to perform, safe and correlates with s-IgE levels to peanut allergens. Before CPT can be an alternative to oral food challenge, a comparison between the two tests must be performed.

383 The Use Of The ISAC Mircroarray Platform In Food Allergic Patients

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RATIONALE: We examined the diagnostic utility of the 112 allergen component Immuno Solid-phase Allergen Chip (ISAC) for the evaluation of food allergy compared to oral food challenges (OFC).

METHODS: We recruited children in our university-based, outpatient practice referred for OFC; children were challenged to egg (31), peanut (39), walnut (26), cashew (18), soy (25), sesame (27), and wheat (27). Challenge outcomes were compared with IgE levels to allergens available on ISAC microarray.

RESULTS: 10 patients reacted to egg, 15 to peanut, 11 to walnut, 5 to cashew, 12 to soy, 12 to sesame and 16 to wheat. The median Gal d 1 and 2 levels were significantly higher in the egg-reactive patients compared to non-reactive patients (P=0.0251 and 0.0269, respectively). The only peanut component that had a significantly higher median IgE level in the peanut-reactive patients compared to the non-reactive patients was Ara h 6 (P=0.0006). An IgE level above 0.5 ISU for Ara h 1, 2, and/or 6, was 73% sensitive and 92% specific in predicting clinical reactivity. The median Jug r 1 level was significantly higher among the walnut-reactive patients (P=0.0078). The median Ses i 1 level was significantly higher in the sesame-reactive patients (P=0.0034). No significant differences were seen for any of the median IgE levels to the cashew, wheat, or soy components between the reactive and non-reactive patients.

CONCLUSIONS: Current ISAC component testing may be a helpful tool in predicting OFC outcomes for some foods, including egg, peanut, walnut, and sesame, but cashew, soy, and wheat need additional components.

384 IgE Antibodies To Ara h 2 and Ara h 6 By Immunocap ISAC Distinguish Peanut Anaphylaxis Children From Asymptomatic Peanut Sensitization

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RATIONALE: Recently Ara h 1 to 3 and 6 are considered major peanut allergens. These components may be more accurate diagnostic tools for the assessment of peanut allergy. We examined the difference of peanut component specific IgE antibody by ImmunoCAP ISAC between in anaphylaxis children and in asymptomatic peanut sensitization with multiple food allergies.

METHODS: Nine convincing history of peanut anaphylaxis (3-17 years old. sIgE to peanut 11～81UA/ml), 7 of multiple food allergy (FA) with asymptomatic peanut sensitization (5 FA and 2 hyper-IgE syndromeHIE. sIgE to peanut 3～100 UA/ml) enrolled this study. The sIgE to Ara h 1,2.3.6.8.9 measured by ImmunoCAP microassay ISAC.

RESULTS: sIgE to Ara h 2 and Ara h 6 were higher than sIgE to Ara h 1 and 3 in the sera of the peanut anaphylaxis. No case of anaphylaxis had Ara h 8 and 9. Four of 5 asymptomatic peanut sensitization with food allergy had sIgE to Ara h 8 (PR -10) and no cases had sIgE to Ara h 2 and Ara h 6. Two cases of HIE had sIgE to Ara h 9 (LTP).

CONCLUSIONS: The measurement of sIgE to Ara h 2 and/or Ara h 6 is useful in a diagnosis of the peanut anaphylaxis. Microassay ISAC technology can distinguish peanut allergy with allergen specific IgE and peanut sensitization with cross reactive IgE.

385 Molecular Component Testing For Peanut Allergy Reactivity Differs Based On Age

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RATIONALE: Peanut allergy is one of the most severe and common food allergies. Peanut molecular component testing for IgE to Ara h 1, 2, 3, 8, and 9 can potentially assist allergists with the stratification of patients.

METHODS: A retrospective review was performed over the first ten months of the testing for IgE to Ara h 1, 2, 3, 8, and 9 (Phadia), utilizing data with de-identified patient health information, compiled into risk groups based on current research utilizing a cutoff of 0.1 kU/L positive test, and evaluated based on patient age.

RESULTS: Overall, 66% of samples were positive for Ara h 2, which carries the highest risk for anaphylaxis. However, in patients <10 years old this percentage rose to 75%. Of these samples, 13% were positive for only Ara h 2; 54% were positive for Ara h 1, 2, and 3. Positivity for Ara h 9 with or without Ara h 8 positivity accounted for 2.2% of samples and did not vary based on patient age. In the complete sample set only 7% of samples were positive for Ara h 8 alone, but samples from patient's >18 this percentage rose to 14%.

CONCLUSIONS: Molecular component allergy testing represents a major step forward in diagnosing and assessing risk for peanut allergic individuals. A significant rate of high risk identification indicates the importance of this information for patients and their families. Additional studies and subsequent practice guidelines are likely to emerge in the near future with component testing now available for clinical use.

386 CD-Sens and Component Resolved Diagnostics In Diagnosing Hazelnut Allergy

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RATIONALE: As a result of shortage of reliable diagnostic tools, the diagnosis of hazelnut allergy highly relies on Double Blind Placebo Controlled Food Challenges (DBPCFC). We aimed to evaluate the clinical significance of basophil threshold stimulation, CD-sens, and component-resolved diagnostics among Swedish children and adolescents with suspected hazelnut allergy.

METHODS: A DBPCFC has so far been performed on 37 children and adolescents with suspected IgE-mediated hazelnut allergy. CD-sens with hazelnut and IgE-antibodies (IgE-ab) against hazelnut and hazelnut components Cor a 1, Cor a 8, Cor a 9 and Cor a 14 were analyzed.

RESULTS: Among the 7 patients (19%) diagnosed with hazelnut allergy (defined as objective symptoms at the DBPCFC), we found statistically significantly higher levels of IgE-ab to components Cor a 9 ($p=0.001$) and Cor a 14 ($p=<0.001$) compared to the non-reacting group. The median level of Cor a 9 and Cor a 14 in those reacting at the challenge was 4.5 kU/l (1.5-97.5) and 6.5 kU/l (2.7-84) respectively, compared to 0.06 kU/l (0-11) and 0.04 kU/l (0-13.9) in the non-reacting group. CD-sens values were also significantly higher in those reacting at the challenge: median 11.9 (5.5-561) compared to 0.1 (0-68) in those not reacting ($p=<0.001$). CD-sens identified all children with objective symptoms on DBPCFC. However, 2/30 children with negative DBPCFC tested positive in CD-sens.

CONCLUSIONS: CD-sens to hazelnut, as well as IgE-ab to the hazelnut components Cor a 9 and Cor a 14 seems to be useful and reliable diagnostic markers in the work-up of suspected hazelnut allergy. The implementation of these tests could reduce the need of DBPCFC.

387 Effect Of Oleic Acid On The Allergenic Properties Of Peanut and Cashew Allergens

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RATIONALE: Oleic acid is the major fatty acid in peanuts and cashews. There is limited information about its effect on peanut and cashew allergens during heating. The objective was to determine if heat treatment with oleic acid changes the allergenic properties of these nut proteins.

METHODS: Peanut and cashew proteins were fixed to solid phase membranes. The membranes were then incubated with a sodium oleate solution (5 mM) at 70°C for 30 min and analyzed for IgE binding. Inhibition ELISA and SDS-PAGE were also performed on sodium oleate-treated allergens solutions under the same condition.

RESULTS: In blots, both peanut and cashew allergens exhibited a marked reduction in IgE binding, indicating that oleic acid may have blocked their IgE-binding sites by cross-linking. Cross-links were confirmed in SDS-PAGE profiles, in which partially-reduced allergen bands and high molecular-weight cross-links were seen. An overall reduction in IgE binding was obtained in ELISA as well.

CONCLUSIONS: Treatment of peanut and cashew allergens with oleic acid (5 mM) at high temperature resulted in a marked reduction of IgE binding in both ELISA and blot assays. This suggests that oleic acid may reduce the allergenic properties of peanut and cashew allergens. Given the health benefits of oleic acid, further investigation is needed to determine its potential for producing less allergenic peanut- or cashew-based products.

388 Identification Of Conformational IgE Epitopes Of Ara h 2 and Ara h 6

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RATIONALE: Although linear IgE-binding epitopes of peanut allergens have been defined, little is known about conformational IgE-binding epitopes that are now felt to be important for IgE cross-linking. This study used phage peptide display system to identify clinically important conformational IgE epitopes of Ara h 2 and Ara h 6.

METHODS: A phage peptide library displaying 12-mer peptides was used. IgE that recognizes Ara h 2/Ara h 6 (IgE-A2/6) was purified from peanut allergic serum. Phage screening strategy included biopanning, immune-screening, and phage-based ELISA. The phage peptides that were identified were then analyzed for sequence homology with Ara h 2/ Ara h 6 protein sequences.

RESULTS: A total of 54 individual phage peptides that bind Ara h 2/6 specific IgE have been identified. Some of the peptides were eluted multiple times with an individual serum and some were eluted with sera from two or more patients. Comparison of the identified peptides with the primary sequences of Ara h 2/6 revealed several alignment hot spots. Among the 54 peptides, 29 have been tested in an ELISA with 2 non-peanut allergic sera and 9 peanut allergic sera from patients with varied clinical histories. Most of the identified peptides are conformational epitopes and are recognized by sera from patients with varied clinical histories. IgE from each patient recognizes a distinct set of peptides.

CONCLUSIONS: Clinically related conformational IgE epitopes can be identified by screening phage peptide library with patient IgE. IgE from each individual allergic serum has distinct recognition pattern.

389 Basophil Response To Storage Proteins and Oleosins From Sunflower Seed

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RATIONALE: Sunflower seeds (*Helianthus annuus*) can trigger anaphylactic reactions, generalized urticaria, angioedema, oral allergy syndrome and other symptoms after ingestion. These reactions have been attributed to 2S albumins (SFA-8) and LTP (Hel a 3). We aimed to characterize the basophil response to storage proteins and oleosins from sunflower seed in patients allergic to sunflower.

METHODS: The proteins 2S, 11S and oleosins were purified from a raw sunflower seed extract by FPLC/HPLC and identified by specific antibodies and peptide mass fingerprinting. We tested the immunological recognition of these proteins by basophil activation test (BAT). Four concentrations (1, 0.2, 0.1, 0.02 μg/ml) of each protein and the sunflower roasted extract were used. Ten patients were selected by clinical history and skin prick test positive to commercial extract. Five subjects with skin prick test negative to commercial extract and not food allergy were included as controls.

RESULTS: All patients showed a positive basophil response to roasted extract. BAT was positive in 87.5% of cases for 2S albumin, 60% for oleosins, and 57.14% for 11S albumin. 50% of patients were positive to the 3 proteins, 37.5% only for 2S albumin and 12.5% for storage proteins (both 2S and 11S albumin). In 40% of controls the concentration 1 μg/ml of 2S albumin induced low basophil activation.

CONCLUSIONS: All the sunflower allergens tested in our group of patients were able to induce basophil activation in a high percentage of cases. Storage proteins and oleosins are responsible for sunflower allergy in 50% of cases.

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390 Utility Of Ovomucoid Specific IgE In Predicting Unheated Egg Food Challenge Outcomes

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RATIONALE: Ovomucoid is the dominant allergen in hen's egg. While several studies have evaluated the utility of ovomucoid specific IgE (sIgE) levels in predicting baked egg food challenge outcomes, studies evaluating ovomucoid sIgE as a predictor of unheated egg (e.g. scrambled or hardboiled) challenge outcomes are limited.

METHODS: Retrospective review of 52 children who underwent unheated egg food challenge and had ovomucoid specific IgE (sIgE) measured.

RESULTS: 44/52 (84.6%) of children passed an unheated egg challenge. Ovomucoid sIgE predicted unheated egg challenge outcome (passed median <0.35 kU/L, range <0.35-0.64 kU/L; failed median 0.40 kU/L, range <0.35-3.13 kU/L, p=0.001). We were able to establish a >90% predictive value for passing unheated egg challenge for ovomucoid sIgE 0.45 kU/L (50.0% sensitivity, 93.2% specificity) and a 100% predictive value for failing unheated egg challenge for ovomucoid sIgE 1.59 kU/L (25.5% sensitivity, 100% specificity). Ovomucoid sIgE correlated with egg white sIgE levels (Spearman correlation coefficient = 0.585, p<0.001). Receiver operating characteristic curve analysis of ovomucoid and egg white sIgE demonstrated areas under the curve of 0.718 and 0.798, respectively. No significant difference was observed among those immunologic parameters in their abilities to predict unheated egg challenge outcome (p=0.382).

CONCLUSIONS: Ovomucoid sIgE level may be helpful in predicting unheated egg challenge outcomes. Funding: This research is supported by grants R01 AI 073964 and K24 AI 106822 from the National Institutes of Health (PI, Dr. Phipatanakul).

391 Allergy Testing In Childhood: Agreement Between Skin Prick Test and Specific IgE In Preschool Children

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RATIONALE: Skin prick test (SPT) and specific IgE (sIgE) are important diagnostic tools for the clinician to assess allergic sensitization. Little is known about the agreement between the two methods in preschool children.

METHODS: 411 children were included from the Copenhagen Prospective Study on Asthma in Childhood ($COPSAC_{2000}$) birth cohort born to mothers with asthma. SPT and sIgE against 14 common allergens were measured simultaneously when the children were 6mo, 18mo, 4yrs and 6yrs old. The allergens were analyzed in two groups: inhalant allergens and food allergens. Agreement between the two methods was analyzed using kappa statistics and visualized by Venn diagrams.

RESULTS: The prevalence of inhalant allergen sensitization increased during childhood for both sIgE (6mo: 0.6%; 18mo: 4.2%; 4yrs: 18.1%; 6yrs: 24.8%, test for trend: p<0.0001) and SPT (6mo: 1.5%; 18mo: 3.8%; 4yrs: 8.4%; 6yrs: 15.4%, p<0.0001). The prevalence of food sensitization increased during childhood for sIgE (6mo: 7.8%; 18mo: 12.1%; 4yrs: 15.0%; 6yrs: 18.9%, p<0.0001) but decreased for SPT (6mo: 5.3%; 18mo: 5.1%; 4yrs: 3.7%; 6yrs: 3.0%, p=0.054). In general, agreement between SPT and sIgE was not good (all κ-coefficients≤0.60); agreement was unchanged for inhalant allergens from 18 months to 6 years (κ=0.45-0.49) but decreased for food allergens to a κ-coefficient of 0.16 and 0.14 at 4 and 6 years.

CONCLUSIONS: There is a substantial disagreement between SPT and sIgE during preschool age, and the choice of assessment method therefore has a major impact on test results.

392 Measurement Of Allergenic Components For Predicting Clinically Relevant Shrimp Allergy In House Dust Mite Sensitized Children

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RATIONALE: Tropomyosin, the major shellfish allergen, is regarded to be responsible for clinical cross-reactivity to inhaled house dust mites (HDMs). The aim of this study was to determine the value of detection of allergenic components in the diagnosis of shrimp allergy in HDM sensitized children.

METHODS: We studied 118 children with allergic disease who had been sensitized to HDM. HDM-sensitized patients were defined as having an allergen-specific history plus a concomitant positive skin-prick tests (SPTs) to natural allergen extracts and/or positive allergen-specific IgE. All subjects underwent SPTs with shrimp. Measurements of specific IgE to shrimp in all subjects were carried out by means of ImmunoCAP. Determination of specific IgE antibodies to allergen components was performed using a customized allergen microarray (ISAC®).

RESULTS: Six patients had a clinical history of shrimp hypersensitivity. IgE measurement to allergen components (Pen m 2, Pen m 4, Pen m 1, and Der p 10) by ISAC was equally positive in 66.7% (4/6) of the patients with shrimp allergy. Of the 112 patients without shrimp allergy, only 1.8% (2/112) had IgE to shrimp component (Pen m 2 and/or Pen m 4) compared with 20.5% (23/112) who had IgE to shrimp and 33.9% (38/112) who had positive SPTs responses to shrimp. IgE to shrimp tropomyosin (Pen m 1) and mite tropomyosin (Der p 10) was both equally positive in 4.5% (5/112) of the patients without shrimp allergy.

CONCLUSIONS: Measurements of allergen components could be beneficial in the diagnosis of shrimp allergy in house dust mite sensitized children.

393 Cross Reactivity Of Alpha Gal Allergy With An Extended Red Meat Panel

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RATIONALE: Galactose-alpha-1,3-galactose (alpha-gal), is a carbohydrate moiety found in red meats such as beef, pork and lamb and is associated with a delayed IgE response, leading to urticaria and/or anaphylaxis. Alpha Gal specific IgE is believed to be responsible for cross reactive allergies to beef, pork and lamb, but little data exists showing the cross reactivity of Alpha Gal and other non-primate mammalian meats such as rabbit and veal.

METHODS: De-identified serum samples from Alpha Gal positive (n=15) and negative (n=15) patients were tested for IgE reactivity with beef, pork, lamb, rabbit, veal, and chicken (as a negative control). Data was compiled into groups based on a cutoff of 0.1 kU/L as a positive test.

RESULTS: Thirteen percent of Alpha Gal negative samples contained IgE antibodies against at least one red meat. In the alpha gal positive subset, 13 percent of samples were negative to veal, and 20 percent were negative to rabbit. However none were negative for beef, pork, or lamb. Twelve percent of beef IgE positive samples were negative for IgE to veal.

CONCLUSIONS: Analysis of results testing for red meat and Alpha Gal reactive IgE revealed that a large portion of samples contained antibodies to both red meats and Alpha Gal, supporting the inter-related nature of these allergies and the need for complete testing to identify source(s). Interestingly, there was not complete agreement between veal and beef positivity indicating there may be different allergens present in the various meat preparations.

394 Prediction Of Tolerance To Food Allergens By The Allergen-Specific IgE/Total IgE Ratio

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RATIONALE: Although allergists typically use allergen-specific Immunoglobulin E (IgE) levels or skin prick test wheal sizes as indicators of tolerance to a food allergen (e.g., readiness to proceed with an oral food challenge), both tests have high rates of false positive results and mislabel patients who are tolerant as allergic to the food. We sought to examine the accuracy of the ratio of allergen-specific IgE to total IgE ("The Ratio") in predicting tolerance to a food allergen.

METHODS: Medical records of food allergy patients participating in an oral food challenge at an allergy outpatient clinic were reviewed for IgE serology and oral food challenge data, which were analyzed for associations using Mann-Whitney U-tests and Receiver Operator Characteristics curves.

RESULTS: The Ratio for participants who failed their challenge was significantly higher than the Ratio of those who passed their challenge (1.94% vs. 1.06%, P<.001, n=195). This was mostly associated with foods for which tolerance typically is not developed, such as peanut, tree nut, shellfish, and seeds (2.58% vs. 0.88%, P<.0001, n=93). Receiver Operator Characteristics curves showed the Ratio was significantly more accurate than allergen-specific IgE alone in predicting tolerance (0.69 vs. 0.55, P=.03). This finding was mostly associated with foods for which tolerance typically is not developed (0.81 vs. 0.54, P<.01).

CONCLUSIONS: The Ratio is more accurate than allergen-specific IgE alone in predicting tolerance to foods for which tolerance typically is not developed. The Ratio may be a useful indicator to identify patients most likely to pass an oral food challenge.

395 T Regulatory Cells and Food Specific Responses In Peanut and Egg Allergic Children

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RATIONALE: We hypothesize that food allergic patients have higher levels of proinflammatory cytokines and that control responses mediated by T regulatory cells are food specific. We report a pilot study investigating this possibility.

METHODS: PBMCs from 36 children with egg allergy or without food allergy were isolated and stimulated *in vitro* for 48 hours with or without ovalbumin 100 μg/mL. Luminex was performed to measure expression of the cytokines IL-5, IL-10, and IL-17. Eleven additional patients with egg and/or peanut allergy, or controls had flow cytometry performed after stimulation with ovalbumin 100 μg/mL and whole peanut extract (WPE) 100 μg/mL. Linear mixed models and the Mann Whitney test were utilized for analyses.

RESULTS: Children with egg allergy demonstrated increased levels of IL-5 and IL-17 expression when compared to non-food allergic children (51.23 vs 6.9 pg/mL, p<0.01, 17.54 vs 15.6 pg/mL, p<0.01). Patients without food allergy expressed higher levels of IL-10 when compared to egg allergic children (1335.2 vs 1295.65 pg/mL, p<0.01). Patients stimulated with non-relevant antigen had higher percentages of FoxP3+, IL-10 producing cells (0.69 vs 0.48%, for egg, 2.85 vs 2.21% for WPE) when compared to antigen stimulation, though this did not reach statistical significance. This finding suggests that tolerance is food specific.

CONCLUSIONS: Our findings suggest that food allergy is a proinflammatory state and that antigen specific T regulatory cells are likely important in tolerance to foods.

396 Surveillance Of Persistent Nut Allergy Including The Use Of Basophil Activation Test In Pediatric Patients

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RATIONALE: 1-2% of the US population is allergic to peanuts and/or tree nuts. Specific IgE (sIgE), skin prick testing and food challenge remain the tools of current food allergy diagnosis and surveillance. We sought to characterize factors associated with persistent allergy to these foods and to evaluate the basophil activation test (BAT) as an emerging diagnostic tool in the evaluation of food allergy.

METHODS: Forty-four nut allergic pediatric patients and fourteen controls enrolled in an ongoing cross-sectional study were analyzed. Historical information regarding foods avoided, preventative avoidance, accidental ingestions, sIgE and SPT were reviewed. Nine patients had blood drawn for BAT to peanut, tree nuts, and/or egg (negative control).

RESULTS: When examining factors associated with persistence of food allergy, we found that peanut sIgE increased significantly in children with peanut accidental ingestion (mean pre-ingestion sIgE: 20.5, mean post-ingestion sIgE: 47.0, P< 0.05). Tree nut sIgE did not change. Analysis of the BAT results showed an overall sensitivity and specificity of 90% and 66% respectively. The test had a positive predictive value of 75% and negative predictive value of 88%. BAT results did not consistently reflect the corresponding specific IgE.

CONCLUSIONS: This study suggests that peanut allergic patients with accidental ingestion may be less likely to develop tolerance to peanut or that development of tolerance is delayed. The BAT may prove a helpful adjunctive diagnostic and surveillance tool in food allergic patients but cannot replace food challenge.

397 Assessment Of a Modified Basophil Activation Test In The Diagnosis Of Peanut Allergy

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RATIONALE: The basophil activation test (BAT) is a flow cytometric test that detects basophil activation to define in vitro allergen specific inflammatory mediator release. Our objective is to establish a modified BAT with improved sensitivity for defining allergic reactivity to foods based on challenge results.

METHODS: The presence of peanut allergy in children ages 2-16 and adults ages 17-65 will be documented by an appropriate history plus positive blood IgE or commercial skin prick testing to peanut. BAT is performed on whole blood stimulated with crude peanut extract and Ara h antigen. We utilize CD123 positive, HLA-DR negative cells to more accurately identify basophils as well as polyclonal anti-IgE antibody as the positive control for a strong activation signal. The patients then undergo oral peanut challenges.

RESULTS: BAT has been performed on 10 subjects with suspected peanut allergy. After *in vitro* peanut challenge with crude peanut extract and Ara h antigen, the basophils from 2 peanut-allergic individuals show activation as up-regulation of CD63 expression as compared to without the addition of allergen. The basophils from 3 non-peanut allergic subjects show no activation of basophils on peanut challenge in vitro. 2 patients were nonresponders and 3 patients had failed tests due to overwhelming number of red blood cells in comparison to white blood cells. Peanut challenges are yet to be performed on these subjects.

CONCLUSIONS: The BAT has potential be become an important tool in the diagnosis and management of severe food allergy by contributing to whether oral challenge can safely be undertaken.

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398 Utility Of Probability Curves Using 3gAllergy For Diagnosis Of Wheat Allergy

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RATIONALE: Previously we presented the utility of allergen specific IgE (sIgE) measurements by 3gAllergy using probability curves for diagnosis of hen's egg and cow's milk allergy. In this study, we assessed the utility of probability curves in the diagnosis of wheat allergy.

METHODS: Serum samples were collected from 330 children (mean age 10.5 months) suspected of having wheat allergy. Allergen sIgE testing for wheat (W) and Gluten (G) was performed using the IMMULITE® 2000 3gAllergy™ and ImmunoCAP® sIgE assays. Final diagnosis of food allergy (FA) was confirmed by oral food challenge (OFC) and convincing allergic reactions to wheat products; acquisition of tolerance was defined as patients who had ingested 100 g of Japanese wheat noodle without any symptoms. Serum samples were drawn 6 months before the FA diagnosis and then stored frozen.

RESULTS: Of the 330 patients, 49 children were diagnosed with W allergy. W and G sIgE measurements by 3g were correlated with CAP W (W: $r=0.91$, G: $r=0.93$, $p<0.0001$); In comparison to CAP, 3g W values were likely to be lower than Cap W. Probability curves using the sIgE values from the FA positive negative groups were established using logistic regression analysis, and the 95% predictive decision point were determined by 3g (W: 234 IU_A/mL, G: 92 IU_A/mL.) The 95% decision point for W by CAP was not determined because sIgE results exceeded the range of the assay.

CONCLUSIONS: Measurement of sIgE to W and G using 3g and the PC are beneficial for diagnosis of W allergy.

399 Skin Prick Test and Specific IgE To Purified Peanut Allergens Are Related To The Age Of Onset Of Sympstons

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RATIONALE: We aim to analyse changes in the skin prick test (SPT) to peanut and IgE levels to Ara h1; Ara h2; Arah3; Ara h9 and Pru p3 in relationship with the age of patients and the onsent of symptons.

METHODS: We studied 167 peanut allergic patients confirmed by SPT or prick by prick to fresh extract, in vitro specific IgE and/or a double blind placebo control challenge with peanut. Patients were divided into 3 ages groups: 15-30, 31-45 and 46-80. IgE levels were determined by ELISA to Ara h1, Ara h2, Ara h3, Ara h9 and Pru p3.

RESULTS: Thirty-three patients evaluated in the first group (54%) were positive to peanut in SPT, 34 (42,50%) in second group and 4 (15%) in the last group. ELISA showed 50% positivity to Ara h1, Ara h2; Ara h3, 60% to Pru p3 and 80% to Ara h9 in the first group. 40% to Ara h1, Ara h3, 50% to Ara h9 and Pru p3 and less than 30% to Arah2 in the second group. In the third group IgE levels fall below 20% to Ara h2, Ara h3 and Ara h9. Ara h1 and Pru p3 were not detected.

CONCLUSIONS: We observed a decrease in the reactivity response of SPT to peanut and IgE levels in the third age group with and onset of symptoms over tan ten years. Levels to Ara h 2 were lower on median ages, although Ara h9 and Pru p3 were similar in the the first groups.

400 The Relationship Between Mitochondrial Haplogroups Variant on Children with Cow Milk Allergy Expressed As Atopic Dermatitis and Gastrointestinal Disease

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RATIONALE: Genotypes associated to cow's milk allergy are unknown. They have not been replicated in independent population, and could be responsible for the marked variability in individual clinical response to cow milk proteins.The objective was to characterize haplogroups of the D-Loop region of mitochondrial DNA in a group of children allergic to coẃs milk in order to arrive to a better knowledge of biological and genetic heritability in the etiology of the disease

METHODS: .We studied 41 children of both sex aged 0 – 2 years, 11 allergic to coẃs milk demonstrated by challenge and 30 healthy subjects (controls), from the urban area of Rio Cuarto City, Córdoba, Argentina. We performed Analysis of variants of D-loop region of the mitochondrial genome. The D-Loop region HVI, II and II of the mitochondrial genome was amplified by PCR, for which we used specific primers. Phylogenetic analysis was calculated using the program CLUSTAL OMEGA, the Neighbor–Joining, BLOSUM62 with data studied and recorded by Jukes-Cantor and then with Kimura-2

RESULTS: The cow milk allergic patients were divided in: (a) Atopic Dermatitis (AD) + Gastrointestinal Disease (GID) (n: 6) and (b) Rhinitis and Asthma (n: 5). We found the non described variant in transition of haplogroups, T16519C associated with (a) in 6/6 cases when compared with (b) negative in 5/5 cases and the control group (6/30), p= 0, 0312, RR: 2,900

CONCLUSIONS: These features suggest that this variant probably increases the possibility of suffering cow milk allergy associated with AD +GID.

401 Detection Of Peanut Allergens In Breast Milk and Saliva

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RATIONALE: Infants have been reported to react to peanut upon their first known exposure and peanut proteins have been detected in breast milk. Identification of the allergens or fragments thereof in breast milk may allow us to determine if they are sensitizing or tolerizing.

METHODS: Various immunoassays were optimized and utilized to analyze for the presence of peanut proteins in breast milk. Breast milk samples from healthy lactating mothers were collected following a 48 hour peanut fast and spiked with known amounts of peanuts and subjected to immunoprecipitation, SDS-PAGE, western blot and ELISA with anti-peanut, anti-Ara h 1, 2 and 3 antibodies. Mass spectrometry was performed to identify peanut peptides in breast milk.

RESULTS: We found that we were able to detect peanut allergen, Ara h 1, Ara h 2 and Ara h3, in peanut-spiked breast milk and saliva at nM levels. We were able to identify specific peanut peptides of Ara h 1, Ara h 2 and Ara h 3/4 using mass spectrometry.

CONCLUSIONS: The fact that allergic proteins or peptides survive in digestive enzymes, and are likely to be secreted in biological fluids indicates they are most likely the sensitizing or tolerizing agents within an allergic food. Developing methods to detect these allergens in breast milk is a preliminary step in identification of allergens or fragments thereof that are secreted and may contribute to the original development of allergic disease.

402 Importance Of High Molecular Weight Proteins In Walnut Allergy

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RATIONALE: Walnut is one of the most commonly reported causes of tree nut allergy, but relatively little is known about the allergens involved, including which allergens are the most important in various populations. The objective of this study was to characterize the profile of IgE reactivity in walnut-allergic subjects from several geographical locations.

METHODS: The extraction of proteins from raw walnuts was optimized using buffers with varying concentrations of NaCl. The optimized extract was used for IgE immunoblotting with sera from walnut-allergic patients from Manchester, UK; Madrid and Barcelona, Spain; and Athens, Greece (n=48). Reactive protein bands were identified by mass spectrometry. An indirect IgE ELISA was conducted with walnut 11S legumin in either a non-reduced or reduced and alkylated state.

RESULTS: IgE immunoblotting indicated that the 11S legumin (Jug r 4), which required 1.5 M NaCl for optimum solubility, showed IgE reactivity in a large proportion (76%) of blot-reactive subjects. This level of reactivity was consistent across populations. For many individuals, however, the intensity of IgE binding decreased dramatically upon analysis under reducing conditions. IgE ELISAs confirmed that reduced and alkylated 11S legumin exhibited decreased IgE reactivity (16-66% decreases in absorbance values) in most sera.

CONCLUSIONS: The 11S legumin-like globulin from walnut is an important allergen in several different walnut-allergic populations. For many individuals the conformation of the allergen, particularly the presence of intact heterodimeric subunit pairs, is important for IgE reactivity. The solubility requirements and structural characteristics of the 11S could have important implications for diagnostic materials and protein allergenicity.

403 Characterizing The Effect Of Sodium Sulfite On Cashew Allergens

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RATIONALE: Sulfites are multipurpose compounds included in the FDAs Generally Recognized as Safe (GRAS) list, but they can cause serious immunologic reactions in some individuals.They are commonly used as preservatives, to prevent enzymatic and non-enzymatic browning, and are found naturally in some foods. Our objective was to characterize the effect of sulfite compounds on cashew allergens.

METHODS: Cashew extracts and purified cashew allergens were treated with sodium sulfite at various concentrations and temperatures and analyzed by SDS-PAGE. LC-MS/MS analysis identified sites of sulfite modification on cashew allergens, and cashew allergen structure was characterized by circular dichroism. IgE binding with cashew allergic patient sera was evaluated by immunoblot and ELISA.

RESULTS: Treatment of cashew extracts with sodium sulfite at concentrations as low as 1 mM (126 ppm) altered migration of Ana o 2 and Ana o 3 on SDS-PAGE. LC-MS/MS and circular dichroism studies indicate that sodium sulfite targets the disulfide bonds of Ana o 3 disrupting its structure. Sodium sulfite treatment of cashew extracts reduced IgE binding to the Ana o 2 and Ana o 3 cashew allergens.

CONCLUSIONS: Treatment of cashew extracts with sodium sulfite disrupts the structure of Ana o 2 and Ana o 3 allergens and reduces IgE binding. It is possible that sulfite treatment may diminish the allergenicity of cashew nuts. The potential creation of sulfite-dependent neo-antigens is unknown. Continued research is warranted to evaluate the inclusion of sodium sulfite or other sulfite compounds in cashew processing steps.

404 Cross-Sensitization To Rosaceae Fruits and Their Molecular Components In Japanese School Children

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RATIONALE: Cross-reactivity between plant allergens is a central pathogenesis of fruit allergy. Cross-sensitization status to both fruits and related pollen in general population is to be clarified. Objectives: To describe the prevalence of allergic sensitization to Rosaceae fruits in general risk children in Japan and to evaluate the cross-sensitization characteristics between the fruit molecular components and its related birch pollen proteins.

METHODS: A cohort of 251 children participated in the study. They had neither acute nor chronic diseases other than having allergic symptoms. Mean age (±SD) was 9.6±2.8. The ISAAC questionnaire was used for the assessment of allergy. Questionnaire asking about food-induced symptoms was also administered. Specific IgE to birch, apple, peach, cherry, and their molecular component families; PR-10 family of Bet v 1, Mal d 1, and Pru av 1, profilin family of Bet v 2, Mal d 4, and Pru av 4, LTP family of Pru p 3 and Pru av 3 was measured by IMMULITE® 2000 3gAllergy™.

RESULTS: Prevalence of self-reported Rosaceae fruit allergy was only 2.8%. However, sensitization to birch, apple, peach and cherry was relatively high at 30.4, 21.6, 26.6, and 29.0%, respectively. The prevalence was especially high in children with rhinitis. Specific IgE levels to each profilin were highly correlated with Spearman's r>0.9 and moderately correlated among PR-10-IgE. There was no correlation between LTP-IgE.

CONCLUSIONS: Although symptomatic Rosaceae fruit allergy is not prevalent, sensitization rate is relatively high, which may indicate future development of fruit allergy. Rosaceae-related profilins seems pan-allergens and LTPs may be specific to each fruit.

405 Food-Specific IgE Panel Testing Commonly Results In Misdiagnosis and Inappropriate Dietary Exclusion

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RATIONALE: Patients are frequently misdiagnosed with food allergy. We examined the frequency with which panels of food-specific IgE identified a previously unknown allergen.

METHODS: A retrospective chart review identified new patient encounters seen in a tertiary Food Allergy Center from September 2011 to December 2012. Patients were selected if a standard panel of food-specific IgE tests was obtained prior to referral and excluded if diagnosed with eosinophilic esophagitis or if test results were unavailable.

RESULTS: Of 797 new patient encounters, 274 met entry criteria. Only 90 of these individuals warranted evaluation for food allergy based on NIAID criteria. The most common reasons for ordering the panel in individuals without a history of food allergy were allergic rhinitis, mild atopic dermatitis (AD), and urticaria. An altered diet was reported by 126 individuals (46%). Only 59 (47%) of these had a history warranting evaluation for food allergy. None of the remaining 67 individuals was determined to have a food allergy. A previously unknown allergen was identified in 42 patients. Milk, egg and peanut were the most commonly identified allergens. No food allergen was known prior to testing in 15 of the 42 individuals. Eleven of the 15 had a history of moderate to severe AD.

CONCLUSIONS: Serum allergy testing is a key tool in the evaluation of food allergy but must be utilized judiciously and only when indicated by history and physical exam. Misdiagnosis may result in inappropriate dietary exclusions for many patients. Food IgE panels have little utility as screening tests.

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406 Epitope Mapping The Peanut Panallergen Ara h 8

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RATIONALE: Ara h 8 is hypothesized to be the panallergen responsible for oral allergy syndrome between birch pollen (Bet v 1) and peanut. We recently determined the crystal structure of Ara h 8. In this work, we probed microarrays of peptides with peanut allergic and peanut sensitized patient sera for IgE and IgG4 reactivity.

METHODS: 15-mer peptides that were offset by 5 amino acids were printed to glass. Patient sera was incubated with the slides. IgE and IgG4 binding was detected with combinations of secondary and fluorescently-labeled tertiary antibodies. The linear epitopes identified were mapped on the 3-D structure and compared with those of birch pollen protein Bet v 1.

RESULTS: The majority of the Ara h 8 IgE epitopes mapped in this work align with those identified with Bet v 1. Considerably more IgG4 epitopes than IgE epitopes were found. Peanut allergic sera were more reactive with regard to IgE and IgG4 than peanut sensitized sera.

CONCLUSIONS: Our results support both the hypothesis that Ara h 8 could be contributing to oral allergy syndrome between birch pollen and peanut.

407 Simulated Roasting Affects Patient IgE Binding To Ara h 2

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RATIONALE: Ara h 2 is a peanut allergen that is a member of the 2S albumins. Recombinant Ara h 2 (rAra h 2) was expressed at high levels and purified in the folded form. This protein was subjected to a simulated roasting model (SRM) and specific amino acid modifications were identified.

METHODS: The cDNA of Ara h 2 was cloned into pET32b and expressed as a thioredoxin fusion, which was then cleaved from the soluble rAra h 2. Proper folding of rAra h 2 was confirmed by circular dichroism. rAra h 2 was then incubated in the presence of 0.25M glucose or xylose for 2, 4, and 7 days in a SRM and tested for IgE binding with patient sera using competitive inhibition ELISA. Mass spectrometry was used to identify chemical modifications that occur following the SRM. Synthetic overlapping peptides of native Ara h 2, on cellulose membranes, were incubated with patient sera to identify patient specific IgE epitopes.

RESULTS: A range of alterations in IgE reactivity towards rAra h 2 was observed following SRM. Mass spectrometry, and patient epitope mapping, allowed us to identify specific modifications to amino acids of rAra h 2 following SRM that may contribute to these observations.

CONCLUSIONS: IgE binding to rAra h 2 before and after processing was altered for some patient sera and specific chemical modifications that contribute to this were identified. Further studies will be done to confirm the contribution of chemical modifications to altered IgE binding.

408 Transfer Of Peanut IgE Sensitization Following Kidney and Pancreas Transplant

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RATIONALE: Peanuts are the leading cause of food-induced anaphylaxis and death. We report a case of peanut sensitization following combined pancreas and kidney transplantation.

METHODS: Circulating specific IgE against peanut and its components were measured in serum samples collected from the transplant recipient 1 month before transplantation, and 1 and 3 months after transplantation. Skin tests were performed 1 and 3 months following transplantation.

RESULTS: A 32-year-old female received organs from an 11-year-old boy following his death from a fatal peanut ingestion. The recipient had no prior history of allergy to peanuts. The patient's pre-transplant peanut and component IgE levels were negative. At 1 month post-transplant, the patient had a 6 mm skin test to peanut and had serum IgE to peanut, Arah1, Arah2 of 0.79kU/L, 0.47kU/L and 0.25kU/L respectively. At 3 months, skin test size and IgE to peanuts, Arah1, Arah2 decreased to 4mm, 0.69kU/L, 0.18kU/L and <0.1kU/L respectively. The donor's other organs were tracked. The liver recipient developed peanut anaphylaxis, while the second kidney recipient remained unsensitized to peanut.

CONCLUSIONS: We report the development of IgE peanut sensitization in a recipient of a combined pancreas-kidney transplantation. There are case reports of transfer of peanut sensitization following organ transplantation. It is unclear at this time, if sensitization develops from transfer of donor T, B or plasma cells and whether sensitization is temporary or permanent. Interestingly, her peanut allergy biomarkers are decreasing, suggesting a possible transient phenomenon. Increasing awareness of this allergen sensitization following transplantation may help prevent serious allergic reactions in transplant recipients.

409 Outcome Measures Of Challenge Testing In Patients With Physically Induced-Urticaria

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RATIONALE: Physical urticaria comprises a subset of chronic urticaria induced by an environmental or physical stimulus. We evaluated the degree of consistency between a history of physical urticaria and the results of standard challenge testing.

METHODS: Seventy-three subjects, ages 3-77, were diagnosed with a physically induced urticaria prior to referral. Prior to evaluation, all subjects refrained from anti-histamines or agents that could affect the outcome of challenge tests. All subjects were evaluated using standard challenge testing for physical urticarias that was directed toward the presenting diagnosis (e.g.,cold urticaria), yet included other stimuli based on the history. The majority of subjects were tested for 3 or more other entities. A subset of subjects (n=13) returned for re-testing one year after initial evaluation.

RESULTS: Of the 73 subjects with a positive history of a physical urticaria, 45 (38%) were challenge negative to the presenting diagnosis and of this group 19 subjects (26%) were negative to all challenge testing and 9 subjects (12%), although negative to the presenting diagnosis, did develop urticaria to a different stimulus consistent with the history upon further questioning. Negative challenge was low (10%) in cold-induced and delayed pressure urticaria (14%), yet high in cholinergic (52%) and solar urticaria (67%). One-year follow-up testing of 13 subjects was consistent with initial results, and included 5 subjects that remained negative to all challenge testing.

CONCLUSIONS: The diagnosis of a physical urticaria should be verified by testing when possible; and particularly when judged to be severe and thus require both life-style changes and significant pharmacologic intervention.

410 Whole Blood Histamine Concentration Response To Omalizumab In Patients With Chronic Idiopathic/Spontaneous Urticaria: Post Hoc Analysis Of Asteria I, Asteria II and Glacial Studies

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RATIONALE: Basopenia is a characteristic feature observed in patients with chronic idiopathic/spontaneous urticaria (CIU/CSU) and is related to disease severity. We evaluated whole blood histamine concentrations as a surrogate for intravascular basophil numbers in a subset of CIU/CSU patients refractory to standard treatment who were enrolled in three phase 3 trials.

METHODS: In two trials, ASTERIA I and II, patients received omalizumab (75mg, 150mg, 300mg) or placebo every 4 weeks; Asteria I (3 doses) and Asteria II (6 doses). In GLACIAL, patients received omalizumab 300mg or placebo every 4 weeks (6 doses). Whole blood histamine levels were measured prior to dosing at baseline and Week 12 in a subset of patients from US study sites (ASTERIA I, n=201; ASTERIA II, n=225; GLACIAL, n=266).

RESULTS: Baseline mean total histamine (SEM) was similar among the treatment groups; 13.41 (1.12) to 19.23 (1.32) ng/mL. Mean percent change (SEM; *P* vs placebo) from baseline in whole blood histamine concentations at week 12 was 12.81 (6.89), 22.85 (7.75; $P=0.215$), 22.50 (6.48; $P=0.171$), 45.11 (8.49; $P=0.002$) for placebo, omalizumab 75mg, 150mg, 300mg in ASTERIA I; 10.76 (4.93), 10.73 (6.12; $P=0.582$), 25.16 (9.67; $P=0.397$), 43.16 (9.31; $P=0.008$) for the same doses in ASTERIA II; and 15.11 (4.84), 41.81 (6.12; $P=0.132$) for placebo and omalizumab 300mg in GLACIAL.

CONCLUSIONS: Increases in whole blood histamine concentrations were observed at 12 weeks on average with the mean greatest change in the highest omalizumab dose of 300mg.

411 Efficacy Of Omalizumab In Patients With Chronic Idiopathic/Spontaneous Urticaria With Different Background Therapy: Post Hoc Analysis Of Asteria I, Asteria II, and Glacial Studies

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RATIONALE: Approximately half of patients with chronic idiopathic/spontaneous urticaria (CIU/CSU) treated with H_1-antihistamines remain symptomatic despite treatment with up to 3-4 times the approved dose. We evaluated the efficacy of omalizumab 300mg every 4 weeks versus placebo in CIU/CSU patients with different background therapies.

METHODS: ASTERIA I and ASTERIA II were randomized, double-blind, placebo-controlled studies of omalizumab 75mg, 150mg, and 300mg as add-on therapy in CIU/CSU patients who remained symptomatic despite treatment with approved doses of H_1-antihistamines. GLACIAL was a randomized, double-blind, placebo-controlled study of add-on omalizumab 300mg in patients who remained symptomatic despite H_1-antihistamines (up to 4X approved dose), H_2-antihistamines, and/or leukotriene receptor antagonists. Data from ASTERIA I and II were pooled, analyzed, and compared with results from GLACIAL.

RESULTS: At Week 12, the mean (95% CI; *P*-value) weekly itch severity score was reduced from baseline (primary endpoint) by 5.28 (4.09, 6.48; $P<0.0001$) points in ASTERIA I/II (N=319) in the pooled 300-mg omalizumab groups and by 4.52 (3.08, 5.97; $P<0.0001$) points in GLACIAL (N=335) in the omalizumab 300-mg group relative to placebo. At Week 12, 33.8% of patients in ASTERIA I/II and 40.0% of patients in GLACIAL in the omalizumab 300-mg groups were complete responders (Urticaria Activity Score over 7 days=0) compared with 6.9% and 4.8% of patients in the placebo group in ASTERIA I/II and GLACIAL, respectively (both $P<0.0001$). Results for other pooled ASTERIA I/II secondary endpoints were comparable with those observed in GLACIAL.

CONCLUSIONS: Omalizumab 300mg every 4 weeks had similar effectiveness in CIU/CSU patients regardless of current urticaria background therapy.

412 Angioedema and Angioedema Management From Asteria I and Asteria II: Phase III Studies To Evaluate The Efficacy and Safety Of Omalizumab In Patients With Chronic Idiopathic/Spontaneous Urticaria Who Remain Symptomatic Despite H1 Antihistamine Treatment

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RATIONALE: Angioedema negatively impacts chronic idiopathic/spontaneous urticariac (CIU/CSU) patients' health-related quality of life. We describe the presence of angioedema and how patients managed it.

METHODS: Subjects (n=640) were randomized and received placebo or omalizumab 75, 150 or 300 mg at Day 1 and every 4 weeks. Angioedema was assessed via the Urticaria Patient Daily Diary. We summarized the proportion of patients with angioedema, the mean number of days with angioedema per week, the mean proportion of angioedema-free days between Weeks 4-12, and how patients managed angioedema. We report data from Baseline to Week 12.

RESULTS: During the week prior to randomization, angioedema was a prevalent symptom (41.3% to 46.9%); patients with angioedema reported it, on average, approximately half of the days during that week (3.0 to 3.8 days). During Week 12, fewer patients reported angioedema across all arms (9.7% to 27.5%) and had it, on average, for fewer days during that week (2.3 to 3.3 days); the reduction in patients and days with angioedema was greater in omalizumab-treated patients. The mean proportion of angioedema-free days between Weeks 4-12 was greater in omalizumab-treated patients (90.1% to 95.8%) than in the placebo group (88.7%). Angioedema management generally consisted of low intensity interventions: most patients reported doing nothing or taking medication; some patients reported having called or visited their healthcare provider; none reported visiting the emergency room or being hospitalized.

CONCLUSIONS: Data from ASTERIA I & II demonstrate that in CIU/CSU patients who were symptomatic despite H1 antihistamine treatment, omalizumab was efficacious in reducing patient-reported angioedema.

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413 Improvements In Health-Related Quality Of Life From Asteria I & II: Phase III Studies To Evaluate The Efficacy and Safety Of Omalizumab In Patients With Chronic Idiopathic/Spontaneous Urticaria Who Remain Symptomatic Despite H1 Antihistamine Treatment

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RATIONALE: To describe changes in health-related quality of life (HRQOL) in patients with chronic idiopathic/spontaneous urticaria (CIU/CSU) using pooled data from the 12-week treatment periods of the ASTERIA I & II studies.

METHODS: Subjects (n=640) were randomized 1:1:1:1 and received placebo or omalizumab 75, 150 or 300 mg (PLB, OMA75, OMA150, OMA 300) at Baseline and Weeks 4 and 8. HRQOL was assessed by the Dermatology Life Quality Index (DLQI; overall score and subscales for symptoms and feelings, daily activities, leisure, work and school, personal relationships and treatment) at Baseline, Weeks 4 and 12.

RESULTS: At Baseline, patients reported substantial HRQOL impairment. All treatment arms improved from Baseline to Week 12 in many of the DLQI domains; however, the largest improvements were seen in the omalizumab-treated patients (change from Baseline to Week 12 in overall DLQI, PLB -6.11, OMA75 -6.93, OMA150 -8.15, OMA300 -10.22). Compared to placebo, change from Baseline to Week 12 in overall DLQI was statistically significant for higher doses of omalizumab (OMA150, p=0.01 and OMA300 mg, p< 0.0001). Improvements in DLQI were observed after one administration of study drug, by the first post-Baseline assessment at Week 4, with additional improvement observed at Week 12. For all DLQI domains assessed, the greatest improvements were seen in patients treated with omalizumab 300 mg.

CONCLUSIONS: In CIU/CSU patients who are symptomatic despite H1 antihistamine treatment, omalizumab was efficacious in improving a broad array of HRQOL domains, as measured by the DLQI instrument.

414 Estimating The Minimal Important Difference (MID) Of The Measures In The Urticaria Patient Daily Diary (UPDD): Updated Findings Using Data From The Asteria I, Asteria II, and Glacial Studies Of Omalizumab In Chronic Idiopathic/Spontaneous Urticaria

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RATIONALE: Conclusive data are available to bolster earlier research providing minimal important difference (MID) estimates of measures derived from the UPDD [weekly itch severity score (ISS); weekly number of hives score (NHS); weekly size of largest hive score (SLH); and the urticaria activity score over 7 days (UAS7)]. MIDs are useful to interpret how much change is clinically meaningful.

METHODS: Patients aged 12 - 75 with CIU/CSU > 6 months duration with UAS7 > 16 and weekly ISS > 8, despite antihistamine use (and H2 blockers and/or LTRAs in GLACIAL), enrolled in one of three Phase III multicenter, randomized, placebo-controlled studies of omalizumab, completed the UPDD and other collateral PRO measures. MIDs were determined through both distribution- and anchor-based approaches. Anchors consisted of the Physician's in-clinic UAS global rating, the Dermatology Life Quality Index, and the EuroQoL-5D. Data from two efficacy studies (Asteria I/II) were pooled for analysis (n=640), while data from the safety study (Glacial) (n=335) were analyzed separately. MIDs were estimated for 4 groups: all participants, all adults, all US adults, and all non-US adults.

RESULTS: After integrating anchor- and distribution-based estimates, the MID for weekly ISS was 4.5-5 points; weekly NHS was 5-5.5 points; weekly SLH was 4.0-4.5 (ranges of 0-21 for these three measures); and UAS7 (range 0-42) was 9.5-10.5 points.

CONCLUSIONS: Both anchor- and distribution-based approaches were used to estimate MID ranges based on widely used and accepted analytic methods. The similarity of results between the 4 groups suggest MID estimates are applicable to CIU/CSU patients globally.

415 Treatment With Off-Label Omalizumab In Chronic Idiopathic Histaminergic Urticaria - Angioedema Resistant To Conventional Treatment

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RATIONALE: Idiopathic chronic spontaneous histaminergic urticaria - angioedema (ICSHU-AE) is sometimes unresponsive to treatment with high doses of antihistamines. Omalizumab has been reported to be beneficial in these patients.

METHODS: A retrospective descriptive analysis was performed in patients diagnosed of ICSHU-AE resistant to high doses antihistamines, in whom off-label omalizumab had been prescribed.

RESULTS: Fourteen patients were included. All the patients had been treated with antihistamines plus corticosteroids and 6 patients also with dapsone (4), hydroxychloroquine (1) and colchicine (1). The initial dose of omalizumab varied from 150mg to 300mg. The initial interval was 4 weeks for 13 patients and 2 weeks for 1 patient. All the patients presented improvement after the first dose, including three patients diagnosed with urticaria-angioedema vasculitis. Six patients were asymptomatic and 8 continued with mild symptoms. Six patients stopped the treatment previous to omalizumab just after the first dose, two patients after the second one. One patient reported initial partial improvement, but failed to go on improving and omalizumab was withdrawn 6 months later. Initial omalizumab dose could not be lowered in any patient, but the interval between doses could be increased in 3 patients. Omalizumab was withdrawn because of improvement after the sixth dose in 1 patient. Mild side effects were seen in 7 patients, being the most frequent drowsiness, followed by cephalea, weight gain and loss of hair. Omalizumab was not stopped in any patient because of them.

CONCLUSIONS: Omalizumab was shown to be effective and safe in patients with ICSHU-AE unresponsive to other treatments.

416 Real-Life Experiences With Omalizumab For The Treatment Of Severe Refractory Chronic Urticaria (SRCIU)

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RATIONALE: Omalizumab, a subcutaneously administered anti-IgE monoclonal antibody, is highly effective in treating patients with SRCIU. We report the treatment of omalizumab in 62 patients.

METHODS: Patients were followed prospectively to evaluate the efficacy of omalizumab 150mg over a 4 year period. Two subgroups were from Toronto and Quebec City. Grading symptoms was based on seven-day urticaria activity score [UAS-7] > 30; history of oral corticosteroid use; and lack of adequate response to standard treatments. The dose interval was 1-3 months.

RESULTS: A total of 62 patients were included in the study. Overall baseline UAS-7 was 28.1 (32.2 in the Toronto group and 24.4 in the Quebec City group). For the Toronto subgroup, mean UAS during four weeks following the last omalizumab treatment was 5.7 (change of 26.5). Fifteen patients achieved remission after the first injection, six after the second, four after the third, and two after the fourth (80%). Six patients (18%) improved, but did not achieve complete remission. One patient was completely refractory to omalizumab and three patients who initially responded became refractory. For the Quebec City subgroup, overall mean UAS-7 declined from 24.2 pre-treatment, 10.1 at one month, 5.4 at 3 months, 4.7 at six months, 2.5 at 12 months, and 2.2 at 18 months. A total of 16/28 patients in this subgroup (57%) achieved complete remission. Eleven more patients improved on omalizumab therapy but not completely. One patient was refractory to the treatment.

CONCLUSIONS: This supports the usefulness of omalizumab 150mg in patients with corticosteroid dependant SRCIU.

417 Outcomes Of Chronic Urticaria Patients Treated With Hydroxychloroquine

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RATIONALE: Chronic urticaria (CU) is a debilitating condition that is often not responsive to anti-histamine treatment alone. Other reported therapeutic medicines have side effects that limit long term use. Hydroxychloroquine is a relatively safe immunomodulatory agent, but published reports of its use for this condition are few and limited to only a small number of patients.

METHODS: A retrospective chart review of patients seen in the Allergy Clinic at the University of North Carolina was performed. Patients who were diagnosed by their physician with chronic urticaria and/or angioedema, refractory to antihistamine therapy, and thus prescribed hydroxychloroquine were identified. Characteristics of these patients and their response to therapy were extracted from their charts.

RESULTS: Ninety-seven patients were identified. Forty-seven patients (48.5%) had autoantibodies, including IgE-receptor antibody (41%), thyroid antibodies, and anti-nuclear antibodies. Overall, 71 patients (73.2%) had complete or partial response to hydroxychloroquine. The mean time to response was 2.3 months. Patients with urticaria and angioedema were more likely to respond than those with urticaria alone (81.3% vs 63.6%, p=0.065), a difference that approached significance. Other characteristics including gender, race, presence of auto-antibodies, duration of CU, or atopy were not found to be related to response to hydroxychloroquine. Fourteen patients (14.4%) attributed minor adverse effects to the medication and discontinued it.

CONCLUSIONS: Hydroxychloroquine appears to be a safe and effective treatment for CU across a large population with varied characteristics. It may be most effective in patients manifesting with both urticaria and angioedema.

418 Polymorphisms Of Genes Encoding Interleukin-4 and Its Receptor Are Associated With Chronic Idiopathic Urticaria

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RATIONALE: Interleukin-4 (IL-4) has pivotal role in promotion of T helper2 responses. Il-4 is also an important regulator of adaptive immune responses. This study is aimed at investigating of association of polymorphisms in *IL-4* and *IL-4-receptor* genes with susceptibility to CIU.

METHODS: A matched case-control study was conducted on 89 patients with CIU and 138 healthy controls. Autologous serum skin test (ASST) was performed according to international standards. Total IgE levels, thyroid peroxidase antibodies (TPO) and anti thyroglobulin antibodies (ATG) were investigated using spectrophotometry and enzyme-linked immunosorbant-assay, respectively. Single nucleotide polymorphisms at following positions were genotyped using polymerase chain reaction: *IL-4* -1098T>G (rs243248), -590C>T (rs2243250), -33C>T (rs2070874), and *IL-4-receptor*+1902A>G (rs1801275). Estimated frequencies were compared between patients and controls.

RESULTS: ASST was positive in 39 (43.8%) and abnormal TPO and ATG were found in 12 (13.4%) and 6 (6.7%) of patients which were significantly higher than controls (p<0.05). Mean serum level of IgE was 140.57 (IU/ml) in CIU patients which fell in normal range similar to controls. Polymorphic site of +1902A>G *IL-4-receptor* gene did not differ between patients and controls. Among polymorphic sites in *IL-4*gene, only C allele at -33C>T (OR 2.39, 95%CI (1.41 to 4.05), p<0.001) was significantly higher in patients compared to controls. CC genotype at -590C>T (OR 4.5, 95%CI (1.9 to 10.82)) and -33C>T (OR 3.46, 95%CI (1.88 to 6.43)), were significantly higher in CIU patients (p<0.0001).

CONCLUSIONS: Polymorphisms in promoter region of *IL-4* but not *IL-4-receptor* gene confer susceptibility to CIU and may predispose patients to immune dysregulation.

419 Chronic Idiopathic Urticaria Index (CIUI) As a Tool For Predicting Response To Cyclosporine In Pediatric Patients With Refractory Autoimmune Urticaria

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RATIONALE: CIUI is an assay that measures autoantibodies to the FcεR1α and/or Fc portion of IgE in chronic urticaria. CIUI has not been reported as a tool for managing medication response, and its clinical utility has not been well established. Subsets of patients with refractory urticaria have been shown to respond better to immunomodulators and it would be helpful to identify these patients.

METHODS: Retrospective medical records review was performed. CIUI was measured by Quest Diagnostics and National Jewish prior to initiating cyclosporine and at variable intervals.

RESULTS: Two female patients (A and B) were followed between the ages of 11-15 and 14-18. They presented with urticaria and elevated CIUI (A 22.7%; B 64.0%). Both initiated treatment with cyclosporine at 100mg/day in addition to cetirizine, baseline ANA (A 1:160, B 1:160), IgE (A 11ng/ml, B 38ng/ml). Patient A had remission of urticaria with CIUI of 6.5% then 3.7%. Patient B had remission of urticaria and an initial decrease of CIUI to 0.3%. Upon discontinuation of cyclosporine CIUI rose to 21.2% within 60 days but subsequently dropped to 3.8% within 30 days of re-initiating treatment.

CONCLUSIONS: These cases suggest that there is utility to obtaining CIUI in pediatric patients refractory to first-line medications. CIUI may be useful serum marker for predicting response to immunomodulators and in deciding whether to continue immunomodulators. CIUI may be a useful management tool in this patient population.

420 Cholinergic Urticaria: Case Report Of Urticaria Induced By Acquired Seasonal Hypohidrosis

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RATIONALE: Cholinergic urticaria due to acquired seasonal generalized hypohidrosis is a rare condition with an unclear pathogenesis. We report a case of seasonal cholinergic urticaria due to acquired seasonal generalized hypohidrosis.

METHODS: A 17 year old male presented with a 3-year history of generalized, erythematous, pruritic macules and papules following hot baths, exercise, and emotional stress. The symptoms spontaneously resolve within 30-90 minutes after the inciting event. Symptoms occur during the Fall, extending through the Spring, but peaking in the Winter. He has never had symptoms in the Summer. The patient also reported hypohidrosis for the last 3 years with the same seasonality as his urticaria. Multiple antihistamines had been used without success.

RESULTS: We identified a patient that develops urticaria during colder seasons of the year, particularly in Winter, related to a hypohidrotic state. There are few published reports regarding urticaria related to hypohidrosis. Koboyashi et al reported 2 patients who developed urticaria thought to be secondary to acquired hypohidrosis due to superficial obstruction of the acrosyringium, the intraepidermal spiral duct of an eccrine gland.

CONCLUSIONS: There are few reports of coexisting cholinergic urticaria and hypohidrosis. Our case was unique in that the hypohidrosis had a seasonal component. We felt that this may be related to a xerotic skin change coupled with a decrease in sweat production during colder seasons. This could lead to occlusion of the acrosyringium in the stratum corneum and cause urticarial lesions.

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421 Relation Between Environmental Allergen Exposure and Chronic Urticaria

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RATIONALE: The definitive cause or trigger is not usually identified in most patients with chronic urticaria (CU). We sought to investigate a potential role of environmental allergens in these patients.

METHODS: Following IRB approval, 36 patients with CU were enrolled. All patients had skin tests (ST) to 14 environmental allergens: tree, grass, weed, ragweed and English plantain pollens, dust mites, feathers, cat and dog epithelium, cockroach, mouse, and three mold spore mixtures. The ST was considered positive if the wheal diameter was 3 millimeters or larger than the negative control. The urticaria severity was assessed using the Urticaria Severity Score (USS).

RESULTS: In the study group, the mean USS was 44. Fifteen patients (Group 1) had more severe urticaria (USS >44). The rate of positive ST to some perennial allergens was higher in Group 1 compared with milder urticaria patients (Group 2) (mouse – 20% vs 10%, mold spores – 13% vs 5%, *A. fumigatus*- 7% vs 5%). Group 1 had significantly higher exposure to both cats and dogs (33% vs 9.5%, p=0.03), but Group 2 had higher rate of positive ST to tree pollen (76% vs 47%, p=0.03) and dust mites (43% vs 7%, p=0.01).

CONCLUSIONS: The patients with more severe CU were more allergic to perennial allergens such as mouse and mold spores, and had greater pet exposure. Our data suggest that environmental allergens may represent potential additional triggers for urticaria and may contribute to the severity of symptoms. Strict environmental control and decreased pet exposure might be beneficial for patients with severe CU.

422 Relationship Of Clinical Characteristics Of Chronic Urticaria In Children To Treatment Outcome

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RATIONALE: Functionally active auto-antibodies have been identified in patients with chronic urticaria against the high-affinity IgE receptor and IgE. This was the rationale for controlled clinical trials demonstrating efficacy of cyclosporine in adults with chronic urticaria not responsive to antihistamines.

METHODS: To evaluate chronic urticaria in children, sequential patients from our Pediatric Allergy Clinic with chronic idiopathic urticaria were identified from our electronic medical record from 2009 through 2012. The urticaria index was examined in those unresponsive to even high doses of either cetirizine or hydroxyzine. Low dose cyclosporine was begun for those found to be antihistamine resistant. Safety measures included cyclosporine blood levels, serum creatinine, and regular examinations of blood pressure.

RESULTS: 46 patients were identified, median age 11.5 years, gender ratio 26:10 with female preponderance; mean duration of hives prior to referral was 14.8 months. 16 of the 47(34%), 12 females and 4 males, median age 12.5 years, were antihistamine-resistant and started on cyclosporine. Antihistamine resistant children were significantly older ($p = 0.0001$). Of the 16, presence of autoantibodies was examined in 12, of whom 5 were positive and 7 were negative. All who started on cyclosporine had complete suppression of their hives after 2 days to 3 months. Serum cyclosporine levels measured at time of resolution ranged from 66 to 200 ng/ml. No effects on renal function or blood pressure were seen.

CONCLUSIONS: Antihistamine resistant chronic urticaria significantly correlated with older age children but not with the presence of autoantibodies. Cyclosporine was highly effective and safe in inducing remissions of even long-standing chronic urticaria in children.

423 Clinical Characteristics Of Elderly Chronic Urticaria

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RATIONALE: Chronic urticaria (CU) is defined as itchy wheals lasting for at least 6 weeks. It is a common disabling disorder occurring in about 0.5–1% of the population. Recently, the aged population is increasing worldwide. Therefore, it is essential to identify the specific features of disease in aged group. We investigated the prevalence and clinical features of elderly CU in comparison with non-elderly CU.

METHODS: We retrospectively analyzed the medical records of 827 CU patients who were followed in the outpatient Allergy Clinic of Ajou University Hospital, South Korea. According to the EAACI/GA^{2}LEN/EDF/WAO guideline chronic spontaneous urticarial patients were included. Elderly was defined as older than 60 years. Severe CU was defined when urticaria activity score was ≥13 at initial visit.

RESULTS: Of the total 827 patients, 37 (4.5%) were elderly. Among co-morbid conditions, the prevalence of atopic dermatitis (AD) was significantly higher (37.8% vs. 21.7%, $p=0.022$), while that of aspirin sensitivity was lower (18.9% vs. 43.6%, $p=0.003$) in elderly CU. Other clinical and laboratory findings were found to have no significant differences between the two groups. However the prevalences of serum specific IgE to staphylococcal enterotoxin A (SEA) and staphylococcal enterotoxin B (SEB) were considerably higher in elderly CU with AD than in those without AD (37.5% vs. 0%, respectively).

CONCLUSIONS: Considering the higher prevalence of AD in elderly CU, it is needed to observe the coexistence of AD in elderly CU. Specific IgE to SEA/SEB may play a role in the pathogenesis of elderly CU, especially who are accompanied by AD.

424 Decreased Serum Vitamin D Level In Patients With Chronic Spontaneous Urticaria

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RATIONALE: Vitamin D is important role in immune system and decreased serum vitamin D level linked to autoimmune diseases. Autoimmune is major caused in chronic urticaria. This study aimed to find vitamin D status in patients with chronic spontaneous urticaria (CSU).

METHODS: A prospective case control study of 60 subjects with CSU compared with 20 healthy subjects. Serum 25(OH)D was measured in all subjects. Urticaria symptom severity and quality of life were assessed as Urticaria Activity Score 7(UAS7) and Dermatology Life Quality Index (DLQI). Serum number of eosinophil and erythrocyte sediment rate (ESR) test were performed in the patients with CSU.

RESULTS: This study was predominately women, 53/80 subjects. The mean age was significantly different between CSU group and controls, 37.17 ± 10.15 VS 62.9 ± 17.07 years ($p < 0.001$). The mean duration of urticaria was 29.88 ± 13.45 weeks. The mean serum 25(OH) D levels were significantly decreased in subjects with CSU (20.57 ± 10.80) compared with controls (31.97 ± 6.56), $p < 0.001$. Vitamin D status in CSU group, 10 subjects, 16.67% were normal, 17 subjects, 28.33% were insufficiency and 33 subjects, 55% were deficiency. Vitamin D levels did not correlate with duration, age, number of eosinophil, UAS7 and DLQI but positive correlated with ESR, $r = 0.43$ ($p = 0.001$).

CONCLUSIONS: This study showed vitamin D deficiency was common in patients with CSU. However, serum 25(OH)D levels cannot use to be biomarker for monitor symptom and severity in CSU.

425 Supplementation With Vitamin D In a Cohort Of Patients With Chronic Urticaria Results In Clinical Improvement

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RATIONALE: Chronic urticaria (CU) is idiopathic in a majority of cases. Recent studies have reported low vitamin D levels in CU patients. Several case reports have demonstrated clinical improvement in CU patients treated with vitamin D. We present 9 patients with CU and vitamin D deficiency/insufficiency placed on vitamin D supplementation.

METHODS: A retrospective chart review of patients diagnosed with CU and vitamin D deficiency/insufficiency was performed.

RESULTS: Nine patients with ages ranging from 8 to 49 years were identified with CU and low vitamin D levels. Despite treatment with long and short acting anti-histamines, all patients had persistent urticaria. 25-hydroxy vitamin D levels ranged from 9-27.8 ng/mL. Treatment was initiated with vitamin D according to current guidelines. Six (66%) patients supplemented with vitamin D reported clinical improvement. Four of the six patients had complete resolution of hives while two reported decreased frequency of hives. One had no change in symptoms, while two patients were lost to follow up.

CONCLUSIONS: Two-thirds (66%) of patients had complete resolution or decreased frequency of hives when vitamin D supplementation was initiated. A large prospective study is warranted to establish a cause and effect relationship between vitamin D deficiency/insufficiency and chronic urticaria. Further research investigating whether routine screening for vitamin D deficiency in this group of patients should become standard is necessary.

426 Clinical and Laboratory Features Of Chronic Urticaria

Dr. Koji Masuda; Kyoto Prefectural University of Medicine, Kyoto, Japan.

RATIONALE: Chronic urticaria is a common skin disorder, but the etiology of chronic urticaria remains incompletely understood. The aim of this study is to determine the clinical and laboratory characteristics of chronic urticaria.

METHODS: We retrospectively investigated 306 patients who suffered from urticaria and visited to our outpatient clinic from 2008 to 2013. Data were collected regarding age, sex, disease duration, severity and laboratory parameters such as total IgE, antinuclear antibodies, autologous serum test, thyroid hormone, anti-streptolysin O antibody, throat swab culture and routine laboratory tests.

RESULTS: From 306 patients with urticaria, there were 93 patients with chronic urticaria. Mean disease duration at first visit of chronic urticaria was 18.2 months. Elevated IgE and abnormal thyroid hormone concentration were found in 46% and 14%, respectively. High antinuclear antibodies titers and anti-streptolysin O antibody were found in 9% and 10%, respectively. Positive autologous serum test and throat swab culture were found in 38% and 33%, respectively.

CONCLUSIONS: Our data showed elevated IgE, positive autologous serum test and throat swab culture had relatively high relevance to chronic urticaria. Because chronic persistent infections are thought to be one of the triggers, throat swab culture may be the good screening programs for causes of chronic urticaria.

427 Serum Specific IgE Response To Thyroid Autoantigens In Aspirin Intolerant Urticaria Patients

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RATIONALE: Thyroid antibodies were frequently observed in urticaria patients, however their role to effector cells such as mast cell or basophil are not clearly elucidated in the mechanism of urticaria. We investigated the role of serum specific IgE (sIgE) to two kinds of thyroid autoantigens in patients with aspirin intolerant acute urticaria (AIAU) and aspirin intolerant chronic urticaria (AICU).

METHODS: We recruited 59 AIAU patients, 96 AICU patients and 69 normal controls (NC). Serum sIgE to thyroid peroxidase (TPO) and thyroid globulin (TG) were measured by ELISA. Basophil activation tests with additions of TPO and TG were performed to prove a direct role in effector cells.

RESULTS: Prevalence of serum sIgE to TPO were significantly higher in AIAU (15.2%) and AICU groups (7.5%) than in NC (0%, ($p<0.001$: $p=0.013$, respectively). Prevalence of serum sIgE to TG in AIAU (11.9%) was significantly higher than in AICU (2.1%) and NC groups (1.5%, $p=0.011$: $p=0.016$, respectively). No significant associations were found between sIgE to TPO/TG and IgG to TPO/TG. Basophil activation test showed increased expression of CD203c in a dose dependent manner with serial additions of TPO in both AIAU and AICU patients having high sIgE to TPO.

CONCLUSIONS: These findings suggest that specific IgE mediated mechanism to TPO and TG may contribute to the pathogenic mechanism of a subset of AIAU and AICU patients.

428 Basophil Receptor Profiles In Chronic Idiopathic Urticaria

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RATIONALE: Patients with chronic idiopathic/spontaneous urticaria (CIU/CSU) have a unique basophil phenotype. Reduced IgE-mediated basophil histamine release (HR) and basopenia are observed in active CIU subjects, and these factors are inversely correlated with disease severity. Basophils are recruited from the blood to urticarial lesions in the skin, but the pathways for basophil migration remain unknown. Given the increased levels of chemoattractants in the serum of CIU subjects, we examined the expression of relevant counter receptors on basophils of CIU subjects relative to control groups without skin disease.

METHODS: Active adult CIU subjects (n=4) and allergic (n=4) and non-allergic controls (n=3) were recruited. Whole blood was analyzed using flow cytometry for CCR1 CCR3, CCR5, CRTH2, CXCR3, and CD11b.

RESULTS: There was no significant difference between basophil receptor expression in individuals with CIU compared with atopic and non-allergic controls. Net MFI ($\pm$ SEM) for adult CIU subjects, atopic subjects, and non-allergic subjects were as follows: CCR1 (17.06 $\pm$ 5.87, 12.03 $\pm$ 7.11, 21.80 $\pm$ 4.74), CCR3 (885.89 $\pm$ 53.36, 827.25 $\pm$ 61.32. 887.79 $\pm$ 70.12), CCR5 (1.80 $\pm$ 0.59, 1.16 $\pm$ 0.50, 1.29 $\pm$ 0.11), CRTH2 (392.98 $\pm$41.86, 395.19 $\pm$ 50.83, 410.33 $\pm$ 59.03), CXCR3 (-0.15 $\pm$ 0.17, -0.09 $\pm$ 0.03, 0.01 $\pm$ 0.07), and CD11b (119.40 $\pm$ 11.97, 117.05 $\pm$ 8.84, 121.97 $\pm$ 26.68).

CONCLUSIONS: The baseline expression of chemoattractant surface receptors on basophils are similar between CIU subjects and non-skin disease controls. Further studies are needed to determine whether functional differences in receptor activation occur between these groups.

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429 Altered Systemic Adipokine Levels In Patients With Chronic Idiopathic Urticaria

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RATIONALE: It becomes increasingly evident that adipokines play a key role in immune response and inflammation. Chronic idiopathic urticaria (CIU) is associated with an altered immune response connecting to chronic systemic inflammation.

METHODS: To investigate the circulating adipokines in patients with CIU and to determine possible mediators of severe CIU or aspirin sensitivity, serum adiponectin, leptin, lipocalin2, interlukin (IL)-10, IL-6 and tumor necrosis factor (TNF)-α concentrations were measured by enzyme-linked immunosorbent assays in 191 (59 with aspirin intolerance) CIU patients and 50 healthy controls (NC). The disease activity of CIU was assessed by the urticarial activity score (UAS), a total score of 0–15.

RESULTS: The mean levels of serum leptin, lipocalin2, TNF-α, IL-6 and IL-10 were significantly higher in CIU patients as compared to NC. Adiponectin, an anti-inflammatory adipokine, was measured at a significantly lower level in the sera of patients with CIU. IL-6 and TNF-α were significantly higher in patients with a UAS≥13. The level of lipocalin2, known as a neutrophil activation marker, was found to be significantly different according to the presence of aspirin intolerance (45.2±42.4 in aspirin-intolerant chronic urticaria *vs.* 80.4±38.8 in aspirin-tolerant chronic urticaria) and anti-thyroid antibody (50.8±30.8 *vs.* 76.0±42.7) after adjusting age, gender and atopy.

CONCLUSIONS: CIU patients have an imbalance of pro- and anti-inflammatory adipokines compared to NC. The modulation of systemic inflammation can be targeted for some subpopulations of CU patients with severe, aspirin tolerant and thyroid autoimmunity.

430 Predicting Clinical Responsiveness To Dapsone In The Treatment Of Chronic Idiopathic Urticaria (CIU)

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RATIONALE: In our office, dapsone has been used successfully to treat patients with CIU unresponsive to antihistamines. Serologic and clinical screening tools may help to determine which patients may benefit from its use.

METHODS: The charts of 19 patients with CIU treated with dapsone were reviewed. Clinical responsiveness (none, partial, or complete) was assessed at 1, 2, 4, 6, 8, 12, 16, and 20 weeks. Records were examined for serologic markers (anti-thyroid antibodies, ANA, CU index) and the presence of angioedema. Predictiveness and time to maximal responsiveness (TMR) were evaluated.

RESULTS: Eighteen females and 1 male (mean age of 46.89 years) were examined, with 2 lost to follow up. Seventeen patients obtained a complete response. 4/17 patients (24%) had a history of angioedema with the average TMR of 4 weeks vs. 6.73 weeks in those without angioedema. 4/15 (27%) patients had elevated CU index values. TMR was 9 weeks in those with elevated CU indexes vs. 5 weeks in those with normal CU indexes. 4/17 (24%) patients had anti-thyroid antibodies, with a TMR of 4 weeks vs. 6.3 weeks in those without. Only one patient had an elevated ANA but was lost to follow-up.

CONCLUSIONS: Angioedema, a normal CU index, and elevated anti-thyroid antibodies were associated with a shorter TMR in our CIU patients treated with dapsone. Further studies examining screening criteria for patients treated with dapsone in CIU are warranted to determine which patients are likely to benefit from this therapy.

431 Chronic Urticaria/Angioedema and Auto-Immunity: Diagnostic Profile Among Patients Attending a Reference Clinic In Brazil

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RATIONALE: Chronic urticaria/angioedema is a challenge to the Allergist/Immunologist. We aimed to evaluate the diagnostic profile among patients with chronic urticaria/angioedema.

METHODS: Two hundred patients with ages 13-80 years-old (161 females, 80.5%), with chronic urticaria/angioedema attending a specialty clinic were evaluated prospectively. Autoimmune basis was suspected by presence of positive autologous test, anti-thyroid or antinuclear antibodies(ANA). Patients were evaluated for triggering of symptoms by medications and/or physical agents, anaphylaxis, skin testing, eosinophilia, IgE, and hepatitis serology. When indicated, a skin biopsy was performed. Use of medications for urticaria was analysed.

RESULTS: Duration of disease ranged from 6 months to 36 years. Fifty-six patients (28%) had urticaria only;130(65%) urticaria and angioedema; and 14(7%) angioedema. Of the 86 patients who underwent autologous skin testing, 36(41.8%) had positive results. Anti-thyroid antibodies and ANA were positive in 12.4% and 12.2% of patients, respectively. Sixty-seven(33.5%) patients reported triggering of symptoms by medications, mostly ASA/NSAIDs(26.5%). Thirty percent of patients reported worsening of symptoms by physical agents. Eight patients had anaphylaxis. Biopsy carried out in 19 patients revealed 4 urticaria vasculitis, 4 eosinophilic infiltrate and 11 non-specific. Sixty-four percent of patients had total IgE>100kU/L; positive skin test were more frequent to mites(44%), cockroach(30%) and shellfish(16%). Ninety-two percent were in use of at least one daily medication, mostly anti-histamines (88%); 13/184(7%) took oral corticosteroids.

CONCLUSIONS: Sixty-nine patients (34%) presented features of autoimmune urticaria/angioedema. Other causes were physical, food-induced, and NSAIDs-induced urticaria. Hereditary and iECA angioedema were diagnosed in 2 and 4 patients, respectively. Despite extensive investigation, 83 patients(41.4%) remained diagnosed as spontaneous urticaria/angioedema.

432 Chronic Spontaneous Urticaria-The Saskatchewan Experience and Questionnaire Survey

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RATIONALE: In this questionnaire study, we examined responses to treatment, effect on lifestyle, beliefs of causation, and satisfaction with treatment method for Chronic Spontaneous Urticaria.

METHODS: 173 patients with CSU had been seen between 2003 and 2013. An autologous serum skin test (ASST) had been performed on 138 patients. 101 participants responded.

RESULTS: Of the respondents, 80 were women and 21 men. The age range was 1 year to 81. The mean age was 36 years. The average duration of symptoms was 9.3 years. They included 40 patients who were ASST positive (M: F; 8:32) and 49 negative M:F; 12:37). 50 participants no longer had hives. Patients reported being most bothered by pruritus, disturbed sleep, anxiety and their physical appearance including facial swelling. Many (71.2%) had missed work or school because of the urticaria. Twenty-nine patients found antihistamines alone gave adequate relief of urticaria. Twenty ASST positive patients with severe uncontrollable hives were treated with intravenous immunoglobulin (IVIG). 85.0% of these patients had improved quality of life, with 13 of these patients now free of urticaria and no longer receiving IVIG. Three patients who did not benefit from IVIG did respond to methotrexate. No ASST negative patients received IVIG.

CONCLUSIONS: Approximately 30% of patients found antihistamines gave effective control. Half the patients had been free of urticaria for at least 3 months. About 40% of patients with CSU had an autoimmune basis as assessed by the ASST and IVIG was a highly effective treatment for this group.

433 A Case Of Incontinentia Pigmenti Masquerading As Urticaria Pigmentosa

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RATIONALE: Incontinentia Pigmentosi (IP) is an X-linked dominant ectodermal dysplasia often associated with opthalmological and neurological complications. Histopathology depends on the stage of skin lesions but mastocytosis is not a recognized feature. This child was referred to us with a diagnosis of Urticaria Pigmentosa (UP) based on the presence of itchy pigmented rash and mastocytes on biopsy. Here we present a patient with skin findings typical of IP, and potentially atypical finding of mast cells on biopsy, misdiagnosed with UP.

METHODS: Punch biopsy of lesion, CD117 and Giemsa stain of tissue sample performed by Quest Diagnostics.

RESULTS: This 14 m/o patient with pruritic hyperpigmented whorled rash following the lines of Blaschko was worked up for mastocytosis. Raised hyperpigmented plaques were present at birth, most of which are now flat. Workup for systemic mastocytosis including C-kit mutation analysis, histamine release and tryptase were normal. Plasma Histamine 2.4 ng/ml (reference range 0.1-1.8). Increased mast cells, verrucous hyperplasia with dyskeratotic cells and pigment incontinence on skin biopsy.

CONCLUSIONS: Only one previous report from 1967 could be found of mast cells in IP. History and physical exam are classic for IP in this patient, yet she was worked up as UP due to the presence of pruritic hyperpigmentation and diagnosis was thought to be confirmed by mast cells on biopsy. This case highlights the need for increased awareness of IP and its features given the importance of ophthalmologic evaluation and genetic counseling, and suggests the need for further study regarding mastocytosis as a histological feature of IP.

434 Acute Urticaria Caused By Infection In 72 Chinese Patients

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RATIONALE: Infection, food and drugs are the most common causes of acute urticaria. To date, the clinical, etiological and prognostic features and the optimal treatment of acute urticaria caused by infection are poorly defined.

METHODS: This retrospective study included 72 inpatients diagnosed acute urticaria caused by infection from 2008 to 2012 at the First Affiliated Hospital of China Medical University. The patients' general information, etiology, clinical manifestations, laboratory examinations, treatment and prognosis were analyzed.

RESULTS: Of 204 inpatients with acute urticaria, 72 cases were caused by infections (35.3%), 38 cases caused by food (18.6%), 26 by drugs (12.7%), 16 by other causes (7.8%), and 52 with no obvious causes (25.5%). The infections included 30 upper respiratory infection (bacterial or viral, 41.7%), 12 gastroenteritis (16.7%), 7 mycoplasma pneumonia (9.7%), 4 wound infection (5.6%). WBC and neutrophil counts, erythrocyte sedimentation rate, serum C-reactive protein and procalcitonin were significantly elevated in the patients with acute urticaria caused by infection. Systemic corticosteroid, antibiotic and antihistamine were administered in 39 cases (54.2%), systemic antibiotic and antihistamine in 19 (26.4%), systemic corticosteroid and antihistamine in 14 cases (19.4%). Of the 72 patients, 65% of the patients healed in 1 week, 80.4% cured in 2 weeks, 91.2% cleared in 3 weeks, and 100% resolved in 6 weeks.

CONCLUSIONS: Infections accounted for a major cause of acute urticaria. WBC, neutrophil count, ESR, CRP, and procalcitonin were useful for monitoring disease activity. Systemic corticosteroid, antibiotic and antihistamine were the cornstone treatment. The prognosis of acute urticaria caused by infection was good.

435 Assessment Of Acute Urticaria In Pediatric Emergency Department

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RATIONALE: Assessment of patients admitted with acute urticaria in the Pediatric Emergency Department(PED).

METHODS: Uncontrolled study of 565 children and adolescents aged from 0 to 15 years, from January 2012 to December 2012, with acute urticaria (ICD L50),treated in the PED.

RESULTS: In this period were performed a total of 64 203 visits due to several causes, and 565 (0.88%) were admitted with acute urticaria. The incidence was higher in males (56%) and in children aged between 1 and 3 years old, which corresponded to 45.5% of the total of cases diagnosed with acute urticaria. Hospital medication was prescribed to 33.27% of the patients. Sixty one cases(10.79%) reported associated diseases, and the most frequent were infectious diseases in 45 cases (75%). The associated pathologies were otitis, acute rhinofaryngitis , acute sinusitis, influenza and acute tonsillitis (see Table I). In a period of less than 72 hours after the hospital discharge, 19 patients (3.36%) were readmitted . Five patients were hospitalized, one case associated with bacterial pneumonia and another with cellulitis.

CONCLUSIONS: Performance measurement of care quality allows the comparison of data between institutions and the creation of incentives for improving care quality standards. After the results, the staff did a protocol for the attendance of acute urticaria and a discharge standard prescription for the patient and his family.

436 OTC Pills and Severe Urticaria

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RATIONALE: A lot of weight loss pill are available over the counter (OTC) and online. Most haven't been proved effective and may contain dangerous substances, causing life-threatening effect. Urticarial Vasculitis (UV) has been reported in association with other drugs but no with OTC diet pills.

METHODS: We describe three patients without antecedents with 2-5 days of generalized urticarial with mild constitutional symptoms. Physical examination revealed hives on head, hands, abdomen, groin, all skinfolds and thighs. They were pruriginous, painful and burning, ≥0.5 cm with an urticarial severity score of 4-6 points. Lesions lasted ≤24 hours, leaving residual pigmentation. They had lymphopenia. The C protein was high, but complement, liver function test, urea and creatinine, plasma sodium, glucose, and thyroid function were in normal ranges. ANA and anti-Tg antibodies were not detected in two of them. They were taking OTC diet pills approximately 15 days ago. The ingredients were: synefrin, guggulsterones, thyroid stimulating matrix, yohimbine and phenylmine. These different ingredients have reported side effect separately but no vasculitis. Skin biopsies, confirmed the diagnosis of Urticarial Vasculitis.

RESULTS: Diagnosis of diet pills induced UV was based on the temporal relationship between intake pills and wheals lesion, systemic manifestation, histologically proven vasculitis and finally by reversal of the clinical signs after discontinuation of the therapy.

CONCLUSIONS: We believe these are the first reported cases of UV with the use of OTC diet pills. Given the widespread use of these pills, allergist and dermatologist should be able to recognize UV which may be under reported.

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437 Hypocomplementemic Urticarial Vasculitis With Elevated Immune Complexes and Nasal Polyposis

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RATIONALE: Hypocomplementemic urticarial vasculitis with elevated immune complexes although uncommon has been been reported after the third decade of life. This is a report of a 34-year-old man with episodes of urticarial rashes, conjunctivitis, knee pain and fever for 7 months and injected conjunctive, generalized lymphadenopathy, and wheals scattered in the trunk, upper and lower extremities who had nasal polyps.

METHODS: Laboratory investigation and detailed medical history were obtained.

RESULTS: Laboratory investigations showed eosinophilia ($1{,}4 \times 10^9$/L); Epstein Barr Virus (EBV) and Human Herpesvirus 6 (HHV-6) IgM and IgG positive; EBNA posiive; PCR (saliva and blood) were HHV6 and EBV positive; positive antinuclear antibody (ANA), anti-neutrophil cytoplasmic antibodies (ANCAs), anticyclic citrulinated peptide (CCP) antibody and antistreptolysion O (ASO) titers; plasma complement levels for C3 low at 44 mg/dL (N: 90-180) and CH50 low. Lymph node biopsy with reactive lymphadenitis. Nasal endoscopy and sinus CAT scan positive for polyps. 3 years of recurrent urticaria and conjunctivitis every 4 weeks for 4-5 days; Nasal polypectomy done. Microscopic hematuria,and at times pyuria and proteinuria. Skin biopsy showed leukocytoclastic vasculitis with many eosinophils. Treated with IV pulses of methylprednisolone followed by oral prednisolone 1 mg/kg/day.

CONCLUSIONS: This case is a systemic hypocomplementemic vasculitis with eosinophilia and nasal polyposis associated with chronic EBV and HHV6 infection with features similar to Churg Strauss Syndrome.

438 Nannies Knowledge, Attitude and Management Of Food Allergies In Children; An Online Survey

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RATIONALE: It is essential that primary caretakers have the knowledge and tools necessary to recognize and treat food allergy reactions. There is limited data in the medical literature regarding this topic.

METHODS: Web-based survey about food allergies in children was emailed to 370 nannies. The IRB at Cleveland Clinic approved the study.

RESULTS: 116 (31%) completed the online survey. 25% of respondents had formal nanny training. 99% recognized food allergy as a potentially fatal event. 36% reported caring for a child with food allergies; of these 32% had food allergy action plans and 28% had epinephrine available. 72% reported training on administering epinephrine. Nannies major concerns included accidental ingestion and discomfort in administering epinephrine. 44% were uncomfortable treating food allergy emergencies, and 48% were uncomfortable administering epinephrine. 4% felt that a small amount of allergenic food may be safely eaten while 13% reported dilution with water may reduce an allergic reaction. 62% desired additional information about recognizing food allergies. 72% agreed that food allergy training should be required for all nannies. Internet search (85%) and pediatricians (82%) were the most used resources for learning about food allergies. 73% expressed preference to attend meetings with their child's doctor as the best way to learn about food allergies.

CONCLUSIONS: This survey identifies significant gaps in knowledge in the nanny population with regard to food allergy in children, and reflects the need for more stringent training on food avoidance and epinephrine use. Increased communication between parents, nannies, and doctors is needed to protect food allergic children.

439 Does Omega-3 Fatty Acid Supplementation During Pregnancy Prevent Childhood Atopic Disease?

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RATIONALE: Making a causal link between exposure and disease is one of the biggest challenges in epidemiology. Certain criteria have been developed to assist judgment of cause-effect relations. We used Hennekens' criteria to attempt to clarify the roll of long chain omega-3 polyunsaturated fatty acids (n-3 PUFA) in maternal diets on the development of atopic disease in children.

METHODS: A literature review was performed using MEDLINE (Ovid and Pubmed) and Google Scholar. Hennekens' criteria were applied to the selected studies.

RESULTS: Three randomized controlled trials of n-3 PUFA supplementation during high risk pregnancies for development of atopy were found and all three were included for review. All three studies were subject to insensitive measure and contamination bias. Significant confounders were well controlled in each. The strength of association for egg sensitization was significant in 2 of 3 studies and eczema or severe eczema in all 3 studies. Peanut sensitization after supplementation was not decreased in any of the 3 studies. The biologic plausibility is well developed in the literature and temporality is clear and appropriate. Across studies, a dose response was observed. Of note, one subanalysis of mothers without a history of atopic disease revealed that no infants developed food allergy in the group who received supplementation, as compared to 25% of the group that did not supplement their diets.

CONCLUSIONS: Application of Hennekens' criteria to these three studies reveals n-3 PUFA deficiency in mothers as a plausible risk factor for the development of allergic sensitization in high risk newborns.

440 Helping Children Cope With Discomfort Associated With Skin Prick Testing In a Pediatric Setting: A Quality Improvement Report

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RATIONALE: Patients undergoing skin prick testing (SPT) in a Pediatric Allergy Clinic can experience significant pain and itching. Incorporating comfort measures into clinical care may help improve the patient experience through a performance improvement process.

METHODS: We administered patient/parent surveys to identify and evaluate parent/nurse-initiated comfort measures used during SPT application ("SPT") and 15-minute waiting period ("wait"). A visual analog scale assessed patient's coping during SPT and wait (0 = very poor, 5= very well). Surveys were emailed to parents within 1 week of testing. After obtaining baseline data, nurse-initiated comfort measures were implemented. Findings were shared with team members monthly to increase use of comfort measures reported most effective.

RESULTS: 44 surveys (59% response rate) have been returned to date. Data are reported as percentage of patients/parents who rate the patient's coping as 4 or 5. Baseline data for comfort during SPT: nothing used by parent, 79%; home comfort measure (electronic device, coloring), 75%. Wait baseline: nothing used by parent, 69%; home comfort measure, 70%. The first nurse-initiated comfort measure implemented, Buzzy® (commercially-available vibrating device) resulted in 63% for SPT and 88% for wait. Other nurse-initiated comfort measures, including fans and electronic tablets, are now being evaluated.

CONCLUSIONS: A nurse-initiated comfort measure, Buzzy®, may improve a child's coping during the 15-minute wait, but not during skin test application. Continued data collection will systematically evaluate additional nurse-delivered comfort measures. Ongoing process evaluation offers promise to improve comfort for children undergoing SPT.

441 Are Total Serum IgE Levels Good Predictors Of Allergies In Children?

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RATIONALE: Atopy is a tendency to produce IgE antibodies in response to low doses of allergens. A considerable number of atopic patients have elevated serum IgE levels. We sought to evaluate the association between different allergic diseases and the serum IgE levels of atopic patients. Objectives: To study the role of total Serum IgE levels as a marker of allergy and its association with different systemic allergies.

METHODS: A cross sectional comparative study conducted at an outpatient clinic for allergy and immunology services in Ponce, Puerto Rico, revision over the period of 5 years (2008-2012). 100 children in the age group 6 months to 18 years meeting the inclusion criteria (atopic and Serum IgE available) were enrolled in the study. Variables included demographic, allergy evaluation and total IgE, and statistics analyses.

RESULTS: Serum IgE levels ranged from 3.7 IU/mL to 15,511.2 IU/mL, mean value of the sample of 1,007.7 IU/mL. 59 patients presented increased serum IgE levels. The largest group in our sample were the allergic rhinitis patients (n=43) with IgE levels mean 672.3 IU/mL. Patients with atopic dermatitis, asthma and angiodema presented the highest mean IgE levels with 2,564.8 IU/mL, 2,249 IU/mL and 2,437 IU/mL respectively. The conditions with the most patients with high IgE levels were allergic rhinitis, urticaria and asthma with over 60% of each group exhibiting increased levels of IgE.

CONCLUSIONS: Atopic patients may present with high serum IgE levels, which varied among the atopic conditions. Asthmatic patients had the highest prevalence of increased serum levels of IgE.

442 Relationship Between Maternal Fat During Pregnancy and Risks Of Allergic and Respiratory Diseases In Early Childhood: The Mothers and Children's Environmental Health Study

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RATIONALE: Recent studies suggest that maternal dietary intake during pregnancy may influence programming of the fetal immune system in favor of development of allergic disease. However, the results for the effect of maternal fat status during pregnancy on development of allergic or respiratory disease are still conflicting.

METHODS: In total, 917 mother–child pairs from a prospective birth cohort in South Korea were studied. Data regarding the children's allergic and respiratory outcomes were obtained from standardized questionnaires completed by the mothers at postnatal months 6, 12, and 24. Serum triglyceride(TG) and high-density cholesterol(HDL) levels were measured in the mothers at mid- and late pregnancy, and in their children at 24 months of age. Atopic biomarkers were measured in the cord blood (CB) and at 24 months after birth.

RESULTS: Serum TG levels during late pregnancy were positively associated with CB eosinophil count ($p<0.016$). However, there was no association between TG or HDL levels during pregnancy and the child's atopic biomarkers at 24 months. Maternal TG levels during mid and late pregnancy were associated with an increased risk of AD at the age of 24 months (adjusted odds ratio [aOR] 2.28, 95% CI (1.25 to 4.16), $p=0.07$; aOR 2.39, 95% CI (1.26 to 4.41), $p=0.07$, respectively). However, there was no association between TG or HDL levels and risks of respiratory outcomes and AD at other different ages in logistic regression analysis.

CONCLUSIONS: High maternal serum triglyceride levels in pregnancy were associated with an increased risk of AD in early childhood.

443 The Efficacy Of Training School and Nursery Personnel On Epinephrine Autoinjector Use

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RATIONALE: Similar to other countries, prescription of the epinephrine autoinjector to children with food allergy has greatly increased in Japan, especially after it received National Health Insurance price listing in 2011. On the other hand, it is not uncommon for schools and nurseries to show concern or even refusal toward the use of the medication at their facilities.

METHODS: We performed a questionnaire survey to school and nursery personnel who attended the food allergy seminar held in our hospital from July to November 2012. This seminar included a lecture about the basics of food allergy and practical training using the epinephrine autoinjector trainer.

RESULTS: 124 attendants answered the questionnaire. A knowledge quiz revealed that the treatment of allergic reactions was less understood compared with the cause or prevention. Before the seminar, when asked "How confident are you to manage an anaphylaxis?" the mean score was 3.8 on visual analog scale. Sixty six percent did not understand the timing to use the epinephrine autoinjector. The average score was 4.0 to the question, "How familiar are you with the skills to apply the epinephrine autoinjector?" The confidence score and skills score right after the seminar increased to 8.3, 9.1 ($p<0.001$) respectively, but declined to 6.1 and 8.1 at the 3 to 6 month follow-up ($p<0.001$).

CONCLUSIONS: Lack of knowledge may be one of the reasons for the reluctance to use the epinephrine autoinjector for school and nursery personnel. Education is efficient in developing understanding of the medication, but regular training is necessary.

444 Referral Patterns To An Outpatient Allergy/Immunology Clinic At a Tertiary Care Pediatric Academic Center

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RATIONALE: There is a lack of literature detailing the referral pattern of pediatric patients to Allergy/Immunology outpatient clinics at academic institutions.

METHODS: We performed a retrospective review of all new referrals scheduled in an outpatient Allergy/Immunology clinic at Nationwide Children's Hospital during three months: October 2012, and January/May 2013.

RESULTS: Of 630 total patients(mean age=5.9 yrs, 52%=male), 72%(N=453) were referred from primary care providers(PCPs) and 66%(N=415) were referred by practitioners at our institution. 78%(N=490) of all referrals lived within 30 miles, with 71%(N=448) within 15 miles and 12%(N=78) between 30-60 miles. Patients>60 miles(10%=62), had a mean number of referral diagnoses=1.1, compared to entire group=1.3. Overall, the most common referral diagnoses were allergic rhinitis(AR)=41%(N=241) and food allergy(FA)=32%(N=189). Patients>60 miles, 34%(21/62) were referred for AR. Overall no show rate=25%(157/630), majority had diagnoses of AR(39%=61/157) and FA(35%=55/157). Of all no shows, 83%(130/157) lived<15 miles. Patients>180 miles had 60%(3/5) no show rate. Of all no show visits, 77%(121/157) came from a PCP, with 57%(N=90) from internal PCPs. The no show rate for internal PCP referrals=38%(90/240), while external PCP referrals had no show rate=15%(31/213).

CONCLUSIONS: Approximately 1/3 of all patients referred to our academic outpatient Allergy/Immunology clinic lived>30 miles away. The majority of patients living>60 miles away had straightforward diagnoses, predominately AR. This suggests a lack of available pediatric allergists in rural areas. In addition, the majority of no show visits to our clinic were referred by PCPs at our own institution, which presents an opportunity to investigate quality improvement strategies targeting this at risk population.

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445 Food Allergy Education Significantly Improves School Personnel Food Allergy Knowledge and Bullying Attitudes

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RATIONALE: Five percent of children have food allergies, 16-18% experience reactions and 28% of fatalities secondary to anaphylaxis occur in school. There are limited and poorly defined studies evaluating the effectiveness of school food allergy education, and none conducted in the private school setting. The purpose of this study was to establish baseline knowledge and attitudes regarding food allergies, and assess the effect of food allergy education in private schools.

METHODS: A validated survey based on the Chicago Food Allergy Research survey for the General Public was given to school personnel before and after a 1-hour didactic educational session. Statistical analysis utilized the t-test, linear mixed effects models, and Wilcoxon rank sum test.

RESULTS: A sample of 50 respondents was obtained from two schools. Greater than 80% of the respondents knew someone with food allergy. Eighty-five percent of responders were women, >80% were Caucasian, and 98% completed at least four years of college, Sixty-five percent had prior experience with food allergy through work. Post-education, correctly answered knowledge-based items increased from 72 to 88%,($P<0.001$). The most improved areas of knowledge included common food allergy triggers (pre:44%;post:79%), prevention of food allergies (pre:56%; post:90%), and of the inheritability of allergic diseases (pre:54%; post:88%). Respondent awareness of food allergy bullying significantly increased ($p=0.0009$).

CONCLUSIONS: Food allergy knowledge among school personnel is inadequate despite a highly educated population with food allergy exposure. Food allergy education significantly improved knowledge and awareness regarding the challenges faced by food-allergic children. School personnel food allergy knowledge and awareness is essential for protecting food allergic children.

446 How Patients Rate Their Allergists Online: Analysis Of Physician-Review Websites

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RATIONALE: Online reviews are increasing in popularity and physician-review websites are becoming more prevalent. The review ratings of allergists were analyzed in this study.

METHODS: A representative subset of 300 allergists was randomly selected from a pool of 3,336 US-based allergists. Numerical ratings from 3 popular physician-review websites were analyzed (ratemds.com, vitals.com, healthgrades.com).

RESULTS: There were 248 allergists (83%) who had at least 1 ratings. Average number of reviews per allergists was 6.6, with large variation (0 to 50 reviews). Most of the reviews were positive with the median rating of 70 (composite scale of 1 to 100). There was a statistical difference in ratings by training completion year, categorized as 1980 or earlier, 1981 to 2000, and 2001 to present ($p=0.03$), with more recent groups receiving higher ratings (76, 82, and 89, respectively). Allergists in faculty/hospital practice received fewer ratings when the number of reviews were analyzed by type of practice, defined as faculty/hospital, solo, and group practice ($p<0.001$). There was no significant difference when comparing ratings by gender, region, or type of practice, or number of reviews when categorized by gender or region.

CONCLUSIONS: The majority of allergists (83%) have reviews on physician-review websites and most of them are positive. However, the number of reviews remains small with large rating discrepancies. Allergists should be cognizant of physician-review websites and possibly take proactive steps such as assuring accuracy of their practice and contact information and using feedback for quality improvement.

447 Improving Allergy and Immunology Education For The Internal Medicine Resident Through Internet-Based Learning Modules

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RATIONALE: Primary care physicians are often the first to encounter patients with allergic diseases, and yet there is a lack of formal Allergy Immunology (AI) education in Internal Medicine residency training. Furthermore, previous studies indicate that primary care physicians are interested in having more AI training. In one recent study, primary care physicians not only desired further education on allergy topics, but also showed a statistically significant improvement in knowledge base and ability to administer epinephrine correctly after participating in teaching modules.

METHODS: We have developed an AI curriculum for the Internal Medicine residents at Brigham and Women's Hospital. Using the previously existing internet-based internal medicine residency education and information portal, we provided case-based didactics in the following areas of need: allergic rhinitis, asthma, environmental, food, and drug allergues, eosinophilic esophagitis, urticaria, atopic dermatitis, angioedema, immune deficiency, anaphylzsi, drug desensitization, and mast cell disorders. Cases and didactics were developed based on previously validated learning tools. Our goal is to reach 150 residents through monthly emails with a link to the portal where an AI icon will be displayed and connect to the learning modules.

RESULTS: The portal will be opened in Fall 2013 and outcomes available Spring 2014.

CONCLUSIONS: Allergy Immunology education should be implemented in Internal Medicine residency training programs as part of the core training experience. We propose a system of internet-based learning modules as a venue for additional specialty exposure. References: 1. JE Yu et al. "Development of a food allergy education resource for primary care physicians." *BMC Medical Education*. 8: 45. 2008.

448 Electronic Patient Data Acquisition Tablet (ePDAT) Provides Customized, Flexible Scheduling For Collecting Patient Reported Outcomes (ePRO) With High Usability and Compliance Ideal For Use In Single and Multicenter Environmental Exposure Chamber Studies

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RATIONALE: The collection of ePRO in EEC studies requires the ability to collect pre-defined data points from multiple patients based upon their own unique start time of allergen exposure. Currently there are no validated ePRO tools available that allow the flexibility to document the moment of allergen exposure so that all data points are re-calculated on-demand.

METHODS: Developed and validated 21CFR, Part 11 compliant system that allows users to create customized, prescheduled, protocol-specific ePRO events that are directly linked to the time-difference from a pre-defined reference point.

RESULTS: Data from a recently completed clinical trial which collected 11,720 data points was examined. It was demonstrated that ePDAT system had the flexibility to pre-schedule patients preferred date and time of exposure in the EEC such that if the actual time of exposure differs, the ePRO system automatically re-calculated the exact time for all ePRO events. Once the actual time of exposure for any patient was entered into the system, the system automatically updated that patient's ePRO schedule to all users, including those who are monitoring ePRO data collection within seconds. 100% data was captured with greater than 95% of all data points occurring as originally scheduled.

CONCLUSIONS: Clinical trials conducted in the EEC are traditionally performed with large cohorts of patients studied simultaneously to reduce variability across patient allergen exposures and to promote clinical trial time and cost efficiencies. These data show that ePDAT can be implemented such that staff can easily manage changing schedules in both single and multicenter EEC and EEC/field hybrid studies.

449 Feasibility and Acceptability Of a Novel Asthma Self-Management Smartphone Application For Children and Adolescents

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RATIONALE: Asthma self-management skills are an important component in achieving optimal asthma control. However, previous data evaluating adherence to these strategies remains discouraging. In recent years, mobile device adoption has rapidly increased amongst children and teenagers, which coincides with an exponential growth in availability of mobile health applications. We aimed to leverage this use of technology and create an evidence-based smartphone application that improves asthma self-management.

METHODS: A personalized, interactive iOS smartphone application (AsthmaCare) was created using Xcode (Apple Inc. Cupertino CA) and distributed to participants on iPod Touch devices. We conducted a prospective, 30-day pilot study of patients with asthma, ages 9-16 years old, who had been prescribed at least one controller medication. Questionnaires were utilized to assess feasibility and acceptability of AsthmaCare.

RESULTS: 21 patients completed the 30-day pilot study. 85% of patients interacted with the application at least on a daily basis, including tracking controller medication use and symptom occurrence. All participants reported comfort in using the app to help instruct them on treatment recommendations in case of worsening symptoms. Almost all(95%) preferred using the app to previously received methods of asthma counseling. All participants reported they would recommend the app to others with asthma.

CONCLUSIONS: Adoption rate of our smartphone application amongst the pediatric population was high. This media not only provides a personalized approach to disease management, but also in an interactive format to which children and teenagers can relate to. Ongoing development of mobile applications offers a promising tool in the promotion of asthma management and medication adherence.

450 Experience In The Development Of a Mobile Diagnosis Support System For Asthma: Intelimed

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RATIONALE: A clinical decision support system in the Estrategia Saude da Familia (Family Health Strategy) through the making of a "smart" decision tree in mobile devices by the engineering, computer sciences.

METHODS: An *ISAAC (International Study of Asthma and Allergies in Childhood)*, *GINA (Global Initiative of Asthma)* and Brazilian Consensus-based questionnaire was created, containing the mains attributes of asthma, having gone through face and content validation. A transversal study was conducted by apllying 113 questionnaires to guardians of children and teenagers (ranging from 5 to 19 years old) with medical appointments in the Allergy and Immunology Clinic of the Hospital das Clínicas of Universidade Federal de Pernambuco (HC-UFPE) and in private practice.The app was tested by using 50 scenarios – 25 asthmatics and 25 non-asmathic patients. The evaluated Standards were accuracy, sensitivity and specificity (validation thourg the Leave-one-out method).

RESULTS: Of The 113 Applied Questionnaires, There Were 72 Asthmatic Patients (Cases) And 41 Non-Asthmatics (Control). The Use Of The Prototype App And Decision Tree Has Presented The Following Results accuracy 76%; sensitivity 72%; specificity 82%.

CONCLUSIONS: The asthma attributes relayed through mobile device can consist in an excellent tool to primary doctors, enabling access to the current medical consensus, with potential improved results in treatment.

451 An Assessment Of Food Allergy Knowledge Among Parents Of Children With Food Allergy and The Role Of An Educational Website

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RATIONALE: This study investigates parents' knowledge of food allergy management in their children and if use of an educational website increases knowledge. The Allergy and Immunology (AI) Clinic at University of Chicago provides a free educational website, AllergyGoAway.com, which aims to educate patients how to prevent, identify, and manage food allergic reactions and anaphylaxis.

METHODS: Parents of patients with food allergy completed surveys during a follow-up visit at AI Clinic. The survey inquired about confidence and knowledge of food allergy topics and use of the website. Confidence was measured using a 5-point Likert scale. Knowledge was assessed using questions pooled from a validated knowledge quiz out of 8 points.

RESULTS: 68 subjects were surveyed and majority (>87%) were knowledgeable in avoidance and recognizing symptom severity. Knowledge was poor (< 56%) regarding timing and management of anaphylaxis. Subjects were confident identifying and preventing symptoms of food allergy and using epinephrine autoinjectors. They were not confident identifying signs of anaphylaxis and following a food allergy action plan. Only 7 subjects (10%) used the website, with no difference in knowledge between users and nonusers.

CONCLUSIONS: Education during the patient encounter remains the standard to improve patient knowledge and should focus on specific warning signs of anaphylaxis, the importance of early intervention with epinephrine autoinjectors, and an allergy action plan to prevent fatality. Website use among the surveyed group was low. An online resource may not be sufficient to replace the education provided by healthcare workers during clinic visits.

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452 The Differences Of TNF-á, Rantes, Interleukin-5 Levels In Nasal Polyps With Allergic, Local Allergic, and Non-Allergic Rhinitis

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RATIONALE: Nasal polyposis is a chronic inflammatory disease of the upper airway which develops in the ethmoidal and middle turbinate area. Nasal mucosal immune-reactivity may occur in varying degrees in polyps with allergic, local allergic, and nonallergic rhinitis. We evaluated the differences of nasal polyps from adolescents with allergic and non-allergic rhinitis for RANTES, TNF-α, and IL-5 contents. The goal of this study was to evaluate the role of allergic rhinitis in the pathogensis of nasal polyposis and the correlation between both.

METHODS: We recruited 29 adolescents with allergic (n=15, mean age: 17.4 yrs old), local allergic rhinitis (n=9, mean age:15.9±5.5 yrs old), and non-allergic rhinitis (n=14, mean age: 15.6 yrs old) undergoing polypectomy. Immunoassays were performed using polyp tissues homogenates to quantify the levels of RANTES, TNF-α, and IL-5 and sera to assess total IgE, eosinophil cationic protein (ECP) from them.

RESULTS: TNF-α, IL-5, and RANTES levels in atopic polyp was higher detected compared with non-atopic polyp tissue homogenates (IL-5: allergic rhinitis: 181.5±143.6, local allergic rhinitis: 211.7±185.3, and non-allergic rhinitis: 82.7±88.3 ng/100mg, RANTES: allergic rhinitis: 174.5± 63.5 local allergic rhinitis: 198.5±102.2, and non-allergic rhinitis: 60.3±38.5 ng/100mg, TNF-α : allergic rhinitis: 180.8±121.9, local allergic rhinitis: 48.3±55.4, and non-allergic rhinitis 64.1±64.5 ng/100mg), but no significant difference in INF-γ levels was observed between them.

CONCLUSIONS: IL-5, and RANTES, and TNF-α play important role in inflammatory recruitment leading to cell activation and directional migration of Th2 specific leukocyte subsets for allergic reaction in allergic polyp. Therefore they are involved in the pathogenesis of allergic rhinitis, local allergic rhinitis with polyp.

453 Cigarette Smoke Promotes Eosinophilic Inflammation, Airway Remodeling and Nasal Polyps In a Murine Polyp Model

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RATIONALE: Exposure to cigarette smoke (CS) is a major risk factor for airway inflammation. However, little is known about the effects of CS exposure on eosinophilic rhinosinusitis with nasal polyps (ERSwNP). Histopathologic and molecular studies were performed to investigate its effects using a murine model of ERSwNP.

METHODS: Mice were assigned to one of the following four groups: control, CS exposure, ERSwNP, and ERSwNP exposed to CS (n=8 for each group). Histopathologic changes were investigated using various stains, including hematoxylin and eosin for inflammation and polyp-like lesions, sirius red for eosinophils, toluidine blue for mast cells, alcian blue for goblet cells, and Masson's trichrome stain for collagen fibers. Serum IgE and systemic cytokine levels were measured by enzyme-linked immunosorbent assays. The expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1-alpha was evaluated by immunohistochemical staining.

RESULTS: In the ERSwNP group, CS exposure increased the number of polyp-like lesions, eosinophil and mast cell infiltration, goblet cell hyperplasia, and subepithelial fibrosis. Total and OVA-specific serum IgE levels were increased in both ERSwNP groups; however, CS exposure had no additive effects. Additionally, the levels of IL-4, IL-6, IL-17, and IFN-γ from splenocytes were elevated significantly by exposure to CS. In the ERSwNP group, exposure to CS enhanced VEGF expression in nasal epithelial cells.

CONCLUSIONS: Chronic exposure to CS aggravated eosinophilic inflammation and promoted airway remodeling and nasal polyp formation in a murine model of ERSwNP. The underlying mechanism might involve upregulated VEGF expression.

454 Evaluation Of Oral Antibiotics Versus Placebo For The Treatment Of Rhinosinusitits With Neutrophilia On Nasal Cytology

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RATIONALE: Rhinosinusitis is defined as inflammation involving both the sinuses and the nasal mucosa. Criteria to distinguish bacterial from viral, vasomotor, allergic or other etiologies are imprecise. Nasal cytology, a possible tool to identify bacterial sinusitis, as shown by the clinical response to amoxicillin-clavulanate over placebo, is under study. Preliminary data are described below.

METHODS: Adults with active sinusitis symptoms for ≥2 weeks and nasal neutrophilia of ≥3 on a scale of 0 (<2 neutrophils/HPF) to 4 (>20 neutrophils/HPF) were included. Among 53 subjects screened, only 7 had sufficient neutrophilia; and 6 enrolled. Subjects were randomized into receiving oral amoxicillin-clavulanate or placebo in a double blind manner for 3 weeks, while all subjects used a daily saline sinus lavage (NeilMed Sinus Rinse™). The primary (SNOT-20 score) and secondary (SF-36 score, nasal neutrophilia and sinus tenderness) outcomes were as indicated.

RESULTS: Of the 6 subjects enrolled to date, 4 received antibiotics, 2 placebo. The antibiotic group showed a substantial improvement in their median SNOT-20 score (2.5 pre to 1.7 post) vs placebo (1.8 pre to 1.8 post). Pre/post or antibiotic/placebo SF-36 scores were not appreciably different. Sinus tenderness and nasal neutrophilia improved in all subjects.

CONCLUSIONS: This ongoing study suggests that chronic sinusitis subjects with nasal neutrophilia have a favorable clinical response to amoxicillin-clavulanate compared to placebo. Sinus tenderness and nasal neutrophilia improved in all, perhaps due to regular use of saline sinus lavage.

455 Pediatric Allergic Fungal Otomastoiditis Improved With Anti-IgE Therapy

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RATIONALE: Reports of allergic fungal disease, including allergic fungal rhinosinusitis (AFRS), allergic bronchopulmonary aspergillosis (ABPA) and mycosis are limited in immunocompetent children. The first case of allergic fungal otomastoiditis (AFOM) was recently reported in an adult (Chen 2013). We present the first pediatric case of AFOM associated with asthma, urticaria, Bipolaris species, and fungal mass.

METHODS: Retrospective chart review was completed. Collected variables included microbial isolates, imaging studies, surgical pathologic descriptions, immune studies, medical/surgical treatment and outcomes.

RESULTS: A 12 year old male without cystic fibrosis or immunodeficiency had a 3 year history of right-sided otorrhea and chronic otomastoiditis requiring multiple surgical interventions (>20). Peanut butter consistency mucus was noted on debridement, and histopathology showed allergic mucin with eosinophils and fungal hyphae. Multiple surgical cultures demonstrated Bipolaris species. Other findings included mild asthma by pulmonary function testing, elevated serum IgE (peak 1060 IU/mL), and positive immediate hypersensitivity skin testing to Bipolaris species. Imaging revealed mastoid involvement with erosion through the right internal carotid artery canal. Treatment included >2 years fungal-specific subcutaneous immunotherapy, oral and topical corticosteroids, parenteral and topical antifungals, topical acetylcysteine, and topical DNase with continued otorrhea and mastoiditis. Omalizumab was started for urticaria to avoid further immunosuppression. The AFOM improved without interval need for surgical intervention after 9 weeks of anti-IgE therapy.

CONCLUSIONS: AFOM is a rare disorder. Treatment of allergic fungal disease is difficult in patients with disrupted anatomic architecture, requiring chronic surgical and medical interventions. In our patient, adjunctive anti-IgE therapy resulted in a diminished need for surgical intervention.

456 Measurement Of Nasal and Exhaled Nitric Oxide In Chronic Rhinosinusitis and Its Comparison According To The Presence Of Nasal Polyps

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RATIONALE: Fractional exhaled nitric oxide (FeNO) and nasal nitric oxide (nNO) are used as a screening study to evaluate the airway inflammation in atopic patients. This study was performed to evaluate whether they can be used for discriminating the chronic rhinosinusitis (CRS), with (CRSwNP) and without nasal polyps (CRSsNP).

METHODS: Thirty-five patients with CRS in Dong-A university hospital were recruited. Sixteen patients were CRSwNP (group I), and 19 were CRSsNP (group II). The NO measurement was performed using a handheld electrochemical analyzer (NObreath®), and the mean value of the 3 consecutive measurements was recorded.

RESULTS: There were no significant differences in gender, age, body mass index and smoking habits between two groups. The FeNO levels were higher in group II (28.78 ± 19.87 v 33.78 ± 52.47 ppb in group I and II, respectively; P=0.655), while the nNO levels were higher in group I (79.42 ± 65.63 vs 70.11 ± 65.65 ppb; P=0.729). However, the difference of FENO and nNO did not reach statistical significance.

CONCLUSIONS: FeNO and nNO was increased in patients with CRS. In CRSwNP patients, FeNO was lower and nNO was higher compared with CRSsNP patients. Further studies are needed to evaluate the role of FeNO and nNO in patients with CRS.

457 Post-Translational Modification By Serine Proteases Controls The CCL23 Activity In Nasal Polyps Of Chronic Rhinosinusitis

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RATIONALE: Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by intense inflammatory cell infiltration including eosinophilia and the accumulation of macrophages and dendritic cells (DCs). We have previously found that the chemokine CCL23 is elevated in CRSwNP. CCL23 recruits monocytes, macrophages and DCs via CCR1. Although mature CCL23(AA22-120) shows low affinity for CCR1, CCL23 is known to be cleaved by synovial fluids and matrix metalloproteinases and truncated forms CCL23(46-120) and CCL23(47-120) have 10-100 times higher CCR1 binding activity than CCL23(22-120). We investigated the potential role of post-translational modification and functional activity of CCL23 in nasal polyps (NPs).

METHODS: We investigated the truncation of CCL23 protein in NP homogenates by western blot and determined the protein sequence of cleaved CCL23 using Edman sequencing and MALDI-TOF MS. We investigated the chemotactic activity of CCL23 on the THP-1 monocytic cell line using a micro-chemotaxis method.

RESULTS: We found that recombinant CCL23 was truncated by NP homogenates and the truncation was inhibited by the serine protease inhibitor Nafamostat, suggesting that CCL23 is truncated by NP serine proteases. CCL23 pretreated with NP homogenates time- and dose-dependently induced migration of THP-1 cells with a potency significantly greater than that of CCL23(22-120) but similar to CCL23(46-120). We further determined that NP homogenates time-dependently generate a novel truncated form, CCL23(47-117). Our data suggests that CCL23(47-117) might be a major active form of CCL23 in NPs.

CONCLUSIONS: CCL23 is post-translationally modified by NP serine proteases. Overproduction and cleavage of CCL23 may play important roles in the inflammation in CRSwNP.

458 Sinusitis In Latino Children Is Associated With Allergic Respiratory Diseases and Inversely Related To Native American Ancestry (GALA II Study)

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RATIONALE: The burden of sinusitis among Latino children is poorly understood at this time.

METHODS: From 2006-2011, Hispanics aged 8-21 years with and without asthma were recruited from 5 cities in the United States and Puerto Rico to participate in the GALA II Study. Procedures included questionnaire, aeroallergen skin prick test, spirometry and blood draw. Logistic regression was used to analyze whether sinusitis was associated with allergy, ethnicity, or genetic ancestry in Latinos.

RESULTS: Of the 4,111 subjects (mean+SD age=13.1+3.4 years, 49% males), 2,022 were asthma cases and 2,135 healthy controls. A total of 691 subjects (16.6%) reported a history of sinusitis, which was more prevalent in males (18.2% vs. females 15.5%, p=0.02), asthmatics (28.2% vs. controls 6.0%, p<0.001), and Puerto Ricans (26.9% vs. Mexicans 7.4%, p<0.001). Logistic regression analysis adjusted for age, gender, center, education level, and income showed that reported sinusitis was associated with total IgE>80kU/L (odds ratio [OR]=1.76, 95% confidence interval =1.42-2.18, p<0.001), with >1 positive allergy skin test (OR=1.53 [1.20-1.96], p=0.01), with a history of physician-diagnosed allergic rhinitis (OR=6.79 [5.57-8.29],p<0.001), and with asthma (OR=6.61[5.26-8.30],p<0.001). Genome-wide measures of genetic ancestry revealed a marked protective effect from Native American ancestry (OR=0.04[0.02-0.08], p<0.001).

CONCLUSIONS: In young Latinos, report of sinusitis was associated with atopy and allergic respiratory diseases. Native American ancestry was associated with marked protection against reported sinusitis.

459 Evaluation Of Olfactory Function In Patients With Chronic Rhinitis

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RATIONALE: Hyposmia represents a common complication of chronic rhinosinusitis, which is usually related to allergic rhinitis and nasal polyposis. The aim of this study is to evaluate olfactory dysfunction in patients with chronic rhinitis.

METHODS: A prospective study was conducted in patients older than 12 years with prior history of chronic rhinitis (more than 8 weeks). Patients underwent clinical examination, nasal cytology, skin prick tests, rhinomanometry and a olfactory disfunction test. Olfactory dysfunction was evaluated through the Connecticut Chemosensory Clinical Research Center test (CCCRC), which consisted of two parts. The first part used butanol at different concentrations and evaluated the butanol smell threshold. The second part was the identification test that consisted in the identification of 8 common substances (talc, chocolate, cinnamon, coffee, mothballs, peanut butter, soap and menthol).

RESULTS: We included 47 patients, 74.5% had diagnosis of allergic rhinitis and 25.5% non-allergic rhinitis. Nasal cytology was normal in 91.5% of the cases. Of the patients with allergic rhinitis, 14.2% (n=5) were sensitized to Dermatophagoides spp., 14.2% (n=5) to pollens and 71.4% (n=25) had a mixed sensitization pattern. After olfactory evaluation, we found that 61.7% of the patients had some degree of olfactory dysfunction as follows: mild hyposmia 38.3% (n=18), moderate hyposmia 19.1% (n=9) and severe hyposmia 4.3% (n=2). There was no difference in olfactory dysfunction between patients with allergic and non-allergic rhinitis (p=0.06).

CONCLUSIONS: Olfactory dysfunction is highly prevalent among patients with chronic rhinitis regardless of the allergy status.

460 A Method For Assessing Regional Determinants Of Eosinophilia In Chronic Rhinosinusitis

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RATIONALE: Biopsy-independent means of identifying regional tissue eosinophilia in chronic rhinosinusitis (CRS) and determining the biological drivers of pathobiology have potential implications for diagnosis and choice of therapy.

METHODS: 0.375 inch discs of polyvinyl alcohol (PVA) sponges were placed in anatomically defined regions (inferior, middle and superior meatus) of 34 patients with CRS (12 CRSwNP, 22 CRSsNP and 31 control patients without CRS). Retrieved nasal secretions and matched adjacent tissue biopsies were analyzed by means of ELISA and Luminex.

RESULTS: Compared to control patients, eosinophilic cationic protein (ECP) was significantly elevated in nasal secretions from CRSwNP patients in all three regions but particularly in the middle and superior meatus (p<0.01). ECP levels in nasal secretions correlated well with levels in adjacent tissue (R^2=0.34; p<0.01) and eosinophilic derived neurotoxin (EDN) levels in nasal secretions (R^2=0.58; p<0.0001) but not ECP levels in nasal lavage. Elevated levels of the cytokines IL-5, IL-6 and IL-13 (p<0.05), but not IL-4 or IL-33, were found in middle meatal secretions of CRSwNP patients and these were significantly correlated with ECP levels (p<0.0001).

CONCLUSIONS: A regionally placed PVA sponge is able to assess biomarkers of eosinophilia in CRSwNP that correlate with adjacent tissue and can sensitively measure cytokines associated with this disease.

461 The Prevalence Of AERD In a Tertiary Care Center

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RATIONALE: Aspirin exacerbated respiratory disease (AERD) is characterized by asthma, chronic rhinosinusitis with nasal polyps (CRSwNP) and aspirin sensitivity. While patients with AERD tend to have more severe sinus disease when compared to patients with CRSwNP alone, factors and mechanisms that account for such differences are not well understood. In order to study AERD pathogenesis, we first identified and characterized patients with AERD, CRSwNP+Asthma, and CRSwNP alone by evaluation of records from a tertiary care center.

METHODS: Electronic health record data from over 1 million entries in the Northwestern Medicine Enterprise Data Warehouse were utilized to identify patients with sinusitis, nasal polyps, and/or history of sinus surgery. From this cohort, we identified 62 patients with AERD defined as having 1) physician-diagnosed asthma; 2) CRSwNP as documented by nasal endoscopy and/or sinus CT; and 3) history of rhinorrhea and/or wheeze following aspirin ingestion. Fifty-four patients with CRSwNP+Asthma and 54 with CRSwNP alone were included who were surgical candidates previously recruited for other CRS studies.

RESULTS: Patients with AERD, on average, underwent significantly more sinus surgeries then patients with CRSwNP+Asthma ($p<0.01$) or CRSwNP alone ($p<0.01$). There was a higher prevalence of women (61%) and physician-reported atopy (87%) in AERD when compared to CRSwNP alone (30% and 53% respectively). Interestingly, there were no statistically significant differences between AERD and CRSwNP+Asthma in regards to gender, atopy, or asthma severity.

CONCLUSIONS: In our population, we found patients with AERD had a similar prevalence of gender, atopy, and asthma as patients with CRSwNP+Asthma yet, on average, underwent more sinus surgeries.

462 Expression Of Hypoxia-Inducible Factor 1alpha In Regulatory T Cells Is Associated With Nasal Polypogenesis

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RATIONALE: Hypoxia-inducible factor 1alpha (HIF-1α) is considered as a key molecule in regulating Th17:regulatory T-cells (Tregs) balance. The aims of this study were to investigate whether HIF-1α is associated with the orphan nuclear receptor gamma (RORγ) expression of Tregs in nasal polyps and to verify whether SEB is involved in this process.

METHODS: Forty patients with Chronic rhinosinusitis with nasal polyposis (CRSwNP) were enrolled and divided into eosinophilic nasal polyps (EPs) and non-eosinophilic nasal polyps (NEPs) according to the proportion of eosinophils. Fifteen subjects who were undergoing septoplasty were enrolled as control subjects. Expressions of HIF-1α in the tissue were measured using RT-PCR, western blot, and flow cytometry. The mRNA expression of RORC and HIF-1α in Tregs separated from tissues were measured by RT-PCR. Double immunofluorescent (IF) staining for RORC/FOXP3 and HIF-1α/FOXP3 were conducted on the tissues. Expressions of RORC and HIF-1α in Tregs from PBMC were measured using flow cytometry after stimulation with SEB.

RESULTS: Expressions RORC and HIF-1α in Tregs were significantly higher in EPs and NEPs compared with control mucosa, and there was a significant correlation between RORC and HIF-1α expression in Tregs. Expressions of RORC and HIF-1α mRNA in Tregs separated from the tissues were also significantly higher in nasal polyps compared with control mucosa. Expression of RORC and HIF-1α in Tregs were increased after 24 hour stimulation with SEB in the PBMCs.

CONCLUSIONS: HIF-1α-induced RORC expressions in Tregs may play a key role in the pathogenesis of nasal polyps.

463 Prognoistic Factors For Olfaction After Endoscopic Sinus Surgery In Chronic Sinusitis With Or Without Allergy

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RATIONALE: Chronic rhinosinusitis (CRS) is one of the most common causes of olfactory dysfunction. After endoscopic sinus surgery (ESS), olfactory function would be improved in most cases or not in some cases. Therefore the purpose of this study was to investigate the prognostic factors for olfaction improvement after endoscopic sinus surgery in CRS.

METHODS: 107 patients with CRS who underwent ESS were studied. We performed olfactory function test for all patients using the Butanol threshold test (BTT) and Cross Cultural Smell Identification test (CC-SIT) preoperatively and postoperatively. The patients‘ subjective symptoms were also recorded using the Visual Analog Scale (VAS) pre-and post-operatively. We also analyzed the duration of disease and preoperative computed tomography.

RESULTS: The improvement of olfactory function after ESS in patients with longer duration of symptoms was significantly lower than in patients with shorter duration. Alllergy, CT scores and involvement of ethmoid sinuses were not influenced the postoperative improvement of olfaction significantly.

CONCLUSIONS: The improvement of olfaction after ESS not depends on the presense of allergy but mainly depends on the duration of CRS. Therefore, for the better outcome of ESS especially in olfactory function, early and proper treatment for CRS will be necessary.

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464 The Association Between Two SNPs GATA3 (rs1269486, rs2229360) Gene and Allergic Rhinitis

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RATIONALE: Allergic rhinitis is caused by the interaction of genetics and environment factors. TH2 has crucial role in atopic disease and GATA3 is known to be a contributing factor to increased TH2. Few studies showed Single Nucleotide Polymorphism (SNP) GATA3 was associated with allergic rhinitis, asthma, increased total IgE, and atopic dermatitis.

METHODS: This research is a case-control study, using two SNPs: GATA3 (rs1269486, rs2229360). These genes have been assessed in patients with allergic rhinitis as well as normal controls (86 patients and 86 normal). Moreover, blood samples of these participants have been genotyped by PCR.

RESULTS: A significant association was found between allergic rhinitis and polymorphisms of alleles, as well as genotypes and hallotype of GATA3 rs1269486 gene. The frequency of A allele and GA genotype were significantly higher in healthy subjects compared to G allele and GG genotype in patients ($P < 0.001$). Furthermore, Haplotypes of AC and GC were found to be significantly higher in normal subjects and patients respectively ($p < 0.001$).

CONCLUSIONS: This study was conducted in the Northeastern parts of Iran. Polymorphisms of GATA3 rs1269486 gene was found to be associated with allergic rhinitis and sensitivity to aeroallergens, however, further research is required to determine polymorphisms in more SNPs, as well as the other regions of the country.

465 Impacts Of Adolescents' Allergic Rhinitis On School Achievement and Quality Of Life

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RATIONALE: Allergic rhinitis is known to negatively affect one with sleep disorder, attention disorder, anxiety, and other mental disorders that may eventually influence one's academic achievement.

METHODS: Raw data of their academic achievements, ISAAC and QOL questionnaires were collected from 662 students from 4 middle and high schools. Depending on academic achievements, which were categorized into high, middle, and low, cross analysis was performed. The correlation between the rating of students' quality of life and the percentile of their academic achievements were analyzed.

RESULTS: There was no significant difference between the allergic rhinitis group and the normal group in terms of total score, sum of math and science, and English; however, high school rhinitis students who had received treatments over the last 12 months had significantly high Korean language scores. Female rhinitis students showed the highest prevalence, especially those who scored high on Korean language. In addition, high school students who had been given treatment over the past 12 months showed a significantly high prevalence, especially those with high Korean language scores. There are significant positive correlations between quality of life represented by physical fitness index and the percentiles for the following subjects: math and science.

CONCLUSIONS: This result contradicts the other research results that support the correlation between allergic rhinitis and low academic achievement. However, considering other research results on how one's academic achievement can represent social status, it is possible to conclude that allergic rhinitis is seen more often among people with high social status.

466 Allergic Rhinitis (AR) Is Sub-Optimally Controlled: The Need For a More Effective Treatment Option

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RATIONALE: AR remains sub-optimally controlled. Our aim was to (i) use patient survey data to explore the unmet need in AR, and (ii) show how a new treatment option (MP29-02; Dymista), a novel intranasal formulation of azelastine hydrochloride (AZE) and fluticasone propionate (FP) in an advanced delivery system can fill this need.

METHODS: AR symptomatology and medication usage data were collected from 746 moderate/severe seasonal AR (SAR) patients who completed a healthcare utilization survey in the UK. The patient characteristics mimicked those of patients in the MP29-02 clinical trials. The clinical efficacy of MP29-02 was compared to commercially available AZE or FP nasal sprays and placebo in a 14-day randomized controlled trial including 610 SAR patients. Time to response was assessed post-hoc. A ≥30% to ≥90% change from baseline in reflective total nasal symptom score (rTNSS) defined response.

RESULTS: 96.2% of patients surveyed were on AR medication; 70.5% taking ≥2 medications. These patients remained symptomatic with a mean rTNSS of 12.8 (range 0-24) and a mean rTOSS of 8.6 (range 0-18). Clinical trial data in a matched population showed that more MP29-02-patients achieved ≥30%, ≥50%, ≥60%, ≥75% and ≥90% rTNSS-reduction, and days faster than either active comparator. FP did not differ from placebo in providing a ≥60% rTNSS reduction.

CONCLUSIONS: AR is often poorly controlled with current therapies (even multiple therapies). Intranasal corticosteroids fail to provide sufficient symptom control in many patients. Treatment with MP29-02 addresses this unmet medical need as it provides faster and more complete symptom control than FP.

467 Short and Long-Term Safety Of MP29-02 In The Treatment Of Allergic Rhinitis

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RATIONALE: MP29-02 (Dymista®), a novel intranasal formulation of azelastine hydrochloride (AZE) and fluticasone propionate (FP) in an advanced delivery system, provides better nasal symptom improvement than firstline treatment in SAR and chronic rhinitis patients. Short- and long-term safety from these clinical trials are presented.

METHODS: 4022 patients were randomized into 4, 14-day double-blind, placebo-controlled SAR trials to MP29-02, AZE, FP or placebo nasal sprays. 612 chronic rhinitis patients were randomized to a 1-year, open-label, parallel-group trial to MP29-02 or FP nasal sprays. For all studies the total daily dose of AZE and FP was 548mcg and 200mcg, respectively. Safety was assessed by incidence, type, and severity of adverse events, vital signs and nasal examinations. Fasting morning serum cortisol concentrations were measured in a sub-group of chronic patients.

RESULTS: In all studies the incidence of treatment-related adverse events (TRAEs) for active groups was low, not exceeding placebo in many instances. The most commonly reported TRAEs for MP29-02 were dysgeusia (2.1-7.2%), headache (0.5-2.6%) and epistaxis (1.0-3.9%). Long-term, there was no evidence of TRAE accumulation over time. A SAE was reported by 3 MP29-02-subjects and 1 FP-subject. None were considered treatment-related. There was no appreciable reduction in serum cortisol from baseline following 12 month's continuous treatment with MP29-02 (-0.08 (SD 5.5) mcg/dL) or FP (-1.04 (SD 5.0) mcg/dL). For all studies, changes in vital signs and nasal examinations were similar in all groups.

CONCLUSIONS: MP29-02 was well tolerated in 14-day studies in SAR patients. MP29-02 was safe in a long-term study with no evidence of late-occurring TRAEs.

468 Anti-Allergic Effect Of Intranasal 1,25-Dihydroxyvitamin D3 Treatment In Allergic Rhinitis Mouse Model

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RATIONALE: The active form of vitamin D (1,25-Dihydroxyvitamin D3) has an essential role in calcium homeostasis and normal mineralization of bone. Recently, many studies identified additional roles of Vitamin D in the immune system as having a potential immunomodulatory effect. However, there is still controversy about the role of vitamin D in allergic inflammation. We aimed to evaluate the effect of intranasal 1,25-Dihydroxyvitamin D3 on allergic symptoms and cytokine profiles using a murine model of allergic rhinitis.

METHODS: An ovalbumin (OVA)-sensitized and –nasally challenged mouse model of allergic rhinitis was established. The active form of vitamin D was intranasally administered 3 hours before each nasal challenge, and multiple parameters of allergic response were measured.

RESULTS: Intranasal vitamin D administration reduced allergic symptoms and total and OVA-specific IgE levels in serum. It also inhibited local Th2 cytokines, IL-4 and IL-5 mRNA expressions in nasal mucosa, and systemic cytokine production by splenocytes. On the contrary, topical vitamin D increased cytokine levels (IL-4, IL-5, IFN-γ and IL-10) in the cervical lymph nodes.

CONCLUSIONS: This study demonstrated that intranasal administration of vitamin D attenuated allergic symptoms, total and OVA-specific IgE levels, and systemic and local allergic inflammations in allergic rhinitis mouse model. Therefore, intranasal vitamin D could be considered as a potential agent in treating allergic rhinitis.

469 B-Cells In Allergic Airways Disease: Inhibition Of Epsilon Transcription By Omalizumab

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RATIONALE: Omalizumab is a monoclonal antibody that binds free IgE for treatment of allergic airway diseases. Recent evidence has shown that omalizumab may also play a role in management of intrinsic non-atopic asthma, raising new questions regarding its mechanism of action. The aim of this study was to assess if omalizumab can modulate B-cell airway responses by determining its effect on epsilon gene expression using explanted nasal tissues.

METHODS: Patients undergoing FESS surgery were questioned regarding symptoms of atopy. Environmental allergy skin testing was performed and inferior turbinate biopsies were obtained. Nasal tissues were cultured for 48 hours with complete media ± aCD40(1mg/mL)+IL-4(200U/mL), allergen extracts [birch(10mg/mL), Timothy-grass(10mg/mL), short-ragweed(10mg/mL), cat(500PNU/mL), dust mites(500PNU/mL)], omalizumab(10mg/mL) or allergen+omalizumab. IgHE, IgHA and $IgHG_4$ mRNA was quantified following RT-PCR and normalized using GAPDH.

RESULTS: Nasal explants stimulated in the presence of relevant allergens demonstrated a 7-fold increase in IgHE mRNA relative expression compared to baseline/media alone. This increase in expression was inhibited when nasal explants were co-cultured with omalizumab. In parallel, these explants exhibited, respectively, a 6-fold and a 17-fold increase in IgHA and $IgHG_4$mRNA relative expression. Control explants with irrelevant allergen did not show changes in immunoglobulin mRNA expression between culture conditions.

CONCLUSIONS: Omalizumab altered the pattern of immunoglobulin gene expression by down-regulating IgHE and up-regulating IgHA/$IgHG_4$ mRNA. This finding may reflect a change in the local airway environment and we hypothesize that omalizumab can lead to diminished T_H2 cytokine production, with a balance towards regulatory cytokines (e.g. TGF-β) supporting more IgHA/$IgHG_4$ mRNA transcription by the B-cell infiltrate.

470 Early Childhood Allergic Phenotypes Are Associated With Internalizing Disorders

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RATIONALE: Few studies have examined the association between allergic disease and childhood emotional outcomes. We hypothesized that allergic phenotypes at age three are associated with internalizing disorders including anxiety, depression, and somatization at age seven.

METHODS: Children enrolled in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS), a birth cohort study, completed skin prick testing (SPT) and clinical examinations at ages 1, 2, 3, 4, and 7. At age seven, parents completed the Behavior Assessment System for Children, Second Edition (BASC-2), a validated psychological assessment. The associations between rhinitis, persistent wheezing, and allergic sensitization at age three and internalizing disorders at age seven are examined by logistic regression adjusting for breastfeeding, maternal education, and race.

RESULTS: A total of 576 children completed the age seven BASC-2 examination; of these the prevalence of rhinitis and persistent wheeze at age three was 35% and 11%, respectively. Aeroallergen and food sensitivity at age three was 37% and 2%, respectively. Rhinitis at age 3 years was significantly associated with internalizing disorders, (aOR=2.4 [1.4-4.1]), anxiety disorders (aOR=1.8[1.1-3.0]), and depressive disorders (aOR=2.0 [1.1-3.6]). Persistent wheeze at age 3 years was significantly associated with somatization (aOR=2.0 [1.0-4.2]). Aeroallergen sensitization at age 3 years was associated with depressive disorders (aOR=2.0 [1.1-3.5]). Food sensitization prior to and including age three was significantly associated with anxiety disorders (aOR=2.4 [1.3-4.6]).

CONCLUSIONS: Early allergic diseases, in particular rhinitis, persistent wheeze, and allergen sensitization, are associated with internalizing disorders such as anxiety, depression, and somatization at 7 years.

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471 Allergic Rhinitis In Puerto Rican Children: Under-Diagnosis and Risk Factors

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RATIONALE: Little is known about allergic rhinitis (AR) in Puerto Rican (PR) children, who are often affected by asthma.

METHODS: Cross-sectional study of children 6-14 years with (n = 288) and without (n = 259) asthma in San Juan (Puerto Rico). Participants underwent a protocol including questionnaires, skin testing to fifteen allergens, and measurement of serum total/allergen-specific IgEs. AR was defined as naso-ocular symptoms apart from colds and ≥1 positive skin test. We examined the sensitivity, specificity, and positive predictive value (PPV) of physician-diagnosed AR (PD-AR) using contingency tables. Next, we identified covariates associated with AR in children with/without asthma using logistic regression. We then used these results to improve the predictive characteristics of PD-AR.

RESULTS: AR was present in 71.9% and 36.7% of children with and without asthma, respectively. PD-AR was reported in only 50 (17.4%) and 13 (5.0%) of children with and without asthma, respectively. Among asthmatics, PD-AR had excellent specificity and PPV (~90%-94%) but limited sensitivity (21.7%). Similarly, PD-AR had excellent specificity and PPV but poor sensitivity (11.6%) in children without asthma. In a multivariate analysis, predictors of AR in children with/without asthma included having dust trigger symptoms and an IgE ≥0.35 IU/ml to Der p 1. A definition of AR combining symptoms, an IgE to dust mite and having dust trigger symptoms had excellent sensitivity/PPV in children with/without asthma.

CONCLUSIONS: AR is markedly under-diagnosed in PR children. Given very few allergists, PR physicians could accurately diagnose/treat most AR cases by inquiring about symptoms and measuring dust mite-IgE.

472 Hematopoietic Prostaglandin D Synthase Is a Useful Target For Treating Nasal Obstruction In Guinea Pigs With Allergic Rhinitis

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RATIONALE: We previously reported that hematopoietic prostaglandin D synthase (HPGDS) is involved in the pathogenesis of allergic rhinitis (AR). The objective of this study is to examine the potency of a novel selective HPGDS inhibitor, TAS-205 which is recently found in our laboratory, on nasal obstruction in guinea pigs with AR.

METHODS: Guinea pigs sensitized to ovalbumin were challenged with intranasal exposure to ovalbumin once a week. To evaluate the effects on biphasic nasal obstruction, specific airway resistance was measured before and after the 3rd antigen challenge. TAS-205 and the other compounds were administered orally once a day for 15 days from the 1st challenge to the 3rd challenge, but only fexofenadine was 1 h before the 3rd challenge.

RESULTS: The challenge with ovalbumin caused a biphasic increase in nasal airway resistance and an increase of prostaglandin D_2 (PGD_2) in sensitized guinea pigs. TAS-205 (1 – 30 mg/kg/day) dose-dependently suppressed nasal obstruction in late phase. The suppressive effect at maximal dose of TAS-205 on nasal obstruction was very potent as well as that of prednisolone (20 mg/kg/day). The nasal obstruction was significantly suppressed by both DP1 and DP2 antagonists. It is suggested that PGD_2 plays an important role in nasal obstruction of AR. Also, combined treatment of TAS-205 and fexofenadine (10 mg/kg) H1 receptor blocker, resulted in additive suppression of both phases.

CONCLUSIONS: The potency of TAS-205 was comparable to that of prednisolone in late phase response in experimental AR model. HPGDS selective inhibitor such as TAS-205 may provide a new therapeutic approach in AR.

473 Efficacy Of MP29-02 In The Treatment Of Nasal and Ocular Symptoms Of Seasonal Allergic Rhinitis (SAR)

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RATIONALE: An allergic rhinitis (AR) treatment ideally should provide relief from nasal and ocular symptoms. The efficacy of MP29-02 (a novel intranasal formulation of azelastine hydrochloride [AZE] and fluticasone propionate [FP] in an advanced delivery system) in providing relief from nasal and ocular symptoms was assessed in patients with SAR.

METHODS: 610 moderate-to-severe SAR patients were randomized into this double-blind, placebo-controlled, 14-day, parallel-group trial to MP29-02, AZE, FP or placebo nasal sprays (all 1 spray/nostril bid [total daily doses: AZE = 548mcg; FP=200mcg]). Change from baseline in the most bothersome symptoms of nasal congestion and ocular itching was assessed secondarily along with the sum total of 7 symptom scores (rT7SS; congestion, itching, rhinorrhea, sneezing, ocular itching, redness and watering) in a post-hoc analysis.

RESULTS: MP29-02 effectively treated the entire rhinitis symptom complex, reducing the rT7SS from baseline by -8.74 versus -6.05 for FP ($p=0.0013$), -5.83 for AZE ($p=0.0004$) and -3.55 for placebo ($p<0.0001$); relative difference 52% to FP and 56% to AZE. MP29-02 provided greater relief from nasal congestion than FP ($p=0.0034$), AZE ($p=0.0001$) and placebo ($p<0.0001$); relative difference of 54% to FP and 70% to AZE. MP29-02 patients experienced significantly better ocular itching relief compared to FP ($p=0.0001$), AZE ($p=0.0127$) and placebo ($p<0.0001$); relative difference 67% to FP and 44% to AZE.

CONCLUSIONS: MP29-02 was twice as effective as two current first-line therapies in providing relief from the entire rhinitis symptom complex, and at least twice as effective as an intranasal corticosteroid in relieving nasal congestion and ocular itching, the most bothersome symptoms.

474 Atypical Symptoms Of Chronic Rhinitis and The Impact On Quality Of Life

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RATIONALE: Several symptoms of chronic rhinitis are underestimated. This study aims to analyze the correlations between clinical presentations, quality of life, and therapeutic response.

METHODS: Patients presenting with chronic rhinitis were asked to score their presenting symptoms based on a Likert scale questionnaire (scale of 0 to 3) and the quality of life by using The 12-Item Short-Form Health Survey version2 on the first visit. Therapeutic response was evaluated 2 months afterwards.

RESULTS: Two-hundred and twenty-eight patients were recruited into this study. The average age was 34.1±14.2 years old, 66.8% of them were females, 72.4% of them were allergic, and 84.4% of them had perennial symptoms. Blocked nose, running nose, sneeze, and itchy nose were the most common "typical symptoms" (average scores were 2.14, 2.05, 1.94, and 1.86, respectively). However, scores of postnasal drip (1.64), fatigue (1.62), daytime somnolence (1.49), dry mouth (1.43), mouth breathing (1.42), and night awakening (1.36) indicates that these "atypical symptoms" were not uncommon. Average physical component summary (PCS) scores and mental component summary (MCS) scores in these patients (N=186) were 40.8±5.3 and 45.3±8.7, respectively, which are lower than those in general normal population. Typical symptoms were correlated with PCS (P value = 0.02) while atypical symptoms were correlated with MCS (P value < 0.01). The decreases of typical and atypical symptom scores at a 2-month follow-up were 38.7% and 22.3%, compared to baseline.

CONCLUSIONS: Atypical symptoms of chronic rhinitis are more difficult to treat and significantly affect mental health status. New therapeutic modalities should also be focused on these symptoms.

475 Clinical Characteristics Of Allergic and Nonallergic Rhinitis In Children

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RATIONALE: Rhinitis is classified as allergic (AR) or nonallergic rhinitis (NAR). They may have different presentation in children. We investigated whether children with AR and NAR manifest different clinical characteristics.

METHODS: A total of 102 children with rhinitis symptoms were enrolled in this study. We performed allergic evaluation including blood eosinophil counts, serum total IgE, specific IgE, and skin prick test and investigated personal and family history.

RESULTS: A total of 69 children (68%) had AR, whereas 33 (32%) had NAR. The mean age was 8.8 ± 2.7 years and there were no significant differences in age and sex between the AR and NAR group. Nasal pruritus was more frequent in children with AR (57% vs. 30%, P= 0.018), whereas other nasal symptoms including sneezing, rhinorrhea, and nasal obstruction were similar between children with AR and NAR. Conjunctivitis was also more frequent in children with AR (49% vs. 24%, P= 0.021), but asthma was similar (32% vs. 15%). Cigarette smoke exposure was more frequent in children with NAR (41% vs. 64%, P= 0.040).

CONCLUSIONS: We showed that children with AR had more nasal pruritus and conjunctivitis and children with NAR had more cigarette smoke exposure.

476 GATA3-Expressing ILC2 Are Selectively Enriched In Allergic Eosinophilic Nasal Polyposis

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RATIONALE: Group 2 innate lymphoid cells (ILC2) are a recently discovered population of lineage-negative cells that may contribute to the pathogenesis of type 2 inflammatory diseases. Nasal polyp ILC2 from patients with chronic rhinosinusitis (CRSwNP) produce Th2 cytokines in a GATA3-dependent manner. Our aim was to determine whether ILC2 are present in greater numbers in eosinophilic compared with non-eosinophilic polyps in order to identify novel cellular differences among CRSwNP endotypes.

METHODS: Nasal polyps were collected from 12 human subjects after UCSD IRB approval. Patient characteristics were obtained by retrospective chart review. Polyps were characterized as eosinophilic based on flow cytometry of single cell suspensions (CCR3+FceR1- granulocytes) and confirmed with cytospin and H&E tissue analysis. ILC2 were identified as lineage-negative CRTH2-positive lymphocytes by flow cytometry and also stained for GATA3 expression.

RESULTS: There were no significant differences in baseline characteristics including age, gender, and medication use between groups. Eosinophilic polyps from allergic subjects (n=5) contained a higher percentage of ILC2 compared to non-eosinophilic polyps that had unknown allergic status (n=7) (0.69% vs. 0.25%, P=0.017). There was a positive correlation between ILC2 and eosinophils (r=0.43, P=0.02), but no correlation between ILC2 and FceR1+CCR3+ cells in the polyp tissue (r^2=0.01, P=0.75). Further, we confirmed ILC2 GATA3 expression in the CRTH2+ lineage-negative cells that was absent in CRTH2-negative cells.

CONCLUSIONS: Eosinophilic polyps in allergic patients demonstrate a greater number of ILC2 compared with non-eosinophilic polyps from patients with unknown allergic status. Thus, ILC2 may preferentially contribute to the eosinophilc endotype of CRSwNP.

477 Innate and Adaptive Lymphocyte Responses In a Mouse Model Of Rhinovirus-Induced Asthma Exacerbation

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RATIONALE: Rhinovirus (RV) is a major precipitant of asthma exacerbations (AEs). Characterising a mouse model of RV-induced AEs would further our knowledge of the immune responses at play, which may be important for understanding disease pathogenesis in man.

METHODS: 6-8 week old female BALB/c mice were sensitized on d-13 intraperitoenally with 40μg OVA/Alum, challenged intranasally on d-2, d-1 and d0 with 40μg OVA/PBS or PBS and infected with RV-1B ($2.5x10^6$ $TCID_{50}$) or UV-irradiated RV-1B immediately after the last challenge. BAL and lung tissue was analysed by cytospins, flow cytometry, qRT-PCR and ELISAs. Data are representative of 2 independent studies (n=5).

RESULTS: Accumulation of activated ($CD69^+$, Granzyme B^+ and IFN-γ^+) NK cells in the BAL and lungs was much greater in RV infected mice with allergic airways, and this was associated with significantly greater expression of IL-15 compared to control mice. A small number of NK cells were observed to express IL-4 in the lungs and BAL at day 1 and 2 post infection respectively however, a much greater number expressed IFN-γ. Numbers of BAL IL-4^+ $CD4^+$ T cells were significantly enhanced in RV infected allergic mice at days 4 and 7 post infection, and numbers of lung and BAL IFN-γ^+ $CD8^+$ T cells were similarly enhanced at days 2-7 and 1-2 respectively compared to UV-RV-1B dosed allergic controls.

CONCLUSIONS: RV infection enhanced both Th1 and Th2 lymphocyte responses in mice with allergic airways inflammation. The presence of IL-4 expressing NK cells may contribute to the increased allergic inflammation observed.

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478 Aspergillus Fumigatus May Promote Th2 Activation By Suppression Of Interferon Signaling

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RATIONALE: *Aspergillus fumigatus*(AF) infection and sensitization are common and promote respiratory diseases. Innate immune responses of bronchial epitheliums are known to play a key role in determination of T cell responses upon encounters with pathogens. To elucidate the impact of AF on human bronchial epitheliums, we hypothesized that AF can modulate the response of epithelium to favor a Th2 response.

METHODS: A bronchial epithelial cell line (BEAS-2B) was stimulated with IFN-beta, poly I:C, IL-4, IL-17A and AF extract for 6 hours. Due to protease activity of the extract, we also examined the cell viability. Cells were collected to measure mRNA (RT-PCR) and supernatants were collected to measure protein (ELISA). Extract was fractionated by filtration based on molecular weight and heat treated for initial characterization of active compounds within the extract. Western blotting was performed to evaluate suppression of IFN-beta activated STAT-1 by AF.

RESULTS: AF extract profoundly suppressed IFN-beta and poly I:C induced CXCL10 and BAFF in a dose dependent manner (69.2% reduction with the most concentrated extract, $p<0.01$). In contrast, heat treated extract did not suppress the chemokine induction. High molecular weight extract (HMW>50kDa) retained the suppressive effect, but low molecular weight (LMW<50kDa) did not. The LMW extract treated cells had reduced viability at 48 hours (71.3% reduction, $p<0.01$) but the HMW extract had a modest effect (9.5% reduction) on viability. Importantly, IFN-beta induced phosphorylation of STAT-1 was inhibited by AF.

CONCLUSIONS: Exposure of bronchial epithelial cells to AF extracts may suppress IFN-signaling and thus skew epithelial cells to promote Th2-related allergic diseases such as CRS and asthma.

479 Heterogeneity Of Specific CD4+ T Cell Responses To Peanut Allergic Components: Prospects For Specific Immunotherapy

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RATIONALE: Peanut allergy is one of the most serious hypersensitivity reactions to foods in terms of persistence and severity. Conventional Specific Immunotherapy using crude peanut extract is not a recommended treatment. There is therefore an urgent need to develop a safe, disease-modifying treatment for individuals with peanut allergy. This requires details characterization of immune response to allergic components of peanut to develop suitable immunotherapeutic strategies.

METHODS: We combined a CD154-based assay and a single-cell transcriptomes analysis to assess ex vivo and at a single cell level the specific CD4+ T cell responses to each peanut allergic components (Ara h) in adults with or without peanut allergy.

RESULTS: Pathogenic responses (Th2 response) were specifically associated with short life terminally differentiated allergen-specific CD4+ T cells, which dominate in allergic subjects but are absent in non-allergic subjects. Protective responses in non-atopic individuals were associated with peanut-specific TH1/TH17 cell responses. Within the peanut allergic group, we observed inter-individual variations of the specific immune response to each peanut allergic component. No direct linkage between CD4+ T cell response and IgE responses against each individual peanut allergic component.

CONCLUSIONS: Ability to identify immunogenicity and type of response elicited by each peanut allergic component appears to be critical to future success in vaccine development against peanut allergy. Understanding the type of cellular response and the role of genetic restriction may allow to target immune response to critical peanut allergen and to uncover the optimal type of cellular immune response necessary for protection.

480 Vitamin D Supplementation Reduces Th17 Cells In The Lung and Spleen Of CRA-Sensitized and Challenged Mice

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RATIONALE: Asthma is a chronic inflammatory airway disease characterized by airway inflammation and airway hyperresponsiveness. Increased infiltration of CD4+ lymphocytes, especially Th17 subsets, in asthmatic lungs suggests their critical role in the pathophysiology of allergic asthma. Vitamin D is a potent immunoregulator modulating functional response of immune cells. There is growing evidence that vitamin D is inversely related to Th17 cell development, differentiation, proliferation and survival.

METHODS: Female Balb/c mice were fed with Vitamin D-deficient (0 IU/kg), -sufficient (2,000 IU/kg) or -supplemented (10,000 IU/kg) diet, respectively and sensitized (i.p.) and challenged (aerosolized) with cockroach antigen (CRA). The mRNA transcripts and protein expression of IL-21R, IL-23R and RORγt on purified $CD4^+CD25^-$ lymphocytes isolated from lung and spleen of the mice were compared using qPCR, $IL\text{-}21R^+$ and $IL\text{-}23R^+$ cells using flow cytometry and immunofluorescence analysis for RORγt. The IL-17 concentration in the bronchoalveolar lavage fluid (BALF) and serum was measured by ELISA.

RESULTS: Vitamin D-deficiency significantly increased the density of $CD4^+CD25^-IL\text{-}21R^+$, $CD4^+CD25^-IL\text{-}23R^+$ and $CD4^+CD25^-ROR\gamma t^+$ in the lung and spleen of mice sensitized and challenged with CRA. This was reversed by vitamin D-supplementation in a dose-dependent manner. The IL-17 levels in the serum and BALF correlated well with the infiltration of Th17 cells in the lung.

CONCLUSIONS: The decrease in $IL\text{-}21R^+$, $IL\text{-}23R^+$, $ROR\gamma t^+$ $CD4^+CD25^-$ lymphocytes and IL-17 levels by vitamin D-supplementation suggests the beneficial effect of vitamin D by inhibiting the differentiation and activation of inflammatory Th17 cells in allergic airway inflammation.

481 Distinct Patterns and Magnitude Of T Cell Responses Are Associated With Seasonal Exposure To Timothy Grass Allergens

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RATIONALE: Timothy grass (TG) pollen is a common seasonal airborne allergen associated with symptoms ranging from mild rhinitis to severe asthma. Our overall goal is to characterize TG-specific T-cell responses as a function of disease severity and also as a function of seasonality. We are further interested in assessing the quantitative differences, in terms of magnitude of the response, but also qualitative differences, in the type of T-cell subsets (TH) involved.

METHODS: PBMCs samples obtained either during the pollen season or out-of-season, from allergic and non-allergic controls were stimulated either with TG extract or a pool of the 20 previously identified antigenic regions. The production of lymphokines associated with different TH subsets was evaluated.

RESULTS: As expected, in PBMC samples obtained in-season, the cytokine production was higher in allergic donors as compared to out-of-season, for IFNg, IL-5, IL-10 and IL-17. Memory cells gave a more robust lymphokine response in-season in allergics. In the case of non-allergic individuals, we observed much lower responses in terms of the Th2 associated cytokine IL5, and expected a robust production of Th1-associated IFNg. Strikingly the Th1 responses in normal individuals were *decreased* in-season when compared to out-of-season. The potential basis of this phenomenon is currently being investigated. It is possible that this phenomenon might reflect differences in tissue homing or in-season allergen stimulation resulting in activation of regulatory cells/mechanisms in non-allergic individuals.

CONCLUSIONS: Our data suggest that the magnitude and functionality of TH responses differ substantially for in-season versus out-of-season in allergic and non-allergic individuals.

482 IgE Production In B Cells Through Up-Regulating CD40L Expression and Mediator Release Via CD1d Expressed In Surface Of Mast Cells Related To Allergic Asthma In Mice

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RATIONALE: Mast cells play important roles via FcεRI-mediated activation in allergic asthma. A nonpolymorphic MHC I-like molecule CD1d, which is mainly expressed in APCs, presents glycolipid Ag to NKT cells and modulates allergic responses. This study aimed to investigate the role of CD1d on IgE production and mast cell activation related to allergic asthma.

METHODS: Bone marrow-derived mast cells (BMMCs) derived from C57BL/6 Wild type (WT) or KO ($CD1d^{-/-}$) mice were activated with Ag/Ab (refer to WT-act-BMMCs and KO-act-BMMCs, respectively) or α-Galactosylceramide (WT-gal-BMMCs, KO-gal-BMMCs) or both (WT-both-BMMCs, KO-both-BMMCs).

RESULTS: KO-act-BMMCs and KO-both-BMMCs reduced intracellular Ca^{2+} levels, expression of signaling molecules (Ras, Rac1/2, PKCs, MAPKs, PLA_2, COX-2, NF-kB/AP-1), release of mediators (histamines, leukotrienes and cytokines/chemokines), and total IgE levels versus the corresponding WT-BMMCs, respectively. KO mice reduced OVA-specific serum IgE levels, numbers of mast cells, recruiting molecules (CCR2/CCL2, VCAM-1, PECAM-1), expression of tryptase, c-kit, CD40L and cytokine mRNA, co-localization of tryptase and CD1d or NKT cells in BAL cells or lung tissues, and PCA responses, compared with the corresponding WT mice. BMMCs-transferred KO mice showed the restoration of all responses compared to those in KO mice. KO-αGal-BMMCs or KO-αGal mice did not show any responses.

CONCLUSIONS: Our data suggest that CD1d expressed in the mast cell surface may exacerbate airway inflammation and remodeling through up-regulating IgE production and mediator release in mast cells activated in OVA-challenged mice.

483 FOXP3 Epigenetic Signature To Distinguish Between Thymic- and Peripherally-Derived Regulatory T Cells During In Vivo Induction Of Immune Tolerance

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RATIONALE: Previous mechanistic studies have documented an increase in FOXP3+CD25+ cells in response to oral immunotherapy (OIT) and with natural resolution of allergy, suggesting a role for "T regulatory cells" (Treg) in the induction of tolerance. However, there are currently no approach that can determine whether these allergen-specific FOXP3+ cells reflect activated T effector cells or are true Tregs, and if so whether they originate from the thymus or are peripherally induced (tTreg vs pTreg). Our aim is to set up and validate an assay that could allow identifying and differentiating the tTreg and pTreg populations in clinical samples.

METHODS: Naïve T cells and tTregs were sorted from whole blood of healthy subjects and FOXP3high pTregs were generated in vitro from naïve T cells. All three populations were then analysed for methylation of main CpG-rich sites in the FOXP3 gene.

RESULTS: Preliminary results showed different methylation patterns for each Treg subsets and activated T effector cells. While tTreg were consistently demethylated at conserved non-coding sequence (CNS) 2, pTreg exhibited a demethylated pattern within the promoter region that differed from $FOXP3^{low}$ activated T cells.

CONCLUSIONS: Our data suggests a difference between the epigenetic control of the FOXP3 master regulator in FOXP3+ activated T cells, tTregs and pTregs in humans. This methylation pattern could be used to possibly determine the proportion of each T cell subpopulation in subjects undergoing OIT.

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484 CD4+ and CD8+ T Cells Of Allergic Humans Express Increased Phosphorylated p38 MAP Kinase (p38MAPK), Substance P Suppresses T Cell Expression Of p38MAPK and Memory IgE Responses

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RATIONALE: Magnetic/electrical stimulation of IgE+ human/rat left TPO cortex increases serum/blood substance P (2 hr) and CD4+/CD8+ T cell numbers (4 hr), while suppressing IgE responses (4 days). Thymus supplied T cells to blood because thymectomy or cutting of spinal cord at T2 (thymic innervation level) abrogated their appearance. Substance P suppresses specific murine IgE responses in vivo/vitro, and a relationship between p38MAPK and IL-4 has been reported. The effect of substance P on p38MAPK expression by human lymphocyte subsets and memory IgE responses has not been studied.

METHODS: Distributions of p38MAPK+ lymphocytes (CD4+,CD8+,CD19+,CD16+) in PBMC obtained from ragweed sensitized IgE+ humans were determined ± 15-30 min incubation with PMA ± substance P (flow cytometry). In addition, PBMC were cultured for 0-12 days ± ragweed antigen ± substance P and IgE levels in supernatants determined (ELISA).

RESULTS: Before PMA stimulation, virtually no p38MAPK+ T cells (CD4+, CD8+), B or NK cells were detected in PBMC (<5%). After PMA, virtually all T cells, and CD4+ T cells which had been purified from PBMC, were p38MAPK+ (90-98%), with no change in p38MAPK+ B or NK cells. When PBMC or purified CD4+ T cells were incubated with PMA + substance P, p38MAPK+ CD4+ and CD8+ T cell numbers were suppressed (50-75%) When PBMC were cultured with ragweed antigen, peak IgE responses were induced on day 8; inclusion of substance P in culture prevented induction of these responses on all days.

CONCLUSIONS: Taken together, our results indicate that brain can suppress IgE responses by releasing substance P.

485 Novel Mechanisms Of Immune Modulation By Alpha-1-Antitrypsin

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RATIONALE: Alpha-1-antitrypsin (AAT) is one of the most abundant circulating serine protease inhibitors. Deficiency results in chronic lung disease, suggesting AAT is critical for the maintenance of respiratory health. The purpose of this study was to investigate novel mechanisms of regulation by AAT.

METHODS: PBMCs and cell sub-sets from healthy adult peripheral blood were cultured for 48hours ± 4 different AAT preparations. Soluble mediators present in AAT and culture supernatants were quantified by cytometric bead array. AAT serine protease activity was assessed using a chymotrypsin assay and characterised by polyacrylamide gel electrophoresis, microscale thermophoresis and surface plasmon resonance.

RESULTS: All AAT preparations induce IL-10 production by PBMCs, including those without serine protease activity. The analyses of isolated cell subsets indicate CD14+ and CD4+ cells are the major responders. The complement component C3a has been shown to induce IL-10 in CD4+ T cells in certain circumstances and all human plasma purified AAT preparations were found to contain C3a, but no C5a. The lack of a defined receptor for AAT to mediate this IL-10 response resulted in the investigation of C3a as a potential intermediate. Binding studies confirmed the physical interaction between the two proteins. The addition of C3a to CD4+ cells treated with C3a-depleted AAT enhanced IL-10 production and anti-C3a neutralising antibody was able to partially inhibit IL-10 production.

CONCLUSIONS: These findings suggest an interaction between AAT and C3a, independent of serine protease activity, could be a potential mechanism by which AAT induces IL-10 and promotes a tolerogenic environment for the maintenance of respiratory health.

486 Prevalence Of Allergic Diseases and/Or Allergic Sensitization In Children and Adolescents With Type 1 Diabetes Mellitus

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RATIONALE: The prevalence of atopic diseases in children with type 1 diabetes mellitus (DM1) has been reported as lower. The aim of this study was to evaluate the prevalence of allergic diseases and allergic sensitization in Brazilian children and adolescents with DM1.

METHODS: 96 DM1 patients (aged 4-18 years, 45 boys) followed for at least one year, were evaluated for allergic disease through a detailed allergologic anamnesis and skin prick tests (SPT) to inhalant allergens (*D.pteronyssinus, D.farinae, B.tropicalis, B.germanica, P.americana*, dog epithelium, cat epithelium, mix fungi), foods (cow's milk, egg-white, yolk, soy, wheat, corn), positive (histamine 1mg/mL) and negative (saline) controls. Wheal with a mean diameter of induration equal or greater than 3mm identified a positive SPT.

RESULTS: The prevalence of rhinitis, asthma and atopic eczema (isolated or associated) were 68.0%, 59.1% and 44.4%, respectively. 20.6% of the patients had no allergic disease. Half of the patients were diagnosed with DM1 for at least four years and there was no relationship between the period of DM1 and the presence of allergic disease, as well as of the gender. 48.0% patients were sensitized with predominance of *D.pteronyssinus, B.topicalis* and *D.farinae*. The frequency of positive SPT was significantly higher among patients with a history of allergic disease (OR=6.98, 95% CI:2.60-18.74, p<0.001).

CONCLUSIONS: The prevalence of allergic diseases and sensitization in patients with DM1 was higher than usually expected and deserves further investigation to identify possible causes for these findings and to evaluate their importance and influence on the metabolic control.

487 Microcytosis: A Risk Factor For Asthma and Pulmonary Inflammation?

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RATIONALE: Limited evidence suggests an association between iron deficiency and allergic inflammation. We investigated whether a hematologic marker of iron deficiency, low mean corpuscular volume (MCV), is associated with asthma/pulmonary inflammation.

METHODS: We analyzed data from 33,709 NHANES participants from 2003-2010. MCV quintiles were created ((Q1) 51.1-82.8fL; (Q2) 82.9-86.4fL; (Q3) 86.5-89.3fL; (Q4) 89.4-92.3fL; (Q5) 92.4-125.3fL). Final models accounted for survey design and were adjusted for age, race, and income, and excluded subjects with evidence of inflammation (CRP>6 mg/L or white count>10.0 x 10^9/L).

RESULTS: Mean MCV was 87.4fL (SD: 6.2), 8.8% reported current asthma, and median eNO was 13ppb (IQR: 8-21ppb). Every 10fL decrease in MCV was associated with a 16% increased odds of self-reported asthma diagnosis (OR [95% CI]: 1.16 [1.03-1.30], p=0.02), and a 1.15ppb increase in eNO (95% CI: 1.11, 1.20, p<0.001). MCV was not associated with wheeze or nocturnal cough. Asthma prevalence increased from highest to lowest MCV quintile (7.4, 8.3, 8.4, 8.9, and 10.7%, respectively, p<0.001), as did prevalence of elevated eNO (>25ppb [adults], >20ppb [children]) (15.8, 18.2, 17.7, 20.4, 19.7% respectively, p<0.001). Relationships with asthma and eNO persisted after adjustment for hemoglobin, serum lead, smoking, folate and B12 levels, and exclusion of those with RBC count >5 million, a surrogate for thalassemia.

CONCLUSIONS: Lower MCV is associated with current asthma/pulmonary inflammation in a sample representative of the US population, even after accounting for multiple other causes of lower MCV, suggesting that iron insufficiency or undetected hemoglobinopathies may be a risk factor for asthma/pulmonary inflammation.

488 Identification and Cloning Of Active CLC3 Promoter

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RATIONALE: We have earlier reported the involvement of CLC3 in TGF-β or eotaxin-induced migration and activation eosinophil. Here, we identified CLC3 promoter and the regulatory elements present on CLC3 gene to identify targets in manipulating the gene response in allergic asthma.

METHODS: Bioinformatics tools were used to identify promoter region and transcriptional sites present on CLC3 gene. Using UCSC genome browser, the promoter region of CLC3 was identified and confirmed with EPD Bioinformatics software. The TSSG, TSSW and EpiTect (SABiosciences) softwares were used to identify transcription-factor binding sites, and translational frame was checked by Expasy software. Plasmid vectors (pGL4.17-promoterless luc2 and pGL4.73-SV40/hRluc) were used for dual luciferase assay. The vectors were co-transfected to HEK293 cells with FuGENE-HD and incubated with TGF-β and eotaxin to monitor CLC3 promoter activity.

RESULTS: Promoter region of CLC3 was identified and successfully cloned from human genomic DNA into a promoterless pGL4.17 vector. Several transcription factor-binding sites, including AP-1, were identified on CLC3 promoter. Dual-luciferase assay on co-transfected HEK293 cells showed CLC3 promoter activity that increased on treatment with TGF-β or eotaxin.

CONCLUSIONS: Successful cloning of genome-amplified CLC3 promoter containing AP-1 binding site provides opportunity to manipulate the promoter sequence to check the active sites, which may be therapeutic in controlling the migration and activation of eosinophils in asthma. Luciferase activity confirms the role of TGF-β and eotaxin in controlling the expression profile of CLC3 on eosinophils in allergic asthmatic individuals at different stages of asthma.

489 Airway Epithelial Cells Exposed To Alternaria Release IL-18 Independent Of NALP3/Caspase-1 Pathway By Inducing Autophagy and Nf-Kb Activation

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RATIONALE: Airway epithelial cells are the first line defense against inhaled allergens like Alternaria. We have previously reported that stimulation of airway epithelial cells with Alternaria extract rapidly releases IL-18. Here we report that *Alternaria* extract activates Autophagy and NF-kB in airway epithelial cells.

METHODS: Caspase-1 activation in airway epithelial cells stimulated with *Alternaria* extract was measured with a specific fluorescent probe. The biological role of caspase-1 was elucidated by stimulating epithelial cells with *Alternaria* extract together with a specific Caspase-1 inhibitor, Z-YVAD-FMK, and quantifying IL-18 in cell supernatants. Autophagy in airway epithelial cells stimulated with *Alternaria* extract was measured by live-cell microscopy using Cyto-ID Autophagy detection kit. Degradation of IkB-a, the cytoplasmic inhibitor of NFkB activation, was measured in whole cell lysate by Western blotting followed by densitometry of bands.

RESULTS: Stimulation of airway epithelia cells with Alternaria extract failed to activate Caspase-1. Furthermore, Caspase-1 inhibitor failed to reduce the release Alternaria extract-induced IL-18. These results suggested that Alternaria extract activates a new pathway that releases IL-18. We discovered that Alternaria extract induces autophagosome formation 15 mins. Furthermore, Alternaria extract rapidly decreased the expression levels of IkB-a.

CONCLUSIONS: Exposure of airway epithelial cells with Alternaria induces IL-18 release independent of NALP3-Caspase-1 pathway, but dependent on autophagy and activation of NF-kB.

490 Generation Of Human Hybridomas Secreting Naturally-Occurring IgE MAbs Using Memory B-Cells From Atopic and Asthmatic Patients

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RATIONALE: Allergen cross-linking of IgE bound to cognate high affinity receptors on mast cells and basophils unleashes a cascade of mediators that result in the wide array of allergic disease. Despite its central role, very little is known about the human IgE molecule. Most of our knowledge of the human antibody response to allergens has come from studies using polyclonal sera.

METHODS: Until very recently, the generation of naturally occurring human mAbs to a specific immunogen has been next to impossible. Here we employ a human B cell hybridoma method, immortalizing activated EBV transformed memory B cells through electrical cytofusion with a non-secreting myeloma partner, to generate naturally occurring full-length human IgE mAbs.

RESULTS: The frequencies of IgE producing memory B-cells in the circulation of atopic and asthmatic patients were very low, averaging one per ten thousand. Despite this, we have generated a panel of 8 human IgE producing hybridomas. Due to the paucity of available methods and reagents, purification and initial characterization has been difficult. Unexpectedly, many commercially available anti-human IgE antibodies bind differentially to our panel of mAbs.

CONCLUSIONS: We have generated the first panel of human hybridomas secreting naturally-occurring IgE mAbs. The variability in commercial anti-human IgE antibody binding could have significant clinical implications, as measurements of polyclonal total and specific IgE may be inaccurate. The goal of our work is to improve upon our molecular understanding of the human IgE antibody response, which will provide insights needed for the design of better immunotherapies and allergy vaccines.

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491 Anti-Viral Innate Immunity Varies Across Different Asthma Inflammatory Phenotypes

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RATIONALE: People with asthma are prone to virus-induced exacerbations and this has been linked to immune dysfunction. Though phenotypic heterogeneity is increasingly recognised in asthma, it is not clear whether dysregulated anti-viral immunity is present in all asthmatics, or only a subset. This study examined the hypothesis that immune dysfunction in asthma varies across different inflammatory phenotypes.

METHODS: Innate immune responses to human rhinoviruses (HRVs) were examined in 85 adult asthmatics (64% female) with an average age of 59 years. All had poorly controlled asthma despite combination therapy with inhaled steroids + long acting β agonists. Inflammatory phenotypes were defined via induced sputum. Blood mononuclear cells were cultured with HRVs for 24h; cytokines were measured by ELISA.

RESULTS: Asthma Control Questionnaire (ACQ) scores averaged 1.7 ± 0.8, confirming poor asthma control. HRV-stimulated IFNα synthesis at 24h was significantly lower in those with neutrophilic asthma (median 45 pg/ml; IQR 23-205), than in those with either eosinophilic asthma (median 582 pg/ml; IQR 163-1114; $p<0.01$) or paucigranulocytic asthma (499 pg/ml; IQR 222-1003; $p<0.01$). HRV-stimulated IL-1β, IL-6 and IL-8 synthesis did not vary across asthma phenotypes. Basal (unstimulated) release of IL-1β and IL-8 was positively correlated with ACQ scores across all asthma phenotypes.

CONCLUSIONS: In asthma the degree of anti-viral innate immune dysfunction varies across different patterns of airway inflammation. The extent to which this predicts subsequent risk of viral infections and asthma exacerbations remains to be determined.

492 Effect Of siRNA Inhibition Of Sialyltransferases and Fucosyltransferases On Siglec-F Ligand Expression By Epithelial Cells In Vitro

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RATIONALE: Siglec-F is highly expressed on mouse eosinophils and binds to Siglec-F ligands expressed by a variety of cells including epithelial cells. In the present study, we investigated the enzymes regulating Siglec-F ligand expression by the murine lung epithelial cell line (MLE-12) in vitro.

METHODS: MLE-12 cells were transfected with SiRNA or controls targeting either sialyltransferases (ST3Gal-III, ST3Gal-IV, or ST3Gal-VI) or fucosyltransferases (Fuc-TIV, Fuc-TVII). Levels of expression of the Siglec-F ligand was quantitated using a Siglec-F-Fc fusion protein and flow cytometry.

RESULTS: Siglec-F ligands were highly expressed constitutively by MLE-12 cells. Transfection with SiRNAs targeting either sialyltransferases or fucosyltransferases did not alter the constitutive levels of existing Siglec-F ligand expression. However, after treatment with sialidase, Siglec-F ligand expression on MLE-12 cells was completely eliminated. After treatment with sialidase SiRNA transfection targeting either sialyltransferases or fucosyltransferases showed different recovery patterns of Siglec-F ligand on MLE-12 cells. Recovery of Siglec-F ligand expression by MLE-12 was delayed with ST3Gal-III, ST3Gal-IV Fuc-TIV, and Fuc-TVII SiRNA transfection but not with ST3Gal-VI SiRNA. Although each SiRNA showed decreased expression of Siglec-F ligand, the expression of Siglec-F ligand on MLE-12 cells showed full recovery within 48 hours.

CONCLUSIONS: ST3Gal-III, ST3Gal-IV, Fuc-TIV, and Fuc-TVII play important roles in the recovery of Siglec-F ligand expression in MLE-12 epithelials pre-treated with sialidase. However, constitutive Siglec-F ligand expression is not reduced by targeting these sialyltransferases or fucosyltransferases for 48 hours in vitro.

493 Allergic Airway Inflammation Can Be Regulated By Semaphorin 4C Through Controlling B-Cell Migration

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RATIONALE: Semaphorins are a group of axonal guidance protein, yet increased evidences have demonstrated their involvement in immune regulation. Our laboratories found that one semaphorin member, Sema-4C, increased markedly on B-cells following exposure to Th2 cytokines. Since allergic airway disease (AAD) is a Th2 mediated condition, we addressed Sema-4C's role in allergic airway inflammation.

METHODS: C57/B6 wild type (WT) and Sema-4C knock out (KO) AAD was induced by i.p. sensitization with OVA on day1&14 and challenge on day28-30, following sacrifice on day 31. Airway hyper-responsiveness to methacholine was assessed by flexiVent. Lung tissue was either digested or fixed for histological analyses. BAL was collected to determine inflammatory cells. Boyden chamber was used to compare the motility between WT and KO B-cells. Immuno-fluorescent staining was performed to localize Sema-4C expression.

RESULTS: Sema-4C KO mice challenged with OVA had increase AHR, bronchospasm. KO mice also had more B-cells and plasma cells infiltrate in the lungs. KO B-cells exhibited increased motility to CXCL12 and CXCL13. Sema-4C colocalized with F-actin in stimulated WT B-cells forming distinct immune synapses, while KO B-cells showed impaired synapse formation.

CONCLUSIONS: Sema-4C may regulate B-cells and plasma cells in AAD through controlling their cytoskeleton and migration, therefore may help them response properly to allergen in the lung.

494 Mouse Bone Marrow Derived Mesenchymal Stem Cells Supress Airway Inflammation In Both Chronic and Acute Murine Asthma Model

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RATIONALE: The aim of the present study was to investigate the efficacy of mouse compact bone (mCB) derived MSCs on lung histopathology and lymphocyte proliferation.

METHODS: mCB-MCS were isolated from BALB/c mice, characterized and marked by GFP. To generate murine models of chronic and acute asthma, mice were i.p. sensitized with OVA and exposed to aerolized OVA. mCB-MSCs ($2.5x10^5$ cells) were administrated i.v. after last nebulization. Mice were sacrified, and splenocytes and lung lymphocytes were isolated and marked with CFSE. Cells stimulated with OVA (40mg/ml) were cultured under suitable conditions for 5 days. Flow cytometric analysis and histopathological examination of lungs were evaluated. In histopathological analysis, the measurements were performed from minimum 5 points of each airway and mean values were calculated. Goblet cells stained with PAS enumarated in 1500 cells.

RESULTS: In sections stained with H&E, the distal [without MCS chronic:29,9mm acute:32,03mm; with MSC chronic:13,3mm acute:12,25mm] and proximal [without MCS chronic:42,6mm acute:28,97mm; with MSC chronic:17,4mm acute:18,9mm] airway epithelial thicknesses were observed to decrease in both mouse models. Likewise, in sections stained with PAS, a significant reduction in number of hyperplasic goblet cells in the proximal [without MSC chronic:140 acute:1200; with MSC chronic:4 acute:211] and distal [without MSC chronic:55 acute:118; with MSC chronic:0 acute:0] airways was observed. Morever, in the CFSE staining experiment, CB-MSCs inhibited lymphocyte proliferation in both asthma model.

CONCLUSIONS: The results reported here provide that mCB-MSCs may provide powerful alternative therapeutic for the treatment of chronic and acute asthma. This study was supported by TUBITAK-SBAG (110S368).

495 Profiling Eicosanoids In Breath Condensates Of Asthmatic and Healthy Children

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RATIONALE: Eicosanoids are lipid mediators implicated in the regulation of allergic inflammation responses and have been considered as potential biomarkers for asthmatic children. The objective of this study was to investigate profiles of 10 selected eicosanoid metabolites in exhaled breath condensates (EBCs) of children with asthma in comparison to those of healthy children.

METHODS: EBCs were from 175 children (aged 9±2.3 years) with stable atopic asthma (58 using inhaled steroids) and 125 healthy controls (10.8±1.2 years). Either high performance liquid chromatography coupled with tandem mass spectrometry (LC/MS) or enzymatic immunoassays (EIA) were used to measure 10 different metabolites. In addition, exhaled nitric oxide levels (FeNO) and bronchial hyperresponsiveness were assessed through a methacholine challenge test (PC_{20}) in all subjects.

RESULTS: Among the ten eicosanoids, the levels of LTB_4 (5.71 pg/ml vs 0.44 pg/ml; P<0.001), LTE_4 (9.13 pg/ml vs 5.38 pg/ml; P<0.001), and PGE_2 (13.29 pg/ml vs 6.77pg/ml; P<0.018) were significantly higher in asthmatics than in healthy children, while 11-dehydro TXB_2 was significantly less abundant (3.55 pg/ml vs 1.0 pg/ml; P=0.045) in asthmatics. The levels of eicosanoids demonstrated no appreciable relationship to asthma severity. From the fasting lipid profiles, we found a slightly higher level of cholesterol, with all other elements being within the normal range. These parameters discriminated asthmatics from healthy children better than FEV_1, FeNO or PC_{20}.

CONCLUSIONS: In the current study, a composite of LTB_4, LTE_4 and PGE_2 levels in EBCs was distinguishable between asthmatic and healthy subjects, suggesting the potential utility of assessing EBC's eicosanoids as inflammatory markers for childhood asthma.

496 Increased IP-10 Expressions In Nasal Fibroblasts From Patients With Refractory Chronic Rhinosinusitis and Asthma

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RATIONALE: Chronic rhinosinusitis (CRS) is characterized by local inflammation of the sinonasal tissues. CRS patients with nasal polyps and asthma often develop acute exacerbation of sinonasal symptoms after upper respiratory tract infections. However, the influence of concomitant asthma on the nasal immune response to viral infection remains unclear.

METHODS: Specimens of nasal polyp and mucosal tissues were obtained from 3 groups of CRS patients (n=14 per group): 1) patients without asthma (CRS group), 2) patients with aspirin-tolerant asthma (ATA group), and 3) patients with aspirin-intolerant asthma (AIA group). Nasal fibroblasts isolated from the specimens were stimulated with poly I:C. IP-10 and I-TAC expressions were analyzed by the quantitative real-time polymerase chain reaction and enzyme-linked immunoadsorbent assay.

RESULTS: Nasal fibroblasts from the ATA and AIA groups showed significantly enhanced expression of IP-10 mRNA and protein after poly I:C stimulation compared with cells from the CRS group and the control group (normal nasal mucosa). However, the expression of I-TAC protein was not detected.

CONCLUSIONS: Our findings suggest that CRS associated with asthma may become intractable through the over-production of IP-10 in response to viral infection.

497 Vitamin D Regulating TGF-β Induced Epithelial-Mesenchymal Transition

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RATIONALE: Subepithelial fibrosis is a hallmark characteristic of airway remodeling in asthma. An important regulator of fibrosis is transforming growth factor β (TGF-β). TGF-β can induce airway remodeling in epithelial cells through induction of epithelial-mesenchymal transition (EMT). This represents a novel therapeutic target in asthma. Vitamin D has immunomodulatory functions that could inhibit subepithelial fibrosis in asthma.

METHODS: Human bronchial epithelial cells (BEAS-2B) were stimulated with the active form of Vitamin D, calcitriol (100 nM). After 24 hours, TGF-β1 (10 ng/ml) or TGF-β2 (10 ng/ml) was added to the cells for an additional 48 hours. Following stimulation, mRNA and protein was isolated and mRNA transcripts for E-cadherin, Snail, MMP2, MMP9 were analyzed by qPCR while E-cadherin and Snail were examined by Western blot. An invasion assay and scratch wound assay were performed to identify the migratory properties of the cells following stimulation.

RESULTS: TGF-β1 and TGF-β2 decreased E-cadherin expression and increased the expression of Snail, MMP2, and MMP9 mRNA transcript levels. TGF-β also increased cell invasion. The effect of TGF-β on these markers and motility was impeded by the presence of calcitriol as ascertained at the mRNA, protein levels, and invasion assays.

CONCLUSIONS: These data suggest that both TGF-β1 and TGF-β2 can regulate EMT expression markers, which can be impeded by stimulation with calcitriol in human airway epithelial cells. Therefore, calcitriol could be a potential therapeutic agent in the prevention and management of subepithelial fibrosis.

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498 Macrophage-Derived Chemokine In Nasal Washes Is Associated With Asthma Exacerbations and Infections With Rhinovirus In Children

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RATIONALE: Macrophage-derived chemokine (MDC; CCL22) is produced by macrophages and dendritic cells in Th2-related diseases, and following stimulation with microbial products. Whether MDC protein expression is increased during asthma exacerbations in children, especially those infected with rhinovirus (RV), is not known.

METHODS: Subjects included 123 children (ages 7-12): 41 with wheezing, 26 with stable asthma, and 56 non-asthmatic controls. Twenty wheezing children tested positive for RV (RV^+) by RT-PCR. MDC was measured in nasal washes (NWs) by ELISA; detection limit = 62.5 pg/ml. The results were compared with Th2 associated biomarkers, eosinophil derived neurotoxin (EDN) in NW's by ELISA and total IgE in serum.

RESULTS: Geometric means for MDC in NW's from children with wheezing (RV^+), wheezing (RV^-), stable asthma, or controls were 150, 85, 92, and 66 pg/ml, respectively (wheeze RV+ vs RV^-, $p = 0.01$; vs stable asthma, $p= 0.029$; and vs controls, $p< 0.001$). MDC and EDN were positively correlated in NW's from RV^- wheezing children, stable asthmatics, and controls; $r_s = 0.48$, $p= 0.03$; 0.68, $p< 0.001$; and 0.35, $p= 0.009$, respectively. Surprisingly, the correlation among RV^+ wheezing children was not significant; $r_s= 0.19$, $p= 0.4$, suggesting a differential effect on MDC expression stimulated by RV. Correlations between MDC and total serum IgE were not significant for any group.

CONCLUSIONS: MDC concentrations were significantly elevated in nasal washes from children treated for wheezing who tested positive for RV. Measuring MDC may enhance our understanding of the pathogenesis of asthma exacerbations in children, especially those infected with RV.

499 Application Of Isoraft Single Cell Isolation For Analysis Of Pediatric Bal Macrophages

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RATIONALE: The Isoraft is a novel device for single cell separation and isolation. Unlike standard cell separation techniques it is ideal for samples that have limited cell yields. Pediatric BAL samples contain important inflammatory/immune cells but have relatively low total cell recoveries thereby limiting the scope of subsequent cell analyses. We tested the feasibility of Isoraft cell isolation on pediatric BAL macrophages.

METHODS: Pediatric BAL samples were obtained from infants and children undergoing clinically indicated bronchoscopy at the UNC Children's Hospital. BAL samples were resuspended in RPMI medium at a concentration of $1x10^6$ cells/ml. The Isoraft wells were pre-coated with an anchor antibody (HLA-DR) to enhance macrophage capture prior to the delivery of sample (1 ml) to the surface of the Isoraft. Following centrifugation (200g, 3 minutes) to enhance macrophage deposition onto the well surfaces and removal of excess sample, the cells on the Isoraft were fixed in paraformaldehyde (0.5%) and allowed to air dry at room temperature. Subsequent immunohistochemical (IHC) staining was performed on the Isoraft wells for INOS (M1) and CD301 (M2) phenotype characterization.

RESULTS: Light microscopy revealed successful adherence of single BAL macrophages onto the surfaces of individual raft wells with enhanced adherence occurring when using an anchor antibody versus not using one. Fluorescence and confocal microscopy revealed HLA-DR+, INOS+, HLA-DR+/INOS+ and HLA-DR+/ CD301+ macrophages.

CONCLUSIONS: Isoraft cell separation and IHC analysis can be used on pediatric BAL samples. Limited cell yield is no longer an obstacle for more detailed phenotype analysis of pediatric BAL samples including genetic assays.

500 7, 4'-Dihydroxyflavone Isolated From Glycyrrhiza Uralensis a Constituent Of ASHMI™ Prevents Dexamethasone Enhancement Of Eotaxin-1 Secretion By Human Lung Fibroblasts

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RATIONALE: Eotaxin-1 is chemotactic for eosinophils, basophils and Th2 cells, and plays a role in allergic inflammation. Dexamethasone (Dex), a potent glucocorticoid anti-inflammatory drug, has dual effects on cultured lung fibroblast eotaxin-1 production. Although Dex initially reduces eotaxin, this effect wanes and reverses so that by 72 hr eotaxin secretion is significantly increased which is associated with STAT-6 up-regulation. This finding is consonant with clinical studies showing that in severe asthma cases, eotaxin production persists during systemic corticosteroid use. Thus, corticosteroids may not be an ideal therapy for eotaxin-1-mediated inflammatory diseases. Effective eotaxin-1 inhibitors would be useful for treating asthma and other inflammatory conditions.

METHODS: We isolated and identified pure compounds from *Glycyrrhiza uralensis* (GU) using chromatographic fractionation and isolation methods and compared their effects on human lung fibroblast (HFL-1 cells) eotaxin-1 production to that of Dex. We also investigated the molecular mechanisms underlying these effects.

RESULTS: Of 9 phenolic compounds and glycyrrhizin isolated from GU, 7, 4'-dihydroxyflavone (7, 4'-DHF) was the most potent inhibitor of eotaxin-1 production (IC_{50}=1.4μM). Dex increased constitutive and IL-4 /TNF-α stimulated eotaxin-1 production after 72 hr culture. In contrast, 7, 4'-DHF inhibited constitutive and stimulated eotaxin-1 production at this time point in a non-toxic manner. Interestingly, the presence of 7, 4'-DHF (10μM) prevented Dex (10μM) enhancement of both constitutive and stimulated eotaxin-1 production. This result was associated with down-regulation of Dex-induced STAT6 and NFκB activity.

CONCLUSIONS: 7, 4'-DHF may prove to be useful in treating eosinophilic inflammatory diseases either alone, or in addition to corticosteroid therapy.

501 Allergen Sensitivities and Obstruction Indices Among Inner City Asthmatic Patients with High IgE (30-700 IU/ml) Vs Ultra-High IgE Levels (>700 IU/ml)

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RATIONALE: Omalizumab treatment has prompted clinicians to focus on asthmatics with serum IgE level of 30-700 IU/ml ("High IgE" cohort, HC) with perennial aeroallergen sensitivity. Current literature has poorly characterized differences in allergen sensitivity profiles or physiologic characteristics of HC vs. serum IgE levels >700 IU/ml ("Ultra-High" IgE cohort, UHC).

METHODS: In a retrospective review, 26 UHC patients (age >18) seen in our University asthma clinic were compared with 27 HC controls, matched for age, sex and race. ImmunoCAP, IgE assays, and spirometry were performed prior to therapeutic intervention. ABPA patients were excluded. Allergens were subclassified into 3 groups: seasonal aeroallergens, epidermal allergens, and molds. Univariate analysis of allergen specific trends and zip code analysis were performed between the two cohorts (Chi square).

RESULTS: Serum IgE within the UHC was 2,575 ±1966 IU/ml (x + SD), versus 213 ±205 IU/ml in the HC. In the UHC, 61.5% were sensitive to individual allergens within both seasonal and mold groups compared to 22.2% in HC (p=0.004); epidermal or seasonal groups were not different. In the UHC 73% were sensitized to at least one mold (except aspergillus) as compared to only 23% in the HC (p= 0.001). Zip code mapping and spirometry did not show significant allergen location prevalence or differences in FEV1%.

CONCLUSIONS: In the UHC, a greater percentage exhibit sensitization to members of all 3 groups of allergens. These results on an inner city asthma population may prompt clinicians to emphasize evaluation of particular allergen exposures in the UHC, and target specific allergen avoidance.

502 Alcohol Exposure and Airway Hyperresponsiveness

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RATIONALE: Alcohol has well-established consequences in the lung including few reports of alcohol-induced asthma. Alcohol can impair ciliary motility and susceptibility to infection, however there is paucity in the literature about the effects of alcohol on airway hyperresponsiveness (AHR). It has been demonstrated that alcohol administration significantly attenuates AHR in a mouse model, but the effects on human studies are sparse. We hypothesize that brief alcohol ingestion attenuates AHR in mild to moderate asthmatics.

METHODS: In this nonrandomized study, treatment naïve, mild to moderate asthmatics, ages 21-65, were subject to Breathalyzer analysis on the control day to confirm alcohol abstinence and on testing day to estimate blood alcohol level after ingestion of distilled alcohol (vodka with fruit juice). Methacholine challenges were conducted to confirm and quantify AHR on both days, using a one-half concentration difference in the $PC20FEV_1$ as a significant change in AHR. Smokers and history of chronic heavy drinking were excluded.

RESULTS: Ongoing preliminary results demonstrate a significant increase in AHR to methacholine following brief consumption of distilled alcohol. The change in AHR was seen at one-half concentration less compared to the control $PC20FEV_1$.

CONCLUSIONS: Although murine models demonstrated that alcohol administration significantly diminishes methacholine-induced AHR, this trend has yet to be observed when applied to humans with mild to moderate asthma. Albeit an ongoing study, preliminary results rather show an opposite trend augmenting AHR. Possible effects observed may be secondary to non-alcohol congeners.

503 Ragweed Or Dust Mite Antigen-Stimulated Human Primary Bronchial Epithelial Cells Differentially Express Cytokines In Response To Formoterol Or Mometasone Or Their Combination

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RATIONALE: It is unknown if different allergens can induce separate cytokines *in vitro*, and if suppression of cytokines may vary with formoterol *vs*. mometasone or their combination.

METHODS: Primary bronchial epithelial cells were cultured *in vitro* until confluence with either ragweed (500 mcg/ml) or dust mite antigens (1000 AU/ml) with or without the addition of formoterol (1.3 x 10-7 M) or mometasone (1 X 10-7 M) or their combination. Supernatants were harvested after 24 hrs and placed in a -80C freezer. Samples were shipped on dry ice to Assaygate (Ihamsville, MD) for ultrasensitive human ELISA cytokines assay.

RESULTS: The combination of formoterol and mometasone was superior to either drug alone in suppressing ragweed-induced IL-6 (P<0.05). This combination also suppressed IL-4 induced by either ragweed or dust mite antigens (P<0.05). The formoterol+mometasone combination also suppressed dust mite antigen-induced GM-CSF and IL-8 compared to either drug alone. (P<0.05) Ragweed-induced IL-8 did not decrease with any combination of these drugs.

CONCLUSIONS: The ability of combinations of formoterol and mometasone to suppress pro-inflammatory cytokines may depend on the inciting allergen used to stimulate bronchial epithelial cells *in vitro*.

504 Release Kinetics Of Soluble ST2 and Proinflammatory Cytokines In Allergic Rhinits

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RATIONALE: The important role of IL-4 and IL-13 in the inflammatory reaction of allergic rhinitis is well established. Increased serum levels of soluble ST2 (sST2) and IL-33 have been found in subjects with allergic rhinitis. The time course of Eotaxin-3 and sST2 release into nasal secretions after nasal allergen challenge has not been previously described.

METHODS: Nasal allergen challenge was performed in six volunteers with seasonal allergic rhinitis out of season. All subjects underwent control challenges. Nasal symptoms were quantified, nasal secretions and serum were collected for 24 hours after allergen challenge. The levels of IL-4, IL-13, IL-33, sST2 and Eotaxin-3 were measured by ELISA-assays or electrochemiluminescent assays. Nasal symptoms and cytokine levels were correlated.

RESULTS: We found a significant increase ($p < 0.05$) of IL-4, IL-13 at 5 h, of Eotaxin-3 at 2, 5, and 24 h, and of sST2 at all measured time points in nasal secretions after allergen challenge. The cytokine levels correlated significantly with nasal symptoms. IL-33 and serum levels showed no significant changes.

CONCLUSIONS: We confirm the release of IL-4 and IL-13 during the late phase after allergen challenge. Furthermore, we describe the time course of Eotaxin-3 and sST2 release during early and late phase into nasal secretions after nasal allergen challenge. We hypothesize that Eotaxin-3 and sST2 are involved in the modulation of the inflammatory reaction of allergic rhinitis.

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505 Interaction Between Dietary Antioxidants and Passive Smoking On The Risk Of Asthma Modified By GSTP1(rs1695) Polymorphism

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RATIONALE: Deficiencies of dietary antioxidants and exposure to smoking have been implicated in the etiology of lung and other cancers. This study investigated relationship between intake of antioxidant vitamin A, C and E, carotene, retinol and passive smoking on the risk of asthma.

METHODS: Children aged 6-13 years in Seoul were surveyed in 2008 and 1,129 children were analyzed. A Korean version of ISAAC questionnaire and food frequency questionnaire (FFQ) were completed by their parents. *GSTP*1(rs1695) polymorphism was genotyped by the TaqMan assay.

RESULTS: Passive smoking increased the risk of wheezing in the past 12 months (aOR, 2.48; 95% CI, 1.29-4.76) and asthma diagnosis (aOR, 1.91; 95% CI, 1.19-3.06). We found no relationship between antioxidants and wheezing in the past 12 months and asthma diagnosis. However, in combined analyses with two risk factors, there were interactions between passive smoking and lower intake of vitamin A on the risk of wheezing in the past 12 months (aOR, 4.43; 95% CI, 1.51-12.96; p for trend 0.0018) and asthma diagnosis (aOR, 2.23; 95% CI, 1.10-4.54; p for trend 0.0046). The relationship was more apparent with AA at *GSTP*1 rs1695 who had been exposed to two risk factors at increased risk of wheezing in the past 12 months (aOR=6.66, 95% CI=1.54-28.85) and asthma diagnosis (aOR=4.44, 95% CI=1.58-12.52) compared to those with AG or GG at this position and had not been exposed to two risk factors.

CONCLUSIONS: Asthma may be modified by Gene-Environment-Environment interaction between *GSTP*1 AA polymorphism and dietary antioxidant/passive smoking.

506 Longitudinal Trends Of Food and Environmental Allergen-Specific IgE In Asthmatic Inner-City Children < 4 Years Of Age

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RATIONALE: The most dynamic changes in allergen- specific IgE happen in early childhood. Yet, little is known about the longitudinal trends of allergen-specific IgE in atopic, inner-city children with asthma aged < 4 years.

METHODS: Preliminary data from children participating as controls in an asthma interventional trial was analyzed. Seventeen atopic inner-city children aged < 4 years with recurrent wheeze, parental history of asthma and/or eczema were included. Specific serum IgE to 7 common food and 8 common environmental allergens was obtained at baseline and then yearly after up to 3 years. We defined sensitization as IgE ≥0.35kU/l.

RESULTS: At baseline, 16 children (median age 33 months; IQR 26, 46 months) were sensitized to at least 1 food and 1 environmental allergen. Six children (35%) lost at least one food sensitization and 14 (82%) had a decrease of specific food IgE over time. In contrast, only 2 (12%) developed new food sensitizations and 10 (59%) had an increase of one or more food specific IgE. Regarding environmental allergens, 2 (12%) lost at least one of their environmental sensitizations and 12 (71%) had a decrease of environmental IgE. Three (18%) patients developed new sensitizations and 12 (71%) showed an increase in one or more environmental IgEs.

CONCLUSIONS: Inner-city children with asthma are unlikely to outgrow sensitizations to environmental allergens and commonly show increase in specific IgE to environmental allergens. Even though they are more likely to outgrow food allergies, a considerable number of children may retain or show increase in food sensitizations.

507 The Effect Of Human Placental Extract In a Mouse Model Of Allergic Rhinitis

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RATIONALE: Effect of placental extract to regulate biological responses and its potential as therapeutic reagents in various diseases have been implicated in numerous studies. In this study, we investigate preventive and therapeutic effects of placental extract in a mouse model of allergic rhinitis.

METHODS: BALB/c mice were divided into control, Derf, Pre-S and Pre-C groups. The allergen was *Dermatophagoides farinae* (Derf). HPE was administered before sensitization (Pre-S) or before challenge (Pre-C). Allergic symptom scores, eosinophil counts and serum Derf-specific IgE levels were measured. Interferone-γ, T-bet, GATA-3, IL-4 and Foxp3 mRNA expression in nasal mucosa were determined by real-time polymerase chain reaction. And Interferone-γ, T-bet, GATA-3, IL-4 were confirmed by western-blotting analysis. Flow cytometry of $CD4^+CD25^+Foxp3^+$ T cells in spleen were analyzed.

RESULTS: Symptom scores, serum Derf-specific IgE, GATA-3 mRNA levels, IL-4 mRNA levels and tissue eosinophil counts were decreased in both Pre-S and Pre-C groups (all, $p<0.05$). Quantitation of western blots showed that expression of GATA-3 and IL-4 were decreased in both Pre-S and Pre-C groups than Derf group. Also, percentage of $CD4^+CD25^+Foxp3^+$ T cells, Foxp3 mRNA levels were increased in Pre-S and Pre-C groups compared with those in Derf group (all, $p<0.05$).

CONCLUSIONS: Both prophylactic and therapeutic treatment with HPE significantly reduces allergic airway inflammation and has potential for induction regulatory T cells in a murine model of allergic rhinitis.

508 Induced Long-Lived Mucosal Mast Cells In The Airways Arise From Circulating Mast Cell Progenitors

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RATIONALE: We used NKT deficient mouse strains to explore the relationship between recruitment of mast cell (MC) progenitors (MCp) to the large airways and the generation and persistence of induced intra-epithelial MCs (IEMC) in the airways in a 7-day ovalbumin challenge model of airway inflammation.

METHODS: MCp recruitment was evaluated by a limiting dilution and clonal expansion assay (LDA) and by FACS analysis. IEMC accumulation in the large airways was analyzed by histochemistry using chloroacetate reactivity.

RESULTS: Compared to WT BALB/c mice, pan NKT-deficient $CD1d^{-/-}$ mice recruited only 43% as many lung MCps by LDA on day 1 ($p<0.05$) and induced only one third as many IEMCs on day 4 post challenges ($p<0.01$) by histology. In contrast, invariant NKT-deficient $J\alpha18^{-/-}$ mice recruited 2.2 fold more MCp by LDA ($0<0.01$) and generated a 2.6 fold increase in IEMCs ($p<0.001$). While the MCps peak 24h post challenge and return to a minimal baseline number by day 4, the IEMCs in the large airways peak around 21 days post challenge and persist for >77 days in WT mice. By FACS, the inducible MCs are characterized by higher expression of β7 integrins and variable side scatter.

CONCLUSIONS: Our findings suggest a direct relationship between MCp influx, IEMC generation and the persistence of IEMCs for weeks. By FACS, the induced MCs are distinguished by their level of expression of the β7 integrins.

509 Despite Inflammation, No Structural Upper Airway Remodelling In Severe Allergic Rhinitis

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RATIONALE: Increased in airway-smooth-muscles, extracellular-matrix, and vascularity are the main features of airway-remodelling in asthma but the extent of such remodelling in allergic rhinitics (AR) patients is unknown. We aimed to test the hypothesis that upper-airway remodelling is a feature of AR.

METHODS: Total nasal symptoms scores, Th1 and Th2 cytokines from nasal lavage and serum, and nasal biopsies were obtained from subjects with moderate/severe AR (n=46) and healthy controls (n=19). Using immunohistochemistry, monoclonal antibodies against CD31 and VEGF (Vascular endothelial), D2-40 (Lymphatic endothelial), HSP-47 (markers of collagen synthesis), MMP7-9 and TIMP1, and α-smooth–muscle-actin (myofibroblasts) were evaluated as markers of activation of upper airway remodelling using image analysis, together with basement membrane (BM) thickness, glandular area, numbers of sub-mucosal eosinophils, basophils and mast cells.

RESULTS: Total nasal symptoms scores, visual analogue scale and total quality of life were significantly higher in rhinitics compared to healthy controls ($p<0.0001$). Nasal cytokine IL-4, IL-5, and IL-13 ($p<0.001$, all respectively) from nasal lavage were significantly higher with a trend for an increase in submucosal eosinophils ($p=0.06$) in AR compared to healthy controls. No statistical difference in terms of angiogenesis, lymphangiogenesis, deposition of extracellular matrix, collagen markers, BM thickness or glandular percentage area was observed between AR and healthy controls.

CONCLUSIONS: Our data suggest that tissue remodelling as judged by changes in vascularity, extracellular matrix and fibrosis is not a feature of AR.

510 Ozone Inhalation Induces Epithelial IL-33 and Thymic Stromal Lymphopoietin (TSLP) and Leads To Eosinophilic Airway Inflammation

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RATIONALE: The mechanisms of ozone-induced eosinophilic airway inflammation in asthma are unclear. The recently discovered group 2 innate lymphocytes (ILC2) are a prominent source of IL-5 and may contribute to airway eosinophilia.

METHODS: Airway inflammation was assessed from Balb/c mice exposed to *Aspergillus fumigatus* and/or ozone (2ppm, 2h) and pulmonary ILC2 were isolated by FACS sorting. A549 cells and human sinonasal air liquid interface (ALI) cultures derived from excised nasal polyp tissue were exposed to ozone *in vitro* (0.9ppm, 1.5h). Freshly harvested atopic and non-atopic nasal tissue was obtained from the Otorhinolaryngology-Head and Neck Surgery Clinic, University of Pennsylvania.

RESULTS: Compared to air exposed controls, ozone significantly amplified airway and lung tissue eosinophilia, increased IL-33, TSLP and IL-5 levels in allergen treated mice ($p<0.05$, n=6) and induced marked IL-5 mRNA activation in isolated pulmonary ILC2 ($p<0.001$, n=8). Ozone stimulated IL-33 and TSLP mRNA 1.5 and 6 h later, respectively, in A549 cells but not cells from ALI cultures, suggesting differential sensitivity. Nonetheless, in freshly harvested atopic nasal tissue IL-33 and TSLP mRNA activation was approximately twice as high as in non-atopic nasal tissue, suggesting that nasal epithelial cells are capable of producing these cytokines and this ability might be enhanced in atopic patients.

CONCLUSIONS: Ozone induced mRNA for IL-33 and TSLP (activators of ILC2), both in mice and human airway epithelial cells and heightened the expression of IL-5 in murine lung ILC2. We speculate that epithelial activation of ILC2 may be important in mediating the ozone effects on airway eosinophilia.

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511 Clinical Efficacy Of Subcutaneous and Sublingual Immunotherapy In Asthma and Rhinitis Children Sensitized To House Dust Mite

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RATIONALE: Specific immunotherapy is the one of the treatment modality with the potential to alter the natural course of allergic diseases. In children, the clinical efficacy of subcutaneous immunotherapy (SCIT) compared with sublingual immunotherapy (SLIT) remains unclear.

METHODS: We performed a prospective trial in 53 patients with house dust mite-sensitized asthma and rhinitis either SCIT (Allergopharma, Germany,n=33) or SLIT (SLITone, Spain, n=20). The patients were followed up with symptom questionnaires and skin prick test at baseline and 3, 6, and 12 months after treatment. Specific IgE levels, eosinophil counts, bronchial hyperresponsiveness to methacholine and adenosine-5'-monophosphate (AMP) were performed at baseline and 12 months.

RESULTS: The improvement in rhinitis symptoms was significant in both groups, whereas asthma symptoms were improved only in SCIT. In both groups, skin reactivity and the bronchial hyperreactivity by methacholine at 12 months after treatment decreased significantly compared to baseline. The development of new sensitization happened in 9% of the SCIT and 23.5% of the SLIT. A significant reduction in eosinophil counts was observed only in SCIT. No changes were observed for specific IgE levels and bronchial hyperresponsiveness to AMP. No serious adverse effects were reported in both groups.

CONCLUSIONS: The symptoms of rhinitis, skin reactivity and bronchial hyperreactivity by methacholine were somewhat improved in both group. SCIT, however, showed better efficacy in the symptoms of asthma, eosinophil counts and new sensitization than SLIT. More studies in children to address the long-term efficacy of these two most often used modes of immunotherapy are needed in a larger scale.

512 A Patient-Reported Symptom-Based Predictor Of Objective Sinus Inflammation

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RATIONALE: Guidelines for diagnosing chronic rhinosinusits (CRS) require symptoms and evidence for objective sinus inflammation on computed tomography (CT) or endoscopy. Identifying patient reported symptoms that correlate with objective inflammation facilitates accurate diagnosis by providers and can significantly advance epidemiological studies of CRS.

METHODS: Consecutive patients presenting with three months of sinonasal complaints without a prior CRS diagnosis were prospectively screened for eligibility. All patients completed a self-administered questionnaire prior to medical evaluation, endoscopy and received a protocolled sinus CT scan to establish objective inflammation. Questionnaire items included medical history (n=12), symptom items drawn from the rhinitis, migraine, and rhinosinusitis literature (n=47), and quality of life (n=32).

RESULTS: Of 531 patients screened for eligibility, 300 enrolled in the study. A total of 274 (91.3%) subjects met guideline-based symptom criteria for CRS but only 112 (37.3%) had objective inflammation on CT (Sensitivity 97%; Specificity 12%). Diagnostically relevant symptoms were established by bivariate analysis and modeled using a stepwise logistic regression that identified frequency of "discolored discharge"; severity of "smell loss" and "difficulty breathing through my nose"; the presence of asthma; and lack of "recurrent headaches" modeled objective inflammation ($p<0.05$). Notably, allergic rhinitis history, symptoms of facial pain and/or pressure, post-nasal drip and migraine symptoms were not associated with objective inflammation. The predicted probabilities generated by multivariable logistic regression of significant symptoms produced an ROC curve that modeled of CRS status c=0.78 (95%CI: 0.73-0.84).

CONCLUSIONS: Three symptoms and two medical history items significantly predict CRS status although further optimization of sensitivity and specificity is needed.

513 Healthcare Providers' Perception Versus Reality In Patient Concerns About Starting Subcutaneous Immunoglobulin

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RATIONALE: Standard treatment for humoral primary immunodeficiency disease (PIDD) is immunoglobulin replacement via the intravenous or subcutaneous route. Most patients begin treatment with intravenous immunoglobulin (IVIG), but may then switch to subcutaneous immunoglobulin (SCIG) because of patient or physician preference. Healthcare providers (HCPs) and patients were surveyed to gain insight into the perceptions and reasons patients cited for remaining on IVIG.

METHODS: Nurses (n=51) and physicians (n=26) experienced with immunoglobulin therapy participated in surveys conducted at professional conferences. Patients (n=131) with PIDD receiving immunoglobulin therapy participated in unbranded surveys distributed online by the Immune Deficiency Foundation.

RESULTS: When asked why some patients were reluctant to start SCIG therapy, physicians (46%) and nurses (33%) stated "reluctance to self-administer treatment," followed by "concern about needle sticks/infusion sites" (physicians, 31%; nurses, 29%). In contrast, when patients receiving IVIG were asked why they did not use SCIG, only 4% cited "concern about learning how to self-infuse" and 6% cited "concern about needle sticks/infusion sites." The most frequent reason (27%) patients reported for not using SCIG was "satisfaction with IVIG." Of patients receiving SCIG, 73% reported that any concerns before starting SCIG did not remain; cost had been the main concern (22%).

CONCLUSIONS: The perception of HCPs regarding reasons why patients with PIDD are reluctant to choose SCIG therapy appears to differ from those stated by patients. Awareness of patient reasons for choosing IVIG or SCIG may allow HCPs to better educate and communicate with their patients when discussing immunoglobulin therapy options.

514 Anaphylaxis During Obstetric Surgery In Latex Allergic Patients

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RATIONALE: Latex is the second most common cause of anaphylaxis in the intraoperative setting. This risk increases substantially during obstetric surgery due to latex contact with mucosal surfaces and may be enhanced by intrauterine oxytocin injection. Increased awareness of the risk in obstetric patients offers opportunities for primary prevention of latex induced anaphylaxis in obstetrics.

METHODS: We present a case series of three obstetric patients, who presented to our allergy clinic after developing an anaphylactic reaction during a cesarean section.

RESULTS: Allergy testing was performed on all patients. Skin tests included general anesthesia, local anesthesia, beta-lactams and opiate panel. Oral challenge to amoxicillin was performed and serum IgE and RAST panel was obtained. Patient 1 had a serum IgE of 332 IU/ml, a positive immunoCAP assay for latex of 9.72 kU/ml at Class 3, and a positive skin test to intradermal propofol at its highest concentration of 1 mg/ml. Patient 2 hadelevated tryptase of 29.4 ng/ml, serum IgE of 449.8 IU/ml and positive immunoCAP assay for latex of 27.9 kU/L Class 4, avocado of 0.67 kU/l Class 1, and banana of 0.85 kU/l Class 2. Patient 3 had serum IgE of 1184 IU/ml and a positive immunoCAP assay for latex IgE of 2.07 kU/l.

CONCLUSIONS: Obstetric patients with latex allergies are at an increased risk for anaphylaxis. Primary prevention would be an important intervention in obstetric patients given their increased risk of latex sensitization. Further studies should be focused on instituting primary latex allergy prevention in obstetric settings as an early intervention.

515 The Impact Of Legislation On Illinois School Nurses

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RATIONALE: The State of Illinois has passed legislation mandating food allergy education including anaphylaxis drills and allowing for the use of undesignated epinephrine auto-injectors in schools. We sought to characterize the impact this legislation had on school nurses.

METHODS: We created a server-mounted questionnaire via SurveyMonkey.com that solicited school description, the nurses experience with allergic reactions and a description of how each school prepares for these type of emergencies. Invitations to participate were emailed to school nurses who had attended state sponsored education (CE) events in the previous year and during a food allergy presentation at the Illinois Associaiton of School Nurses (IASN) fall conference in 2012.

RESULTS: 70% of the school nurses felt confident in their ability to respond to a food allergy reaction and this confidence was due at least in part to their schools policies. The nurses supported the undesignated epinephrine policy (92%) and 83% of the nurses felt confident or very confident in their ability to provide food allergy education, while only 30% felt confident in their ability to provide an anaphylaxis drill. Although 79% of the nurses provided food allergy education in the past year, 92% reported that their school has never conducted an anaphylaxis drill.

CONCLUSIONS: Illinois school nurses reported positive effects of recent legislation. Although school nurses provide and feel confident in providing food allergy education they do not feel confident in providing anaphylaxis drills despite current recommendations.

516 Socio-Demographic and Environmental Correlates Of Exhaled Nitric Oxide Levels

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RATIONALE: The fraction of exhaled nitric oxide (FeNO) is a biomarker of airway inflammation; however, its clinical utility in asthma management has not been established. Asthma disproportionately affects Black children. We hypothesize that variation in FeNO may underlie these differences. Socio-demographic and environmental factors that influence FeNO have not been well described, and may help improve the utility of FeNO as a biomarker.

METHODS: We used data from the National Health and Nutrition Examination Survey 2007-2010 to determine the relationship between socio-demographic and environmental factors and FeNO in a nationally representative sample of 1,592 children.

RESULTS: After adjusting for covariates, the average FeNO of Blacks was 8.7 ppb higher (95%CI 6.7-10.7, p<0.01) than that of Non-Hispanic Whites. Recent food or drink ingestion, recent steroid use, and recent upper respiratory infection increased FeNO levels by 2.32 ppb (95%CI 0.44-4.19, p=0.02), 4.82 ppb (95% CI 0.35-9.29, p=0.04), and 4.08 ppb (95% CI 1.59-6.58, p<0.01), respectively. Female gender and environmental tobacco smoke exposure decreased FeNO levels by 6.12 ppb (95%CI -8.25 – -3.98, p<0.01), and 2.43 ppb (95%CI -4.19 – -0.67, p<0.01), respectively. We did not identify a statistically significant association between poverty income ratio, recent exercise, recent ingestion of foods rich in nitrogen, and FeNO levels (p>0.13).

CONCLUSIONS: In this large, nationally representative sample of children, socio-demographic and environmental factors were associated with FeNO levels. Black children have higher FeNO levels which may contribute to disparities in asthma prevalence and control. Our results have implications for future studies which rely on FeNO as a biomarker in asthma.

517 Relationships Between The New Biomarkers Induced By Interleukin-13 and Bronchial Hyperresponsiveness In Asthmatic Children: Periostin and Squamous Cell Carcinoma-Related Antigens

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RATIONALE: It was found that interleukin (IL)-13 induces expression of squamous cell carcinoma antigens (SCCAs) in asthmatic primary epithelial cell cultures. Also, IL-13 induces bronchial epithelial cells to secrete periostin, a matricellular protein. This study addressed the relationships between the biomarkers of IL-13-driven airway inflammation and bronchial hyper-responsiveness (BHR) in asthmatic children.

METHODS: The study enrolled 79 children aged 6–12 years in asthmatic (n = 54) and healthy control (n = 25) groups. We established new enzyme-linked immunosorbent assays (ELISAs) to detect SCCA1, SCCA2, and periostin. Mannitol and methacholine provocation challenges were performed. The response to mannitol was expressed as a provocative dose causing a 15% fall in FEV_1 (PD_{15}) and the response-dose ratio (RDR).

RESULTS: The children with asthma had significantly higher periostin (78.6 ± 17.4 vs. 70.7 ± 14.1 ng/mL; P = 0.034) and SCCA1 (0.92 ± 0.60 vs. 0.77 ± 0.26 ng/mL; P = 0.039) levels than the controls. The serum periostin levels were significantly correlated with the methacholine PC_{20}, mannitol PD_{15}, and RDR to mannitol. Both the serum SCCA1 and SCCA2 levels were significantly correlated with the mannitol PD_{15} and RDR to mannitol, but not with the methacholine PC_{20}.

CONCLUSIONS: The serum levels of new biomarkers induced by IL-13, periostin, and SCCAs, increased in asthmatic children, and were related more to the BHR to mannitol than that to methacholine.

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518 Exhaled Nitric Oxide Performance Compared To Methacholine Challenge In Asthma

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RATIONALE: Exhaled nitric oxide (FeNO) and Methacholine challenge (MCH) are both utilized in the detection and management of asthma. We hypothesize that FeNO can decrease the need for MCH testing.

METHODS: Retrospective chart review of patients ≥ 18 years presenting to a tertiary referral center seen between 11/01/2009 - 8/31/2013 who received FeNO and MCH within 2 weeks and diagnosis of asthma was used for both procedures.

RESULTS: 197 patients were identified. Demographics: 129 (65.5%) females and 68 (34.5%) males; 188 (95.4%) Caucasian, 6 (3.0%) Black, 3 (1.5%) Asian. Average age was 49.6 years (SD +/- 17.1 years). Mean BMI 30.6 (SD +/-7.0). 29 patients were positive for both MCH and FeNO, 31 patients had a positive MCH but negative FeNO, 22 patients had a negative MCH but positive FeNO, and 115 patients had both a negative ($p<0.01$). Directly comparing FeNO to MCH yielded: sensitivity 48.33% (95% CI: 35.23% to 61.60%), specificity 83.94% (95% CI: 76.70% to 89.65%), positive likelihood ratio 3.01 (95% CI: 1.89 to 4.79), negative likelihood ratio 0.62 (95% CI: 0.48 to 0.79), positive predictive value 56.86 (95% CI: 42.25% to 70.65%), and negative predictive value 78.77% (95% CI: 71.24% to 85.09%)

CONCLUSIONS: FeNO and methacholine responsiveness measure different biological phenomenon. In patients suspected of asthma, a strategy of FeNO at the point-of-care may reduce but not eliminate the need for MCH testing.

519 Serum Interleukin 13 (IL-13) and Surfactant Protein D (SP-D) Expression Is Differentially Associated With Disease Status In Pediatric Asthma Patients

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RATIONALE: Allergic airway inflammation is associated with a negative regulatory feedback between IL-13 and the immunoprotective SP-D in mice but the relevance of this to human asthma is not known. We hypothesized that serum IL-13 and SP-D expression would reflect disease status in asthmatic children.

METHODS: SP-D and IL-13 serum levels were measured in 17 atopic and 8 nonatopic children (7 to 16 years old) with inadequately controlled asthma, and in 15 non-asthmatic controls matched for age and atopic status, in duplicates of dilutions (1:5 and 1:10) by ELISA (BioVendor and RayBiotech, Inc., respectively). The inter-assay and inter-experimental variability was <10% for both assays. Measurements were repeated in asthmatic children 4 to 6 months after initiation or escalation of inhaled glucocorticoid therapy ($n=10$) and after initiation of inhaled Sodium Cromoglycate ($n=7$) given according to the American Thoracic Society Criteria and European Consensus Guidelines.

RESULTS: Expression of IL-13 (ranged between 0-171 pg/ml) was significantly greater in the asthmatic samples than in controls (25.6 ± 2.7 vs. 12.6 ± 2.0 $p=0.0001$). In contrast, SP-D levels (ranged between 19-373 ng/ml) were significantly higher in the controls than in asthmatics (148.2 ± 5.9 vs. 200.2 ± 20.2; $p=0.0219$). Inhaled glucocorticoids or sodium cromoglycate did not change IL-13 or SP-D serum levels significantly although the sodium cromoglycate-treated patients showed a trend for reduced IL-13 (41.0 ± 21.1 vs. 19.2 ± 7.6 pg/ml).

CONCLUSIONS: Serum levels of IL-13 and SP-D inversely associated with presence of moderate to severe asthmatic airway inflammation in children suggesting that these biomarkers may be useful noninvasive indicators of disease status in childhood asthma.

520 Correlation Of Exhaled Breath Temperature With Age In Chronic Respiratory Diseases

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RATIONALE: Asthma exacerbations may be associated with increased exhaled breath temperature. Chronic obstructive lung disease may be influenced by vascular inflammation. There may be a relationship between exhaled breath temperature and patient age in asthma and COPD.

METHODS: 124 patients with COPD and asthma (72 male and 52 women) were studied, ranging from 42 to 81 years old, mean age 58.4 years. 30 healthy control subjects were assessed. Patients with acute respiratory infection or disease exacerbation in the last month were excluded. All exhaled breath tests were performed in the same room, at similar times of day with ambient temperatures between 20 and 23°C and humidity 55% and 70%. Exhaled breath temperature was measured using an X-Halo device (Delmedica,Singapore). Mean temperature value was calculated by 3 consecutive measurements 1 day apart.

RESULTS: EBT values were almost 2.0°C higher in the control group (EBT = 33.91°C), compared to patients with chronic obstructive respiratory disease (EBT= 31.69°C) and asthma (EBT= 30.89°C). Inverse correlation was seen between patient age and EBT ($r=0.54$; $p<0.05$).

CONCLUSIONS: An inverse correlation exists between patient age and EBT in asthma and COPD patients not in exacerbation. COPD and asthma patients had lower EBT at baseline then healthy controls. This may reflect underlying chnages in vascularity in both diseases compared with healthy controls without chronic respiratory diseases.

521 Peripherally Induced Foxp3+ Regulatory T Cells Mediates The Immunomodulatory Effect Of Intravenous Immunoglobulin In An Experimental Model Of Allergic Airway Disease

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RATIONALE: IVIg is a polyclonal IgG preparation with potent immunemodulating properties. We demonstrated that IVIg protects against airway hyperreactivity (AHR) and airway inflammation in mouse models of allergic airway disease, accompanied by peripheral induction of $Foxp3^+$ regulatory T-cells (iT_{reg}). The requirement of IVIg-induced iT_{reg} and their antigen-specificity in attenuation of AHR and airway inflammation remains unknown.

METHODS: We utilized DEREG mice, carrying a transgenic diphtheria toxin receptor under the control of the Foxp3 promoter, allowing for selective depletion of $Foxp3^+T_{reg}$ by the application of diphtheria toxin (DT). Mice were sensitized and challenged with ovalbumin (OVA) and treated with IVIg. AHR was measured using a FlexiVent small animal ventilator. Total and antigen-specific IgE, as well as pro-inflammatory cytokines levels were determined in serum and alveolar lavage, using ELISA.

RESULTS: In the absence of T_{reg}, due to multiple DT doses before and after the treatment, IVIg was not able to attenuate AHR, diminish IgE levels and Th-2 type cytokine production, nor alleviate airway inflammation. However, mice in which the pre-established T_{reg} cells (nT_{reg}) were depleted before but not following IVIg treatment demonstrated an induction of $Foxp3^+T_{reg}$ to IVIg therapy and did not develop AHR and airway inflammation to allergen-challenge. Adoptive transfer of enriched IVIg-induced iT_{reg} from OVA-IVIg treated mice failed to transfer protection to mice exposed to ragweed, but was protective in OVA-sensitized and challenged mice.

CONCLUSIONS: T_{reg} can be induced from effector $CD4^+$T-cells in the absence of nT_{reg}. IVIg-induced antigen specific T_{reg} are capable of suppressing all aspects of antigen-driven airway inflammation in an antigen-specific manner.

522 Differential DNA Methylation In Mothers Increases The Prevalence Of Atopic Dermatitis In Their Offspring

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RATIONALE: Allergic diseases are increasing in developed countries with early manifestations as atopic dermatitis and food sensitivities. To determine the effects of prenatal diet on DNA methylation and transgenerational inheritance of atopy, we studied a birth cohort of children born to mothers at high and at a low risk of having an atopic child based on parental history.

METHODS: Three hundred three newborns born to 159 high- and 144 low-risk mothers were followed biannually for two years for development of atopy. Genome-wide DNA methylation patterns were determined on maternal peripheral blood mononuclear cells (PBMC) using Comprehensive High-throughput Arrays for Relative Methylation (CHARM). Prenatal intake (NIH NCI Diet History Questionnaire) and serum concentrations of the methyl donors folate and vitamin B12 were measured.

RESULTS: Atopic dermatitis was more prevalent in children of mothers at high risk of atopy compared to children of low-risk mothers at age 6 months (36.1% versus 20.9%; p=0.0096), 12 months (41.3% versus 17.9%; p=0.0001), 18 months (31.4% versus 17.4%; p=0.023) and 24 months (38.8% versus 15.6%; p=0.0119). Greater than 95% of mothers in both groups took prenatal preparations containing folate. While dietary intake and serum levels of folate and vitamin B12 did not differ between high- and low-risk mothers, DNA methylation analyses showed significant differences in PBMC from high- and low-risk mothers, with 64 gene loci being differentially methylated (increased methylation at 22 and decreased methylation at 42 loci).

CONCLUSIONS: These data suggest that DNA methylated/demethylated gene loci in mothers at high-risk may affect the development of atopy in their offspring.

523 Hypereosinophilia In Children and Adults: A Retrospective Comparison

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RATIONALE: Although the differential diagnosis of eosinophilia is similar in children and adults, the clinical presentation, ultimate diagnosis, treatment, and prognosis of marked eosinophilia in children have not been well characterized.

METHODS: A retrospective chart review was conducted of 297 subjects (39 children, 258 adults) referred to the NIH for unexplained eosinophilia ($> 1.5 \times 10^9$/L) between 1983 and 2012. Demographic, clinical and laboratory data pertaining to baseline characteristics, diagnosis, and treatment responses were collected. Means and proportions were compared between children and adults using the Mann-Whitney U and Fisher Exact tests.

RESULTS: In the pediatric group, 33% of subjects were ultimately diagnosed with hypereosinophilic syndrome, 15% with eosinophilic gastrointestinal disease, 8% with parasitic disease, and 8% with malignancy or myeloproliferative disease. This was similar to the diagnostic breakdown in the adult subjects. There was a significant male predominance in the pediatric group (72%) as compared to the adults (52%; p=0.02). Peak eosinophil count (16123 v. 8646; p<0.005) and serum B12 levels (1347 v. 1147; p<0.001) were significantly increased in pediatric patients; whereas adults were more likely to have a clonal lymphocyte population (p<0.006). F1P1L1/PDGFRA status, absolute eosinophil count at presentation, hemoglobin, platelet count, serum IgE, IgM, and tryptase levels were comparable. Pediatric patients were less likely to have cardiac complications of HES (3% v. 18% in adults, p<0.01) and had lower overall mortality.

CONCLUSIONS: Despite higher peak eosinophil counts, pediatric patients with hypereosinophilia appear to have a decreased prevalence of cardiac involvement and a better overall prognosis than adults.

524 Interleukin 35 Modulates TSLP, IL-25 and IL-33 Primed Dendritic Cells and Inhibits Naive T Cell Differentiation and Grass Pollen-Specific T Cell Proliferation

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RATIONALE: Interleukin-35 (IL-35) is a novel heterodimeric cytokine consisting of IL-12p35/EBI3 subunits. Here we examine its role in modulating Thymic Stromal Lymphopoietin (TSLP)-, IL-25- and IL-33-induced native T cell activation/differentiation. We hypothesised that TSLP-primed DCs differentiate naïve T cells into potent grass pollen-specific Th2 cells. We further hypothesized that IL-35 inhibits Th2 responses induced by TSLP-primed DCs.

METHODS: Dendritic cells obtained from grass pollen-allergics (n=14) were stimulated with TSLP, IL-25, IL-33 or all three cytokines in the presence of 5μg/mL of *P. pratense* (Phl p) for 24 hour. Primed DCs were co-cultured with naive T cells for 6 days (1:10 ratio) in the presence/absence of rhIL-35. Phl p-driven proliferation and Th2 cytokines were measured by ^{3}H tritiated thymidine incorporation and Luminex MagPix assay, respectively.

RESULTS: TSLP-primed DCs when stimulated with Phl p allergen resulted in a 97-fold (p=0.003) increase in naive T cell proliferation compared to non-TSLP primed DCs. IL-25- and IL-33- primed DCs did not increase proliferation of native T cells (p=0.43; p=0.50). The increase in TSLP-induced allergen-driven naïve T cell proliferative responses was associated with an increase in IL-4 (p=0.008) and IL-5 (p=0.008) production. In parallel co-culture experiments, IL-35 inhibited naive T cell proliferation (42%, p<0.0001) and Th2 cytokine production (IL-5, p=0.02; IL-13, p=0.02). Neutralizing rhIL-35 with monoclonal antibodies resulted in recovery of allergen-driven naïve T cell proliferative responses (p=0.0009).

CONCLUSIONS: IL-35 inhibits TSLP-induced naïve T activation and Th2 differentiation. The mechanism of this suppression remains to be further determined.

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525 Fc-Gamma-Receptor-IIb Is Required For The Immunomodulatory Actions Of Intravenous Immune Globulin In An Antigen-Driven Murine Model Of Allergic Airways Disease

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RATIONALE: Intravenous immunoglobulin (IVIG) is a potent immune modulator but the cellular receptors mediating immune tolerance are not conclusively defined. We have shown that IVIG induces regulatory T cells (Treg), which inhibit inflammatory changes associated with antigen-driven murine allergic airway disease (AAD). We investigated the contribution of the inhibitory Fc-gamma-receptor-IIb (Fcγr2b) to the regulatory effects of IVIG.

METHODS: Activating FcγRI/IIIA/IV knockout (Fcγr-KO), inhibitory Fcγr2b knockout (Fcγr2b-KO), and wild-type (WT) mice were subjected to ovalbumin (OVA) sensitization and challenge. IVIG was administered intraperitoneally 24 hours prior to challenges. Airway hyper-responsiveness (AHR) to methacholine was measured by flexiVent, and lung histopathology was examined. Bone-marrow-derived dendritic cells (BMDC) from KO and WT were primed *in vitro* with OVA and/or IVIG and adoptively transferred into naive WT recipients. Recipients were subsequently challenged with OVA and lungs were assessed for Treg.

RESULTS: IVIG inhibited the OVA-induced increase in AHR in WT and in Fcγr-KO; however, AHR in Fcγr2b-KO was similar to untreated WT. Histopathological changes consistent with airway inflammation were also unaffected by IVIG in Fcγr2b-KO. IVIG treatment of OVA-primed BMDC from WT or Fcγr-KO led to *in vitro* and *in vivo* induction of Treg, but OVA/IVIG-primed Fcγr2b-KO BMDC were unable to induce Treg.

CONCLUSIONS: IVIG appears to require the inhibitory Fcγr2b for its anti-inflammatory actions in murine AAD. This may be due to downstream upregulation of Fcγr2b on DC and subsequent interaction with effector T cells. IVIG ligates the C-type lectin receptor DCIR on DC: subsequent studies will examine the cross-talk between Fcγr2b and DCIR.

526 Endotoxin In Size-Specific Airborne Particles Induces Differential Nitrative Stress In Human Bronchoepithelial Cells

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RATIONALE: To characterize inflammatory responses and corresponding nitrative stresses in human bronchoepithelial cells (BEAS 2B) after exposure to endotoxin in airborne particles with aerodynamic diameters (d_a) <1.0μm and >1.8μm.

METHODS: In vitro, BEAS-2B cells were exposed to endotoxin extracts from airborne particles with d_a<1.0μm or from particles with d_a>1.8μm. Thereafter, differential induction of nitrative stress and expression of genes regulating inflammatory pathways were determined in these cells. Size-specific airborne particles (d_a<1.0 and >1.8μm) were collected using a bio-aerosol cyclone sampler (BC221, NIOSH, CDC) for 24 hours in a domestic home. Endotoxin in size-specific particles was separately extracted and then quantified with Limulus Amebocyte Lysate Assays (AOCC, MA). BEAS-2B cells (ATCC, VA), grown on glass cover-slips, were loaded with nitrative stress indicator, DAF-FM (Invitrogen, NY). At 488 nm excitation light wavelength, DAF-FM fluoresces higher when bound to nitric oxide. Nitrative stress induced by endotoxins from size-specific particles at similar concentrations were then detected by comparing post-exposure fluorescence (F) with baseline fluorescence (F_0). Using real-time PCR, the relative gene expression of the human inflammatory pathways was determined for both groups.

RESULTS: Cells exposed to endotoxin in particles [d_a<1.0μm] had comparatively less nitrative stresses than cells exposed to endotoxin in particles [d_a>1.8μm] at similar concentrations. The former cells over-expressed anti-inflammatory genes (NR3C1, ITGB1) and down-regulated pro-inflammatory genes (HRH1, MC2R, LTB4R, PLA2G7, PTGIS).

CONCLUSIONS: Airway epithelial cells exposed to endotoxin in airborne particles with d_a<1.0μm suffer less nitrative stress than those exposed to iso-concentrate endotoxin in particles with d_a>1.8μm while over-expressing anti-inflammatory genes and down-regulating pro-inflammatory genes.

527 High Rates Of Sensitization To Selected Metals and Bone Cement In Joint Replacement Failure Patients and Preoperative Evaluations

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RATIONALE: Of the million joint replacements performed annually in the US, approximately 10% will fail. Infection and biomechanical problems are common, but do not explain all cases. Orthopedic implants and bone cement contain known sensitizers, and we hypothesized that allergic reactions might be associated with "unexplained" implant failures.

METHODS: We enrolled 311 consecutive patients referred for evaluation of potential implant sensitization into an IRB-approved study following informed consent. We obtained demographic and medical information, and patch tested to a standard panel of metals and bone cement components used in joint replacements. We describe the patient characteristics and patch test results.

RESULTS: The most common reason for referral was implant failure (77%) after other causes were excluded (n=240); the remaining 23% (n=71) were pre-operative evaluations, primarily for history of reaction to metal jewelry. The mean age was similar in both groups, approximately 60 years. Women comprised 93% of the pre-operative group and 60% of the implant failures. Knees (69%) and hips (20%) were the most commonly replaced joints. Sensitization to nickel, cobalt, chromium, and/or bone cement was found in 50% of implant failure patients and 59% of preoperative patients. Metal allergy was more common in pre-operative patients (52% vs. 25%), whereas allergy to bone cement was twice as high in the implant failure group (30% vs. 15%).

CONCLUSIONS: Sensitization to metal and bone cement is common in selected patients with "unexplained" implant failures. Pre-operative screening based on history may help prevent implant failures. These results should help inform selection of patients and patch test panels.

528 Component Resolved Diagnosis In Baker's Asthma

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RATIONALE: Subjects with bakeŕs asthma recognize several allergens identified in the water/salt-soluble fraction of wheat flour. The aim of our study was to characterize the allergenic profiles of baker's asthma patients from three different regions in Spain by using a panel of wheat allergens purified from natural sources and printed on a protein microarray.

METHODS: Forty five patients from 3 regions in Spain (Madrid n=17, Malaga n=10, Valladolid n=18) with a consistent history of bakeŕs asthma, positive results to skin-prick test and bronchial challenge with wheat flour were recruited. Twelve wheat allergens (WDAI-0.19 and WDAI-0.53, WTAI-CM1, WTAI-CM2,WTAI-CM3,WTAI-CM16,WTAI-CM17, Tri a 14, profilin, ω-5-gliadin, Tri a Bd 36 and Tri a TLP) were purified and applied on epoxy-activated glass using a MicroGrid II TAS arrayer. The IgE binding of each allergen spot was calculated as the final fluorescence intensity, measured by GenePixTM software. The Ethics Committee of each hospital approved the study.

RESULTS: WTAI-CM16 and Tri 14 were defined as the most prevalent allergens (54 and 45% on average, respectively) covering a total of 64% of the baker's asthma population. On the other hand, ω-5-gliadin and Tri a Bd36 were recognized by less than 10% of the bakeŕs population.

CONCLUSIONS: The highest prevalence of IgE binding was observed for WTAI-CM16 and Tri a 14, since more than 60% of patients with baker's asthma recognized at least one of these markers.

529 Endotoxin Exposure May Protect Against The Development Of Rhinoconjunctivitis and Respiratory Symptoms In Non-Atopic Individuals With Occupational Exposure To Mice

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RATIONALE: Endotoxin exposure is associated with the development of respiratory symptoms in individuals with occupational mouse exposure. It is unknown whether atopic status modifies this relationship.

METHODS: Adults (18-74y) newly employed in a mouse facility were enrolled. Participants were skin prick tested (SPT), administered questionnaires about rhinoconjunctival and respiratory symptoms, and wore personal monitors for collection of breathing zone air samples every 6 months. Mus m 1 (MA) and endotoxin (ET) content in air samples were quantified by ELISA and limulus amebocyte assay, respectively. Atopy was defined as ≥1 +SPT (net wheal ≥3mm). Relationships between MA exposure, ET exposure, and incident symptoms were examined using Cox proportional hazards models adjusted for age, gender, total serum IgE level, level of education, smoking status, and respiratory protection usage.

RESULTS: 193 participants were enrolled. 54% were female, and 52% were atopic. Median MA and ET concentrations were 1.4 ng/m^3 and 3.2 EU/m^3, respectively, and MA and ET were correlated (r_s 0.41, p<0.001). Median follow-up time was 24 months, and 38 participants developed symptoms. In a model adjusting for ET, MA exposure was associated with developing symptoms (crude HR [95% CI]: 1.58[1.08-2.31], p=0.02). Increasing endotoxin exposure was associated with lower risk of developing symptoms among non-atopic (0.09 [0.02-0.42], p=0.002), but not atopic participants (2.16[0.53- 8.77], p=0.28; interaction p=0.02).

CONCLUSIONS: Both the level of MA exposure and the level of ET exposure influence the development of upper and lower respiratory symptoms among individuals with occupational exposure to mouse. Endotoxin exposure may be protective for non-atopic individuals.

530 Sensitization To Occupational Allergens and Allergic Diseases In Workers Of 5 Havana Bakeries

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RATIONALE: Bakers are a professional group at risk of developing occupational allergic diseases. There are several allergens that may sensitize them, especially wheat flour and dust mites, which are present in their workplace. Our objectives were to identify the frequency of sensitization to dust mites and occupational allergens in 80 workers at Havanaś bakeries, and to determine the prevalence of allergic diseases in these workers.

METHODS: A cross-sectional analytical study was carried out. The study group included 80 workers of 5 Havanaś bakeries, mean age 37 (range 18-67 years). For each subject, a clinical and occupational history was compiled and skin testing was performed.

RESULTS: 86% subjects showed a positive response to at least one allergen by SPT. 46,3% of workers reported allergic diseases and 18,8% current symptoms, mostly, respiratory symptoms. The highest percentage of positivity was reported to D. farinae (61.3%), as well as the largest wheal size (mean 4.6 mm). 42.5% of workers showed positive response to wheat. A significant association (Spearman, p<0.05) was found between the reaction size to wheat, yeast and soy, respective to both storage and house dust mites.

CONCLUSIONS: There is a high prevalence of respiratory diseases and sensitization to mites and wheat flour in bakers, which represents a risk factor to consider for their occupational safety.

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531 Safety Of Propofol Use In Patients With Food Allergies

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RATIONALE: Propofol (2,6-diisopropylphenol) is a commonly used intravenous drug for induction and maintenance of anesthesia during endoscopic procedures. The drug, though generally considered safe, has been considered a relative contraindication in egg and soy allergic patients. Our aim was to determine whether patients with evidence of food allergy, particularly to egg, soy and/or peanut had an allergic reaction to propofol when used during their endoscopies.

METHODS: Records of 100 patients that had endoscopies performed at the Mount Sinai Center of Eosinophilic Disorders were reviewed. Egg, peanut and soy allergy was confirmed based on finding elevated food-specific serum IgE levels, positive skin prick tests and/or convincing allergic reaction history. Patients were included if anesthesia records indicated propofol as the main anesthetic administered.

RESULTS: 45 patients (mean age=13.2 years, range=2-64) were identified with one or more food allergies, which included 6 patients with history of anaphylaxis. Of those, 16 patients had evidence of egg allergy (median egg-IgE=16.2 kIU/L, range=0.8-100). Two of these patients had history of anaphylaxis to egg. Of the 10 patients with confirmed soy allergy, 6 had serum soy-IgE levels obtained (median soy-IgE=8.5 kIU/L, range=3.6-100). Fourteen patients had peanut allergy (median peanut-IgE=100 kIU/L, range=0.35-100). Three of these patients had history of anaphylaxis to peanut. There were no reported reactions to propofol in any of the patients.

CONCLUSIONS: In this food allergic population undergoing endoscopies under anesthesia with propofol, no allergic reactions to propofol were seen. Propofol (2,6-diisopropylphenol) can be considered generally safe for use in patients with history of severe food allergies.

532 Risk Stratification Protocol For Carboplatin and Oxaliplatin Hypersensitivity Reactions With Repeat Skin Testing Improves Care

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RATIONALE: A risk stratification protocol utilizing repeat skin testing (ST) was previously published in a small number of patients with carboplatin hypersensitivity reactions (HSRs). We studied this strategy in a larger number of patients with carboplatin or oxaliplatin HSR.

METHODS: In a 5-year retrospective review, patients referred to Allergy/Immunology with carboplatin or oxaliplatin HSR were treated with a risk stratification protocol using 3 repeat STs with intervening desensitizations. If repeat ST remained negative three times, patients received subsequent infusions without desensitization.

RESULTS: From 2008-2012, 144 patients (92 carboplatin, 52 oxaliplatin) completed 577 desensitizations. Carboplatin HSR patients were classified as ST positive (n=32), negative (n=37), or converters (n=23) when initial negative ST converted to positive on repeat ST. ST positive patients had more severe initial HSR than ST negative patients ($p<0.05$). Of the 52 oxaliplatin patients, 22 were ST positive, 25 were ST negative, 3 were ST converters, and 2 did not receive ST. For both carboplatin and oxaliplatin, ST converters had a longer time interval between HSR and initial ST compared to ST positive patients (carboplatin median 42 (IQR 2-96) vs 3 (IQR 2-8) weeks, oxaliplatin median 78 (IQR 40-92) vs 3 (IQR 1-5) weeks, $p<0.05$). Twenty-two carboplatin and 18 oxaliplatin HSR patients remained ST negative after three serial STs with intervening desensitizatons, and the majority (82% and 89%, respectively) completed their chemotherapy regimens as outpatient infusions without desensitizations.

CONCLUSIONS: Repeat STs improve care for patients with carboplatin or oxaliplatin HSR. Our novel risk stratification protocol identifies patients that can receive infusions without desensitization.

533 Added Value Of Skin Testing In Hypersensitivity Reactions To Taxanes

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RATIONALE: Taxanes hypersensitivity reactions (HSRs) occur in 1-10 % of ovarian cancer patients treated with these drugs and can be anaphylactic precluding their re-adminsitration. Desensitization has provided a treatment option for these patients but the mechanisms of taxane hypersensitivity have not been elucidated. Skin testing (ST) was recently introduced in the evaluation of these patients and we sought to determine its value in the management of taxane HSRs.

METHODS: We reviewed the skin test results of all patients undergoing desensitization or challenge to taxanes between 01/2012 and 06/2013.

RESULTS: Seventy-one patients reported a total of 87 HSRs (57 immediate and 30 delayed; 73 to paclitaxel and 14 to docetaxel). Paclitaxel ST was performed on 64 patients and was positive (+) in 44/56 patients (79%) with a paclitaxel HSR, in 3/6 (50%) with a docetaxel HSR and in 2/2 (100%) with HSRs to both. Fifteen patients had negative (-) paclitaxel ST and one of them was ST+ to docetaxel. Eleven ST- patients with a delayed or mild to moderate immediate HSR to paclitaxel were challenged. All tolerated the procedure and resumed regular infusions. One patient had a recurrent HSR and was found to have converted from ST- to ST+. Sixty-two patients (50 ST+, 4 ST-, 1 ST converter and 7 not ST) underwent a total of 471 desensitizations. Mild HSRs occurred in 8% of all desensitizations.

CONCLUSIONS: Taxane ST can identify patients with immediate and delayed HSRs who can safely undergo a challenge (ST- with a non-severe initial HSR) and, if successful, resume regular infusions.

534 Risk Stratification For Paclitaxel-Induced Hypersensitivity Reactions Improves Quality Of Care

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RATIONALE: Paclitaxel-induced hypersensitivity reactions (HSRs) limit its use in standard therapy. While desensitization is a successful strategy, evidence-based risk stratification can improve quality of care.

METHODS: We performed a retrospective review of all paclitaxel-induced HSRs referred to Allergy/Immunology between 2010 and 2013. The initial HSR and clinical outcomes were reviewed and graded. Utilizing these results, a risk stratification strategy was developed to improve quality of care for patients with paclitaxel-induced HSRs.

RESULTS: We identified 23 patients with paclitaxel-induced HSRs who underwent desensitization. Initial HSRs were sub-divided into grade 1 (N=7), grade 2 (N=9), and grade 3/4 (N=7). 28% (N=2) of initial grade 1 and 55% (N=5) initial grade 2 patients experienced HSRs (six grade 1, one grade 2 HSRs) during desensitization compared to 86% (N=6) of initial grade 3/4 patients. Paclitaxel was advanced to 50% of the standard rate without desensitization in four patients (three initial grade 1, one initial grade 2). All four patients tolerated the infusions and returned to the outpatient setting. Based on these findings, a risk stratification protocol was implemented. Grade 1 initial HSR are infused at 50% of the standard rate while grade 2 and grade 3/4 HSR are administered paclitaxel utilizing 8- and 12-step desensitization protocols respectively. All five patients studied to date using this risk stratification tolerated re-treatment safely including 2 patients with initial grade 1 HSR administered paclitaxel without desensitization.

CONCLUSIONS: Our risk stratification strategy for paclitaxel-induced HSRs improves quality of care and allows patients to safely receive paclitaxel while reducing the number of unnecessary desensitizations.

535 Healthcare Utilization and Serious Infection Prevalence Associated With Penicillin "Allergy" In Hospitalized Patients: A Cohort Study

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RATIONALE: Penicillin is the most common inaccurate drug "allergy" noted at hospital admission. Determine hospital utilization and prevalence rates of Clostridium difficile, methicillin resistant Staphylococcus aureus (MRSA), and vancomycin resistant enterococcus (VRE), in patients with and without penicillin "allergy".

METHODS: A retrospective, matched cohort, study of individuals admitted to Kaiser Foundation hospitals in Southern California during 2010 through 2012.

RESULTS: It was possible to match 51,582 (99.6% of all possible cases) unique penicillin "allergic" hospitalized individuals to 2 unique control subjects each. Penicillin "allergic" cases averaged 0.59 (9.9%) [95% CI, 0.47 to 0.71] more total hospital days during 20.1 ± 10.5 months of follow-up, compared to discharge diagnosis category-, gender-, age-, and date of admission-matched controls. Cases were treated with significantly more fluoroquinolones, clindamycin, and vancomycin, $p < 0.0001$, compared to controls. Cases had 23.4% [95% CI, 15.6% to 31.7%] more Clostridium difficile, 14.1% [95% CI, 7.1% to 21.6%] more MRSA, and 30.1% [95% CI, 12.5% to 50.4%] more VRE infections than expected, compared to controls. When adding matching for the number of drug "allergies", differences between cases and controls disappeared, but a strong positive correlation was seen for all 4 outcome variables and increasing drug "allergy" number in both cases and controls.

CONCLUSIONS: A drug "allergy" history though often inaccurate, is not a benign finding at hospital admission. Drug "allergies" are associated with increased hospital utilization and increased Clostridium difficile, MRSA, and VRE prevalence.

536 Length Of Avoidance Period Following Peanut Oral Immunotherapy Influences Effector Cell Suppression and Clinical Outcomes

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RATIONALE: Desensitization of peanut allergic subjects can be achieved with oral immunotherapy (OIT), however, changes in clinical outcomes and effector cell reactivity are unknown after OIT is stopped for varying lengths of time.

METHODS: Subjects on OIT underwent double-blind placebo-controlled food challenges (FC) to peanut while continuing daily therapy to assess desensitization (FC1), and then avoided peanut in the diet for either one or three months, at which time a second FC was administered (FC2). Basophil activation and skin prick tests (SPT) to peanut were measured.

RESULTS: Basophil assays were captured at FC1 and FC2 on 20 subjects, making up the cohort used in this analysis. All 20 subjects were desensitized as evidenced by passing FC1. Tolerance at FC2 was achieved in 16 of 16 subjects that avoided peanut for one month following FC1, whereas only 1 of 4 subjects passed FC2 after avoiding peanut for three months. Basophil activation was increased to peanut antigen and anti-IgE stimulation during the FC1 to FC2 period in subjects avoiding peanut for three months ($p<0.05$), but not in subjects avoiding peanut for one month. Between-group comparisons demonstrated significant differences at FC2, but not FC1, indicating that desensitization effects were similar between the groups but the length of avoidance had a measurable effect on basophil responses. SPT to peanut returned to baseline levels in 3 of 4 subjects during the three month avoidance phase.

CONCLUSIONS: Prolonged avoidance of peanut exposure following OIT may have detrimental effects on suppression of effector cells, leading to re-establishment of clinical reactivity.

537 Basophil Hyporesponsiveness To Peanut Following Immunotherapy May Be Transient and Correlates With Clinical Response

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RATIONALE: To evaluate how peanut oral (OIT) and sublingual (SLIT) immunotherapy alter measures of basophil reactivity.

METHODS: Spontaneous (media alone) and stimulated (peanut, anti-IgE) histamine release (HR) (by automated fluorimetry) as well as CD63 expression (by flow cytometry) were measured from basophil-enriched suspensions isolated from blood of children at baseline and multiple time-points during a trial comparing OIT and SLIT.

RESULTS: Peanut-induced upregulation of CD63 correlated strongly with HR ($r = 0.59$, $p < 0.01$); both measures of basophil reactivity were suppressed after 6 months of maintenance dosing (OIT 31.5% to 1.2% and SLIT 29.6 to 7.7% CD63 expression at 1ng/mL peanut). No significant difference was seen between OIT and SLIT. Spontaneous HR and CD63 also decreased during this period on both treatments. However, both spontaneous and peanut-induced CD63 and HR increased after 24 months of treatment compared to levels after 6 months of maintenance (OIT $p=0.027$, SLIT $p=0.016$ for spontaneous CD63 expression). No change in basophil reactivity was observed following incubation with anti IgE antibody. Peanut induced basophil CD63 expression correlated with the total amount of peanut tolerated by subjects at all time-points (rho = -0.38, $p<0.01$). Basophils from subjects who passed a peanut food challenge after 4 weeks off therapy had among the lowest CD63 expression.

CONCLUSIONS: Peanut OIT and SLIT induce an initial state of basophil hyporesponsiveness specific for peanut, which is partially lost at later time-points. Measures of basophil reactivity show moderate correlation with clinical tolerance to peanut, suggesting this may have some utility as a prognostic biomarker.

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538 Safety Of Pediatric Peanut Oral Immunotherapy Is Complicated By High Adverse Event Rates

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RATIONALE: Though peanut oral immunotherapy (OIT) is a promising investigational therapy, its safety for clinical use is limited by significant adverse event (AE) rates. This retrospective analysis pooling three pediatric peanut OIT trials is the largest analysis of peanut OIT safety to date.

METHODS: We pooled 98 peanut-allergic children from three studies, an unpublished peanut OIT trial in toddlers and two previously reported studies (one open label and one randomized controlled trial). We catalogued all home AEs via parental report and daily symptom diaries. We characterized events by likelihood of being related to study product, and calculated AE rates using events likely related to OIT (by parental report or diary).

RESULTS: AE rates were 1.4% (650 of 46,062 dosing days, affecting 62% of subjects) during the buildup, and 0.5% (297 of 60,473 dosing days, affecting 36% of subjects) during maintenance. Doses were skipped in response to 41% of parent-reported AEs (79% of these due to infection), and decreased in response to <1% of AEs. Symptom diaries showed 54% of subjects received treatment at home for likely related events at some point during OIT, 53% with antihistamines, 18% with albuterol, 9% with epinephrine, and 15% had an emergency room visit. Over the course of OIT, 37% of subjects should have received epinephrine based on symptom severity yet were not given any.

CONCLUSIONS: Peanut OIT is associated with high AE rates during home dosing. Many subjects require epinephrine administration at home at some point during OIT, with even higher rates failing to administer epinephrine when needed.

539 Course and Outcome Of Patients With Asthma During Oral Immunotherapy To Cow's Milk Protein

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RATIONALE: Although asthma patients with cow's milk allergy (CMA) are at increased risk for anaphylactic reactions upon accidental exposure to cow's milk, they are often excluded from oral immunotherapy (OIT) programs. Thus, the risks and benefits of OIT in these patients should be examined.

METHODS: Children ≥6 years with IgE-CMA, with (n=101) and without (n=93) asthma, undergoing milk-OIT were compared. Milk dose escalations were performed until patients reached full (>7.2 gram CMP) or partial desensitization. Skin prick tests in all patients, and spirometry in those with asthma, were performed.

RESULTS: Prior to OIT, patients with asthma experienced more accidental exposure-induced anaphylactic reactions (p=0.003), ER visits (p=0.02) and admissions (p=0.03) and reacted to a lower dose of CMP on oral food challenge (p<0.001). Asthma patients had more reactions during induction (p<0.005) and home OIT treatments (p<0.012), including the use of injectable epinephrine (p<0.0001). Patients with asthma were less likely to reach full desensitization (49.5% vs. 65.6%, p=0.03), but most (85%) reached a protective dose. A subgroup of asthma patients in whom controller therapy was initiated during OIT, had higher CMP sensitivity and lower lung function pre-MOIT and were less likely to reach full desensitization at the end of treatment.

CONCLUSIONS: Patients with asthma experience more adverse reactions and are less likely to reach full desensitization, but most reach a protective dose during milk-OIT. Given their higher risk for anaphylactic reactions upon accidental exposures, asthma patients with CMA should not be excluded from milk-OIT, but their asthma should be well controlled prior to their enrollment.

540 B-Fahf-2 Pretreatment Reduces OIT Adverse Reactions and Improves Outcomes In a Murine Model Of Multiple Nut Allergy

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RATIONALE: Adverse reactions during OIT for food allergy are a central concern and barrier to wide clinical use. The TCM herbal formula B-FAHF-2 completely protects mice from anaphylaxis. We questioned whether pretreatment with B-FAHF-2 could improve safety profile and outcome of OIT for multiple nut allergy (MNA).

METHODS: C3H/HeJ mice were concurrently sensitized to peanut (PN), walnut (WN) and cashew (CSH). Oral challenge with individual nuts induced anaphylactic reactions. Some multiple nut allergic mice then received twice daily B-FAHF-2, control mice received sham treatment. OIT using a PN/WN/CSH mixture was then performed. After completing OIT all mice were orally challenged twice with a PN/WN/CSH mixture, and then with individual nuts. Symptom scores and core body temperatures were recorded on day 1 of OIT and after each challenge. Histamine, nut-specific IgEs, and cultured splenocyte cytokines were measured by ELISA.

RESULTS: BFAHF-2 treated mice exhibited fewer OIT adverse reactions, lower symptom scores and serum histamine levels than sham-treated mice (P<0.05-0.01 for all). At post-OIT challenges treated mice exhibited lower levels of nut-specific IgEs, fewer and milder reactions and lower histamine levels than control mice (P<0.05-0.01). IFN-gamma/IL-13 and IL-10/IL-13 ratios in cell cultures from B-FAHF-2 treated MNA mice but not control mice were elevated (P<0.05).

CONCLUSIONS: BFAHF-2 pretreatment reduced adverse reactions during OIT, and produced greater post-OIT protection and a beneficial immunregulation. Addition of BFAHF-2 to an OIT regimen for human food allergy may improve its safety and efficacy.

541 Adherence Documentation During Asthma Encounters At a Pediatric Tertiary Care Referral Center

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RATIONALE: Non-adherence to asthma controller medications is a significant cause of morbidity and urgent health care utilization. Assessment regarding documentation of adherence during physician encounters has not previously been reported.

METHODS: We performed a retrospective review of the electronic medical record(EMR) for all patients ages 2-18 with encounters at a tertiary care pediatric academic referral center with primary diagnosis 493.xx during September 2012. Site of encounter was identified as: urgent care(UC), emergency department(ED), inpatient(IP), pulmonary and allergy/immunology clinics(SP), and primary care(PCC). Documentation of adherence was defined by specific mention regarding patient use or disuse of controller medication at any part of the EMR for that unique encounter.

RESULTS: 62%(N=676/1090) of EMR encounters reviewed had at least one controller medication prescribed at the time of encounter. Of the 676 encounters, documentation of adherence was observed in 78%(N=525). UC=36%(31/87) and ED=58%(54/93) had significantly less documentation of adherence compared with IP=92%(48/52), SP=87%(244/282), and PCC=91%(148/162)$p<0.05$. Use of an EMR asthma encounter template=60%(407/676) was associated with a significant increase in documentation of adherence=97%(394/407) vs. 49%(131/269)$p<0.05$. An EMR template was used less frequently in UC=16%(14/87) and ED=34%(32/93) compared with IP=77%(40/52), SP=65%(182/282), and PCC=86%(139/162).

CONCLUSIONS: Documentation of adherence at sites of urgent health care utilization (UC/ED) occurred less frequently compared with other sites of asthma care. Utilization of a prepopulated EMR template was associated with a significantly higher rate of adherence documentation. The use of an EMR template within the UC/ED setting could offer an important opportunity to address non-adherence with asthma controller medications.

542 High-Risk Asthma Multidisciplinary Care Clinic Adherence Linked To Asthma Control Test (ACT) Score Improvement

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RATIONALE: Children with high-risk asthma are at greater risk of having uncontrolled asthma flares, resulting in missed school days, ER visits/hospitalizations, decreased quality of life, and increased mortality. Psychological and social co-morbidities are associated with difficult-to-treat asthma, particularly urban populations. We hypothesized that attendance at a referral clinic with a multidisciplinary care approach, inclusive of allergist, pulmonologist, psychologist, social work and nursing specialists, would result in improved asthma control in a high-risk pediatric urban population.

METHODS: Visits between January 1, 2010 and December 31, 2012 were reviewed. McNemar's test compared the change from first to last visit of categorized variables. Mann-Whitney test was used to compare continuous variables and Chi-square for categorical variables. Linear regression was done with dependent variable: documented Asthma Control Test (ACT) score at last visit, and independent variables: number of visits, average time between visits, gender, and baseline ACT score.

RESULTS: No statistical difference was found comparing baseline ACT score for patients who did not follow up (clinic non-adherent) and those who followed up at least once (clinic adherent). Comparing first versus last visit ACT scores, more patients changed from uncontrolled (ACT<20) to controlled (ACT>19), compared to vice-versa ($p<0.02$). ACT score improvement was also more likely in females ($p<0.002$) or if the first visit ACT score was <20 ($p<0.045$). Regression analysis also showed that as time between visits increases, the final visit ACT score decreases.

CONCLUSIONS: Visit adherence to a multidisciplinary, high-risk, urban asthma clinic leads to improved ACT score. Further study is needed to identify barriers of visit adherence.

543 Understanding Asthma Medical Nonadherence In Adult and Pediatric Populations

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RATIONALE: Medical nonadherence has been identified as a significant reason for poor asthma control. The Medical Adherence Report Scale for Asthma (MARS-A) questionnaire, a 10 item self-reported measure of adherence with inhaled corticosteroids, provides insights to nonadherence behavior. We hypothesized that medical nonadherence among both adult and children are more likely due to unintentional nonadherence than intentional nonadherence.

METHODS: 115 adult patients between the ages of 46-77 and 159 pediatric patients between the ages of 4-11completed the study. 283 MARS-A surveys from the adult group and 254 from the pediatric group were collected during multiple visits from June 2010 to May 2011. Cronbach's alpha was calculated to assess the internal validity of the data. Nonparametric one-way ANOVA was used to globally compare the mean responses to each question. Unintentional medical nonadherence was defined by question 1 and intentional medical nonadherence was defined by Questions 2-9. A pairwise comparison using the Bonferroni approach was used to determine any differences between the questions.

RESULTS: Cronbach's alpha was 0.84 for the adult group and 0.80 for the pediatric group. ANOVA showed a very large significant difference ($p<10^6$) between the mean values of the 10 questions for both groups. Bonferroni approach showed question 1 was statistically lower than other questions except question 9 in the adult and question 10 in the pediatric populations.

CONCLUSIONS: This study supports the importance of addressing unintentional medication nonadherence to improve overall asthma medication adherence for both adult and pediatric patients.

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544 Improved Education and Self-Management In Children and Adolescents With Asthma Using a Personalized Smartphone Application

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RATIONALE: Electronic media consumption and mobile device use has rapidly expanded amongst children and adolescents. Engaging this population in asthma self-management is challenging, and suboptimal disease control is associated with increased healthcare utilization, cost and poor outcomes. We created an interactive, personalized smartphone application aimed to teach and improve asthma self-management skills directly to children and teenagers.

METHODS: A personalized, interactive iOS smartphone application (AsthmaCare) was created using Xcode (Apple Inc. Cupertino CA) and distributed to participants on iPod Touch devices. We conducted a prospective, 30-day pilot study of patients with asthma, ages 9-16 years old and who had been prescribed at least one controller medication, to assess if users engaged in self-management behaviors.

RESULTS: 21 patients completed the 30-day pilot study. Of all participants, 85% reported improved medication adherence by using AsthmaCare. Upon completion, asthma trigger avoidance skills were acquired by 42% of children and implemented by 100%. All participants preferred using AsthmaCare's interactive action plan compared to a written plan. Of all participants, 95% preferred smartphone applications to other methods they previously received for asthma monitoring and education.

CONCLUSIONS: Asthma educational and self-management content presented in our interactive smartphone app was highly accepted in the pediatric population. Electronic media is emerging as a superior platform to educate and engage this age group and should be considered for future asthma initiatives. Mobile health applications should be further developed to promote and improve asthma management and medication adherence.

545 Misuse Of Medical Devices Among Patients In a Tertiary Care Allergy/Immunology Practice

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RATIONALE: Medical devices are essential to managing numerous diseases. Inhalational devices are first line therapy in asthma and COPD, and epinephrine autoinjectors are first line treatment of anaphylaxis. Misuse of these devices translates into reduced clinical efficacy. Furthermore, improving technique improves clinical outcomes. We sought to determine if misuse of epinephrine and metered dose inhalers (MDIs) was a significant problem among patients previously verbally instructed in use of the device in our academic Allergy/Immunology practice.

METHODS: Patients were observed using their EpiPen or MDI with spacer and scored based on comparison to an accepted standard. For EpiPen, the manufacturer's instructions for use were the standard. In the case of MDI, a previously published standard was used. Performance of each step was scored as correct: yes or no. Data for possible confounders were collected.

RESULTS: 61 patients using EpiPen and 31 using MDIs were enrolled. 18% of patients used EpiPen properly. Of the remaining 82%, 48% missed >=3 steps. The most common error was not holding the unit in place for at least 10 seconds after triggering. 10% of MDI users demonstrated perfect technique. Of the remaining 90%, 74% missed <=4 steps. The most commonly missed step was exhaling to residual volume prior to actuating canister.

CONCLUSIONS: Misuse of EpiPen and MDIs was common in our clinics. This may interfere with delivery of effective therapy and may potentially be life-threatening. It is imperative to develop effective means of improving patients' long-term ability to use these devices properly. We are considering innovative strategies for this.

546 B Cells and Plasma Cells Populations Suffer Changes Along The Time After Dermatophagoides Pteronyssinus Specific Immunotherapy

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RATIONALE: The mechanism involved in the immunotherapy is still not well known, but it is established that immunological changes appear in a chronologic way and are related to B-lymphocytes and IgE production. We aim to monitor the immunological changes occurring in different subpopulations of B-lymphocytes and plasma-cells during the immunotherapy in allergic rhinitis patients sensitized to Dermatophagoides pteronyssinus (DP).

METHODS: Peripheral blood was collected during the immunotherapy (before and after 1, 3, 6 and 12 months) and the phenotypical analysis of different cell subpopulations performed using a FACSCanto II cytometer.

RESULTS: After 6 months of treatment we found a decrease of total B-cells ($p=0.021$) with an increase of DP-specific-B-cells along the time ($p=0.012$ at 12 months). We found also an increase of DP-specific-B-cells expressing IgE in the first month ($p=0.036$) that rapidly decrease after 3 months. Total plasma-cells increased after 1 month ($p=0.005$) returning to basal levels at month 12. Similarly an increase in plasmablasts has observed at 1 month ($p=0.012$) that return to basal levels at 3 months. However, both peripheral ($CXCR3^+$) and long-lived ($CXCR4^+$) plasma-cells show a decrease after 3 months ($p=0.036$ and $p=0.012$) that is maintained along immunotherapy. Regulatory B-cells decrease at 3 and 6 months ($p=0.005$ and $p=0.012$) but those producing IL10 increase ($p=0.035$ at 6 month).

CONCLUSIONS: DP-specific immunotherapy induces changes in the different B-cell subpopulation in very early stages. With a significant decrease of plasma-cells both peripheral and long-lived and an increase of IL10 regulatory B-cells which will influence the low production of IgE antibodies and treatment effectiveness.

547 A Subset Of Novel Timothy Grass Antigens Is Associated With Marked Th1/Th2 Shifts Following Specific Immunotherapy

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RATIONALE: T cells play an important role in the pathogenesis of allergic diseases. Here we tested a set of 822 novel Timothy grass (TG) peptides previously found to elicit Th2 responses in allergic donors for T cell reactivity in patients who received specific immunotherapy (SIT) treatment.

METHODS: A set of recently identified TG peptides from 93 novel TG proteins were screened for T cell reactivity in PBMCs from allergic and SIT treated donors by ELISPOT and intracellular cytokine staining. IL-4, IL-5, IL-13, IFNg and IL-10 production was determined.

RESULTS: Strong Th2 cytokine production detected in response to peptides from several novel antigens in allergic donors was significantly reduced in donors who had received SIT. This data was used to select 20 strongly modulated peptides that were tested as a pool in an additional independent cohort of 20 allergic and 20 SIT treated donors by ELISPOT and ICS. A significant decrease in Th2 cytokine production and an increase of INFg was detected in SIT donors. Further analysis demonstrated that the selected pool of peptides and associated antigens is predicted to provide substantial population coverage in diverse ethnicities.

CONCLUSIONS: Our findings demonstrate that the significant decrease in Th2 responses reported to accompany specific immunotherapy is not limited to responses targeting major known IgE-binding allergens but is also observed for recently discovered novel Timothy grass antigens. Since most of these antigens lack IgE reactivity in allergic donors, they may present promising candidates for specific immunotherapy.

548 Interleukin IL-27+ Dendritic Cells Modulate Ex-Vivo Th2 Responses In a Pdl-1-Dependent Manner and Increase In-Vivo Following Grass Pollen Immunotherapy

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RATIONALE: Interleukin (IL)-27 belongs to the IL-12 superfamily and comprises IL-12p28 and EBI3 subunits. We hypothesized that IL-27: (i) up-regulates PDL-1 on myeloid-derived dendritic cells (mDC) and inhibits Th2 responses following *ex-vivo* grass pollen allergen stimulation. ii) polarizes naïve T cells into IFN-γ+ T cells and IL-10+ iTregs. iii) IL-27+ mDC are induced following immunotherapy.

METHODS: IL-27-primed DCs (n=10) stimulated with *P. pratense*were co-cultured with CD4+ T cells. T cell proliferative and cytokine responses were measured by 3H-thymidine incorporation and Luminex MagPix assay, respectively. We quantified IL-27+ mDC in PBMCs obtained from I4 immunotherapy-treated (6 sublingual (SLIT), 8 subcutaneous (SCIT)), 14 untreated allergic (SAR) and 14 non-atopic participants (NA).

RESULTS: IL-27 significantly up-regulated PDL-1 expression mDCs (p=0.0004). Grass pollen-driven T effector cell proliferation were significantly supressed when allergen-stimulated IL-27-primed DCs were co-cultured with $CD4^{+}$T cells (p=0.02). Th2 cytokines were decreased in culture supernatants (IL-4, p=0.01; IL-5, p=0.02; IL-13, p=0.04). PDL-1 blockade by a specific monoclonal antibody resulted in loss of reduced proliferation (p=0.02). Exogenous exposure of naïve T cells (n=7) to IL-27 induced up-regulation of T-bet (p=0.02), IFN-γ (p=0.03) and IL-10 (p=0.02). IL-27+ mDCs and IL-27 levels in culture supernatants were significantly reduced in SAR compared to NA (p=0.005; p= 0.016). SLIT and SCIT-treated patients had elevated IL-27+ mDCs and IL-27 levels compared to SAR (p=0.05, p=0.02).

CONCLUSIONS: IL-27 supresses Th2 allergic responses in a PDL-1-dependent manner. AIT is associated with induction of IL-27+ mDCs. It's relevance in the induction of long-term tolerance after immunotherapy remains to be to be determined.

549 Local 'Protective' IgG4 Antibodies In Nasal Fluid Are Elevated Following Grass Pollen Immunotherapy

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RATIONALE: Grass pollen immunotherapy (AIT) is associated with induction of serum IgG4-associated blocking antibodies that prevent IgE-facilitated allergen binding to B cells. We hypothesised that inhibitory IgG4 antibodies are also induced locally in nasal fluid following AIT.

METHODS: Nasal fluid and sera were obtained from 7 untreated seasonal grass pollen allergics (SAR) and 8 AIT-treated (7 SCIT, 1 SLIT) and 8 non-atopic control (NA) participants. Specific-IgE and IgG4 to *Phleum pratense* (Phl p) components were measured by ISAC microarray technology. Inhibitory activity in nasal fluid and serum was measured using the IgE-FAB assay.

RESULTS: SAR participants had elevated levels of nasal and serum specific IgE to rPhl p 1 (p=0.001; p=0.0002) and rPhl p 5 (p=0.001; p=0.0002) compared to NA whereas nasal specific IgE to irrelevant allergens was undetectable. There was no difference in sIgE levels between SAR and AIT-groups in either serum or nasal fluid. Specific IgE against rPhl p 2, 4, 6, and 11 were detected in some but not all SAR and AIT groups. IgG4-associated inhibitory activity in nasal fluid and serum was significantly elevated in AIT compared to SAR groups (nasal fluid, p=0.0002; serum, p=0.0002). Functional nasal blocking antibodies correlated inversely with overall seasonal symptoms (all 3 groups combined, spearman, r= -0.43, p=0.04) and a trend was observed for measurements in serum (r= -0.37, p=0.08).

CONCLUSIONS: IgG4 antibodies with inhibitory activity for IgE-FAB were elevated in nasal fluid and serum following AIT. Further studies are needed to validate whether local nasal antibodies have potential as biomarkers for monitoring AIT.

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550 Grass Pollen Immunotherapy: Impaired Allergen-Induced Nasal and Cutaneous Responses Correlate With Overall Seasonal Symptom Scores and Are Associated With Suppressed Local Th2 Cytokines In Nasal Fluid

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RATIONALE: We previously optimised grass pollen nasal allergen challenge and measurement of Th2 cytokines in nasal fluid (Scadding et al, J Immunol Methods 2012;384:25-32). Here we test the utility of this methodology as surrogate for clinical response to immunotherapy.

METHODS: In a cross-sectional study we compared 14 grass pollen allergics, 14 non-atopic controls, 14 patients receiving grass pollen immunotherapy, and 4 having completed immunotherapy. Participants underwent nasal challenge and recorded symptoms and peak nasal inspiratory flow (PNIF) to 8 hours. Nasal fluid was collected using absorptive sponges and analysed for Th2 cytokines/chemokines by multiplex assays. Intradermal skin tests were recorded and participants completed retrospective seasonal symptom scores. Clinical and laboratory measurements were performed blind to clinical status of subjects.

RESULTS: Participants receiving immunotherapy had reduced nasal symptoms (45% lower combined early and late phase scores, $p=0.04$) and higher PNIF (54%, $p=0.02$) following challenge compared to untreated allergics; they had reduced early (15 minutes, 27% lower, $p=0.0007$) and late (8 hours, 51% lower, $p<0.0001$) skin responses and seasonal symptom scores (60% less, $p=0.003$). Skin late response and nasal challenge scores correlated with seasonal symptoms ($r=0.61$, $p<0.001$; $r=0.45$, $p=0.012$). Nasal fluid levels of IL-4, -5, -9, -13, and eotaxin peaked at 6-8 hours post-challenge in untreated allergics (all $p<0.001$ versus non-atopics at 8 hours). All five were lower in immunotherapy-treated participants, significant for IL-4, -9, and eotaxin at 8 hours (all $p<0.05$).

CONCLUSIONS: Grass pollen nasal allergen challenge represents a useful clinical surrogate and adjunct for assessing clinical outcome of allergen immunotherapy.

551 IL-4 and IL-13 Differentially Regulate TLR-Induced Eosinophil-Basophil Differentiation Of Cord Blood CD34+ Progenitor Cells

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RATIONALE: Intrauterine environmental exposures have been shown to influence neonatal immunity and subsequent atopy. The effect of "pro-allergic" T_H2 cytokines on neonatal cord blood (CB) hematopoietic progenitor cells, the phenotype and differentiation of which are associated with atopic risk, is currently unknown. Since we have previously shown that high-atopic risk infants have decreased eosinophil-basophil (Eo/B) differentiation after stimulation with LPS, we investigated whether a T_H2 milieu (IL-4 or IL-13) could influence LPS-induced Eo/B colony forming units (CFU), and the mechanism(s) through which this might occur.

METHODS: CB $CD34^+$ cells from healthy donors were stimulated with IL-4 or IL-13 (in combination with LPS) and assessed for Eo/B differentiation, using methylcellulose cultures and Eo/B differentiation-related intracellular signalling pathways, using flow cytometry. Additionally, we investigated the effects of pharmacological inhibitors of intracellular signalling in $CD34^+$ cells in relation to Eo/B CFU formation.

RESULTS: We found that stimulation of $CD34^+$ cells with IL-4, but not IL-13, reduced Eo/B CFU formation in the presence of LPS; this was found to be dependent on IL-4Rα and not IL-13Rα1. IL-4, but not IL-13, reduced the expression of ERK 1/2 after LPS stimulation, which was recovered by inhibition of IL-4Rα. Inhibition of ERK 1/2 likewise significantly reduced Eo/B CFU formation.

CONCLUSIONS: The responsiveness of CB $CD34^+$ progenitor cells to LPS is differentially regulated by the T_H2 cytokines IL-4 and IL-13. This may have implications for *in utero* interactions between placental (maternal)-derived T_H2 cytokines and neonatal hematopoietic progenitor cell contribution to the development of atopy.

552 Basophils Act As a Cellular Switch to Drive Eosinophilic Inflammation after IgE Activation

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RATIONALE: Hallmark pathologic findings in atopic dermatitis include elevated serum IgE and eosinophilic tissue infiltration. However, whether these associations are merely coincident or causal remains unclear. The goal of this study was to establish a model system to investigate the mechanistic role of IgE in driving eosinophilic dermatitis.

METHODS: We developed a passive sensitization system with intravenous IgE. After waiting for monomeric IgE to be cleared from the bloodstream, antigen was painted onto the ears of mice. Eosinophilic infiltration was assessed by both flow cytometry of skin homogenates and whole mount confocal microscopy. To investigate the role of specific IgE-binding cells, we utilized mice with a global deletion in the high-affinity IgE receptor (FcεrI) as well as specific ablation of mast cells and basophils using lineage-specific cremouse strains.

RESULTS: We established a requirement for IgE to drive eosinophilic dermatitis in our model system. This eosinophilic dermatitis was dependent on FcεrI+ cells. Mast cells were not required to drive eosinophilic infilitration. By contrast, lineage ablation of basophils also abrograted eosinophilic infiltration. Imaging analysis suggested an overlapping distribution of basophil and eosinophil infiltration.

CONCLUSIONS: In a model of IgE-driven eosinophilic inflammation, basophils act as a cellular switch that biases inflammation toward a type 2, allergic immune response. This action is engendered through IgE/Antigen activation on basophils. Co-localization of basophils and eosinophils also supports a hierachical relationship between these two myeloid cells. Further studies will focus on elucidating how activated basophils drive eosinophilic inflammation.

553 The SNARE VAMP-7 Contributes To Eosinophil Degranulation, In Vivo

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RATIONALE: Eosinophil degranulation is associated with allergic inflammation, and occurs through regulated exocytosis. Regulated exocytosis in eosinophils is dependent on *vesicle associated membrane protein* (VAMP), a prominent component of the universal SNARE (*soluble NSF attachment protein receptor*) complex. We have previously demonstrated a role for VAMP-7 in human eosinophil activation. We hypothesized that VAMP-7 is critical for eosinophil degranulation *in vivo*.

METHODS: Gene deletion of VAMP-7 in mouse eosinophils was carried out by crossing an eosinophil-specific *Cre* recombinase-expressing strain of mice (*eoCre*) with "floxed" VAMP-7 mice to achieve VAMP-7 gene deficiency exclusively in eosinophils (*eoCre/V7*). Eosinophil degranulation was determined by release of eosinophil peroxidase (EPX), major basic protein (MBP), and eosinophil-associated ribonucleases (Ears) using: (i) platelet-activating factor (PAF), interleukin-33 (IL-33) with or without Ca^{2+} ionophore; and (ii) adoptive transfer of eosinophils into airways of double transgenic interleukin-5 (*IL-5*)/human eotaxin-2 (*hE2*) mice that were deficient in EPX gene expression (*IL-5/hE2/EPX*$^{-/-}$).

RESULTS: *eoCre/V7* mice exhibited gene deletion in eosinophils, with no evidence of deletion in other cell types. Following stimulation of *eoCre/V7* eosinophils with PAF, IL-33 and ionomycin, degranulation was significantly reduced relative to wild-type eosinophils as assessed by EPX, MBP, and Ears release. Inhibition of EPX release was confirmed *ex vivo* following adoptive transfer of *eoCRE/V7/EPX*$^{+/+}$ eosinophils into the airways of *IL-5/hE2/EPX*$^{-/-}$ mice.

CONCLUSIONS: These data suggest that VAMP-7-mediated granule exocytosis is a key component of eosinophil degranulation, which provides us with a unique opportunity to test the importance of SNARE-mediated granule protein release in animal models of eosinophilic diseases.

554 Microbiota Regulates Eosinophils In The Small Intestine

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RATIONALE: Eosinophils are common residents in the small intestine (SI) under healthy, homeostatic conditions. However, relatively little is known about the mechanisms that regulate their presence and activity in the gastrointestinal tract. The microbiota has emerged as a critical component for immune development and homeostasis. Whether the microbiota regulates the number and/or function of SI eosinophils is unknown at this time.

METHODS: Germ free (GF) and specific pathogen free (SPF) mice on a BALB/c and C57BL/6 background were used to collect SI, bone marrow and spleen for histological evaluation and comprehensive flow cytometry analysis. GF mice were subjected to either full colonization after one month of co-habitation with SPF mice or specific colonization with altered Schaedler flora (ASF). Eosinophil peroxidase activity of intestinal homogenates from GF mice and SPF mice was assessed.

RESULTS: The number of eosinophils in the lamina propria of the SI of GF mice was significantly greater compared to SPF mice while no differences were found in the bone marrow and the spleen. Full colonization of GF mice reduced eosinophils in the lamina propria back to levels observed in SPF mice, while ASF colonization induced a partial decrease. Histology and eosinophil peroxidase assays suggested higher activation of SI eosinophils from GF mice.

CONCLUSIONS: The intestinal microbiota regulates the number and arguably, activity of eosinophils populating the lamina propria of the small intestine, and this effect is dependent on the complexity of the microbiota.

555 The Airway Mucins Muc5b and Muc4 Are Endogenous Ligands For Siglec-F and Induce Mouse Eosinophil Death

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RATIONALE: Siglec-F is a glycan binding protein expressed on mouse eosinophils and its engagement induces apoptosis, suggesting a pathway for controlling eosinophil-associated diseases. Siglec-F recognizes sialylated, sulfated glycans in vitro, but the identities of endogenous glycoprotein ligands in vivo are unknown.

METHODS: Lungs from normal and mucin-deficient mice, as well as tracheal epithelial cells from mice (mTEC) were examined. Western blotting and immunocytochemistry was completed looking for Siglec-F-Fc binding glycoproteins. Liquid chromatography-tandem mass spectrometry analysis of Siglec-F-Fc binding glycoproteins was performed, and mouse eosinophil mucin binding and cell death assays were done using flow cytometry.

RESULTS: We characterized glycoproteins isolated from mTEC and mouse lung tissue homogenates that bind to Siglec-F-Fc in a sialic acid dependent manner. Binding of Siglec-F-Fc to mTEC was sialidase-sensitive and was increased after treatment with IL-4 or IL-13. Sialidase-sensitive, PNGaseF-resistant binding of Siglec-F-Fc to glycoproteins of apparent MW $\approx$ 500 kDa and 200 kDa was detected by western blotting of mTEC lysates and culture supernatants, indicating the importance of sialylated O-linked glycoprotein glycans for Siglec-F binding. The expression of these glycoprotein ligands was increased during mouse allergic airways inflammation. Liquid chromatography-tandem mass spectrometry-based proteomics, cross-immunoprecipitation, and histochemical analysis of lungs from mucin-deficient mice assigned and validated the identity of the glycoproteins as Muc5b and Muc4. Purified Muc5b/Muc4 carried sialylated and sulfated glycans, bound to eosinophils and induced their death in vitro.

CONCLUSIONS: These data identify a previously unrecognized endogenous anti-inflammatory property of mucins by which their sialylated glycans can control lung eosinophilia through Siglec-F engagement.

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556 Impact Of Asthma Exacerbations On Lung Function In a Large Cohort Of Patients With Severe Or Difficult-To-Treat Asthma

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RATIONALE: Asthma exacerbations contribute to morbidity and mortality, but limited evidence exists about the extent to which such exacerbations may lead to worsening airway obstruction.

METHODS: Patients with severe or difficult to-treat asthma were followed observationally for three years in The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR). Percent predicted post-bronchodilator forced expiratory volume in one second ($ppFEV_1$) was collected annually, and asthma exacerbations, defined as an overnight hospitalization, emergency room visit, or steroid burst, were assessed bi-annually. Patients with chronic obstructive pulmonary disease and current smokers were excluded from analyses. Annual change in $ppFEV_1$ was modeled using repeated measures as a function of asthma exacerbations during that year, baseline $ppFEV_1$, age, sex, race/ethnicity, and body mass index.

RESULTS: A total of 2,429 patients (n=1,803 adults ≥18 years; n=394 adolescents ages ≥12-17 years; n=232 children ages 6-11 years) met the entry criteria. After adjustment for covariates, the 12-month change in $ppFEV_1$ was lower in patients with any asthma exacerbation compared with those with no asthma exacerbation (-1.27±0.26 vs. 0.70 ±0.22; net difference: 1.97±0.36; p<0.001); in adults (-1.14±0.30 vs. 0.68±0.25; net difference: 1.82 ±0.41; p<0.001); in adolescents (-2.46±0.74 vs. 0.46±0.59; net difference: 2.92±0.98; p=0.004); in children (-1.05±0.79 vs. 0.99±0.76; net difference: 2.04±1.14; p=0.081).

CONCLUSIONS: Asthma exacerbations are associated with lung function decline in patients with severe or difficult-to-treat asthma. Together with prior evidence, this research suggests that prevention of asthma exacerbations may limit airway remodeling and declines in irreversible airway obstruction.

557 Analysis Of Severe Asthma Phenotypes By Using High-Resolution Computed Tomography: Relation To Clinical Assessment

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RATIONALE: Recent studies suggest that severe asthma is a heterogeneous syndrome with different phenotypic characteristics. However, there have been few attempts to classify severe asthma according to the structural differences of airway and lung parenchyma.

METHODS: We enrolled 71 patients with severe asthma who underwent chest computed tomography and reviewed medical records. Two radiologists blinded to clinical information performed visual analysis in consensus for the extent and severity of bronchial wall thickening (BT), mucus plugging (MP), and the extent of bronchiectasis (BE). The association between radiologic findings and clinical characteristics in severe asthma subjects was evaluated.

RESULTS: In radiologic analysis, most subjects were classified into one of three types: near-normal type (n=19), large airway remodeling type, which mainly exhibited diffuse BT combined with MP and BE (n=29), and small airway remodeling type, representing prominent low lung attenuation (n=15). Patients with large airway remodeling type had a tendency to use more controller medications and oral steroids as a maintenance therapy compared to patients with near-normal type. However, there were no differences in mean age, disease duration, atopic status, sputum/peripheral blood eosinophil and total serum IgE level between these two groups despite overt radiologic differences. Small airway remodeling type on CT were associated with older age, late onset, male-predominance, more smoking history, lower FEV1/FVC with fixed airway obstruction, and more frequent healthcare use due to acute exacerbation than other groups.

CONCLUSIONS: Severe asthma can be classified into 3 different types based on the radiographic imaging and the radiologic types may enhance characterization of phenotypes in addition to the clinical features.

558 Associations Between The Expression Of Corticosteroid Regulated Genes By Peripheral Blood Mononuclear Cells (PBMCs) In Children From The NIH/NIAID Sponsored Asthma Phenotypes In The Inner City (APIC) Study

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RATIONALE: Development of peripheral blood markers to predict/monitor therapeutic responses to corticosteroids and LABA in asthmatic children is of great interest.

METHODS: APIC study seeks to define phenotypic characteristics of Difficult-to-Treat asthma among children (ages 6-17 years), receiving one year of asthma guidelines-based therapy. As part of the study, PBMC were collected from 112 asthmatics children after one-month of NAEPP guidelines-based therapy. Based on initial controller medication regimen, patients were categorized as mild (no ICS or Flovent 50mcg bid), moderate (Flovent 100mcg or 250mcg bid) or severe (Advair 250mcg/50mcg or 500mcg/50mcg bid). Based on initial asthma control scores, patients were categorized as controlled or uncontrolled. PBMC expression of corticosteroid-regulated genes (glucocorticoid receptor alpha (GRalpha), mitogen-activated kinase phosphatase 1 (MKP-1), IL-8, TNFalpha) at baseline and in response to 10^{-8}M fluticasone and the expression of vitamin D-regulated cyp24a mRNA were determined by RTPCR.

RESULTS: Based on medication regimen and asthma control 22, 33, 43 and 14 patients were categorized as mild/controlled, moderate/controlled, severe/controlled and severe/uncontrolled, respectively. Severe asthmatics (controlled+unconcontrolled) had the lowest PBMC cyp24a expression (p<0.001 and p=0.05 as compared to mild and moderate groups) and significantly lower expression of GRalpha (p=0.05 and p<0.01 as compared to mild and moderate groups). PBMC from patients in severe/controlled group had the highest expression of MKP-1 and IL-8 (p=0.05 as compared to mild/controlled group) at baseline. PBMC in severe/uncontrolled group had the lowest IL-8 and TNFalpha suppression by fluticasone among the study groups.

CONCLUSIONS: Preliminary studies indicate that expression of corticosteroid-regulated targets in PBMCs varies with clinical disease severity.

559 Bronchial Mast Cell Markers and Clinical Asthma Severity In Steroid Refractory Asthmatics

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RATIONALE: Mast cells are thought to play a significant role in asthma and are resistant to corticosteroid effects. Bronchial mast cells express chymase, tryptase and the c-kit receptor. We investigated the relationship between these mast cell markers and clinical asthma severity measurements in patients with steroid refractory asthma.

METHODS: We recruited adult asthmatics with severe asthma symptoms (ACQ>1.5) on continuous treatment with high-dose inhaled corticosteroids (ICS) and at least one additional controller therapy. All subjects had confirmed airway hyperresponsiveness with methacholine challenge (PC_{20}< 10 mg/ml). Endobronchial biopsies were obtained from these subjects (n=27) and mast cells were immunostained for tryptase and chymase. Bronchoalveolar lavage (BAL) fluid (n=28) was processed and c-kit mRNA was measured in the BAL cell pellet using qPCR.

RESULTS: Intramucosal chymase positive mast cell numbers show a positive correlation with log PC20 (r=0.45, p=0.02); suggesting decreasing methacholine hyperresponsiveness with increasing chymase positive mast cells. Intramucosal tryptase positive mast cell counts did not show any significant association with log PC20. FEV1/FVC ratio had a negative correlation with c-kit mRNA in the BAL cell pellet (r=-0.37, p=0.05).

CONCLUSIONS: Increased c-kit mRNA in BAL is related to worsening airway obstruction suggesting mast cell activity contributes to poor asthma control in steroid unresponsive asthmatics. It has been previously reported that chymase positive mast cells in severe asthma correlate with better lung function. The negative correlation between chymase positive mast cells and airway hyperresponsiveness in our study supports a unique pathobiological role for this mast cell phenotype in severe asthmatics.

560 Antagonistic Effects Of Ozone (O3) Exposure and Glucocorticoid Treatment On Airway Hyperresponsiveness (AHR) and Surfactant Protein D (SP-D) Production In Mice

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RATIONALE: Balb/c mice express relatively low levels of the immunoprotective SP-D and are highly susceptible to O_3–induced exacerbation of allergic airway inflammation. We hypothesized that therapeutic induction of SP-D would alleviate this susceptibility.

METHODS: Primary human lung type II alveolar epithelial cells and A549 cells were studied for SP-D mRNA and protein expression and signaling pathways in response to O_3 (0.9ppm for 1.5h) dexamethasone and budesonide (0-100nM). Balb/c mice were sensitized and challenged with *Aspergillus fumigatus* (*Af*) and were treated with (1μM) budesonide immediately, exposed to O_3 or air 96h later and studied 12h after O_3 inhalation. Lung function was studied by methacholine responsiveness, (Flexivent) and pulmonary inflammation was assessed by FACS.

RESULTS: Airway epithelial cells produced SP-D to budesonide or dexamethasone treatment, in a dose-dependent manner. The stimulatory effects of glucocorticoids on pSTAT3, SP-D mRNA and protein production were diminished in O_3 exposed airway epithelial cells *in vitro*. Treatment of allergen sensitized Balb/c mice with budesonide enhanced SP-D expression in the airways, reduced eosinophil and CD4 T cell influx and inhibited AHR (p<0.05, n=8). In O_3-induced exacerbation of allergic inflammation however budesonide treatment failed to increase SP-D or reduce AHR while its inhibitory effects on eosinophil and CD4 T cell number were preserved.

CONCLUSIONS: O_3 exposure induced differential glucocorticoid insensitivity in airway epithelial cell SP-D production and in airway cells responsible for physiological changes leading to methacholine AHR. This mechanism may have high clinical significance in the pathogenesis of glucocorticoid resistant asthma.

561 Impaired Glycosylation Due To Autosomal Recessive PGM3 Mutations Results In Atopy, Immune Deficiency, Autoimmunity, and Neurocognitive Impairment

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RATIONALE: Understanding how specific genetic mutations lead to atopy and immune dysregulation can provide new insights into the orgins of allergic disease and illuminate novel therapeutic targets.

METHODS: Eight patients from two ethnically distinct families were identified with a common syndrome of severe atopy, elevated serum IgE, immunodeficiency, autoimmunity, and neurocognitive impairment. Comprehensive clinical evaluations were conducted, including brain MRI and sensory evoked potentials. Immunophenotyping and intracellular cytokine staining of circulating lymphocytes were performed by flow cytometry, and humoral and proliferative immune responses were assessed. Whole exome sequencing was performed to identify common genetic variants. Immunoblotting, qRT-PCR, and MALDI-TOF mass spectrometry were used to assess the molecular consequences of the mutations.

RESULTS: Novel mutations in a single gene, phosphoglucomutase 3 (PGM3) segregated with disease in an autosomal recessive manner. Increased Th2 and Th17 cytokine production by CD4+ T cells correlated clinically with atopic disease and autoimmunity. T cell lymphopenia, particularly of CD8+ T cells, and reduced memory B cells were associated with increased viral and bacterial infectious susceptibility, respectively. Consistent with clinical neurocognitive abnormalities, patients had markedly delayed evoked potentials and a pattern consistent with hypomyelination on MRI. While PGM3 protein expression was variably diminished, impaired enzymatic function was demonstrated by decreased O-linked protein glycosylation. Additionally, the abnormal cellular phenotype could be corrected *in vitro* with exogenous N-acetylglucosamine.

CONCLUSIONS: Hypomorphic PGM3 mutations result in abnormal protein glycosylation and result in an autosomal recessive disorder of elevated serum IgE, atopy, immunodeficiency, autoimmunity, and neurologic impairment.

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562 Expansion Of Circulating T Follicular Helper Cells In CVID Patients With Autoimmune Cytopenias

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RATIONALE: Peripherally circulating T cells, resembling germinal center T follicular helper cells, have been proposed as a biomarker in rheumatologic diseases. We have examined the peripheral blood of CVID patients with and without autoimmune cytopenias for the presence of these cells.

METHODS: We enumerated circulating $CD4^+CXCR5^+PD\text{-}1^{hi}$ T cells in the blood of CVID patients and healthy donors by flow cytometry. When present, we compared this cell population to classical T follicular helper cells from the tonsils of healthy donors by measuring the expression of characteristic co-activation markers and the transcription factor BCL6. For each patient we correlated the frequency of $CD4^+CXCR5^+PD\text{-}1^{hi}$T cells with other laboratory and clinical features relevant to CVID.

RESULTS: Many CVID patients displayed abundant circulating populations of $CD4^+CXCR5^+PD\text{-}1^{hi}$ T cells that expressed co-activation molecules (ICOS, CD40L) and the transcription factor BCL6. The frequency of circulating $CD4^+CXCR5^+PD\text{-}1^{hi}$ T cells was linearly related to the the frequency of $CD21^{-/lo}$ B cells (R^2=0.62, p<0.0001) and inversely related to the frequency of FOXP3+ T regulatory cells (R^2=0.59, p<0.0001) in peripheral blood. An elevated frequency of circulating T follicular helper-like cells in CVID patients accurately predicted the presence of comorbid autoimmune cytopenias.

CONCLUSIONS: Circulating $CD4^+CXCR5^+PD\text{-}1^{hi}$ T cells in CVID patients resemble conventional T follicular helper-like cells and may be a useful biomarker of CVID related autoimmunity.

563 Treatment Of Murine Chronic Granulomatous Disease (CGD) With The PPARγ Agonist Pioglitazone Enhances Phagocyte Mitochondrial Reactive Oxygen Species (ROS) Production and Antimicrobial Responses

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RATIONALE: PPARγ agonists enhance mitochondrial biogenesis and ROS production via "starvation signaling." Mitochondrial ROS contribute to bactericidal activity of phagocytes. It was hypothesized that PPARγ agonist treatment of CGD mice would enhance phagocyte mitochondrial ROS production and microbicidal activity.

METHODS: Following pioglitazone treatment (5 days), blood and exudate neutrophils and monocytes/macrophages from wild type (WT) and $gp91^{phox-/-}$ (CGD) mice were characterized for ROS production and killing of *Staphylococcus aureus*(SA).

RESULTS: Blood neutrophils and monocytes from WT and CGD mice treated with pioglitazone (or vehicle) were tested for ROS production by dihydrorhodamine fluorescence following *ex vivo* PMA stimulation. Pioglitazone (but not vehicle) treatment of CGD mice resulted in a subpopulation of neutrophils (30%) and monocytes (38%) that produced ROS to levels comparable with WT phagocytes. Similar results were demonstrated for exudate phagocytes lavaged from zymosan-inflamed peritonea. Pretreatment of phagocytes from both genotypes and treatment groups with the inhibitor diphenyleneiodonium ablated stimulated ROS production indicating an alternative flavochrome(s). Further characterization of ROS from the phagocytes of pioglitazone-treated CGD mice showed that superoxide was produced (cytochrome c reduction inhibitable with superoxide dismutase), and the source was mitochondria. Phagocytes from pioglitazone-treated CGD mice showed significantly enhanced killing of SA *in vitro*: 44% of WT killing (vs. 11% for vehicle-treated). *In vivo* killing was also enhanced: 24h SA recovery following peritoneal injection of 2x10e7 CFU was 4.2x10e5 for WT and 2.6x10e6 in pioglitazone-treated CGD mice (compared to 4.2x10e7 with vehicle treatment).

CONCLUSIONS: PPARγ agonism significantly enhances mitochondrial ROS production and antimicrobial responses in CGD phagocytes warranting further investigation.

564 Patient Specific Targeted Gene Therapy In The Treatment Of X-Linked Hyper-IgM Syndrome

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RATIONALE: X-linked hyper-IgM Syndrome (XHIM) is a primary immunodeficiency with absent IgG, IgA, IgE and normal/elevated IgM due to defective CD40 ligand (CD40L). Although stem cell transplantation is curative, it is frequently complicated by significant risks. Previous gene therapy trials with a constitutively expressed CD40L transgene in mice resulted in lymphoproliferation. An alternative approach is targeted gene repair with TAL effector nucleases (TALENs), which target specific DNA sequences and create double-strand breaks (DSBs), combined with homologous donor sequences serving as repair templates to allow homology directed repair of DSBs and physiologic expression of CD40L.

METHODS: TALEN pairs are created following a previously published protocol. After electroporation of K562 cells with TALEN plasmids, allelic disruption is quantified by a surveyor endonuclease assay (Cel-1). Donor molecules containing the correct DNA sequence are introduced with TALEN plasmids into hematopoietic cells from CD40L deficient patients to assess for restored CD40L expression.

RESULTS: T cells were isolated from an XHIM patient, transformed with HTLV-1, and has been in continuous culture for nine months. This patient was found to have a spice site mutation in the first base pair of intron 3 and three TALEN pairs targeting this location have been assembled. Preliminary studies using TALENs targeting a different locus of the CD40L gene have demonstrated allelic disruption in up to 31% and targeted gene insertion of GFP in up to 10% of K562 cells.

CONCLUSIONS: This approach allows site-specific correction and physiologic expression of the endogenous CD40L gene to safely provide permanent immune reconstitution.

565 Digeorge Syndrome Found By SCID Newborn Screening In California

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RATIONALE: DiGeorge Syndrome (DGS) includes a wide spectrum of congenital abnormalities; particularly hypocalcemia, cardiac defects, and T cell lymphopenia (TCL). Screening 1.5 million newborns in California over 3 years for T cell receptor excision circles (TRECs), yielded 18 DGS patients with <1500 T cells/uL.

METHODS: We reviewed SCID newborn screening (NBS) data, including flow cytometry for all infants with low TRECs or 2 incomplete TREC tests. We also reviewed charts of 4 NBS-detected DGS patients followed at UCSF. CGH array, FISH or TBX1gene sequencing confirmed DGS.

RESULTS: Of 88 TCL infants found by NBS, 18 had DGS (6% of total predicted DGS in California, assuming 1/5000 incidence). Two complete DGS infants had undetectable T cells, one of whom received a thymus transplant. Cardiac anomalies affected 75% of patients, one of whom died. Median cell values were: CD3, 1060/μL (0-1447); CD4, 660/μL (0-1045); CD8, 317/μL (0-558); CD19, 412/μL (74-3720); CD16/56, 336/μL (20-1447); CD4/CD45RA, 566/μL (0-825); CD8/CD45RA, 298/μL (0-520). Two cases unsuspected prior to the NBS alert had only one clinical symptom: nasopharyngeal reflux or hypocalcemia. Another patient with the classic DGS triad but a normal CGH array had a novel intragenic TBX1truncation mutation. All others had 22q11.2 deletion. Three of the four UCSF patients had normal mitogen blastogenic responses and specific antibodies following killed vaccines.

CONCLUSIONS: NBS successfully detected the DGS patients with TCL, who are at risk for infections. Live rotavirus vaccine was avoided and parents were alerted to seek medical attention early for suspected infections.

566 Urinary Levels Of Phytoestrogens Are Inversely Associated With Wheezing, Asthma, and Atopy

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RATIONALE: Previous in vitro studies support an immunomodulatory effect for some phytoestrogens, but human evidence is limited. We were interested in examining the relationship between these seed- and legume-derived antioxidants and markers of asthma and allergy.

METHODS: Data were obtained from the National Health and Nutrition Examination Survey 2003-2010. Urinary phytoestrogen levels and a history of wheezing and asthma, total IgE levels, and atopy were obtained from 10,708 subjects. Atopy was defined as having ≥1 positive specific IgE level (≥0.35 kU/L) to an aeroallergen. Logistic and linear regression were used to determine associations between phytoestrogen levels and outcomes, adjusting for creatinine, age, sex, ethnicity, and poverty index ratio.

RESULTS: Urinary levels of daidzein, O-DMA, enterodiol, and enterolactone were inversely associated with wheezing (e.g. OR for third vs. first tertile of O-DMA, 0.76, 95% CI 0.64-0.92; P=0.004 for trend). In a model including all phytoestrogens, only enterolactone was independently associated with reduced risk of wheezing (OR for third vs. first tertile 0.76, 95% CI 0.70-0.83). Enterolactone was also inversely associated with asthma (OR for third vs. first tertile 0.77; P=0.01 for trend). The odds of atopy significantly decreased with increasing daidzein levels (OR for third vs. first tertile 0.68, 95% CI 0.52-0.90; P=0.01 for trend). O-DMA and daidzein were associated with lower levels of total IgE (P=0.01).

CONCLUSIONS: In this large, nationally representative sample, increased urinary phytoestrogen levels were inversely associated with markers of asthma and allergy. Increased consumption of phytoestrogens may help prevent or treat asthma and allergic disease.

567 Vitamin D Treatment Is Protective Of Inhalant Organic Dust-Induced Bone Loss

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RATIONALE: Skeletal health consequences associated with inflammatory airway diseases contribute to disease morbidity. We recently reported that repetitive inhalation exposure to non-infectious inflammatory agents such as complex organic dust extract (ODE), endotoxin, and peptidoglycan resulted in bone loss in mice. We sought to determine whether vitamin D treatment would be protective against adverse airway and bone consequences following subchronic airway injury.

METHODS: C57BL/6 mice were maintained on diets with either low (1 IU D/g) or high (10 IU D/g) vitamin D for 5 weeks, and intranasally treated with ODE or saline daily for 3 weeks per established and institutionally approved animal protocols. Bronchoalveolar lavage fluids and lung tissues were collected to quantify airway inflammatory responses. Hind limbs were isolated, processed, scanned, and analyzed using a micro-CT system that allows for quantifying 3D changes in bone morphology.

RESULTS: Serum 25-hydroxy vitamin D levels were decreased between the low (~ 7.5 ng/ml) vs. high (75-80 ng/ml) vitamin D treatment group with no difference between saline/ODE treatments. There was no difference in ODE-induced influx of neutrophils, macrophages and lymphocytes or lung histopathology between vitamin D treatment groups. ODE-induced IL-6 and CXCL2 were significantly reduced in animals on high vitamin D diet. Micro-CT analysis of trabecular bone showed the loss of bone mineral density, volume and deterioration of bone micro-architecture and mechanical strength, induced by inhalant ODE, were profoundly reduced in animals on high vitamin D diet.

CONCLUSIONS: High concentration vitamin D treatment protects against systemic bone loss resulting from subchronic airway injury induced by organic dust exposures.

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568 Antigenic Determinants On Der p 1 Identified By Mutagenesis Analysis Based On The Structure Of Allergen-Antibody Complexes

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RATIONALE: Der p 1 is a major dust mite allergen, cross-reactive with the homolog Der f 1. Two structures of Der p 1 in complex with Fab fragments of either monoclonal antibody (mAb) 4C1 or 5H8, that partially inhibit IgE antibody binding, were solved by X-ray crystallography. The goal was to identify the residues involved in allergen-antibody interactions, and IgE antibody binding sites by site-directed mutagenesis.

METHODS: Single or multiple mutants of Der p 1 residues involved in mAb 5H8 and/or 4C1 binding were expressed in *Pichia pastoris* and purified by affinity chromatography. IgE and mAb binding assays were performed by ELISA using sera from mite allergic patients.

RESULTS: The capacity of the mutants to bind the mAbs was reduced up to 40 times for 5H8 and almost completely for 4C1, depending on the mutation, and according to the displacement of the mAb direct binding or inhibition curves. This result confirmed the importance of specific residues in the allergen-antibody interaction. Specific mutations in each epitope also reduced IgE antibody binding in different degrees (up to 40%) depending on the patient's serum and the mutation, and proved an overlap between IgE and mAb binding sites.

CONCLUSIONS: Site-directed mutagenesis analysis of residues from epitopes identified by X-ray crystallography revealed mechanisms of antibody recognition. Amino acids important for allergen-antibody interaction were identified for the expression of allergen mutants with reduced IgE antibody reactivity with potential use for immunotherapy.

569 Development Of IgE Against a Cimex Lectularius Allergen After Being Bitten By Bed Bugs Was Common Among Children In NYC

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RATIONALE: *Cimex lectularius*, the bed bug, has made a resurgence in New York City (NYC). Previously we demonstrated that IgE antibodies against allergens from *C. lectularius* including a salivary *C. lectularius* nitrophorin protein (cNP) were common among adults reporting recent bed bug bites. We hypothesized that some children would develop IgE antibodies against cNP subsequent to being bitten by bed bugs.

METHODS: As part of the New York City Neighborhood Asthma and Allergy Study, an asthma case-control study, 7-8 year-old children were recruited through a middle-income health insurance plan and are being followed to age 10-11. Serum samples were collected at ages 7-8 and 10-11. Parents were queried about the child being bitten by bed bugs between ages 7-8 and 10-11. IgE against cNP was measured by ImmunoCAP as described [JACI 2012;129:863-5].

RESULTS: Ten children had a report of being bitten by bed bugs between the baseline and follow-up serum collection. Of these, one child had measurable IgE against cNP (>0.1 IU/ml) at baseline (and at follow-up). Among the other nine children without measurable IgE at baseline, five had developed IgE against cNP by follow-up. In this group of 5 incident IgE cases, anti-cNP IgE ranged from 0.14-10.4 IU/ml (geometric mean=1.2 IU/ml). Two of the five incident IgE cases did not have IgE against other common inhalant allergens, including the child with the highest IgE against cNP.

CONCLUSIONS: To our knowledge, this is the first study to demonstrate the development of an IgE response to *C. lectularius* following bed bug bites.

570 Divergent Effects Of Endotoxin and Mold Exposure On Asthma Exacerbations In The Childhood Asthma Management Program (CAMP)

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RATIONALE: Both endotoxin and mold exposures have been associated with asthma severity. No study has evaluated their effects on long-term lung function and exacerbation risk. We used the recombinant Factor C (rFC) assay instead of the Limulus Amebocyte Lysate (LAL) assay to distinguish endotoxin exposure from molds (LAL detects both endotoxin and mold glucans); hence independent effects of home endotoxin and mold exposure on asthma outcomes were evaluated.

METHODS: From CAMP, 951 participants with mild/moderate asthma had both baseline house-dust samples to measure endotoxin (rFC), and mold counts (settled plates). Asthma outcomes from the first 4 years of the study: spirometry, methacholine challenges, and exacerbations (using number of prednisone courses, number of prednisone days, ER/hospitalization). Log transformed endotoxin and mold counts were analyzed separately and combined.

RESULTS: No association was found between baseline endotoxin concentrations and mold counts and spirometry measures of lung function or FEV_1 log PC_{20} during the first four years of the CAMP study. A significant inverse relationship was found between log endotoxin and the number of prednisone days during the trial; a log change in endotoxin corresponded to 5.2% relative decrease in prednisone days ($p<0.0001$). In contrast, high mold counts were associated with 10.6% relative increase in prednisone days ($p<0.0001$). Site-specific differences in the association between endotoxin concentrations or mold counts and exacerbations during the CAMP trial were found.

CONCLUSIONS: In children with mild-to-moderate persistent asthma, endotoxin and mold exposures impact future exacerbation risk; however, their effects on exacerbation are divergent and affected by locality.

571 Inhibition Of Epidermal Tight Junction Function By Histamine Is Mediated By H1 and H4 Receptors

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RATIONALE: Atopic Dermatitis (AD) is characterized by stratum corneum and tight junction (TJ) barrier defects. Histamine is increased in lesional AD skin. Recently, histamine has been shown to inhibit human keratinocyte (KC) terminal differentiation and promote proliferation. KC express histamine receptor 1 (H1R), H2R and H4R.

METHODS: Ca^{+2}-differentiated primary KC and epidermal explants were treated with histamine (100 μM) to determine the effect this had on TJ function. TJ integrity was assessed by trans-epithelial electrical resistance (TEER) and paracellular fluorescein flux. Selective antagonists were used for each receptor: H1R (Cetirizine, 10 μM), H2R (Cimetidine, 100 μM), or H4R (JNJ7777120, 10 μM). Keratinocyte differentiation was assessed by examining filaggrin, loricrin and keratin 10 protein expression by Western blot. Expression level of HRs were evaluated by PCR in skin biopsies of AD (n=6-8) and non-atopic (n=10) subjects.

RESULTS: Histamine significantly reduced TEER, in both KC (0.6 fold, $P<0.001$, n=7) and *ex vivo* skin explants (0.7 fold, $P<0.05$, n=3), and enhanced fluorescein permeability flux of PHK (2.2 fold, $P<0.05$, n=7) and skin explants (1.3 fold, $P<0.05$, n=3). In KC, H1R and H4R, but not H2R, antagonists blocked histamine-mediated TEER reduction. We confirmed that histamine selectively reduced filaggrin. Only H1R antagonist was able to prevent the histamine-mediated reduction of filaggrin expression. H1R was reduced in AD skin lesional and non-lesional ($P<0.01$). No significant changes in H4R expression were found.

CONCLUSIONS: Our studies revealed that histamine might contribute to epidermal barrier impairment observed in AD skin, by reducing TJ integrity (H1R and H4R -dependent) and filaggrin expression (H1R-dependent).

572 The Natural History and Clinical Predictors Of Egg Allergy In The First 2 Years Of Life: A Prospective, Population-Based, Cohort Study

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RATIONALE: There is a paucity of data examining the natural history and risk factors for persistent IgE-mediated egg allergy in infants. recruited from a population based cohort.

METHODS: The HealthNuts study is a prospective, population-based cohort study of 5276 infants. Infants underwent skin prick testing (SPT) to four allergens, including egg, and those with a detectable wheal were offered oral food challenge (OFC) to raw egg, irrespective of SPT wheal size. Infants with challenge-confirmed egg allergy at age 1, were offered baked egg OFC, also at age 1, and follow-up at age 2 with repeat OFC to raw egg.

RESULTS: 140 infants with egg allergy at age one participated in follow-up. Egg allergy resolved in 66 infants (47%, 95%CI 37-56%) by 2 years-of-age, however resolution was lower in children with baked egg allergy at age 1, compared to baked egg tolerance, 13% versus 56% respectively, (aOR 5.27 95%CI 1.36-20.50, p=0.02). In the subgroup of infants who were tolerant to baked egg at age 1, frequent ingestion of baked egg (≥5 times/month) compared to infrequent ingestion (0-4 times/month) increased the likelihood of resolution (aOR 3.52, 95%CI 1.38-8.98 p=0.009). Mutation in the filaggrin gene was not associated with the resolution of either egg allergy or sensitisation.

CONCLUSIONS: Phenotyping of egg allergy (baked egg tolerant versus allergic) should be considered in the management of egg allergy as it has prognostic implications. Randomised controlled trials for oral immunotherapy should consider stratifying at baseline by baked egg sub-phenotype to ensure the differential rate of tolerance development is accounted for.

573 Mendelian Inheritance Of Elevated Tryptase Associated With Atopy and Connective Tissue Abnormalities

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RATIONALE: Mendelian diseases of atopy are rare, but have revealed important insights and novel pathways in the pathophysiology of allergic disease.

METHODS: A large cohort of patients and their families were referred for evaluation of suspected mast cell disorders, severe atopy, and/or eosinophilic inflammation. Comprehensive clinical evaluations were performed and fractionated serum tryptase levels were obtained. Bone marrow biopsies were performed as clinically indicated, and mast cell number was quantified in the marrow. *In vitro* basophil activation was assayed and patients were subjected to cutaneous vibratory challenge.

RESULTS: Nine families were identified with inherited elevations in basal serum tryptase (mean, 21.9 ng/mL) following an autosomal dominant pattern. Episodic flushing, abdominal pain, gastrointestinal distress and/or urticaria were seen in 26/32 individuals with elevated tryptase. Atopic disease, anaphylaxis, connective tissue abnormalities, chronic musculoskeletal pain, eosinophilic esophagitis, autonomic dysregulation and neuropsychiatric illness were additional common features. Upon cutaneous vibratory challenge, all tested individuals (n = 16) rapidly developed pruritis and expansive erythema. Bone marrow biopsies were performed in 5 index patients revealing an increase in mast cell number without pathologic changes or evidence of clonality. Basophil activation was also significantly reduced *in vitro*, among all affected individuals assayed (n = 10).

CONCLUSIONS: This is the first clinical description of a dominantly inherited atopic syndrome characterized by elevated basal serum tryptase, allergic disease, connective tissue abnormalities, and diminished in vitro basophil activation.

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574 Expression Of TSLP and TSLP-R In Chronic Idiopathic Urticaria

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RATIONALE: In mice, TSLP elicits a distinct subset of basophils with increased cytokine expression and decreased degranulation responses relative to IL-3 elicited basophils. In humans, TSLP expression is noted in skin lesions of atopic dermatitis subjects and thought to promote Th2 responses. Since human basophils show suppressed IgE receptor degranulation responses in chronic idiopathic urticaria (CIU), we explored TSLP expression in skin and TSLP-R expression on basophils of CIU subjects.

METHODS: Biopsies of CIU lesions, psorasis, and normal skin were obtained from the Johns Hopkins (JH) Pathology and JH Histology core while CIU non-lesional skin was obtained by a JH IRB protocol. Immunohistochemistry (IHC) on skin tissue was performed using anti-human TSLP and matched controls. Blood basophils were isolated from CIU, normal, eczema, and psoriatic donors and examined for surface expression of TSLP-R by flow cytometry before and after culture.

RESULTS: TSLP was strongly expressed in the epidermis of CIU lesional skin (n=3) and modestly in non-lesional CIU tissue (n=3), but not in normal and psoriasis skin. CIU blood basophil surface expression of TSLP-R was not detected at baseline (n=4, -0.24±0.31 standard error of mean (SEM) net median fluorescence intensity (MFI)) but was present after overnight culture with IL-3 (1 ng/ml, n=3, 12.3±8.03 MFI), and enhanced with IL-3 and anti-IgE co-culture (1 ng/ml IL-3 + 0.01 ug/ml anti-IgE, n=3, 35.14±12.15 MFI).

CONCLUSIONS: TSLP is expressed in CIU lesional and non-lesional skin whereas it's receptor is detected on blood basophils only after IL-3 exposure. The functional role of TSLP on CIU basophils requires further study.

575 Preclinical Study Of Rapidly-Disintegrating Sublingual Tablets (RDST): Effect Of Epinephrine (E) Incorporated As Nano-Crystals

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RATIONALE: E auto-injectors are recommended for anaphylaxis treatment in the community, however they are under-utilized when anaphylaxis occurs. We developed E taste-masked, RDST as a potential alternative dosage form *(J Allergy Clin Immunol 2013;131:236-8).* Into these new-generation tablets, manufactured by direct compression without moisture or heat, we subsequently incorporated E as nano-crystals (NC) *(AAPS Journal 2013;15 (2) Abstract T3243).* We hypothesized that this would enhance E sublingual absorption.

METHODS: In a preclinical study in a validated animal model, we investigated the rate and extent of E absorption from E-NC 20 mg and E 40 mg RDST, using E 0.3 mg IM (EpiPen, positive control) and placebo RDST (negative control). Blood samples were collected at frequent intervals to 1 h. E concentrations were measured using HPLC with electrochemical detection.

RESULTS: The mean±SD $AUC_{0\text{-}60}$ and C_{max} from 20 mg E-NC RDST (942±244 ng/ml/min and 38±10 ng/ml) and 40 mg E RDST (678±149 ng/ml/min and 32±10 ng/ml) did not differ significantly ($p>0.05$) from each other or from E 0.3 mg IM injections (592±122 ng/ml/min and 28±7 ng/ml); however, all these values were significantly higher than the endogenous E values (220±78 ng/ml/min and 8±3 ng/ml) ($p\leq0.05$) after placebo RDST.

CONCLUSIONS: E-NC RDST improved E absorption two-fold, therefore the E-RDST dose could be reduced by 50% using E-NC. These E-NC RDST have the potential for the first-aid treatment of anaphylaxis in community settings and are suitable for Phase I human studies.

576 A 24-Month Randomized, Controlled Trial Of An Automated Speech Recognition Program To Improve Adherence In Pediatric Asthma

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RATIONALE: Nonadherence with pediatric asthma treatments remains an enormous challenge. Interventions to improve adherence have been only partially effective, and most have not been designed to reach large patient populations.

METHODS: 1187 children, ages 3-12, treated for persistent asthma at Kaiser Permanente of Colorado (KPCO) were randomized to a computerized speech recognition (SR) or usual care (UC) condition and followed for 24 months. Telephone calls in the SR condition pulled information from the electronic health record (EHR) enabling the automated call to provide personalized patient and medication information. The call reminded the parent when the inhaled corticosteroid fill was overdue, and assisted with automated mail order refills or transfer to a KPCO pharmacy or asthma nurse specialist.

RESULTS: In the intention-to-treat analysis, adherence measured by medication possession ratio was 25.4% higher in the SR condition than the UC condition over the 24-month interval. Utilization of urgent care did not differ between the two groups. The intervention effect was consistent in subgroups stratified by age, gender, race, body mass index, or disease-related characteristics.

CONCLUSIONS: The SR intervention's significant impact on adherence demonstrates strong potential for a system-based, large-scale adherence program integrated with an EHR. The absence of change in urgent care visits may be attributable to the already low number of asthma urgent care visits within KPCO. Application of this and similar technology-based interventions may reduce healthcare utilization when applied in a less-well-controlled population of asthma patients or patients with other chronic conditions.

577 Asthma Carepartners: A Home-Based Asthma Intervention Embedded Within Medicaid Managed Care

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RATIONALE: The Asthma CarePartners (ACP) program, an innovative partnership between the Sinai Urban Health Institute (SUHI) and a Medicaid managed care organization, aims to improve the health of Chicago children and adults with asthma. Four previous asthma interventions with rigorous results and demonstrated cost savings have proven the effectiveness of SUHI's Community Health Worker (CHW) model, leading to this venture to incorporate the model within standard healthcare delivery.

METHODS: The ACP program utilizes CHWs making home visits to educate patients with poorly controlled asthma about the disease, its triggers and proper management. Participants receive six home visits during the year-long intervention. Education focuses on improving medical management while simultaneously addressing environmental triggers. CHWs conduct structured home environmental assessments, working collaboratively with families to reduce exposure to home triggers.

RESULTS: Since 8/16/2011, 469 patients have been referred to the program. Of the 52 participants who completed the 12-month intervention, ED visits were reduced by 78% between the year prior to and the year following the intervention ($p=0.0004$). Similarly, nights where sleep was disturbed by asthma decreased from 7.1 to 2.3 over the intervention period ($p<.0001$). Caregiver Asthma-Related Quality of Life Scores improved from 5.1 at baseline to 6.4 at the 12-month follow-up ($p=<.0001$), a clinically and statistically significant improvement.

CONCLUSIONS: Preliminary data demonstrate an improvement in asthma control, reduction in symptom frequency and a dramatic reduction in asthma-related health resource utilization. Not only is this partnership model extremely effective, it also has great potential to reduce Medicaid costs for patients with uncontrolled asthma.

578 The Osia Platform: An Extensible Tool For Improving Individual Allergy and Asthma Control and Understanding Environmental Drivers

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RATIONALE: Asthma control is significantly improved with the use of control instruments, but compliance drops precipitously when digital, rather than paper formats, are used. The OSIA platform is a cloud-computing-based tool that improves individual allergy and asthma control, and helps to understand individual and population-level relationships between allergy and asthma events and associated environmental triggers by increasing patient participation.

METHODS: Data have been collected for roughly two years using the OSIA platform. Participating patients use a cell phone application to log information regarding individual asthmatic episodes, as well as composite measures of asthma control based on assessment instruments developed by the National Institutes of Health. Additional data collected and appended to logged events include geo-referenced air quality data (ozone, particulate matter sizes 2.5 μm and 10 μm [PM10]) and weather data (temperature, relative humidity, wind speed, wind direction, and barometric pressure).

RESULTS: The environmental factors most strongly related to allergy events, and explaining approximately 24% of the observed variation ($p < 0.001$), were PM10 and relative humidity. Controlled asthma events were not significantly related to environmental variables, but uncontrolled asthma events were most strongly related to atmospheric ozone concentrations, relative humidity, and wind direction, these variables explaining approximately 15% of the observed variation ($p < 0.001$).

CONCLUSIONS: The OSIA platform enables patients and healthcare providers to better understand allergy and asthma outcomes related to specific environmental triggers on an individual basis and potentially population basis, likely resulting in improved asthma and allergy control and quality of life.

579 Allergy Immunotherapy Significantly Reduces Outpatient Services Use For Allergy and Respiratory Conditions In Patients With Newly-Diagnosed Allergic Rhinitis

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RATIONALE: We previously reported that patients with newly-diagnosed allergic rhinitis (AR) initiating allergy immunotherapy (AIT) use significantly less healthcare services than matched controls throughout 18-months. The present study examined targeted allergic and respiratory outpatient-treated conditions that may benefit from AIT.

METHODS: Among Florida Medicaid (1997-2009) enrollees newly-diagnosed with AR and AIT treatment-naive, we matched (1:1 on demographics, comorbidities, atopic conditions) patients receiving AIT to controls not receiving AIT. Chi-square tests compared within- and between-group differences in the proportion of patients receiving outpatient services for allergic and respiratory conditions during the 18 months before (pre-period) and after (post-period) AIT initiation.

RESULTS: 4,967 AIT-treated patients were matched to 4,967 controls. Among AIT-treated patients, the proportion receiving outpatient care for chronic upper respiratory infections (URIs) declined 24.6% ($p<0.0001$) from the pre- to post-period; the proportion of controls declined 14.3% ($p=0.0005$). The decline was significantly greater for AIT-treated than control patients ($p<0.0001$). Similarly, at 18-month post-period follow-up, there was a significantly greater decline in the proportion of AIT-treated patients receiving outpatient services for nasal polyps, influenza (both $p<0.0001$), allergic reactions ($p=0.004$) or emphysema ($p=0.03$) compared with controls.

CONCLUSIONS: At 18-month follow-up, compared with controls, AIT was associated with significantly reduced proportions of patients receiving outpatient services for chronic URIs, nasal polyps, influenza, allergic reactions or emphysema. Consistent with the unified airway model, by improving AR control, AIT may mitigate the development and severity of other allergic and respiratory diseases. Further analyses are underway to examine the impact of AIT on healthcare use and costs for these targeted conditions.

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580 Real-Time Asthma Outreach Reduces Excessive Short-Acting Beta-Agonist (SABA) Canister-Dispensing: A Randomized Study

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RATIONALE: Excessive SABA use indicates impaired asthma. We hypothesized that real-time outreach to excessive SABA users reduces SABA canister-dispensing.

METHODS: After real-time determination, using information technology, of a 7th SABA canister-dispensing in the prior 12 months, 12-56 year-olds with physician-coded asthma and inhaled corticosteroid dispensing were block-randomized by prior asthma specialist care and step-care level into intervention (N=1001) and control groups (N=998). Intervention included real-time letter notification to patient and electronic message to their physician with management suggestions including facilitated allergy referral for patients without prior asthma specialist care. The control group received standard Managed Care Organization (MCO) asthma care without research contact. Frequency and time to 7thSABA canister-dispensing in the follow-up year were the primary outcomes.

RESULTS: Intervention compared to controls reached 7 SABA canister-dispensings less frequently (50.7% versus 57.1%; risk ratio, 0.89; 95% CI, 0.82-0.97; P=0.007) and later (median 246 versus 225 days; Hazard ratio, 0.80; 95% CI, 0.71-0.91; P<0.001). SABA canister-dispensings were less in intervention (7.5±4.9) than controls (8.6±5.3) (rate ratio, 0.87; 95% CI, 0.82-0.93; P<0.001). Allergy visits were more frequent in intervention (30.9%) than controls (16.8%) (risk ratio, 1.83; 95%CI, 1.54-2.16, P<0.001). Controller-to-total medication ratio ≥0.5 was more frequent in the intervention group (P<0.001). The subgroup without prior specialist care achieved these benefits [P for interaction: frequency of (P=0.04) and time (P=0.007) to 7thSABA canister-dispensing; time to allergist visit (P<0.001)]. Exacerbations were unaffected.

CONCLUSIONS: A novel administrative-based outreach program improves impairment in patients without prior asthma specialist care and could be adaptable to MCOs with electronic medical records.

581 Activation Of TLR4 Induces VEGF Expression Via Akt Pathway In Nasal Polyps

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RATIONALE: Nasal polyposis is characterized by tissue remodeling and edematous nasal mucosa. Vascular endothelial growth factor (VEGF) plays a significant role in the regulation of remodeling in nasal polyps. TLR4 activation is associated with VEGF expression in murine macrophages and odontoblasts.

METHODS: Nasal polyp-derived fibroblasts (NPDFs) were isolated from 10 patients with nasal polyps and exposed to LPS. LPS from *Rhodobacter sphaeroides* (LRS) was used to inhibit the expression levels of TLR4, MyD88 and VEGF. Messenger RNA (mRNA) expression levels of *TLRs, MyD88*and *VEGF* were determined by gene expression microarray and semiquantitative reverse transcription-PCR. Protein expression levels of TLR4 and VEGF were analyzed by western blot, immunofluorescence staining, and enzyme-linked immunosorbent assay (ELISA). Activation of MAPKs (ERK, p38, and JNK) and Akt was examined by western blot analysis. The expression level of VEGF was measured by ELISA and western blot analysis in *exvivo* nasal polyp organ culture.

RESULTS: The protein expression level of VEGF was increased in nasal polyp tissues compared with inferior turbinate tissues. LRS inhibited the mRNA and protein expression of TLR4, MyD88, and VEGF in LPS-stimulated NPDFs. LPS activated MAPKs and Akt signals, whereas MAPK inhibitors did not inhibit VEGF expression, and only Akt inhibitor blocked VEGF production. LRS reduced the production of VEGF in LPS-stimulated *exvivo* organ culture.

CONCLUSIONS: These results suggest that LPS stimulates the production of VEGF through the TLR4-Akt signaling pathway in nasal polyps. LPS may be involved in the pathogenesis of nasal polyp remodeling.

582 Non-Eosinophilic Nasal Polyps In Second-Generation Asian Patients In The U.S. With Chronic Rhinosinusitis; Evidence For Genetic Influence On Eosinophilia

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RATIONALE: Nasal polyps in CRS from Asian patients residing in their country of origin are known to be less eosinophilic compared to polyps from patients of European descent in Belgium or the US. In an attempt to test if this is due to environmental or genetic factors, we evaluated the eosinophilic marker ECP and evidence for eosinophilia in the pathology report of polyp and sinus tissue in a group of second-generation Asian CRS patients in Illinois compared with patients of other ethnicities.

METHODS: A consecutive series of 168 chronic rhinosinusitis with polyp(CRSwNP) patients who underwent surgery at Northwestern-University were included. Patients with Asian ancestry(Chinese, Korean, Japanese....), born in the US were identified as second-generation Asian. Tissue homogenates from polyp and uncinate tissue(UT) were analyzed for ECP. All the original pathology reports of polyp and sinus tissue obtained during surgery were reviewed for eosinophilia.

RESULTS: There was a significant difference in ECP level in both polyp(mean199vs1146;P<0.001) and UT(mean136vs.693;P<0.001) between Asian versus non-Asian patients. Per pathology reports, 18% of polyps/sinus tissue from Asian patients were eosinophilic as opposed to 70% in patients of European descent, 66% in Hispanics and 59% in African-American patients(P<0.0001for all comparisons). ECP in both polyp and UT was significantly higher in cases with eosinophilia in the pathology-report.

CONCLUSIONS: The evidence for predominance of non-eosinophilic inflammation in polyps in second-generation Asian patients compared to other ethnicities is in-line with the reported higher prevalence of non-eosinophilic polyps in native-born Asians and may be indicative of a genetic predisposition in formation of non-eosinophilic polyps in this group of patients.

583 Regulation Of Expression Of Pendrin Protein In CRS With Nasal Polyps and In Airway Epithelial Cells

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RATIONALE: Pendrin is an anion transporter expressed by airway epithelial cells. Pendrin functions as a regulator of inflammation, mucus production and in the maintenance of air-surface liquid (ASL). We and others reported that expression of pendrin mRNA was elevated in nasal polyps (NP) of patients with chronic rhinosinusitis (CRS). In this study we analyzed the regulation of pendrin protein expression in NPs of patients with CRS and also in nasal epithelial cells (NECs).

METHODS: Tissue samples were collected and analyzed by real-time PCR and/or immunoblot analysis for expression of pendrin and various cytokines. NECs were stimulated with various cytokines, dsRNA and rhinovirus to analyze the regulation of pendrin expression.

RESULTS: Immunoblot analysis indicated that pendrin protein was increased in NP of patients with CRS compared to control uncinates (3.5 fold, $p<0.01$, $n=9$-16), although the molecular size of pendrin was less than the expected 80-100 kDa of the fully glycosylated form. In cultured NECs, glycosylated pendrin expression was increased by treatment with IL-13 and IL-17, and profoundly so when these two cytokines were combined. IL-13 and IL-17 also potentiated pendrin expression induced by dsRNA or rhinovirus. Pendrin mRNA expression in vivo correlated with IL-13 and IL-17 mRNA in sinonasal tissues.

CONCLUSIONS: Our findings confirm in vivo expression of pendrin protein in NP of patients with CRS and also indicate that pendrin expression is induced synergistically by Th2/Th17 cytokines in vitro. Profound induction of pendrin by rhinovirus and Th2/Th17 cytokines suggests that pendrin may be involved in viral induced exacerbations in CRS and/or asthma

584 Sex-Specific Differences In Disease Severity In Patients With Chronic Rhinosinusitis With Nasal Polyps

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RATIONALE: Up to 50% of patients with chronic rhinosinusitis (CRS) have comorbid asthma, and we have reported that a subset of CRS patients who have nasal polyps (CRSwNP) have elevated autoantigen-specific antibodies within their nasal polyps (NP). While increases in the prevalence and/or severity of asthma and autoimmunity in women are well characterized, it is not known whether CRSwNP demonstrates a similar trend. We sought to determine whether CRSwNP demonstrated sex-specific differences in prevalence and/or severity.

METHODS: Using a prospectively collected database of patients undergoing nasal surgery ($n>1200$), we evaluated the effect of sex on the prevalence of CRSwNP, aspirin sensitivity and asthma status. We further compared levels of eosinophil cationic protein (ECP) and anti-dsDNA antibodies in NP extracts from men and women by ELISA.

RESULTS: Although women comprised about 50% of control and CRS patients without NP (CRSsNP), a significantly smaller proportion of CRSwNP patients were female (35%, $p<0.05$). Interestingly, women with CRSwNP were more likely to have comorbid asthma ($p<0.01$), and 65% of patients with aspirin-exacerbated respiratory disease (CRSwNP plus asthma and aspirin sensitivity) were women ($p<0.001$). Asthmatic women with CRSwNP had the highest levels of autoantigen-specific IgG ($p<0.01$) and ECP ($p<0.05$), and were more likely to have revision surgeries ($p<0.05$).

CONCLUSIONS: These data suggest that women with CRSwNP have more severe disease than men. Future studies are needed to elucidate the mechanisms that drive disease in men and women, and may pave the way for the development of improved therapeutic strategies for treatment of CRSwNP in both women and men.

585 The Role Of Innate Cytokine In Non-Asthmatic, Non-Eosinophilic Nasal Polyps: IL-25, IL-33 and TSLP

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RATIONALE: Chronic rhinosinusitis with nasal polyps (CRSwNP) in Asian showed the different histological, immunological, and remodeling features comparing to Western CRSwNP. It suggested that other major immunologic responses may exert different impacts on the pathogenesis of non-eosinophilic Asian CRSwNP. Recently; some studies described the role of innate cytokines (IL-25, IL-33 and TSLP) in CRSwNP. We hypothesized that innate cytokines would be associated with the development of Asian CRSwNP.

METHODS: In analysis, we used only non-asthmatic, non eosinophilic NP. Non-eosinophilic was defined as eosinophils comprised less than 10% of total inflammatory cell. 23 CRSsNP, 55 CRSwNP and 10 controls were enrolled into this study. Samples were collected from polyp and uccinate process in the respective groups and assessed for IL-25, IL-33 and TSLP by immunohistochemistry; IL-25, IL-33, TSLP, GATA-3, Foxp3, RORC and T-bet by real time RT-PCR.

RESULTS: Patients with CRSwNP showed significantly higher the number and concentration mRNA of IL-25, TSLP compared with patients with CRSsNP, controls. However, there was no significant difference between IL-33 in CRSwNP and CRSsNP patients. The number and concentration mRNA of IL-25, TSLP correlated positively with the expression of RORC and T-bet, whereas IL-33 correlated negatively with the expression of GATA-3.

CONCLUSIONS: The development of non-eosinophilic NP would be related with up-regulation of IL-25, TSLP and down-regulation of IL-33. Also, IL-25 and TSLP is associated with T_{H1}/T_{H17} polarization; however, IL-33 may be related with T_{H2} polarization in non-eosinophilic NP.

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586 IL33 and Type 2 Innate Lymphoid Cells (ILC2) But Not Th2 Cells Are Essential For Persistence Of Chronic Experimental Asthma

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RATIONALE: We have developed a mouse model of chronic asthma where the disease persists longer than 6 months after cessation of allergen exposure. The objective was to delineate the contribution of IL33, Th2 and type 2 innate lymphoid cells (ILC2) to the persistence of asthma.

METHODS: Chronic asthma was induced by semi-weekly intranasal exposures to dust mite, ragweed, and Aspergillus for six weeks. Mice underwent immune ablation by irradiation 3 weeks after the last allergen exposure and received bone marrow from naïve, $Rag1^{-/-}$, or $Rag2^{-/-}\gamma c^{-/-}$ mice. Airway hyperreactivity and immunohistological features were measured 6 weeks after irradiation with and without anti-IL33 treatment. Lung ILC2 and their contribution to total IL5 and IL13 production was measured by flow cytometry. The direct effect of IL33 on airways was studied 4 weeks after administration.

RESULTS: Immune ablation eliminated allergen-specific T cell responses but failed to resolve airway hyperreactivity and remodeling. All features of chronic asthma completely resolved in mice that received bone marrow from $Rag2^{-/-}\gamma c^{-/-}$ (lacking T and B cells, and ILC) but not in mice that received marrow from $Rag1^{-/-}$ mice (lacking T and B cells only). Chronic asthma resulted in a 2.5 fold increase in ILC2 (lin-CD25+Sca1+c-kit+CRTH2+ST2+IL5+IL13+). Anti-IL33 inhibited ILC2 and induced resolution of asthma. Conversely, intranasal IL33 upregulated ILC2 and induced airway inflammation and hyperreactivity, persisting for 4 weeks. IL33 was autoinduced, partially facilitated by IL13.

CONCLUSIONS: Airway epithelial cell-derived IL33 is critical for ILC2 generation. ILC2, not Th2 cells, are essential for persistence of chronic asthma in the absence of allergen exposure.

587 PGE2 Deficiency Causes a Phenotype Of Aspirin Sensitive Asthma That Depends On Platelets and Cysteinyl Leukotrienes

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RATIONALE: Aspirin exacerbated respiratory disease (AERD) is characterized by asthma, tissue eosinophilia, overproduction of cysteinyl leukotrienes (cysLTs), and respiratory reactions to nonselective cyclooxygenase (COX) inhibitors. Ex vivo studies suggest that functional abnormalities of the COX-2/microsomal prostaglandin (PG)E2 synthase (mPGES)-1 system may underlie AERD. Previously we have demonstrated that mPGES-1-null (*ptges*$^{-/-}$) mice develop a remarkably AERD-like phenotype in a model of eosinophilic pulmonary inflammation. We hypothesized that the AERD-like phenotype in mice depends on platelets and cysLTs.

METHODS: *ptges*$^{-/-}$ mice were treated intranasally with low-dose of house dust mite extract on 6 occasions, and then were challenged with aerosol Lys-aspirin (Lys-ASA) and airway resistance was measured. Montelukast, zileuton, a T prostanoid (TP) receptor antagonist, or anti-CD41 (to deplete platelets) was given to some mice to determine their effects on Lys-ASA induced airway resistance (R_L). Lung mast cell (MC) activation and cysLT production were measured by ELISA.

RESULTS: Lys-ASA-challenged *ptges*$^{-/-}$ mice exhibit sustained increases in R_L, along with lung MC activation and cysLT overproduction. The increases in R_L and MC products were blocked by montelukast or zileuton, implying that bronchoconstriction and MC activation were both cysLT-dependent. Lys-ASA induced cysLT generation and MC activation depended on platelet adherent granulocytes and TP receptors.

CONCLUSIONS: Lesions that impair the inducible generation of PGE_2 remove control of platelet/granulocyte interactions and TP receptor-dependent cysLT production, permitting MC activation in response to COX-1 inhibition. The findings suggest applications of anti-platelet drugs or TP receptor antagonists for the treatment of AERD.

588 CCR8 Is a Receptor For CCL18 On Human Th2 Cells

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RATIONALE: The CC chemokine ligand 18 (CCL18) is one of the most highly expressed chemokines in chronic allergic diseases. The study of CCL18 has been hindered by the lack of an identified chemokine receptor.

METHODS: We performed chemotaxis assays and calcium flux assays using a CCR8-transfected mouse pre B-cell line. We also isolated and cultured naïve CD4+ T cells in Th2 polarizing conditions and performed chemotaxis assays, calcium flux assays, RT-qPCR, and flow cytometry with intracellular cytokine staining. Expression of chemokine and chemokine receptor mRNA in esophageal biopsy specimens from patients with eosinophilic esophagitis (EoE), EoE in remission, or GERD was quantified by RT-qPCR.

RESULTS: CCL18 induced chemotaxis and calcium flux of human CCR8-transfected cells. Highly polarized human Th2 cells expressed CCR8, secreted IL-4 and IL-5, and exhibited chemotaxis and calcium flux to CCL18. Furthermore, CCL1, a known CCR8 ligand, and CCL18 induced heterologous cross-desensitization of human Th2 cells, suggesting that they share a common receptor. Expression of PITPNM3, a previously identified CCL18 receptor relevant in breast cancer metastasis, was not detected on human naïve T cells or Th2 cells by PCR or flow cytometry. CCL18 and CCR8 were coexpressed in esophageal biopsy tissue from individuals with active EoE and were present at markedly higher levels compared with tissue from patients with EoE in remission or controls with GERD.

CONCLUSIONS: Identifying CCR8 as a chemokine receptor for CCL18 will help clarify the biological role of this highly expressed chemokine in human allergic diseases.

589 Increased Frequency Of Dual Positive Th2/Th17 Cells In Bronchoalveolar Lavage Characterizes a Population Of Severe Asthmatic Patients

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RATIONALE: Th2 cells can further differentiate into dual positive Th2/Th17 cells. The presence of dual positive Th2/Th17 cells in the airways and its impact on asthma severity are unknown. We studied dual positive Th2/Th17 cells in bronchoalveolar lavage (BAL) from asthmatic patients, examined their response to glucocorticoids, and defined their relevance for disease severity.

METHODS: Bronchoscopy and lavage were performed on 38 asthmatic patients and 15 disease controls. Th2 and Th2/Th17 cells were analyzed by multi-color flow cytometry and confocal immunofluorescence microscopy. Cytokines were assayed by ELISA.

RESULTS: Dual positive Th2/Th17 cells were present at a higher frequency in BAL from asthmatic patients as compared to disease controls. Th2/Th17 cells co-expressed GATA3 and RORgT as well as pSTAT6 and pSTAT3. Increased presence of Th2/Th17 cells was associated with heightened levels of IL17 in the lavage fluid. Th2/Th17 cells and IL17 correlated with PC20 for methacholine, eosinophils and FEV1. Th2/Th17 cells, unlike Th2 cells, were resistant to dexamethasone-induced cell death. They expressed higher levels of MEK1, a molecule that induces glucocorticoid resistance. Based upon the dominance of Th2 or Th2/Th17 cells in BAL, we identified three distinct subgroups of asthma—$Th2^{high}$, $Th2/Th17^{high}$ and $Th2/Th17^{low}$. The $Th2/Th17^{high}$ subgroup manifested the most severe form of asthma whereas the $Th2/Th17^{low}$ subgroup had the mildest asthma.

CONCLUSIONS: Asthma is associated with a higher frequency of dual positive Th2/Th17 cells in BAL. The $Th2/Th17^{high}$ subgroup of asthmatic patients manifests glucocorticoid resistance in vitro. They also have the greatest airway obstruction and hyperreactivity as compared to $Th2^{high}$ and $Th2/Th17^{low}$ subgroups.

590 Cmrf-35-Like Molecule 1 (CLM-1) Is Required For IL-4-Induced Cellular Responses and Development Of Allergic Airway Inflammation

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RATIONALE: IL-4 is critically involved in the development of Th2-immune responses. Surprisingly, endogenous molecular mechanisms regulating IL-4-induced responses are largely unknown. CMRF-35-like molecule-1 (CLM-1) is an immunoreceptor tyrosine-based inhibitory motif-containing receptor. Yet, its role Th2 immune responses is unclear.

METHODS: Wild type (WT), and *Clm1*$^{-/-}$ mice were intranasally challenged with *Aspergillus fumigatus* extract or IL-4. Lung cellular expression of CLM-1 (Flow), cytokine/chemokine and IgE levels were assessed (ELISA). IL-4/IL-13-induced mediator secretion and STAT-6 phosphorylation were assessed in bone marrow (BM)-derived macrophages (Mφ) and eosinophils (BM-Eos). CLM-1 expression was assessed in human peripheral blood monocytes and eosinophils from healthy and allergic patients.

RESULTS: CLM-1 was specificllay upregulated by lung eosinophils and macrophages following aeroallergen-challenge, a phenomenon, which was recapitulated in-vitro and in-vivo by IL-4. IL-4/IL-13-stimulated *Clm1*$^{-/-}$ BM-Mφ and BM-Eos displayed decreased cytokine/chemokine secretion and STAT-6 phosphorylation compared with WT cells. Furthermore, IL-4-induced "priming" of eosinophil chemotaxis was absent in *Clm1*$^{-/-}$ BM-Eos. Consistently, activation of CLM-1 by antibody cross-linking enhanced IL-4 induced responses in BM-Mφ. In-vivo, IL-4-challenged *Clm1*$^{-/-}$ mice displayed decreased cellular infiltration and reduced cytokine/chemokine secretion compared with WT mice. Similiarly, aeroallergen-challenged *Clm1*$^{-/-}$ mice were protected from allergic airway inflammation and exhibited reduced IgE levels, decreased lung cellular infiltration and decreased chemokine levels. Finally, CLM-1 expression was increased in monocytes and eosinophils obtained from allergic patients compared to healthy individuals.

CONCLUSIONS: Our data demonstrates that CLM-1 is required for the development of allergic airway disease by co-activating IL-4-induced responses. These data highlight CLM-1 as a potential novel therapeutical target for asthma treatment.

591 Quality Of Life Improves In Children Undergoing Peanut Immunotherapy

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RATIONALE: This analysis sought to determine if parent and child perspectives on food allergy on quality of life (QOL) changed following a peanut immunotherapy study.

METHODS: 21 children 7-13 years of age enrolled in a peanut immunotherapy study and their parents were given a QOL questionnaire at baseline and prior to the post-treatment food challenge. The questionnaires assessed food allergy related QOL on a six-point scale where 0="not at all", 1="barely", 2= "a little bit", 3="fairly", 4="quite a bit", 5="very much" and 6="extremely". Differences in responses between the two questionnaires and between parents and children were evaluated by the Wilcoxon sign rank test.

RESULTS: 15 children and their parents completed the pre- and post-immunotherapy QOL questionnaire. At baseline, parents reported greater fear of an allergic reaction than children (64.2% vs. 40% rated 5 and 6, $p=0.03$). However, fear of consuming unfamiliar food ($p=0.97$) and feelings of social isolation ($p=0.51$) were rated similarly among parents and children. Following immunotherapy, median fear of an allergic reaction decreased among children (3 vs. 1, $p<0.01$) but increased among parents (5 vs. 5, $p=0.048$), with 85.6% of parents rating their fear 5 or 6. Fear of consuming unfamiliar food decreased in children (2 vs. 1, $p=0.03$) but not parents (2 vs. 3, $p=0.58$). Feelings of social isolation decreased, but did not change significantly in either group (children: 2.5 vs. 2, $p=0.07$; parents: 5 vs. 3, $p=0.40$).

CONCLUSIONS: Following peanut immunotherapy, food allergy related quality of life improved significantly among children but not their parents.

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