1 A Mutation in the Human Uncouordinated 119 Gene Impairs TCR Signaling and is Associated with CD4 Lymphopenia.
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RATIONAL: Idiopathic CD4 Lymphopenia (ICL) is an immunodeficiency syndrome of unclear etiology. Lck is a major TCR-linked kinase. Lck activity was reported to be reduced in ICL. We hypothesized that ICL was associated with a defect of the recently described Lck activator -Uncouordinated 119 (Unc119).
METHODS: CD4 T cells from three ICL patients were analyzed for Lck activity (immune-complex kinase assay), the Unc119 protein level (western blotting) and sequence of the Unc119 cDNA and exons. The identified mutant cDNA was expressed in normal CD4 T cells by retroviral infection and the cells were examined for Lck activation and proliferation.
RESULTS: All ICL patients demonstrated reduced Lck activity. The level of the Unc119 protein and its molecular weight were normal. One patient had heterozygous missense mutation (codon 22 GCC→GTC; V22G) in the Unc119 cDNA and gene. The patient was a 32-year-old female with <300 CD4 T cells/μl, recurrent sinusitis/otitis media, recurrent shingles, fungal skin and nail infection, oral herpetic lesions and BOOP following two episodes of bacterial pneumonia. The reduced Lck activity was accompanied by decreased T cell proliferation. Transduction of the mutant but not wild type Unc119 into normal T cells reproduced signaling and proliferation defects. The mutation disrupted the Unc119-Lck interaction, which is essential for Lck activation. The mutant also caused mislocalization of Lck to endosomes. The mutation was not present in two other ICL patients, patients with secondary CD4 lymphopenia or 60 healthy subjects.
CONCLUSIONS: We identified a novel genetic defect -a dominant negative missense mutation in the Unc119 gene in ICL.

2 Delayed Food Challenge Reactions Correspond Temporally to the Appearance of CD63+ Basophils in Subjects with IgE to alpha-Gal
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RATIONAL: Allergen-stimulated basophil activation can be assessed using the activation markers CD63 and CD203c. Patients who develop IgE to the oligosaccharide galactose-alpha-1,3-galactose (alpha-gal) report reactions that occur 3-6 hours after eating mammalian meat. The purpose of this study was to document the clinical delay and assess in vivo activation of basophils.
METHODS: Following local IRB approval, informed consent was obtained from subjects (n=6) who reported delayed urticarial reactions and tested positive for IgE to alpha-gal. After baseline blood work was obtained, 86 grams of beef was consumed. Peripheral blood was drawn hourly to assess for in vivo activation by flow cytometry using CD63 and CD203c expression.
RESULTS: In each challenge, symptoms did not appear until ≥3 hours after eating meat (range 3.5-5.5 hrs). Symptoms included pruritus, GI upset, urticaria, flushing and anaphylaxis. Basophil activation, as increased CD63 expression, was seen 4-5 hours post-meal consumption. The average maximum CD63 expression was 20.3 % above unstimulated basophils (range 2.2%-60.2%), whereas the expression of CD203c was not increased during the food challenge. Patterns of activation were not affected by sIgE titer to alpha-gal nor by ratio of sIgE:total IgE.
CONCLUSIONS: In the recently described red meat allergy, we report not only documentation of the clinical delay but also the first experiments showing that basophils are activated in vivo during a food challenge. Moreover, these results support the utility of CD63 as a marker for food allergy-induced basophil activation and imply that the form of alpha-gal which causes basophil activation and urticaria takes 4 hours to enter the bloodstream.

3 Rapid Induction of Tolerance To Peanut By Antigen-coupled Cell Transfer
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RATIONAL: Food allergy is an increasing worldwide health concern. Immunotherapy is an effective therapeutic approach but has a high risk of adverse reaction. New methods to efficiently and safely induce antigen-specific tolerance could improve the clinical approach to food allergy. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI) is a non-toxic chemical used to link antigens to cells (Ag-SP) and has been used in clinical trials and shown to promote tolerance in Th1/Th17 autoimmune diseases. However, the tolerizing effect of Ag-SP on Th2-associated diseases is still unclear.
METHODS: For the peanut (WPE)-induced murine food allergy model, mice were orally sensitized with WPE/SEB or SEB and were tolerized with Ag-SP before or after sensitization. Anti-CD25 antibody (PC61) was given right after tolerance induction. Disease severity was determined after challenge. Murine bone marrow derived mast cells (BMMCs) were generated in vitro by culturing with IL-3. BMMCs were cultured with Ag-SP or free WPE alone and degranulation was analyzed by β-hexosaminidase assay.
RESULTS: In vivo, we show that Ag-SP tolerance prevented WPE-specific responses including WPE-specific IgE and mMCP-1 production and core body temperature decrease upon oral challenge. Ag-SP tolerance also reduced disease severity prophylactically without inducing anaphylaxis during tolerization compared to mice received free antigens. Ag-SP induced tolerance was partially dependent on CD25+ Tregs since PC61 treatment restored allergic responses. Furthermore, Ag-SP did not induce mast cell degranulation in vitro.
CONCLUSIONS: Ag-SP tolerance can be rapidly, safely and efficiently induced and highlights a potential new Ag-specific tolerance immunotherapy for food allergic diseases.

4 Increased FceRI Expression on Basophils at Birth Predicts Subsequent Allergic Sensitization
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RATIONAL: Animal models have demonstrated that basophils can act as antigen presenting cells and serve as important initiators and amplifiers of a Th2 response, in part through high-affinity IgE receptor (FceRI)-mediated antigen uptake. We sought to determine whether FceRI expression on mononuclear cells and basophils in early life is associated with the subsequent development of allergic disease.
METHODS: Children at high-risk for asthma and allergy based upon parental histories were enrolled at birth and followed prospectively in the Childhood Origins of Asthma (COAST) study. FceRI expression on basophils, plasmaicytid dendritic cells (pDCs), myeloid dendritic cells (mDCs), and monocytes was determined by flow cytometry of cord blood from 49 children. In vitro IgE was assessed at 1, 3, and 6 years by ImmunoCap®; values ≥0.35 KU/L were considered positive.
RESULTS: Cord blood basophil FceRIα median fluorescence intensity (MFI) was higher in children who developed aeroallergen sensitization by age 1 year compared to those without aeroallergen sensitization (median, 2300 vs. 996; p=0.005). Similar relationships were seen for the development of aeroallergen sensitization at ages 3 years (2306 vs. 876; p=0.0008) and 6 years (2191 vs. 996; p=0.04).
CONCLUSIONS: Increased expression of FceRIα on the surface of basophils in cord blood predicts the subsequent development of allergic sensitization to aeroallergens. Whether this is a predictive biomarker or actually causal in the development of allergic sensitization warrants further study.
AB2 Abstracts

5 Bronchial Hyperresponsiveness in sportschildren; Different methods to reach a diagnosis


RATIONALE: There are several methods for the diagnosis of bronchial hyperresponsiveness (BHR). Eucapnic voluntary hyperventilation test (EVHT) is proposed as the gold standard to diagnose exercise-induced asthma (EIA). We have compared the utility of three techniques in asthmatic sportschildren.

METHODS: We studied 17 patients (11 boys, 6 girls) aged between 10 and 17 (average 13.65 ± 2.49 years), 12 of them belonging to official sports teams. On the first visit skin prick tests with a panel of common inhalant allergens were performed, basal spirometry and fraction of exhaled nitric oxide measurements were taken. On separated visits, and after parents gave written informed consent, bronchial challenges with Methacholine, Mannitol and eucapnic voluntary hyperventilation were performed.

RESULTS: No patient had the three tests positive, and 3 patients had all tests negative. 14 patients had positive Methacholine test (PC20 < 16 mg/ml) with an average PC20 of 2.91 + 4.09 mg/ml; 3 patients of this group also had positive EVHT and negative Mannitol Test. 8 patients with positive Methacholine test also had positive Mannitol test (PD15 < 512 mg) with an average PD15 of 217.16 mg + 147.51 mg, but negative EVHT. 3 patients with positive Methacholine test had negative results for the other two tests.

CONCLUSIONS: Methacholine test showed much higher sensitivity in our group of patients for the BHR diagnosis than EVHT. Mannitol test didn’t provide any additional information.

6 Probable Asthma Is Associated With Reduced Lung Function Among Recurrent Wheezing Infants

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RATIONALE: The association between wheezing phenotypes and early lung function remains not fully understood. The better definition of asthma risk factors allowed the diagnosis of probable asthma in infants.

METHODS: Forced expiratory flows (FEF; raised volume rapid thoracic compression technique) e volumes (whole body plethysmography) were measured in recurrent wheezing (RW) infants (at least three previous episodes of medical-diagnosed wheezing in the last year) and in control infants. Probable asthma was defined by the presence of known risk factors: atopic eczema, parental history of asthma, rhinitis, wheezing without colds and eosinophilia. Lung function tests were performed under sedation with chloral hydrate in the absence of viral infections and wheezing exacerbations.

RESULTS: Technically acceptable measures were obtained in 64 RW infants (35 with probable asthma and 29 without) and in 37 normal infants. Normal infants showed significantly higher FEF than both groups of RW infants. RW infants with probable asthma had values significantly lower than those without probable asthma for zFEF25-75 (-0.44 x 0.41; p=0.03) and significantly higher for the ratio between residual volume and total lung capacity (%RV/TLC: 125 x 113; p=0.02). None significant difference in clinical parameters was observed between those with and without probable asthma.

CONCLUSIONS: Probable asthma is associated with reduced lung function among RW. Those infants showed a clear obstructive pattern with reduced forced flows and signs of air trapping. The reduced lung function impairment observed in RW without probable asthma might be related to their better clinical prognosis.

7 Mannitol Bronchoprovocation in the Evaluation of Airway Reactivity in a High-Risk Pediatric Cohort

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RATIONALE: The aim of this study was to examine the properties of a new indirect bronchoprovocation test using inhaled mannitol (AridolTM) for evaluating airway hyperreactivity and its relationship with various phenotypic characteristics in children.

METHODS: Children at high risk for development of asthma and allergic disease based on parental histories were enrolled at birth into the Childhood Origins of Asthma (COAST) study and followed prospectively. At age 11 years, 171 children underwent a mannitol challenge. A positive challenge was defined as the provocative dose that caused a 15% fall in FEV1 (PD15). Relationships among mannitol challenge with clinical asthma diagnosis at age 11, allergen sensitization (as determined by specific IgE), and total IgE were evaluated. Total and specific IgE were measured by fluoroenzyme immunoassay (UniCap 100, Pharmacia Diagnostics).

RESULTS: Of the 170 children who began the mannitol challenge, 146 children (86%) completed the procedure successfully. 57 of 144 children in the cohort were diagnosed with asthma at age 11. Of the children with asthma, 40% had a positive challenge while 79% of non-asthmatics had a negative challenge (p = 0.01). The procedure demonstrated a specificity of 79% with a sensitivity of 40%. Although allergen sensitization was not associated with mannitol hyperresponsiveness, total IgE levels were inversely associated with the PD15 (r = -0.19, p = 0.03).

CONCLUSIONS: In children, mannitol challenge has low sensitivity but relatively high specificity for current asthma. In addition, total IgE levels, but not the presence of allergic sensitization, were inversely associated with the PD15.

8 Comparison Between Methacholine And Mannitol Tests For The Study Of Bronchial Hyperresponsiveness In Asthma Induced By Exercise In Pediatric Athletes

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RATIONALE: Mannitol challenge has been proposed as a method to demonstrate bronchial hyperresponsiveness (BHR) in athletes. The aim of this study was to compare BHR to mannitol and methacholine in a group of pediatric athletes with symptoms of exercise induce bronchoconstriction (EIB).

METHODS: 23 children between 7-17 years- old (12.9 ±2.9 years) with symptoms of EIB were enrolled. Children belong to various Federative Teams (football-soccer, tennis, gymnastics, judo, swimming, volleyball and hockey). Pulmonary function tests, skin prick test with aeroallergens and measurement of the fraction of Exhaled nitric oxide (eNO) (NOXMINO-®) were also performed.

RESULTS: All but two children were atopic. Methacholine test was positive in 18 children (mean PC20 2.20 ± 3.44 mg/ml) while mannitol test was positive in ten patients (mean PD15 216 ±253mg). In 10 patients both tests were positive. In 5 children both tests were negative. In 8 patients methacholine test was positive and mannitol negative, while all patients with positive mannitol also had positive metacholine test. Mean eNO was 32.9 ± 21.2 ppb. eNO>30 ppb was present in 11 children (eight with both tests positive, one with both tests negative and two with positive methacholine test and negative mannitol test).

CONCLUSIONS: The methacholine test appears to be more sensitive than the mannitol test to demonstrate the HRB in this population of children athletes with EIB.
9 Association between Atopy and Bronchial Hyperresponsiveness in Preschool children with Recurrent Wheezing

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RATIONALE: Atopy is one of the important factors affecting bronchial hyperresponsiveness (BHR). Some preschool children with BHR show persistence of BHR at their school age. Atopy would be associated with persistence of BHR in those children.

METHODS: We reviewed the medical records of 113 preschool children who presented with recurrent wheezing and had regular check up by their school age. Methacholine bronchial challenge was performed at preschool age, using a modified auscultation method. The end-point was defined as the appearance of wheezing and/or oxygen desaturation. Atopy was determined to be present when a child had at least one positive reaction to a panel of 13 common airborne allergens in the presence of positive and negative controls. Methacholine bronchial challenge with spirometry was performed at their school age. Positive BHR was defined as end-point concentration ≥8 mg/mL at preschool age and as ≥PC20 ≥8 mg/mL at school age.

RESULTS: Atopic children (n=76) and non-atopic children (n=37) showed similar end-point concentration and similar prevalence of positive BHR at preschool age (1.32 mg/mL vs. 1.44 mg/mL, p=0.486 and 66/76 vs. 31/37, p=0.662, respectively). By contrast, at school age, atopic children showed a significantly higher prevalence of positive BHR than non-atopic children (56/76 vs. 17/37, p=0.004, respectively). Atopic children also showed a significantly lower PC20 than non-atopic children (1.87 mg/mL vs. 2.87 mg/mL, p=0.009, respectively).

CONCLUSIONS: Atopic in preschool children with recurrent wheezing is associated with increased risk of persistent and stronger BHR at their school age.

10 Is Methacholine Challenge Sufficient To Rule Out Bronchial Hyperresponsiveness In Patients With Suspected Asthma?

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RATIONALE: Asthma is confirmed by either evidence of reversible airway obstruction or assessment of bronchial hyperresponsiveness by bronchial provocation challenges, of which methacholine is regarded as the gold standard. The Spanish Guideline on the Management of Asthma (GEMA 2009) recommends considering the diagnosis of asthma in steroid-naive patients with suggestive symptoms and FeNO (fractional exhaled nitric oxide) values higher than 30 ppb. The aim of this study was to establish to what extent performing an additional inhalation challenge with adenine is necessary in patients with suspected asthma who had a negative methacholine challenge, in function of their airway inflammation status as measured by FeNO.

METHODS: Thirty patients (13 males, mean age 37.3 years [13-69]) were consecutively recruited among those reporting persistent respiratory symptoms consistent with asthma. Inclusion criteria: normal spirometry, negative bronchodilator test, negative methacholine challenge (provocative concentration inducing a 20% fall in FEV1 [PC20] >16 mg/mL). In all subjects FeNO (NIOXMINO, Aerocrine; Sweden) was measured, thus dividing them into two groups: group I (FeNO≥30 ppb: 23 patients) and group II (FeNO<30 ppb: 7 patients). Demographics and clinical information were recorded in both groups. One week after methacholine, adenosine challenge was performed in all patients.

RESULTS: All the patients from group II had a negative adenosine challenge (PC20>400 mg/mL), while in group I, 623 patients (26%) exhibited a positive result, being diagnosed with asthma.

CONCLUSIONS: In patients with asthma-like symptoms who had a negative methacholine challenge, but FeNO≥30 ppb, we recommend performing an adenosine challenge before definitely ruling out asthma.

11 Mannitol Challenge for Diagnosis of Exercise -Induced Bronchoconstriction: Experience in Practice

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RATIONALE: Mannitol (Aridol, Pharmaxis) challenge provides a easy to perform, commercially available and FDA approved hyperosmolar surrogate to formal exercise test for diagnosis of exercise induced bronchoconstriction (EIB) adaptable to the office setting.

METHODS: Individuals presenting with symptoms of EIB and/or asthma were evaluated using mannitol inhalation powder bronchial challenge test (Aridol, Pharmaxis, Australia) a boarded allergy/immunology private practice setting. History, physical exam, prick skin testing to inhalant allergens, and pulmonary function testing pre and post bronchodilator were also performed. IRB approval was obtained.

RESULTS: There were 16 individuals with symptoms of asthma and 13/16 with symptoms of EIB. There were 7 males and 9 females, 15 caucasian and 1 non-Caucasian. Subject age range was 7-36 years with mean age of 17 years and median age 15 years. All had allergic rhinitis documented by inhalant sensitivity by skin testing and none had a positive response to bronchodilator on pulmonary function testing. Response to mannitol (Aridol) was positive in 8/16 (50%) with a provocative dose PD15 or two consecutive PD10 ranging from 10 mg to 635 mg mean 272 mg and median 315 mg. The specificity of Mannitol (Aridol) was 100%. Negative predictive value of 100%, but sensitivity 57% for diagnosis of EIB.

CONCLUSIONS: Mannitol (Aridol, Pharmaxis) provides a highly specific but less sensitive office adaptable surrogate challenge for diagnosis of EIB for pediatric and adult populations. Mannitol permits evaluation of EIB without the necessary competency and less office adaptable equipment of formal exercise challenge.

12 Exercise-Induced Bronchoconstriction in Thai Pediatric Asthma: Prevalence and Risk Factors

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RATIONALE: Exercise-induced bronchoconstriction (EIB) among childhood asthma worldwide occurred 70-90%. Compared to the previous study in Thailand, 10 years ago, 52%. Our purpose is to identify the current prevalence and risk factors of EIB in Thai asthmatic children.

METHODS: A prospective study was performed in 56 asthmatic children, 6-15 years old, who presented at our allergy clinic, during 25 January - 25 August, 2011, with and without EIB symptoms. They did not have underlying heart disease, chronic lung disease, respiratory tract infections, asthma attacks within 2 weeks or take controllers within 4 weeks. 15% reduction in PEFR or having wheezing during exercise-challenge test was considered as having EIB.

RESULTS: 56 asthmatic children (37 boys) with mean age 9.4±2.4 years old were completed questionnaires, physical examinations, and ran on treadmill following the standard protocol. Prevalence of EIB was 41% (n=23), 16/23 (69.5%) had EIB history; whereas, 7/23 (30.4%) had no EIB history. EIB history (p=0.021) and family history of asthma (p=0.04) correlated with EIB. Durations of asthma, intervals from the last asthmatic attack, associated atopic diseases, temperatures 25.4±1.0°C, humidity 66.5±6.3% and Borg scale, did not related to EIB (p>0.05).

CONCLUSIONS: Prevalence of EIB among Thai asthmatic children currently is 41%, lower than in previous study. EIB history and family history of asthma were clues for an exercise-challenge test, the crucial examination for pediatric asthma.
13 Usefulness of Impulse Oscillometry in Children With Eosinophilic Bronchitis

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RATIONALE: Eosinophilic bronchitis (EB) has been shown to resolve, whereas it has been shown to progress to asthma in some patients, despite treatment with inhaled steroids. It is controversial whether EB is a pre-form of asthma. We evaluated pulmonary function by impulse oscillometry (IOS) and airway inflammation by measuring fractional exhaled nitric oxide (FeNO) of children with EB in comparison of those with asthma and healthy children as a control.

METHODS: A total of 232 children with asthma, 109 with EB, and 115 control subjects were enrolled. We compared pulmonary function test parameters and FeNO levels among three subject groups, and designated a cutoff value of FeNO combined with IOS parameters to distinguish EB from the control group.

RESULTS: Pulmonary function and bronchodilator response for EB in spirometry were of normal range as well as for the control. In IOS, the percentage change in reactance at 5 Hz (Δ X5) and the percentage change in reactance area (Δ AX) of the EB as well as the asthma groups decreased significantly compared to the control (P < .0001). A cutoff value to distinguish EB from control was Δ X5 as is 20% (sensitivity, 75.5%; specificity, 49.6%), and Δ AX is 30% (sensitivity, 75.0%; specificity, 46.0%) when FeNO is 20 ppb.

CONCLUSIONS: Reversible airway obstruction in IOS and elevated FeNO levels can be useful for evaluation of EB in children. This would support that EB shows airway characteristics similar to those of asthma.

14 Eucapnic Voluntary Hyperventilation Screen for Bronchospasm Risk During a SCUBA Dive

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RATIONALE: Eucapnic Voluntary Hyperventilation (EVH) may be used to duplicate the cold dry air exposure of a SCUBA dive. It should be useful to evaluate the likelihood of bronchospasm in potentially susceptible individuals who wish to dive.

METHODS: A 15 year old female student who sought enrollment in a SCUBA diving class had an undocumented history of wheezing after a respiratory infection in the past, was on no medications presently and had normal pulmonary functions. Using a previously described simplified method, she was given an EVH challenge with a target ventilation of 25 times her FEV1 (62.5% of her estimated Maximum Voluntary Ventilation) for 6 minutes.

RESULTS: The applicant had a 24.8% decrease in her FEV1 from baseline at 15 minutes after the challenge and required inhaled bronchodilator to restore her pulmonary function to baseline values. She was informed that she would be at risk of exercise induced bronchospasm triggered by the cold dry air of SCUBA exposure during actual dive conditions. This in turn would create the risk of arterial gas embolism, rupture of lung membranes, mediastinal emphysema or pneumothorax due to the expansion of trapped air upon ascent from depth and, accordingly, she was advised not to SCUBA dive.

CONCLUSIONS: A simplified method for EVH may be conveniently used to evaluate individuals for the risks attendant to exercise induced bronchospasm triggered by the cold dry air typically ventilated during a SCUBA dive.

15 BMI Does Not Correlate with PFTs in Asthma Screening

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RATIONALE: There is a positive association between asthma and obesity, but the mechanism is unclear. Studies have compared this relationship based on clinical symptoms without correlating objective pulmonary function tests (PFTs) with body mass index (BMI).

METHODS: Retrospective chart reviews were performed on 36 patients who had been evaluated for respiratory symptoms within the past 3 years. Initial PFT and BMI measurements were obtained prior to any intervention by the health care provider.

RESULTS: Average age of subjects was 41.3 years with a range of 10 to 86 years. There were 10 males and 27 females. Knudson predive values were used for PFTs; BMI was calculated using the following formula: (wt(lbs)/ (ht(in)xht(in))x703. Average BMI was 24.5 with a range of 15.0-34.9. Average FEV1 was 82.8% predicted with a range of 30.8 to 146.6. Regression analysis using FEV1, FVC, and FEF25-75 as dependent variables showed no correlation with BMI. (FEV1, p=0.783; FVC, p=0.275; FEF25-75, p=0.874) Correlation between FVC and BMI was 1.284), but did not reach statistical significance.

CONCLUSIONS: The relationship between obesity and asthma is based primarily on clinical symptoms, and objective measurements of pulmonary obstruction cannot be predicted by BMI. This lack of correlation would suggest multiple factors are involved in the relationship between asthma and obesity depending on the phenotype of each disease under study. Screening for obesity and asthma in the same clinical setting is unlikely to yield more positive results than either test alone.

16 Patterns of Aeroallergen Sensitization and Development of Sputum Eosinophilia and Airway Hyperresponsiveness

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RATIONALE: Specific aeroallergen sensitization may have more influence on the development of airway inflammation and airway hyperresponsiveness (AHR). This study was aimed to investigate the relationship between aeroallergen sensitization pattern and development of sputum eosinophilia and AHR.

METHODS: We retrospectively evaluated the data of skin prick test to aeroallergens, methacholine bronchial provocation test and induced sputum analysis from the patients who had performed all of three tests for the evaluation of their allergic and respiratory symptoms.

RESULTS: Mean age of 1207 enrolled subjects was 49.9 ± 16.4 and 43.2% were male. 51.9% of them had positive skin test response to at least one allergen, 38.8% had sputum eosinophilia (eosinophil ≥ 5%), and 23.3% had AHR (PC20 ≤ 16μg/ml). Both sputum eosinophilia and AHR were significantly associated with sensitization to at least one perennial allergen(OR=1.8, 95%CI: 1.4-2.3; OR=2.6, 95%CI: 1.9-3.5, respectively) and individual allergen sensitizations to house dust mite, indoor mold, cat, and dog, after adjusting for age and gender. There was no significant relationship in seasonal allergens such as tree, grass and weed pollen, outdoor mold, and cockroach.

CONCLUSION: Increased sputum eosinophilia and AHR are associated with specific allergen sensitization, especially sensitization to indoor perennial allergens.
Pitfalls In The Diagnosis Of Allergic Bronchopulmonary Aspergillosis In Patients With Asthma In Real Clinical Practice

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RATIONALE: We evaluated roles of various diagnostic tests in the diagnosis of allergic bronchopulmonary aspergillosis (ABPA) including, skin prick test (SPT) for Aspergillus fumigatus (Af), serum Af specific IgE and IgG antibody.

METHODS: A total of 50 asthma patients with more than 500 cell/μL of peripheral blood eosinophils were prospectively collected between March 2010 and August 2011. Evaluations using skin prick test (SPT) for Af, serum total and specific IgE antibody to Af by CAP system, IgG antbody to Af by enzyme immunoassay (EIA) or CAP system were performed according to the essential minimal criteria for the diagnosis of ABPA.

RESULTS: Among 50 patients, 2 patients (4.0%) were compatible with ABPA who had 5 items of the essential minimal diagnostic criteria for diagnosis of ABPA-seropositive. Six patients (12.0%) showed negative responses to Af in SPT, but positive responses in specific IgE by CAP system. Eight patients (16.0%) showed negative responses to IgG to Af by CAP system, but positive responses by enzyme immunoassay (EIA).

CONCLUSIONS: SPT and serum IgE to Af measurement by CAP system should be performed simultaneously. It is reasonable to set up cut off values in various diagnostic methods for differentiation of ABPA in Af sensitized asthma patients.

A rare case of Allergic Bronchopulmonary Mycosis secondary to Penicillium

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RATIONALE: Allergic bronchopulmonary mycosis (ABPM) is a hypersensitivity reaction that occurs in asthmatic patients contributing to worsening of disease. An immune response to Aspergillus fumigatus colonization of the airway is the most common cause of ABPM but other fungi such as A. flavus, Candida albicans, Schizopothum and Penicillium have been implicated.

METHODS: We report a 53 yo male with ABPM secondary to Penicillium presenting as asthma.

RESULTS: A 53 yo Korean male presented to Allergy Immunology Clinic with a diagnosis severe asthma. He had 4 year h/o asthma symptoms requiring 30 ER visits, over 10 hospitalizations and 2 intubations. He has a 35 pack/year smoking history, quitting 1 year prior to initial visit. Patient symptoms included thick brown mucus secretions and outside chest CT revealed “bronchiectasis”. Absolute eosinophil count 1500 and IgE 623 kU/L (on steroids). SPT specific IgE to Aspergillus was negative. IgE testing to penicillium notatum was positive (1.05 kU/L). Skin prick testing was positive to penicillium (5mm wheal and flare). A diagnosis of ABPM secondary to Penicillium was made. We initiated therapy with oral steroids and 16 week course of Itraconazole 400mg daily.

CONCLUSIONS: Penicillium is rare but possible cause of ABPM, and should be considered in severe asthma with bronchiectasis.
21 Cord Blood Cellular Proliferative Response as a Predictive Factor For Atopic Dermatitis At 6 Months of Age

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RATIONALE: Allergen exposure in sensitized asthmatics has been shown to induce an immediate allergic response. In this study, we investigated the allergen induced response in cord blood from infants who were non-sensitized and had a negative challenge (sens/chall-). The median lymphocyte O2- was 911,615 relative luminescence units (RLU) at baseline and 3,544,645 RLU at 24hr (p = 0.12) among sens/chall- subjects. Eosinophil% and lymphocyte O2- remained stable among sens/chall+ subjects (p=0.11, p=0.45, respectively). Neither lymphocyte nor neutrophil percentage increased in (sens/chall+) subjects 24hr post challenge.

CONCLUSIONS: Asthmatic responses to mouse allergen exposure may be associated with the development of a systemic inflammatory response characterized by increased blood eosinophils and lymphocyte activation 24 hours following exposure. These findings suggest that the respiratory allergic response results in systemic inflammatory changes.

22 Allergic Airways Responses Are Associated With A Late-phase Systemic Inflammatory Response In An Environmental Allergen Challenge Model

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RATIONALE: Allergen exposure in sensitized asthmatics has been associated with pulmonary eosinophil infiltration; however, whether there is a systemic inflammatory response is unclear.

METHODS: 21 stable asthmatics who were non-smokers (18-50 y) were skin prick tested (SPT) and underwent environmental mouse allergen challenge (EMAC) in a controlled environmental chamber. A positive challenge was defined as a 15% drop in FEV1. Peripheral blood eosinophils and superoxide anion (O2-) production by peripheral white blood cells were measured before and 24 hours after EMAC. O2- was measured by a luminol chemiluminescence assay. Outcomes for participants who were mouse sensitized (3mm+ net wheal to mouse epithelial extract) and had a positive challenge (sens/chall+) were compared to outcomes for participants who were non-sensitized and had a negative challenge (sens/chall-).

RESULTS: The median blood eosinophil percentages were 3.5% and 5.1% at baseline and 24hr, respectively (p=0.04), among sens/chall- subjects. The median lymphocyte O2- was 911,615 relative luminescence units (RLU) at baseline and 3,544,645 RLU at 24hr (p=0.12) among sens/chall+ subjects. Eosinophil% and lymphocyte O2- remained stable among sens/chall- subjects (p=0.11, p=0.45, respectively). Neither lymphocyte nor neutrophil percentage increased in (sens/chall+) subjects 24hr post challenge. "Fever" was observed in 24% of sens/chall- subjects (p=0.04). Boys had higher levels of total IgE at year 9 (median, 56 vs 28 KU/L; p=0.01) and year 9 (median, 63 vs 45.2 KU/L; p=0.04). Median PHA-induced IFN-γ responses were higher in boys when compared to girls at year 6 (median, 1131 vs 777; p=0.005). PHA-induced IL-5 and IL-10 responses were also greater in boys at year 6 (median, 289 vs 236; [p=0.029] and median, 1040 vs 920; [p=0.034], respectively).

CONCLUSIONS: There are gender differences in atopic phenotype expression and in vitro immune responses between boys and girls during the pre-pubertal school age years.
25 Asthma Phenotypes in School-aged Children from the Population Study: Cluster Analysis

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RATIONALE: We evaluate the childhood asthma phenotypes from the population study using cluster analysis.

METHODS: A questionnaire survey, blood tests for total IgE and eosinophil fraction, skin prick test, spirometry and methacholine bronchial challenge test were performed in 2,491 primary school children. Among the various factors affecting to childhood asthma, representative variables were extracted by principle component analysis. And we performed two step cluster analysis in the subjects with a history of doctor-diagnosed asthma. We then compared differences in demographic characteristics, lung functions, atopic status and bronchial hyperresponsiveness between clusters.

RESULTS: In the questionnaire survey, 235 children (10.1%, 235/2,337) had a history of doctor-diagnosed asthma. After excluding subjects with missing values, three clusters were extracted in the 193 children with asthma. The first cluster (atopic asthma, n = 77) was more likely to have atopy (77/77, 100%), higher total IgE (380.80 ± 21.97 IU/mL), and higher blood eosinophil % (7.76 ± 7.76 %). The second cluster (non-atopic, n = 90) was less likely to have atopy (0%) and had low IgE and blood eosinophil.

CONCLUSIONS: Three phenotypes were classified among subjects with childhood asthma in Korea. Cluster analysis can be a useful statistical method for identifying asthma phenotypes.

26 Ectoparasite Induced Elevations of alpha-gal Specific IgE are Associated with Increased Total Serum IgE and Cat Sensitization but not with Asthma


RATIONALE: In the southeastern United States, IgE antibodies to galactose-alpha-1,3-galactose (alpha-gal) are common. Induced by ectoparasitic ticks, these antibodies can cause anaphylaxis both to the mononclonal antibody cetuximab and red meat. In keeping with the distribution of alpha-gal amongst non-primate mammals, these IgE antibodies also bind extracts derived from mammals, including cats. Despite elevated total IgE and cat specific IgE, these patients describe relatively few symptoms of bronchial hyperreactivity.

METHODS: Study 1: 208 subjects presenting to central Virginia allergy clinics with symptoms of urticaria, angioedema, or anaphylaxis, 68 asthmatics, and 59 controls were assayed for total serum IgE and IgE to alpha-gal and several inhalant allergens. Spirometry and eNO were performed as markers of asthma.

RESULTS: The mean total serum IgE amongst those in the anaphylaxis group was not different from that of asthmatics (1771±1660 IU/mL, p=0.63), and markedly higher than that of the control group (281±537 IU/mL, p<0.0001). While most alpha-gal subjects have IgE to cat epithelium, living with a cat did not increase risk for asthma. Mean eNO and FEV1/FVC amongst those living with a cat was 18±5 ppb [CI. 15-20] and 0.80 [0.78-0.82], respectively, and 24±5 ppb [17-31] and 0.79 [0.77-0.81] for those not living with cats; mean eNO and FEV1/FVC for our asthmatics were 51±11 ppb [38-64] and 0.70 [0.68-0.73], and controls 20±10 ppb [13-27] and 0.80 [0.78-0.83].

CONCLUSIONS: Subjects with alpha-gal specific IgE and allergic symptoms after ingestion of red meat have elevated total IgE and cat specific IgE, but lung function that is no different from that of our control group.

27 Helicobacter Pylori, A Protective Agent For Asthma Or Not?

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RATIONALE: Helicobacter pylori is a Gram-negative gastric bacillus, whose diagnosis is made by endoscopic biopsy. H. pylori has been associated to the inhibition of Th2 responses in asthmatic patients activating Th1 response. Asthma is inversely associated with serologic evidence of the presence of cagA+ H. pylori strains. We will explore the association between Helicobacter pylori status and asthma prevalence among a pediatric population, that may suggest a protective factor conferred by the bacteria.

METHODS: Review of medical records of pediatric patients (N=855) that underwent an upper endoscopy on 2009 at HESL in Ponce, Puerto Rico. Study includes patients between 1 to 21 years of age. Demographic, age, gender, history of asthma and allergies were obtained. H. pylori status was obtained from gastric biopsy results.

RESULTS: From 831 gastric biopsy results, 52 were positive for H. pylori. 63% were (33/52) females and 37% (19/52) males. 54% (28/52) were teenagers. Twenty-nine percent (15/52) of patients have history of asthma, 17% (9/52) have history of allergies, 10% (5/52) have history of both, and 54% (28/52) did not have history of asthma or allergies.

CONCLUSION: More females are diagnosed with H. pylori infection and teen patients suffer more infections with such bacteria. There appears to be an association between infection and the absence of asthma and allergies, since 54% of our patients with H. pylori infection did not have a history of asthma or allergies. To evaluate H pylori as a protective factor for asthma and allergies, a control population with negative cultures will be required.

28 The Role of Atopy as a Predictor of Childhood Atopic Asthma

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INTRODUCTION: The diagnosis of childhood asthma remains difficult. Atopy is a surrogate marker for determining asthma in a preschool child with chronic respiratory symptoms. However, local studies have brought this relationship into question. Two studies conducted at the Children’s Chest and Allergy Clinic, Steve Biko Academic Hospital, investigated the role of atopy as a predictor of childhood atopic asthma.

METHODS: Study 1 (Atopy in asthmatic children attending a tertiary hospital in Pretoria) enrolled 100 asthmatic children and an age and sex-matched control group of 50 non-asthmatic children. Skin prick tests to standard allergen extracts were performed. Study 2 (An investigation into maternal factors of asthmatic children for predicting the allergic basis of childhood asthma) enrolled 100 asthmatic children and their mothers. The mothers completed a questionnaire which included demographic details, a history of symptoms suggestive of allergic diseases and a history of asthma. Skin prick testing was performed on the mothers.

RESULTS: In study 1, 45% of asthmatic children and 16% of the control group, had a positive skin prick test. In study 2, 14 of the 16 mothers with asthma, had atopic children (p=0.045). Maternal atopy or a history suggestive of maternal allergic disease, weren’t good predictors of childhood atopic asthma.

CONCLUSION: Atopy occurs less commonly in our population of asthmatic children than previously thought. Maternal asthma is the only valuable predictor of childhood asthma, suggesting that asthma must be associated with other environmental exposures. A more extensive study is suggested.
Assessment of Repeated Measures of Fractional Exhaled Nitric Oxide in Clinically Stable Persistent Asthma

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RATIONALE: The recent American Thoracic Society guidelines on interpretation of fractional exhaled nitric oxide (FeNO) levels suggest monitoring changes in children with asthma. The goal of this study was to determine if changes in FeNO levels are observed in children with persistent asthma during clinical stability to determine the need for repeated FeNO levels once baseline values are established.

METHODS: This is a prospective control cohort study of clinically stable asthmatic children (n=54) aged 4 to 18, followed in the allergy and immunology division. Patients were assessed at two visits six months apart. FeNO (Aerocrine, Sweden) and spirometry (Koko Pneumotach) were conducted and clinical history was obtained at each visit. Controls (n=12) were healthy with no history of atopy or asthma. Statistical analysis was performed using Prism (Graphpad Software, California).

RESULTS: Significant difference in FeNO (p<0.02, Mann-Whitney U 55.75) was observed between the asthma (28.3 ppb±4.59) and control group (9.4 ppb±1.42). We found no significant change in FeNO values between visits (p=0.78, Mann-Whitney U 153, 22.98 ppb±5.24 on initial measurement and 24.66 ppb±5.77 on follow up). Two subgroups were identified in the asthma group, subjects with elevated FeNO (54.73 ppb±6.38) and those with levels comparable to controls (11.66 ppb±1.36). There was no significant difference on follow up levels in each of these two subgroups (p>0.05).

CONCLUSIONS: FeNO measurements remain unchanged in children with clinically stable persistent asthma suggesting FeNO levels should be repeated based on changes in clinical symptoms.

Identification of Vocal Cord Dysfunction by Methacholine Laryngoscopy Reduces Inhaled Corticosteroid and Rescue Inhaler Use in Children and Adults

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RATIONALE: Vocal cord dysfunction (VCD) is associated with chest tightness, shortness of breath and wheezing, often masquerading as asthma. The gold standard for diagnosis of VCD is direct laryngoscopic visualization during a symptomatic episode. Methacholine (MCT) is a short-acting anticholinergic shown to be of benefit in acute asthma exacerbation. Vocal cord dysfunction (VCD) is paradoxical adduction of the vocal cords during inspiration that can mimic asthma. We examined if ipratropium could reverse the decline in FEV1 following methacholine challenge in patients with demonstrable vocal cord dysfunction.

METHODS: A retrospective chart review of 103 consecutive patients (65% female, mean age 22.5 years) who underwent methacholine laryngoscopy by a board-certified allergist for evaluation of VCD from 9/2008 to 1/2011 was performed. Baseline spirometry, response to methacholine, presence of VCD, and change in FEV1 and FVC following nebulized ipratropium was recorded.

RESULTS: Seventy patients were diagnosed with VCD, of which 52.8% (n=37) had a positive methacholine challenge. Of those having a positive methacholine challenge, 13/29 received nebulized ipratropium as their rescue therapy. Ten of these patients had spirometry documenting response to ipratropium therapy which was compared to their pre-procedure baseline spirometry. Ipratropium treatments demonstrated a mean improvement to 94% of their baseline pre-methacholine FEV1.

CONCLUSIONS: Inhaled ipratropium is a viable rescue treatment choice to reverse respiratory symptoms in those diagnosed with VCD.
Gastroesophageal Reflux in Patients with Chronic Cough
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RATIONALE: A major cause of chronic cough is gastroesophageal reflux disease (GERD). Its diagnosis is based on symptoms and diagnostic tests, such as upper gastrointestinal endoscopy (UGE), 24-hour pH monitoring, and manometry. Many patients also present chronic posterior laryngitis in fibrolaryngoscopy (FNL). The objective of the present study was to evaluate the diagnosis of esophagitis, by FNL and UGE in patients with chronic cough.

METHODS: Patients followed up for chronic cough, over 18 years of age, were asked about the presence of GERD symptoms and submitted to the FNL and UGE, some of them with esophageal biopsy.

RESULTS: Fifty-one patients participated in the study. The average age was 56.8 years (±13.2 years). 90.2% were female and the average duration of cough, 12.2 years (±14.9 years). Of these, 46 (90.2%) had dyspepsia, and partial or complete improvement of symptoms of cough with proton pump inhibitor. Of the 46 symptomatic patients, only 18 (39.1%) had esophagitis on UGE; however, 36 patients (78.3%) had posterior laryngitis on FNL. Seventeen patients also underwent esophageal biopsy, and 15 examinations identified esophagitis. Nine (60%) of these patients had only posterior laryngitis on the FNL (UGE without esophagitis).

CONCLUSIONS: Fibrolaryngoscopy was more sensitivity than upper gastrointestinal endoscopy to confirm gastroesophageal reflux disease. Although the indication for biopsy of esophagus follows standardized criteria, this study suggests that in patients with chronic cough, if there is an indication for the performance of UGE, it would be interesting to complement with biopsy of the esophagus.

Development and Preliminary Validation of the Asthma Intensity Manifestations Score (AIMS) Derived from Asthma Control Test, FEV₁, Exhaled Nitric Oxide, and Step Therapy Assessments

RATIONALE: Inherent asthma severity is difficult to assess clinically. The purpose of this study is to develop an Asthma Intensity Manifestations Score (AIMS) as a surrogate for asthma severity.

METHODS: 304 patients treated with inhaled corticosteroids completed the Asthma Control Test (ACT), underwent spirometry and fractional exhaled nitric oxide (FENO) testing, and reported their current medications. These parameters (ACT < 16, FEV₁ < 80% predicted, FENO > 300 % predicted, and EPR3 step care level > 3) were related to prior year outcomes to develop the AIMS and to follow-up year outcomes to validate it.

RESULTS: FENO was independently related to prior year short-acting beta agonists (SABA) ≥ 7 (OR 2.9) while ACT (OR 4.9), FEV₁ (OR 3.3), and Step Care (OR 3.9) were independently related to prior year systemic corticosteroids (SCS) ≥ 2. Thus, all four items were chosen for the AIMS (0-4 points). AIMS scores were linearly related to follow-up year SABA ≥ 7, any OCS, OCS ≥ 2, and emergency hospital care (all p < 0.01). Compared to patients with AIMS scores < 2, patients with AIMS scores ≥ 2 were at more than a 1.5-fold greater risk of requiring ≥ 2 SCS during the following year and 2.5 times as likely to receive ≥ 7 SABA during the following year.

CONCLUSIONS: The AIMS is linearly related to future year adverse asthma outcomes. Further studies will be necessary to confirm its utility as a surrogate for asthma severity in clinical practice and clinical research.

The Role Of Induced Sputum Cytology In Clinical Monitorizing Of Childhood Asthma
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RATIONALE: The role and characteristics of airway inflammation in childhood asthma are less well established than in adults. The aim of this study was to investigate the airway inflammation degree in children with mild and moderate persistent asthma on inhaled corticosteroid therapy by using sputum induction technique.

METHODS: Sputum samples were collected from 30 children with asthma aged between 7-18 years and 40 healthy controls in same ages. After sputum processing, slides were prepared with cytospinrifuge and differential cell count was obtained by counting 400 non-squamous cells.

RESULTS: An adequate sample was obtained in 71.4% of subjects. Patients with asthma and healthy subjects were differed significantly in respect to sputum eosinophil counts (p = 0.03). Sputum eosinophils of the partly controlled asthmatics were significantly higher than the controlled asthmatics (p = 0.004). There was a strong negative correlation between the disease control and the sputum eosinophilia (r = -0.66, p < 0.001). There were strong relationships between the sputum eosinophilia and daytime symptoms (r = 0.51, p = 0.007), nocturnal symptoms (r = 0.6, p = 0.001), activity limitations (r = 0.66, p < 0.001) and need for relievers (r = 0.66, p < 0.001). Exacerbations were also correlated with sputum eosinophilia (r = 0.51, p = 0.007).

CONCLUSIONS: These findings suggest that, unsuppressed eosinophilic inflammation of airways can reflect poor control of clinical symptoms in asthmatic children on standard dose inhaled corticosteroids.

A Retrospective Analysis of Distinguishing Features Between Asthma and Vocal Cord Dysfunction
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RATIONALE: Vocal cord dysfunction (VCD) is an important and often overlooked clinical problem that has a variety of causes, all of which can lead to the accentuation of glottic closure that defines VCD. Symptoms overlap can make differentiating asthma and VCD difficult. Our present study aimed to adequately define the relationship between asthma and VCD.

METHODS: A retrospective study of 263 patients with VCD compared to 50 patients with asthma but without a diagnosis of VCD was performed. For binary variables, statistical significance was quantified using the chi-square test, and the sensitivity and specificity were also calculated. For continuous variables, we used the median value as a cut-off for determining whether the value was elevated or not, which was subsequently used to calculate the sensitivity and specificity.

RESULTS: The following variables were significantly associated with VCD status: throat tightness (66% sensitivity, 100% specificity), wheezing (61% sensitivity, 80% specificity), sensitivity to odors (60% sensitivity, 94% specificity), anxiety (11% sensitivity, 100% specificity), use of inhalers (17% sensitivity, 98% specificity), oral steroid use (24% sensitivity, 90% specificity), gastrointestinal conditions (67% sensitivity, 58% specificity), cough (42% sensitivity, 74% specificity), exercise (68% sensitivity, 62% specificity), short-acting beta agonists (29% sensitivity, 88% specificity), proton pump inhibitors (48% sensitivity, 72% specificity), and leukotriene modifiers (39% sensitivity, 78% specificity).

CONCLUSIONS: Even though a number of individual factors are significantly associated with VCD, very few (throat tightness, wheezing, and sensitivity to odors) have reasonable discriminatory ability on their own, thus stressing the need for multivariable predictor models.
**Phenotypes of Rhinitis and Difficult Asthma**

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**RATIONALE:** In asthma, phenotyping is well advanced. Such phenotyping is lacking in rhinitis. We applied an unsupervised statistical methods (cluster analysis) to characterize the clinical phenotypes of rhinitis associated with difficult asthma (DA) in children and in adults.

**METHODS:** Following approval by the local ethics committee 117 children mean aged 7.91±2.02 years old and 164 adults aged 36.14±12.08 years old with rhinitis and asthma were included. The following phenotype traits were considered: sex, atopic, obesity; predominant symptom pattern for rhinitis (rhinorrhea or nasal obstruction), nasal polyposis (NP); DA divided into frequent asthma exacerbations and fixed airway obstruction.

**RESULTS:** 3 clusters were distinguished for the paediatric population: male, atopic, rhinorrhea, no DA; male, atopic, no predominant rhinitis symptom, frequent asthma exacerbations and female, non-atopic, obese, no DA. Adults segregated into 4 clusters: female, obese, atopic, rhinorrhea, fixed airway obstruction; female, atopic, nasal obstruction, no DA; male, obese, atopic, nasal obstruction, NP, no DA.

**CONCLUSION:** In children male sex, atopy, lack of obesity and no predominant symptom pattern for rhinitis can distinguish the frequent exacerbations phenotype of difficult asthma. In adults female sex, lack of obesity and predominant rhinorrhea point to the fixed airway obstruction phenotype of difficult asthma. Further prospective validation is however warranted.

**Spry2 is a Novel Regulator of T Cell Activation and Differentiation**

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**RATIONALE:** Spry2 (Spry2) is a regulator of receptor ubiquitination and recycling, and ERK activation. Its function in T cells is unknown. The objective was to delineate the role of spry2 in T cell function and differentiation.

**METHODS:** We generated CD4 targeted spry2 knockout (KO) mice. CD4 T cell functional and signaling studies were done by flow cytometry, ELISA, ICC and western blotting.

**RESULTS:** Spry2 was expressed at a low level in resting T cells. Its expression increased upon sustained TCR stimulation and co-stimulation. We generated CD4 targeted spry2 KO mice, which were grossly normal and had normal peripheral CD4 and CD8 T cells. Spry2 KO T cells responded poorly to co-stimulation (CD28, IL-2) in proliferation (CFSE dilution) and functional assays (CD25 and CD62L expression). Spry2 interacted with the endosomal regulators—Rab5, Rab7 and Rab11. The downregulation of surface TCR after TCR stimulation due to endocytosis was augmented in KO cells. Spry2 KO T cells poorly differentiated into Th2 cells. The development of airway inflammation and hyperreactivity (airway resistance by Flexivent) in a mouse model of acute asthma was significantly impaired. Th1 differentiation was also impaired but was compensated by a stronger stimulus. Mechanistically, the activation of ERK1/2, STAT5 and STAT6 was impaired in KO T cells. Spry2 interacted with the mTOR complex, a regulator of Th1/Th2 differentiation. Spry2 KO T cells showed reduced phosphorylation of mTORC1 and mTORC2 regulated kinases—AKT and S6 kinase, respectively.

**CONCLUSION:** We have identified spry2 as a novel regulator of T cell activation, differentiation and development of asthma.

**Stat5b (Signal Transducer and Activator of Transcription 5b), Not Stat5a, Is a Critical Modulator of Human Treg Development and Function**

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**RATIONALE:** Human STAT5b deficiency is a rare, autosomal recessive primary immunodeficiency characterized by autoimmune disease, chronic lung disease, growth failure, and other immunodeficiencies. Unlike mice, STAT5a sufficiency in humans cannot compensate for STAT5b defects. Our study aims to determine the relative contributions of STAT5a and STAT5b to Treg function by analyzing samples from homozygous STAT5b-deficient patients and evaluating knock down of STAT5a or STAT5b expression in control human Treg.

**METHODS:** CD4+ cells from STAT5bneg/neg patients were analyzed for T cell excision circles (TRECs), phenotype (CD45RA, CD45RO), and FoxP3 (Forkhead box P3) expression, and in 1H thymidine suppression assays. IL-2, IL-10 and TGF-b plasma levels were determined by multiplex. To knock down STAT5a or STAT5b, CD4+ healthy control cells were transfected with STAT5a or STAT5b RNAi siRNA, and STAT5a, STAT5b, FoxP3, and CD25 protein levels were determined by NanoPro. Transcript expression for FoxP3, IL-2R, BCL-X (B cell lymphoma X), and IGF-I (insulin-like growth factor I) were assessed by Q-PCR.

**RESULTS:** Treg from patients with STAT5bneg/neg deficiency (n=4) demonstrated decreased TRECs, FoxP3 expression, CD45RO suppression, and suppressive function compared to age-matched control Treg. Specific knock down of STAT5b decreased transcriptional expression of IL-2R, FoxP3, and IGF-I, while knock down of STAT5a decreased only expression of BCL-X.

**CONCLUSIONS:** Gene silencing was employed successfully to differentiate the roles of STAT5a versus STAT5b in human Treg functions. The results supported clinical analysis of STAT5b deficient patients and provide strong evidence, for the first time, that STAT5b, not Stat5a, is a critical contributor to normal Treg development and function in humans.

**Hyperthermia enhances Th1 differentiation and downregulates Fox3 expression in Tregs**

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**RATIONALE:** Fever is a primordial response to infection. There is an increased incidence of allergy and an obsession to suppress fever early during infection and vaccination. Our hypothesis is that fever plays a crucial role in optimizing vaccine and infectious responses by driving Th1 differentiation and abrogating Treg function.

**METHODS:** FACS analysis of Tregs in peripheral blood of 5 adult cancer patients enrolled in an IRB-approved protocol for 6h thermal therapy (TT, 40°C). For in vitro studies, Tregs (CD4+CD127-CD25hi) and naive T cells (CD4+CD127+CD25-CD45RA+) were FACS-sorted from PBMC of healthy donors. Tregs were stimulated with anti-CD3/CD28/IL12 for 6h at 37 vs. 40°C and analyzed by FACS for FoxP3 expression. Naive T cells were stimulated with anti-CD3/CD28/IL12 for 3h daily x 5d and analyzed for intracellular IFNg.

**RESULTS:** Patients treated with TT had a significant (p<0.0001) decrease in geometric mean fluorescent intensity of FoxP3 expression in Tregs concentration between 15-40% after 6h treatment and persisted after 18h later. There was a rise in the percentage of Tregs after 6h that fell below preheat after 18h postheat (p<0.001). In vitro studies demonstrated a decrease in the gMFI of FoxP3 in Tregs after 6h hyperthermia. Hyperthermia also enhanced Th1 differentiation based on increased percentage (25-50%) and gMFI of IFNg-producing cells.

**CONCLUSIONS:** Hyperthermia decreased Foxp3 in Tregs via a ubiquitin-proteosome degradation pathway. The concentration of Foxp3 has been shown to be critical for Treg suppression. Hyperthermia also promotes Th1 differentiation. The results suggest that fever is beneficial for infection by transiently abrogating Treg function and enhancing Th1 response.
41 Critical Role Of T Follicular-helper Cells In B Cell Autoimmunity During Lymphopenia
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RATIONALE: Lymphopenia-induced proliferation (LIP) is critical for the maintenance of T-cell homeostasis but may have a pathogenic role in the development of systemic autoimmune diseases characterized by the presence of autoantibodies.
METHODS: Lymphopenic mouse transfer model is a well known model for autoimmune diseases, in which various autoantibodies are observed. We transferred CD4+CD25+ T cells into T-cell-deficient recipients, and investigated the mechanisms of antinuclear autoantibody production during LIP.
RESULTS: LIP of CD4+CD25+ T cells provoked enhanced antibody production, class switching, antinuclear antibody production, and generated lymphoid folicles and germinal centers in the recipients. We identified a new subset of T follicular-helper cells (TFH cells) which develop from conventional T cells during LIP. Compared with traditional TFH cells, TFH cells generated by LIP have a distinctive cell surface profile, as CXCR5-CXCR4+ICOS+PD1+CD200+CD4+ cells. Moreover, TFH cells generated by LIP expressed lower levels of Bcl6 and Helios, which are transcriptional factors expressed in traditional TFH cells, and help B cells to produce IgG through IL-21 production and ICOS-ICOSL interaction. LIP-TFH cells develop independently of cognate antigen recognition under the control of regulatory T cells, and have a critical role in breaking B cell tolerance.
CONCLUSIONS: Regulatory T cells maintain B-cell tolerance during T-cell homeostasis by preventing novel TFH cell development.

42 Per a 10 Protease Activity Induces Th2 Polarization That Is Amplified By Allergic Status
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RATIONALE: Proteases induce Th2 polarization mediated by dendritic cells (DCs). The present study investigates effect of serine protease from Periplaneta americana in T cell polarization depending on allergic status.
METHODS: Cockroach-sensitive patients with high specific IgE against P. americana/Per a 10 were selected. Spirometric plus exhaled nitric oxide measurements were done for both patients and healthy donors. Monocyte-derived DCs obtained from cockroach-sensitive patients or healthy donors were stimulated with proteolytically active or inactive Per a 10. The effect on antigen presenting function was assessed by flow cytometry, ELISA and T cells polarization analyzed.
RESULTS: Per a 10 induced a significant increase in costimulatory molecules CD86 and CD80 on DCs from cockroach-sensitive patients as compared to healthy donors. This was associated with increased capacity to induce T cell proliferation, production of proinflammatory cytokines, and the type 2 cytokine IL-5 (p<0.01). No differences were observed in IL-12 release by DCs after Per a 10 stimulation. Purified CD4+ T cells from cockroach-sensitive patients stimulated by autologous Per a 10-pulsed DCs preferentially produced IL-4 and IL-5 rather than IFN-γ. Further, IL-12 production by T cells was significantly low (p<0.05) with cockroach-sensitive patients than healthy individuals. Such effects are reduced in presence of inactive protease, although significant differences exist between cockroach-sensitive patients and healthy donors.
CONCLUSIONS: The study indicates that DCs from cockroach-sensitive patients plays pivotal role in amplification of Th2 response and is dependent on immune status of the donor.

43 IgE and Atopy Are Associated With Phosphorylated P38 MAPK Expression By CD4 and CD8 T Lymphocytes
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RATIONALE: P38 MAPK is associated with Th2 cytokine responses. We determined if phosphorylated P38 MAPK (p-38) is associated with IgE production and atopy, and if inhibition of p38-38 suppresses IgE responses.
METHODS: Phosphorylated P38, ERK (p-ERK) and JNK (p-JNK) MAPK expression by blood leukocyte subsets, serum immunoglobulin levels and skin prick testing (SPT) of atopic adults with asthma or rhinoconjunctivitis (n = 32) were determined (flow cytometry [MESF], ELISA). Peripheral blood mononuclear cells (PBMC) were cultured for 0-12 days ± anti-human CD40/recombinant human interleukin-4 ± varying concentrations of inhibitors of p38-P38 (SB202190 and SB203580) and p-JNK (SP600125). Phos-p38, p-ERK and p-JNK expression by leukocyte subsets, and IgE levels in supernatants were determined.
RESULTS: Phos-P38 MAPK expression by CD4+ T and CD8+ T, CD19+ B cells, and CD14+ monocytes was associated with serum IgE levels (linear regression, P<0.001) and SPT (P<0.04), even when adjusting for cell counts, age, sex, race and current smoking status (P<0.04). In contrast, p-ERK and p-JNK expression by any of the cell types was not (P>0.09 - 0.99). Instead, p-ERK was associated with serum IgG. P38 MAPK inhibitor SB202190 suppressed induction of IgE responses by PBMC in vitro (P=0.0001), without significant cytotoxicity; whereas SB203580 and SP600125 were associated with significant cytotoxicity.
CONCLUSIONS: Our results indicate the P38 MAP kinase mediates IgE production and atopy, and that specific inhibition of p38-P38 MAPK activity suppress IgE production.

44 Regulatory T cell regulation by STAT5B
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RATIONALE: We reported that STAT5A and STAT5B show peptide sequence similarities of >90% and the STAT5A levels of the STAT5A deficient patients were normal. This indicates STAT5B plays a specific role in differentiation into Treg. To reveal the regulation mechanism on Treg, we performed to detect STAT5B specific binding sites.
METHODS: This study was in adherence with the Declaration of Helsinki, and approved by the Ethics Committee of School of Medicine, Stanford University. We used healthy human CD4+ cells for this analysis. The samples for chromatin immunoprecipitation followed by sequencing (ChIP-seq) were followings: 1) wild type CD4+ cells for STAT5A and STAT5B, 2) STAT5A knock down (KD) CD4+ cells, 3) STAT5B KD CD4+ cells, and 4) wild type CD4+ cells as control for IgG. The KD cells were obtained by the Neon™ electroporation system and cultured for 3 days. After sequencing with Illumina Genome Analyzer, the results were mapped back to the reference genome for determination of the binding sites, following analyzing the peak signal in order to detect STAT5B specific binding sites.
RESULTS: We detected several candidates binding sites specific to human CD4+ cell for STAT5B.
CONCLUSIONS: To our knowledge, this is the first report detecting the specific binding sites for STAT5B by using human CD4+ cells. The ChIP-seq technique enables us the genomewide identification of binding sites of transcription factors. Our findings would contribute to one step closer to reveal of Treg regulation.
45 The Effect Of Phototherapy On Lymphocyte Subsets In Newborn Infants
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**Rationale:** Phototherapy is the mainstay treatment of neonatal hyperbilirubinemia. Previous studies found that neonatal jaundice was associated with increased parental perception of vulnerability. The study was to investigate the influence of the use of phototherapy on different lymphocyte subsets; CD3+, CD19+ and CD56+ cells after 72 hours of exposure and correlate the finding to the frequency of infections in the first six months of life.

**Methods:** Thirty term neonates with indirect hyperbilirubinemia were sampled before and 72 hours of exposure to phototherapy. The percentages of CD3+, CD19+ and CD56+ cells were assessed by flow cytometry. The study group were followed-up for a period of 6 months with monitoring of the growth pattern, frequency of infections and the need for hospitalizations.

**Results:** The percentage of CD3+ and CD19+ lymphocyte subsets were significantly lower at 72h of exposure to phototherapy. CD56+ cells were decreased as well at 72h of exposure to phototherapy yet the decline was statistically insignificant. Patients with a higher decline in CD3+ cells had an increase in the number of hospital visits (r=0.64, p=0.05).

**Conclusion:** Phototherapy affects the immune system by affecting the lymphocyte subsets percentages even after a short duration of exposure. The effect on T cells number might contribute to the vulnerable child syndrome.

46 **CD27** Developing B Cells are Common in Human Fetal Liver
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**Rationale:** CD27 is generally accepted as a marker of post-germinal center (GC) memory B cells. However, several observations challenge this consensus, including expression of CD27 on naïve, IgM+ fetal-origin B cells in umbilical cord blood and the presence of CD27+ peripheral blood B cells in Type 1 IgM syndrome patients, who cannot form GC.

**Methods:** To determine the origin of these CD27+ B cells, we analyzed the earliest source of human B cell ontogeny, fetal liver.

**Results:** In 13 and 19 week human fetal liver, most (285%) of CD19+B cells express CD10, a marker for developmental immaturity. Surprisingly, 6%-8% of these CD19+CD10+ B cells express CD27, further analysis revealed that CD27 expression could be detected at the earliest stage of B-cell commitment, CD19+CD10+IgM+IgD+CD34+ pre-B cells. This CD27 expression subsequently continued in the B-lineage at least until the appearance of IgM+ immature/transitional B cells. Similar CD27+CD19+ B cells with a pro-B, pre-B, and immature/transitional B-cell phenotype are present, albeit less frequently (1.6 %–0.5 %), in adult bone marrow. Consistent with patterns of gene expression in conventional (CD27+) pro-B, pre-B and immature/transitional B cells, high levels of recombination activating gene-1 (RAG-1), terminal deoxynucleotidyl transferase (TdT), and Vpre-B mRNA are present in CD27+ pro/pre-B cells from fetal liver and adult bone marrow, but undetectable in CD27+CD19+CD10+IgM+ immature/transitional B cells. When CD27+ pro-B cells are cultured, these cells efficiently differentiate into pre-B and IgM+ immature/transitional B cells.

**Conclusions:** Our findings suggest a distinct, fetal origin for a subset of CD27+IgM+ B cells that is minimized in adults.

47 Intravenous Immunoglobulins Suppress Antibody-Dependent Effector Functions of Human Peripheral Blood Cells

**Rationale:** Several studies have shown the importance of self-reactive antibodies in the maintenance of autoimmune diseases. Therefore, we asked whether IGIV, which is therapeutically effective in some of those diseases, directly modulates antibody-mediated effector functions.

**Methods:** We used a human in vitro system to analyze potential modulatory effects of IGIV on effector functions in antibody-dependent cellular cytotoxicity (ADCC). Human peripheral blood mononuclear cells (PBMC) from 47 healthy volunteers were pre-incubated with IGIV and then added to human breast cancer cells (SK-BR-3) opsonized with a specific antibody. The cytotoxic damage to SK-BR3 cells was determined by measuring lactate dehydrogenase release into supernatants. IGIV-mediated effects on viability and Fc gamma receptor (FcγR) expression of PBMC were analyzed by flow cytometry.

**Results:** Pre-incubation of PBMC with IGIV for 48h resulted in a marked inhibition of ADCC, ranging from 15% to 74% (mean 42%) which could not be mimicked with human monoclonal IgG1 and IgG2 antibodies. Flow cytometric analysis indicated that IGIV induced natural killer (NK) cell death, which significantly correlated with ADCC inhibition. The IGIV-induced decrease in NK cell viability was not triggered by FcγR ligation since the addition of FcγR blocking antibodies could not prevent cell death induction. Furthermore, IGIV enriched for sialic acid bearing glycans by lectin affinity chromatography with Sambucus nigra agglutinin showed no differences in the effects on NK cell viability and ADCC compared with non-enriched IGIV.

**Conclusions:** IGIV modulates antibody-dependent effector functions of human immune cells, which could contribute to the modulatory activities of IGIV in inflammatory and autoimmune diseases.

48 Physiologic DNA Breaks Activate Non-canonical NFkB Signaling in Developing B Cells
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**Rationale:** Previous data demonstrated that DNA breaks generated during antigen receptor recombination activated an NFkB-dependent gene cohort. One of these genes was p100, the key regulator of the alternative NFkB pathway. Non-canonical NFkB participates in early B cell development but its mechanism of activation remains unknown. We hypothesized that DNA breaks activate alternative NFkB to regulate specific genes.

**Methods:** Gene expression was assessed by QRT in pre-B cell lines deficient in p100. Cells were reconstituted with p100 to confirm gene targets, and mutations of p100 were introduced to assess its regulation by DNA breaks.

**Results:** Activation of the non-canonical NFkB pathway in early B cells was dependent on induction of DNA breaks. p100 regulates the expression of a defined gene cohort in a manner dependent on DNA damage responses.

**Conclusions:** Non-canonical NFkB is activated in early B cells by DNA breaks. DNA break signals can integrate with surface receptor signals to tune NFkB signaling and direct lymphocyte development.
**49** Phenotypic Characterization Of Immune Cells Isolated From Adenoids And Tonsils Of Children With Adenoid And Tonsil Hypertrophy

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**RATIONALE:** The composition and immunophenotype of the lymphocyte subsets isolated from adenoids and tonsils of children with adenotonsil pathology may provide insight into tonsil and adenoid disease.

**METHODS:** Samples tissues of adenoids and tonsils were obtained from 15 children undergoing adeno-tonsillectomy due adenoid and tonsil hypertrophy. Adenoids and tonsils were minced and the tissue digested with collagenase IV and DNase I. Released cells were filtered through 100-μm nylon cell strainer. Immunophenotypic analysis of mononuclear cells was performed using FACSCalibur cytometer.

**RESULTS:** CD19+ B-lymphocytes were present as a major lymphoid cell fraction (median 60.69%; range 51.95 to 61.64%), while CD3+ T-lymphocytes averaged 30.8 (range 22.27 to 34.55) %. The majority of cells among CD3+ T-lymphocytes were CD4+ T-cells helpers. Correlation existed between intensity of expression of CD3+ T-lymphocytes and CD19+ B-lymphocytes (R²=0.7, p<0.05). Myeloid DC were 0.3 (range 0.22 to 0.44) % of the total isolated lymphoid cells and plasmacytoid DC were 0.44 (range 0.21 to 0.66) %.

**CONCLUSIONS:** CD19+ B-lymphocytes are a major component of the total lymphoid cells isolated from adenoids and tonsil tissue in children with adenoid and tonsil hypertrophy. Relatively small numbers of DC may be involved in the pathogenesis of adenotonsil hypertrophy.

**50** B Cell Phenotype in Patients with Common Variable Immunodeficiency (CVID) and its Relation to their Clinical Characteristics

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**RATIONALE:** Common variable immunodeficiency (CVID) is a primary antibody immunodeficiency characterized by decreased serum levels of immunoglobulins and increased susceptibility to infections. Most patients show impaired B cell differentiation. The aim of the present study was to analyze the B cell phenotype in patients with CVID from our population and relate it to their clinical characteristics.

**METHODS:** Patients with diagnosis of CVID were included. Peripheral blood mononuclear cells (PBMC) were isolated from 11 patients and 10 normal controls. We analyzed the B cell subpopulations by four-colour flow cytometry (BD FACSCalibur): phenotyping of naive B cells (R0), CD27+IgM-IgD- (switched) memory B cells and class-switched memory B cells was performed with combinations of CD19, CD27, IgM and IgD monoclonal antibodies (BD Biosciences). Clinical manifestations were related to the B cell phenotype.

**RESULTS:** Among diagnosed with CVID, a significant decrease in the number of CD19+CD27+IgM-IgD- (switched) memory B cells compared to normal controls, and its percentage might relate to their clinical characteristics.
Factors Affecting Infusion of High (20%) vs Lower Concentration (16%) SCIG in Primary Immunodeficiency Disorders

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RATIONALE: Subcutaneously infused human immunoglobulin (SCIG) is increasingly used in treatment of PID patients. Higher concentration Ig solutions (e.g., 20% Hizentra) may be perceived as being more viscous than lower concentration SCIGs (e.g., 16% Vivaglobin) and slower to infuse. However, the viscosity of 16% and 20% SCIG (14.4 vs 14.7mPa s) are only marginally different due to stabilisation of 20% SCIG by L-proline. Infusion duration can also be affected by dose, infusion rate, subcutaneous fat, choice of pump and tubing, volume per injection site and number of sites. Here we evaluate infusion characteristics of 16% and 20% SCIG.

METHODS: SCIG infusion rates, infusion duration and Ig trough levels achieved with 16% and 20% SCIG during four open-label, multinational pivotal trials (EUB, NA, US, H-Eur) were compared. Methodology varied between studies. Data are mean values from the efficacy period of each study unless otherwise stated.

RESULTS: SCIG 16% studies: infusion rates were 18.2mL/h (median) and 37.2mL/h; infusion durations were 1.23h and 2.60h, respectively. IgG trough levels achieved with SCIG were 8.7g/L vs 7.7g/L in patients previously receiving IVIG (EUB study), and 10.4g/L vs 7.9g/L at baseline (NA study). SCIG 20% studies: infusion rates were 39.1mL/h and 25.5mL/h; infusion durations were 1.6-2.0h and 1.14-1.27h, respectively. IgG trough levels were 12.53g/L vs 11.24g/L on previous IVIG (H-US study) and 7.94g/L vs 6.78g/L on previous IVIG (H-Eur study).

CONCLUSIONS: The increased concentration of 20% SCIG is not associated with an increase in viscosity or reduction in infusion rate and may permit more rapid Ig infusion.
Tolerance and Efficacy of Facilitated-Subcutaneous Infusion of Immune Globulin (Human), 10% and Recombinant Human Hyaluronidase (IGHy) in a Subset of Patients With Primary Immunodeficiency Disease (PIDD)

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RATIONALE: The limited volume of subcutaneously administered IgG (IGSC) per site necessitates weekly infusions, each using several sites. rHuPH20 is a permeation enhancer that improves dispersion of SC-infused (IGSC) per site necessitates weekly infusions, each using several sites. In a previous study rHuPH20 facilitated infusions of IgG (IGHy) enabled infusions at rates and frequencies equivalent to IGIV, with tolerability similar to IGSC.

METHODS: Infusion parameters, tolerability and efficacy were compared in 31 PIDD patients who received IGIV, IGSC, and IGHy in two phase III studies of IGSC alone (NCT00546871) and IGHy (NCT00814320). IGIV and IGHy were infused every 3 or 4 weeks and IGSC weekly.

RESULTS: Mean volume/site: 268.3 (6-716) mL for IGHy, 22.6 (8-51) mL for IGSC; 300.8 (75-652) mL for IGIV. Median (range) number of infusion sites/infusion: IGHy 1.0 (1-2) every 3-4 weeks; IGSC 5 (2-10) weekly; IGIV 1 every 3-4 weeks. Mean maximum infusion rate: IGHy 268.3 (6-716) mL for IGHy; 22.6 (8-51) mL for IGSC; 300.8 (75-652) mL for IGIV. Median (range) number of infusion sites/site: IGHy 3.7 (1-6) every 3-4 weeks; IGSC 2.6 (1-3) weekly; IGIV 1 every 3-4 weeks. Mean duration of maximum infusion rate: IGHy 244.66 (90-300) for IGHy; 27.08 (30-300) for IGSC; 198.83 (25-668) for IGIV. Rate of local ADRs/infusion: 0.12 for IGHy; 0.017 for IGSC; 0.008 for IGIV. Related systemic AE rate/infusion: 0.094 for IGHy; 0.037 for IGSC; 0.326 for IGIV. Efficacy: Annualized rate of all infections was 2.41 infections/subject-year (95% CI: 2.08-2.70) for IGHy; 1.24 (1.02-1.46) for IGSC; 3.77 (95% CI: 2.80-4.94) for IGIV.

CONCLUSIONS: IGHy was infused at a frequency similar to IGIV, at a faster rate and shorter time compared with both IGSC and IGIV. Local and systemic ADRs were similar to IGSC. The overall rate of infections was lower in IGHy-treated patients compared with IGSC or IGIV.

Subcutaneous Hizentra® (20%) Is Better Tolerated And Shares Similar Efficacy Compared To Subcutaneous Vivaglobin® (16%)

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RATIONALE: Replacement Subcutaneous IgG (SCIG) therapy is effective in treating infections in patients with primary immunodeficiency diseases (PIDD), but there are few comparative studies using different SCIG preparations. This study examines the tolerability and efficacy of Hizentra® (20%) subcutaneous immune globulin (SCIG) product compared to Vivaglobin (16%).

METHODS: A prospective, single-center, open-label cohort of 32 PIDD subjects, who received 16% Vivaglobin for at least 6 months and transitioned to 20% Hizentra for 24 weeks. Number of acute serious bacterial infections (ASBI) and overall tolerability on Vivaglobin was assessed for 8 weeks prior to switch and compared to Hizentra over 24 weeks. Average Hizentra dose was higher than Vivaglobin at 161.6 ± 99.8 and 145.9 ± 88.2 mg/kg/week, respectively (p < 0.0001).

RESULTS: The study is ongoing and preliminary findings for all subjects through 12 weeks on Hizentra are reported. ASBI/subject/year while receiving Vivaglobin was 0.2 compared to 0.14 on Hizentra. Per-person annual rates of other infections were lower for Vivaglobin at 1.2 versus 2.63 for Hizentra (p = 0.0167). There were no infusion-related serious adverse events in either group. Average infusion time decreased from 108 minutes (3.2 sites) with Vivaglobin to 72 minutes (2.1 sites) with Hizentra. Mean Vivaglobin IgG were similar to Hizentra, 1096.1 (+/- 231.2) and 1105.2 (+/- 233.3) mg/dL, respectively (p = 0.77). Both groups had similar titers to varicella (103.1 versus 107.9 EU/mL) and tetanus (2.8 versus 2.9 IU/mL) on Vivaglobin and Hizentra, respectively.

CONCLUSIONS: Hizentra (20%) achieves better tolerability and similar efficacy to Vivaglobin (16%).

TARGETING IL-18 BY EMPLOYING A RECOMBINANT IL-18 PEPTIDE-BASED VACCINE AMELIORATES TNBS-INDUCED MURINE ACUTE AND CHRONIC COLITIS

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RATIONALE: IL-18 plays important roles in the pathogenesis of Crohn’s disease. Blocking IL-18 with its mAb or binding protein results in an attenuation of murine colitis. We sought to develop IL-18 peptide-based virus-like particle vaccines which induce long-lasting autoantibodies to IL-18 and to test the effects of the vaccine in the down-regulation of trinitrobenzene sulfonic acid (TNBS)-induced murine colitis.

METHODS: A vaccine against IL-18 was constructed by inserting a peptide derived from mouse IL-18 into the carrier protein hepatitis B core antigen using gene recombination methods. Recombinant protein was expressed using E. coli and purified by chromatography. Mice were subcutaneously immunized 3 times with the vaccine, vaccine carrier or saline. Two weeks after the final immunization, mice were intra-rec tally administered with increasing doses (1.0-2.3 mg) of TNBS for 2 or 7 times to induce acute or chronic colitis. One week after the last TNBS administration, mice were sacrificed. Sera and colon were collected and analyzed.

RESULTS: The vaccine induced high levels of IL-18-specific IgG antibodies that inhibited IL-18-induced IFN-γ secretion from splenocytes dose-dependently. In both acute and chronic colitis, mice receiving vaccine exhibited a significant decrease in intestinal inflammation (H&E staining), collagen deposition (Masson’s trichrome staining), soluble collagen production, and levels of IL-18, IL-12/IL-23p40, and TNF in colon tissue (enzyme-linked immunosorbent assays), compared to saline and carrier groups.

CONCLUSIONS: IL-18 vaccine induces high levels of IL-18-specific IgG antibodies leading to the improvement of acute and chronic intestinal inflammation. This strategy may provide a potential therapeutic approach in the treatment of Crohn’s disease.

In Patients With Hereditary Angioedema, Self-administration Of Intravenous C1 Esterase Inhibitor Decreases The Number Of Days Spent In An Emergency Room

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RATIONALE: Hereditary Angioedema (HAE) is a rare, inherited, autosomal dominant disease caused by a deficiency in C1-esterase inhibitor. It affects one in every 50,000 to 100,000 individuals. There were no approved treatments for HAE in North America until C1-esterase inhibitor (Berinert®), an intravenous medication, was released. However, it requires patients to present to an emergency room (ER) for treatment.

RESULTS: Cases of two patients with HAE in North America were no approved treatments for HAE in North America until C1-esterase inhibitor (Berinert®), an intravenous medication, was released. However, it requires patients to present to an emergency room (ER) for treatment. The cases of two patients with HAE were presented. Both decreased their number of emergency room visits substantially after self-administering the medications.

METHODS: Cases were obtained from office visits with an allergist and continued communication with the patients.

RESULTS: The first patient averaged around 17 visits to an ER annually. She and her husband were both paramedics and were able to start intravenous lines for the required infusion at home. After she began self-administering C1 esterase inhibitor, she had no further visits to an emergency room. The second patient suffered from very frequent attacks, including 37 ER visits in 2009, and 48 visits in 2010 alone. Given her poor venous access, a Hickman catheter was placed in her internal jugular vein for easy access. In 2010, she had no further visits to the ER. C1 esterase inhibitor (Berinert®), an intravenous medication, was released. However, it requires patients to present to an emergency room (ER) for treatment. The cases of two patients with HAE are presented. Both decreased their number of emergency room visits substantially after self-administering the medications.

CONCLUSIONS: Self-administration of C1-esterase inhibitor (Berinert®) dramatically improved the lives of these two young patients, and resulted in a considerable decrease in the number of days spent in an emergency room.
Subcutaneous Immunoglobulin Replacement Therapy with Hizenta® is Safe and Effective in Two Infants with Immunodeficiency

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RATIONALE: Administration of subcutaneous IgG (SCIG) is an effective and safe treatment for children and adults because it does not require venous access, has fewer side effects than intravenous IgG (IVIG) administration, and can be administered in a home setting. Hizenta® (IgPro20, recently approved in the US) is a 20% liquid IgG product for subcutaneous administration that has been studied in adults and children greater than two years of age. There is limited data on the safety and efficacy of Hizenta® in infants less than two years of age.

METHODS: We describe two infants, one with Comèl-Netherton syndrome and impaired response to vaccines and another with Turner syndrome and hypogammaglobulinemia, receiving IgG replacement therapy.

RESULTS: Due to poor venous access, both infants were switched after three doses of IVIG to dose-equivalent weekly SCIG administration of Hizenta® for 31 and 20 weeks respectively. Individual infusion time for both patients was one hour. Local reaction was mild in the infant with Comèl-Netherton syndrome and absent in the infant with Turner syndrome. No serious adverse events were reported. IgG levels achieved with Hizenta® increased an average of 25% compared to levels with IVIG. One serious bacterial infection (Escherichia coli urinary tract infection) occurred in the infant with Comèl-Netherton syndrome. The rates of non-serious infections were 1.19/year and 0.53/year in the infants with Comèl-Netherton syndrome and Turner syndrome, respectively.

CONCLUSIONS: Hizenta® is both safe and effective in infants less than two years of age, and is a suitable alternative in infants with difficult intravenous access.
64 Exposure to Smaller-Sized Fungal Fragments in Homes with a Childhood Asthmatic

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RATIONALE: Much scientific evidence shows positive associations between moldy environments and respiratory illnesses and/or symptoms. Recently, submicron fungal fragments (< 1.0 µm) have been suggested as a potential contributor to adverse health effects due to their biological composition (e.g., antigens, mycotoxins, (1-3)-β-D-glucan), as well as their small size. However, the contribution of fungal fragments to exposure and adverse health outcomes are poorly characterized, in particular homes with childhood asthmatic. We characterized exposure to smaller-sized fungal fragments between homes with and without child asthmatic.

METHODS: We visited all 30 homes with (n=15) and without asthmatic in Seoul, Korea, and sampled fungal fragments in a living room and a child’s bedroom(s) along with outdoor sampling using NIOSH two-staged samplers. (1-3)-β-D-glucan of fungal fragments analyzed by Laminaria Amebocyte lyase assay (LAL) has been used for quantifying the mold exposure.

RESULTS: The mean age of asthmatics was 8.1 years, and they lived in an apartment or a collective housing. The overall geometric mean of (1-3)-β-D-glucan of fungal fragments was approximately three times higher in homes with asthma (93.1±2.6 pg/m³) than without asthmatic (33.9±2.5 pg/m³) (p<0.001). In particular, the GM of fungal fragments for a child bedroom in homes with asthmatic (203±2.0 pg/m³) was around 4 times higher than this for a living room (52.0±1.9 pg/m³) (p<0.001). Similarly, the indoor measurement was around 2.6 times higher than the outdoor level, but results were not significant (p>0.05).

CONCLUSIONS: These results indicate that much exposure to smaller-sized mold may occur in homes with asthmatic. However, further research including seasonal samplings may be necessary.

66 Profiling of Endotoxin Induced Immune Regulatory Network in Ovalbumin Sensitized Mice

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RATIONALE: The role of endotoxin exposure in immune regulatory network of airway allergy is still largely unexplored. We hypothesize a significant alteration of immune regulatory cell populations [(e.g., regulatory B cells (Breg), regulatory T cells (Treg)] and various subsets of dendritic cells (DC) in lungs and spleen of ovalbumin (OVA) sensitized mice upon endotoxin exposure.

METHODS: Age-matched C57Bl/6 female mice (6-8 week old) were immunized with intranasal delivery of 100 µg endotoxin (pure lipopolysaccharide in PBS) alone or with same amount of LPS plus 100 µg of OVA. After 24 h of the last immunization, both groups of mice were sacrificed and spleen, lung, and blood were collected. Single cell suspension from harvested tissue was prepared for flow cytometry analysis.

RESULTS: We found slightly higher trend for the proportions of Tregs, eosinophils, and DCs (Gr-1+, CD11c+, CD4+) in mice treated with endotoxin plus OVA treated mice compared with mice treated with endotoxin only. Breg (CD19+CD5+foxp3+) population were relatively high in both groups (17.8 ± 1.4% in endotoxin plus OVA treated mice and 19.1 ± 3.8% in mice treated with endotoxin only) than Treg (corresponding proportions: 4.6 ± 1.4% and 6.05 ± 1.5%). Spleens from endotoxin plus OVA treated mice had significantly higher (p<0.05) amount of DCs (Gr-1+, CD11c+, CD4+) than mice treated with endotoxin only.

CONCLUSIONS: Endotoxin exposure is associated with airway allergy and inflammation by alteration of immune regulatory network particularly with reference to regulatory T and B cells.

65 Effect of meteorological parameters on airborne fungal spore counts: Sixteen Year Study (1995-2010) in Sarasota, FL

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RATIONALE: Fungal spores are associated with atmospheric bio-pollution and have long been known to trigger allergic respiratory diseases in sensitive individuals; yet little is known about the change in fungal spore counts (FSC) over the last 16 years in relation to global warming.

METHODS: Fungal spores were sampled daily between January 1995 to December 2010 in Sarasota by using a Burkard volumetric trap. The same person (MJ) personally identified the spores microscopically based upon morphological structure. Weather data for Sarasota were obtained from the National Climatic Data Center. Daily averages of temperature, relative humidity and wind speed were used to investigate change in spore concentrations in relation to meteorological parameters.

RESULTS: Using the Pearson’s correlation test, FSC are positively correlated to daily mean temperature (r =0.31, P<0.001), relative humidity (r=0.23, P<0.001) and negatively correlated to wind speed (r=-0.04, P>0.001). Using linear multivariate regression analysis with meteorological parameters as independent variables, daily mean temperature and relative humidity remained statistically significant to FSC over the last 16 years (p<0.001 for both).

CONCLUSIONS: Daily mean temperature and relative humidity are positively correlated with FSC. These observations predict that exposure to airborne fungal allergen will likely increase with continued global warming.

67 Airborne Fungus Diversity and Concentrations in Inner City Elementary Schools

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RATIONALE: Fungal sensitization is a risk factor for increased asthma morbidity. Students spend a large portion of their day at school and school classrooms may be a source of fungal exposure. There are few studies that describe fungus concentrations and species diversity in inner-city school classrooms.

METHODS: Airborne fungal spore samples were collected from 12 inner-city elementary schools twice during the school year over two days using a Burkard sampler. A total of 180 classroom samples were evaluated. Slides were microscopically analyzed at 1000X magnification and results were reported as spores per cubic meter of air (spores/m³) for the 8-hour school day. Two 8-hour collection days were averaged for each classroom. A “total fungus” category was calculated as the sum of the geometric means of all species.

RESULTS: The “total fungus” per classroom was 270.54 ± 3.62 spores/m³ (geometric mean ± standard deviation) and ranged from 15 to 15,845 spores/m³. The species with the highest concentrations included Cladosporium (33.13 ± 3.65, range 0-1459), Penicillium/Aspergillus (26.51±4.62, range 0-8858), Basidiobospori (25.76±8.23, range 0-11135), Smuts (18.76±2.91, range 0-394), and Ascospori (13.63±4.48, range 0-956). The species found most commonly in classrooms included: Cladosporium (found in 96% of rooms), Smuts (89%), Penicillium/Aspergillus (87%), Basidiobospori (67%), Rasts (33%) and Alternaria (27%).

CONCLUSIONS: There is variability in the concentrations and types of fungal spores found in schools.

All abstracts are strictly embargoed until the date of presentation at the 2012 Annual Meeting
**68** Evaluation Of The Proinflammatory Activity Of Basidiospores And Spore-bearing Tissues From The Mushroom Chlorophyllum Molybdites Using Human Whole Blood

**F. E. Rivera-Mariani**, T. Hartung, P. N. Breyssse; Johns Hopkins University, School of Public Health, Baltimore, MD.

**RATIONALE:** Little is known about the inflammatory potential of basidiomycetes ("true mushrooms"). As a result we analyzed the proinflammatory activity of spores and spore-bearing tissue from the *Chlorophyllum molybdites* using a novel pyrograms assay.

**METHODS:** Mushrooms were collected from a recreational park. Spores were collected by cutting the stem from the fruiting body and leaving the basidiocarp of the fruiting body overnight on aluminum foil with gills (spore-bearing tissue) facing downwards. Also, small squares of gills were cut with a sterile scalpel. Blood was collected from adult subjects with no history of inflammatory diseases (e.g. asthma, COPD, autoimmune disease) and cryopreserved at -80°C. Dry spores (7.2, 4.2, and 0.6 mg), spore-bearing tissue (9.8, 4.2, 1.4 mg), and spore dilutions (14 x 10^6 to 10^10) were incubated for 18 hours with cryopreserved whole blood. Endotoxin dilutions (0.5 - 10 EU/ml) and pyrogen-free water were also incubated with cryopreserved blood as positive and negative controls, respectively. After incubation, the supernatants were collected and the concentration of the proinflammatory cytokine interleukin 1β (IL-1β) determined with ELISA using monoclonal antibody pairs.

**RESULTS:** Dry spores induced 951, 758, and 480 pg/ml, dry spore-bearing tissue 767, 515, and 385 pg/ml, and spore dilutions induced 443, 378, and 375 pg/ml of IL-1β, respectively. Endotoxin induced 286, 127, 88, and 13 pg/ml, while the blank was below the limit of detection (>0.5pg/ml).

**CONCLUSION:** To our knowledge, this is the first study to evaluate the proinflammatory activity of spores and spore-bearing tissues from basidiomycetes, which induced more IL-1β than endotoxin alone.

**69** Inactivation of Stachybotrys Antigen by Mold Remediation Chemicals

**A. Dixit**, B. R. Tumala; Saint Louis University School of Medicine, St. Louis, MO.

**RATIONALE:** Building materials exposed to moisture may grow varied fungi, including *Stachybotrys chartarum*. We tested *Stachybotrys* chartarum-antigen inactivation capacity of select antimicrobial products currently marketed for use in mold remediation.

**METHODS:** *Stachybotrys* chartarum antigen test kit (SchX) was purchased from Indoor Biotechnologies, Inc. Manufacturer’s protocol for Enzyme Linked Immunosorbant Assay (ELISA) was followed, except SchX antigen standard prepared from culture extract of *Stachybotrys* chartarum was also exposed at various concentrations to three (3) antimicrobial products comprising of quaternary ammonium compound-, phenol-, and thymol-based formulations in microcentrifuge tubes prior to the start of each assay. To avoid interference in ELISA, active ingredients of antimicrobial products in the reaction mixtures were subsequently chemically quenched prior to the addition in microplate wells. Commercial antimicrobial products were used in ready-to-use concentrations based on technical specifications from the manufacturer or distributor.

**RESULTS:** Active ingredients of all antimicrobial products were completely neutralized by a universal quenching agent that had no detrimental effect on ELISA components. The antigen concentrations in pre-antigen exposure neutralization treatments were comparable to the results of negative controls without the test chemicals. Reduction (37% to 77%) in SchX antigen concentration following treatment with phenol and quaternary ammonium formulations was proportional to the concentration of SchX antigen (125 units/ml to 750 units/ml). Greater reductions were observed at higher antigen concentrations but thymol-containing botanical formulation had minimal detrimental effect on any concentration level of SchX antigen.

**CONCLUSION:** Inactivation of *Stachybotrys* chartarum antigen is dependent on active ingredients of mold remediation chemicals.

**70** Differences of Indoor Endotoxin Levels from South Florida versus Latin American Bedrooms of Hispanic/Latino Children

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**RATIONALE:** Indoor house dust endotoxin (HDE) can increase allergy/asthma inflammation in children. There is scant data on its effect on Hispanic/Latino (HL) atopic children.

**METHODS:** HDE sampling was collected by families of atopic children referred to our Allergy clinic. Two groupings were based on location of residence, either locally from South Florida (SF) or from Latin American (LA). All SF children were of HL descent from various LA countries. LA children came from Dominican Republic, Ecuador, Venezuela, or Central America. A dust collection device was used to vacuum the bedroom. These samples came from mattresses, pillows, floors, rugs, and A/C vents. After collecting, samples were sieved, weighed, extracted, vortexed, and incubated. Endotoxin concentrations were measured using Limulus Amebocyte Lysate assay and quantified in EU/mL. Spirometry, exhaled NO (eNO) and labwork were collected.

**RESULTS:** Samples were returned from 30 SF and 40 LA homes. There was a significant difference in HDE concentrations between both locations. The geometric mean HDE from SF was 336 EU/mL versus 507 EU/mL from LA. Using a small sample size, our results showed HL children from LA had higher HDE exposures and eNO levels.

**CONCLUSIONS:** Although this was a small sample size, our results showed HL children from LA had higher HDE exposures and eNO levels.

**71** Metagenomic Analysis Of Bacteria And Bacteria-derived Nanovesicles Collected From Indoor Dust

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**RATIONALE:** Bacteria in indoor environment are causative agents of bacterial pneumonia. Moreover, our recent experimental study showed that bacteria-derived nanovesicles from indoor dust induced inflammatory pulmonary diseases, such as asthma and COPD. Here, we aimed to evaluate bacterial population indoor environment and bacterial source of nanovesicles present in indoor dust.

**METHODS:** Indoor dust was collected from a bed from two western-style houses during fall season. To assess the distribution of bacteria and EVs’ origin of mattress, we collected dust from mattress and isolated bacteria and EVs’ portion using centrifugation and ultracentrifugation respectively. Culture-independent methodology using conserved bacterial 16S rRNA sequence performed to analyze bacterial contents and source of nanovesicles focused on bacteria.

**RESULTS:** The distribution of bacteria in mattress dust was exceedingly diverse and dominated by gram-negative bacteria. A total of 120 clones of bacterial species were identified. Bacteria from mattress dust were distribution a sample containing over 90% gram-negative bacteria such as Acinetobacter (46%), Enterobacter (17%), Erwinia (5.7%), Pseudomonas (2.89%), Pantoea (1.93%) and Escherichia (1.83%). The 27 of bacteria were identified as source of nanovesicles and mainly consisted in gram-negative bacteria including Enterbacter (94%) and Escherichia (4.67%).

**CONCLUSIONS:** Our study indicates that gram-negative bacterial content in indoor dust are dominant in bacteria from mattress and the origin of nanovesicles focused on bacteria is mainly occupied by gram-negative bacteria. We need to find other nanovesicles’ source such as fungus and will also find out each bacteria and nanovesicles produced each bacteria to find evidence for relationship between bacterial contents/ component and asthma/allergy.
1. Ambient Air Pollution and Allergic Sensitization: Results from the National Health and Examination Survey (NHANES) 2005-2006

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RATIONALE: Allergic sensitization is a risk factor for asthma and allergic disease. To date, no large-scale population-based studies have examined the relationship between air pollution and allergic sensitization in the U.S. We hypothesized that exposure to NOx, PM2.5, and O3 would be associated with increased odds of allergic sensitization.

METHODS: We analyzed air pollution estimates from monitored data and the Community Multiscale Air Quality Model linked to participants ages 6 and older from NHANES. We assessed sensitization to five categories of allergen-specific IgE: 1) any of 19 allergens; 2) inhalant allergens; 3) outdoor allergens; 4) indoor allergens; and 5) food allergens.

RESULTS: Logistic regression was used to produce odds ratios per 10 parts per billion for NO2 and ozone, per 10 μg/m3 for PM2.5 and per 1 μg/m3 for PM2.5.

CONCLUSIONS: Exposure to ambient air pollution is associated with allergic sensitization.

2. Time Trends In Residential Indoor Polycyclic Aromatic Hydrocarbon Exposures Among Inner City Children

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RATIONALE: Polycyclic aromatic hydrocarbons (PAH) has been associated with adverse respiratory health. From 1998 to 2006, levels of nonvolatile PAHs in New York City (NYC) measured using personal air monitors, declined. Our objective was to analyze more recent annual trends in PAH levels measured from children's residences.

METHODS: Residential indoor levels of 16 PAH were measured from 2005 to 2011 in a cohort of children from age 5-6 (n=390) and again at 9-10 (n=39) living in NYC Northern Manhattan and the Bronx. Gas and particulate phase PAH were analyzed as the ∑PAHsemivolatile (MW 178-206) and ∑PAHnonvolatile (MW 228-278).

RESULTS: Of the 39 children analyzed at both time points, median levels of ∑PAHsemivolatile, but not ∑PAHnonvolatile, increased from age 5-6 to age 9-10 (32.6 to 55.2 ng/m3, p<0.001). Between age 5-6 and 9-10, 77.5% and 48.7% of children experienced a rise in ∑PAHsemivolatile and ∑PAHnonvolatile exposure, respectively. Examination of annual trends, merging all data (n=429 monitors), showed that levels of ∑PAHsemivolatile, but not ∑PAHnonvolatile, increased between 2005 and 2011 (linear regression, β=0.076, p<0.001). The increasing trend in ∑PAHsemivolatile levels occurred mostly in the heating season. Decreases in ∑PAHnonvolatile levels were observed during the nonheating season (β=-0.122, p<0.001).

CONCLUSION: As PAH exposure has been shown to be associated with respiratory symptoms, the rise in indoor ∑PAHsemivolatile may have public health implications. Further policy initiatives against traffic and other sources of combustion products to decrease environmental PAH exposure may be necessary.

3. Urban Heat And Humidity Islands And The Preferential Deposition Of Airborne Pollen

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RATIONALE: Juniperus ashei, a highly allergenic winter pollinating tree, common across the Edwards Plateau in Texas. Pollen from these populations travel long distances when atmospheric and meso-scale weather patterns prevail and affect downwind communities. Increasing urbanized areas are affected, perhaps preferentially. Whereas it is assumed that entrained pollen travels over these areas, the advent of urban heat and humidity islands may significantly affect the characteristics of the pollen causing increased deposition.

METHODS: Burkard volumetric pollen traps were established during winter pollination seasons along an east/west transect. Samplers ran from 1997/1998 to 2000/2001 and again during the 2009/2010 and 2010/2011 seasons. Standard methods were used to prepare and analyze slides. Weather service records for the stations closest to each sampler were used for the analysis.

RESULTS: Initial results show rural areas have greater pollen concentrations. Characterization of pollen records suggests significant pollen concentrations move across areas, having been entrained upward earlier in the day. Humidity changes in urban areas add to the deposition rate because Juniperus pollen is hygroscopic and absorbs moisture from the atmosphere. Data shows Juniperus ashei pollen increases 25 weight percent across humidity gradients. Added water weight thus increases downward velocity conditions for creating greater deposition.

CONCLUSIONS: Increased humidity in urban areas occurs especially in dryer climatic zones. The increase in humidity is often accompanied by increasing temperatures. Changes in the temperature and humidity of urban areas compared to more rural locations show correlation with pollen fluctuations along the west to east humidity transect studied.
AB20 Abstracts

75 Risks Factors for Exercise-Induced Wheeze Among Asthmatics in NYC Include Neighborhood Asthma Prevalence and Differ by Serotype

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Rationale: Exercise-induced wheeze (EIW) is a common but not defining characteristic of asthma and may identify a specific asthma phenotype. Neighborhood asthma prevalence (NAP) varies (3-19%) in New York City (NYC). Among middle-income asthmatic children, we previously found no associations of asthma severity indicators (e.g., wheeze frequency, lung function, exhaled NO) with lower (3-9%) vs. higher (11-19%) NAP. In the same sample, we investigated the associations of EIW with NAP and other risk factors.

Methods: The NYC Neighborhood Asthma and Allergy case-control study recruited families of 7-8 year-old children who had the same health insurance coverage through a parent’s employment. Asthma cases (n=140) were defined by symptoms and medication use. Asthmatics with EIW were compared to asthmatics without EIW in logistic regression models that included NAP, sex, race/ethnicity, environmental tobacco smoke, maternal asthma, and household income. Analyses were stratified by seroatopy (measurable IgE to inhalant allergens).

Results: Overall, EIW prevalence among asthmatics increased with increasing NAP quartile (38%, 36%, 49%, 65%, P trend=0.013). In multivariable analyses, EIW varied directly with NAP (P=0.002) and inversely with FEV1/FVC (P=0.004). EIW was associated with abdominal circumference among non-atopics (n=53, P=0.028) and with FEV1/ FVC (P=0.002) and maternal asthma (P=0.044) among seroatopics (n=87). EIW was associated with NAP among the serotoacic (P<0.001) but not the non-seroatopic (P=0.38) children (Pinteraction=0.053).

Conclusions: Seroatopic asthmatics with higher NAP were more likely to report EIW than seroatopics with lower NAP and non-seroatopics with either higher or lower NAP. These findings suggest that community and individual factors may interact to affect risk for EIW.

76 Seasonal Variation of Pollen and Spore Counts Does Not Influence Severity of Sleep Apnea

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Rationale: Obstructive sleep apnea (OSA) is the most common form of sleep-disordered breathing. Nasal congestion, by narrowing airways, has been shown to contribute to sleep apnea; nasal congestion can be caused by a number of environmental factors including allergens (pollen, mold).

Methods: A retrospective cross-sectional study was conducted comparing environmental factors with sleep studies. All diagnostic sleep studies performed at an urban safety-net hospital in 2010 were included. Seasons were defined in 3-month intervals. Patient characteristics were compared by season using chi-squared or ANOVA analyses. Days of the top 10 percentile pollen counts were compared to days of the lowest 10 percentile pollen counts, and mean apnea-hypopnea indices (AHI) of studies done on these days were compared using Student’s t-tests.

Results: 1941 patients were included. No differences in gender, body-mass index or Epworth sleepiness score noted between studies by season, but age was different (mean 48.1y in Jan-Jun, 50.3y Jul-Dec, p=0.02). No differences in AHI noted by season. AHI did not differ in patients studied on the highest pollen count days versus lowest pollen count days, nor by highest spore count days versus lowest spore count days.

Conclusions: Overall seasonal variation of pollen and spore counts in the environment may not influence severity of obstructive sleep apnea.

77 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: The majority of 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 and BM were measured. Subjects were then challenged to BM.

Results: Nine subjects were recruited. Mean CMSPT wheal was 10.5mm(range 4-15mm) compared to BMSPT wheals of 10.7mm(range 3-20mm), 9.1mm(range 0-20mm), and 6.4mm(range 0-15mm) at 5, 10, and 20min, respectively. There was a trend toward significance comparing CMSPT and BMSPT at 20min(p=0.07). Mean CM specific IgE was 13.2 kU/L(range <0.35-43.9). Mean BM specific IgE was 12.5 kU/L(range <0.35-44.9). The Pearson correlation coefficient for the CM and BM IgE was 0.998(p<0.0001). Three subjects completed a BM challenge. One subject passed and CMSPT wheal was 8mm and BMSPT wheals at 5, 10, and 20min were 10mm, 5mm, and 3mm. The subject’s specific IgE to both CM and BM was 0.35. Two subjects failed the OFC and mean CMSPT wheal was 12.5mm and BMSPT wheals at 5, 10, and 20min were 15mm, 10mm, and 9mm. The mean specific IgE to CM was 29.1 and BM was 28.8.

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. SPT to BM at 20min may be clinically useful.

78 Characterization Of Immunologic Parameters In Children With Variable Milk Protein Tolerance

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Rationale: We have previously reported that about 75% of children with cow’s milk allergy tolerate baked milk in a form of a muffin (BM); baked milk diet appears to accelerate the development of unheated milk tolerance. In addition, BM-reactive children have more persistent milk allergy and higher risk for anaphylaxis. In this follow-up study we sought to characterize milk-allergic children in regard to tolerance to variable baked milk products.

Methods: At baseline, 143 children underwent oral food challenges to baked products that contained increasing amounts of milk protein and were baked under different conditions (muffin, pizza, and rice pudding) as well as unheated milk to determine the degree of milk tolerance. We performed skin prick tests (SPT), and measured serum cow milk-, casein-, and beta-lactoglobulin-specific IgE levels (UniCAP).

Results: Forty children reacted to a muffin (BM- reactive), 85 tolerated BM (31 muffin-tolerant, 11 pizza-tolerant, 43 rice pudding-tolerant), and 8 tolerated unheated milk. These five groups were significantly different (P for trend <0.001) when compared for: (1). age (median 8; 7; 7; 7, and 6.5 years); (2) cow milk extract SPT wheal (10; 9; 8; 9; 6 mm); (3) cow milk-IgE (11.9; 5.0; 7.7; 3.5; 0.7 kU/L); (4). casein-IgE (12.2; 4.1; 5.6; 1.6; 0.4 kU/L); and (5). beta-lactoglobulin-IgE (1.8; 0.6; 1.3; 0.3; 0.2 kU/L).

Conclusions: The majority (85/125, 68%) of milk-allergic children tolerate milk in baked products. BM-reactive children with more severe milk allergy were slightly older and had significantly larger SPTs and higher levels of serum cow milk, casein, and beta-lactoglobulin-specific IgE than BM-tolerant children.
79 Case-specific Regulatory T Cells and Effector T Cells in Patients with Variable Milk Protein Tolerance

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Rationale: About 75% of children with cow’s milk allergy tolerate baked milk (BM), and addition of BM to the diet appears to accelerate unheated milk tolerance. BM-reactive children have more severe milk allergy. We sought to examine the contribution of case-specific regulatory T (Treg) cells and effector T cells to milk tolerance among milk allergic children.

Methods: We examined 92 subjects classified as BM-reactive (28), BM-tolerant (21 muffin-tolerant, 7 pizza-tolerant, 30 rice pudding-tolerant), or unheated milk-tolerant (6) based on oral food challenge (OFC). From blood obtained at the baseline OFC, PBMCs were cultured with purified caseins and controls for 7 days; proliferating Treg cells and effector T cells were identified by flow cytometry. Treg cells were defined as CD127/CD25/FoxP3 cells derived from CD3/CD4/CFSE+T cells. Effector T cells were defined as CD3+/CD4+/FoxP3+ T cells that were CD127+/CD25+/FoxP3+. Differences between clinical groups in Treg cell and effector T cell frequencies were analyzed using the Mann Whitney rank sum test.

Results: There was an upward trend in the Treg cell frequency from those who were BM-reactive (median 27.6%) to those who had outgrown their milk allergy (median 41.8%; P = 0.086), though differences were not significant between the 5 clinical groups. There were no significant differences in the median effector T cell frequencies between the groups.

Conclusions: There was a higher frequency of case-specific Treg cells in children with mild clinical disease. Further longitudinal studies examining the development of tolerance to less-baked products will elucidate the role that Treg cells and effector T cells play in this process.

80 Predicting Food Challenge Outcomes for Baked Milk: Role of Specific IgE and Skin Prick Testing

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Rationale: Most children with cow’s milk allergy tolerate baked milk products, but little data exists on which children are candidates for baked milk challenge. We examined the correlation of specific IgE (sIgE) and skin prick testing (SPT) levels with baked milk challenge outcomes.

Methods: We performed a retrospective chart review of children who underwent baked milk challenges at Children’s Hospital Boston from September 2009 to August 2011. We evaluated SPT results, sIgE levels, demographics, and food challenge outcomes.

Results: 32 children underwent open challenges to baked milk and 26 (81%) passed. Of those who failed, 3 (50%) passed the initial clinic challenge but developed symptoms at home, days to months later. One child who ultimately failed at home required epinephrine. Compared to those who passed, children who failed were younger (median 8.52yrs and 3.69yrs, respectively; p = 0.006), more likely to have eczema (50% and 13%, respectively; p = 0.009) were found to be risk factors for persistence of IgE-CMA on univariate analysis.

Conclusions: Resolution occurs in most infants with IgE-CMA. Infants who react in the first month of life, or react to < 10 ml of milk are at increased risk for persistence. These parameters can be applied into clinical care.

81 Reaction in the First Month of Life and a Lower Eliciting Dose are Risk Factors for Persistence of IgE-Mediated Cow’s Milk Allergy

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Rationale: IgE-mediated cow’s milk allergy (IgE-CMA), albeit transient in most patients, persists in some infants beyond the first years of life. However, risk factors for persistence that could be applied to clinical care are not well described.

Methods: In a prospective population-based cohort study including 13,019 children followed from birth, 66 infants were diagnosed with IgE-CMA based on history, skin prick test (SPT) and an oral food challenge (OFC) when indicated. Infants with IgE-CMA were followed for 48-60 months. A telephone questionnaire regarding recent exposures to milk was performed every 6 months and recovery was evaluated by repeated OFC. Persistence was defined as IgE-CMA at the end of the study period.

Results: Forty three infants (65.2%) recovered from IgE-CMA during the study period. Most infants (76.7%) recovered within the first 2 years. A reaction in the first 30 days of life (p = 0.026) and a reaction to an amount of milk of less than 10 ml on OFC (or on initial exposure if OFC was not performed) (p = 0.009) were found to be risk factors for persistence of IgE-CMA on multivariate analysis.

Conclusions: Resolution occurs in most infants with IgE-CMA. Infants who react in the first month of life, or react to < 10 ml of milk are at increased risk for persistence. These parameters can be applied into clinical care.
AB22 Abstracts

83 Ovalbumin and Ovomucoid IgE/IgG4 Epitopes in Patients Ingesting Baked Egg Products by Peptide Microarray Immunoassay

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RATIONALE: We have previously demonstrated that the majority of egg allergic patients can tolerate baked egg (BE) products. Using peptide microarray immunoassay (MIA), we sought to identify IgE and IgG4 epitopes of the egg white major allergens, ovomucoid (OVm) and ovalbumin (OvA) and to determine how peptide binding diversity differs between BE-reactive and tolerant patients.

METHODS: Twenty-four BE-reactive and ten BE-tolerant subjects (median age, 6 and 5.9 yrs, median egg white-specific IgE, 26.2 and 13.7 kUA/L, respectively) were selected from a larger study investigating the effects of ingesting BE. Clinical reactivity to BE was determined by oral food challenge. Serum samples were collected at baseline, and analyzed using MIA according to the previously published protocol.

RESULTS: We identified several IgE and IgG4 OVm and OvA sequential epitopes. The OVm regions were consistent with those previously established using SPOTS membranes. We found overall higher intensity and diversity of binding to IgE and IgG4 epitopes of OVm and OvA in the BE-reactive group as compared to the BE-tolerant group, who demonstrated minimal binding to the same epitopes. These findings were consistent when OVm and OvA were evaluated separately.

CONCLUSIONS: Ingestion of BE products has been shown to be safe for most egg-allergic patients. The identified differences in recognition patterns of IgE and IgG4 epitopes BE-reactive and tolerant groups may be helpful in the future for determining which egg-allergic patients may tolerate BE. Use of peptide MIA may provide an important tool in the proactive treatment and management of egg allergy.

84 Identifying Characteristics in Egg-Allergic Subjects to Predict Heated Egg Tolerance: A Retrospective Review

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RATIONALE: Studies have shown that up to 73% of egg allergic individuals can tolerate heated egg. We seek to identify criteria predicting the likelihood of passing a heated egg challenge.

METHODS: A retrospective chart review of egg allergic subjects who participated in a heated egg challenge at the CMC Food Allergy Center was conducted. All subjects underwent a heated egg challenge to sponge cake containing 3.8g of egg protein per serving. Skin prick test (SPT) wheal diameters, egg white-specific IgE and ovomucoid-specific IgE were evaluated if recorded. Data was correlated with the outcomes of the challenge and analyzed using Mann-Whitney test.

RESULTS: Twenty-four subjects (Age range: 11 months - 11 years) underwent a heated egg challenge. 15/24 (63%) subjects tolerated heated egg. There were no significant differences between subjects who passed the challenge versus those who failed in the following parameters: egg white-specific IgE (mean 5.14kUA/L vs. 11.3kUA/L, p=0.08), ovomucoid-specific IgE (mean 0.57kUA/L vs. 4.5kUA/L, p=0.42), SPT wheal diameter (mean 12.9mm vs. 18.3mm, p=0.20).

CONCLUSIONS: Our data reveals the majority of egg allergic children tolerate heated egg, consistent with previous studies. It does not identify clear predictors of heated egg tolerance. However, our data suggests that subjects with higher levels of egg white-specific IgE, ovomucoid-specific IgE and SPT wheal diameters may be less likely to pass a heated egg challenge. Future studies are required with larger sample sizes.

85 Association To Other Food Allergies And Persistence Of IgE-mediated Hen’s Egg Allergy

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RATIONALE: Egg allergy usually is a temporary condition, the association with other food allergies (e.g: milk allergy) is established. The aim of the study is to identify in children with egg allergy the association to other food allergies and the differences among groups.

METHODS: Prospective study of egg allergic children studied from March 2008 to July 2011. Egg allergy was determined by a positive history of reaction to egg, skin prick testing (SPT), egg specific IgE or a positive open challenge. Medical records were analyzed regarding: demographic, atopic disease, age of the onset, number of episodes, amount intake and interval before clinical presentation, symptoms, sensitization to other foods, acquisition of tolerance.

RESULTS: 163 children were included, 40.5% had other food allergies: milk (60%), legumes (11%), white fish (8%), tree nuts (6%), fruits (4%) and crustaceans (1%). 11% had more than one food allergy. 56% were male, 47% with atopic eczema, 56% with a family history of atopy, 29% without previous egg intake. Mean age at first symptoms was twelve months (R:2-24), 41% studied in less than one month after reaction. Symptoms were immediate in 92%, (cutaneous (79%); gastrointestinal (19%) or anaphylaxis (2%) manifestations), 15% after yolk intake.

In the course of follow-up, 23 infants (38%) became tolerant: 83%, 4,5%, 4,5%, 8% at the different analysed moments (6,12,18 and 24 months).

No statistically significant differences in clinical records or in persistence were found among egg allergic children with or without other food allergies.

CONCLUSIONS: In this study the presence of other food allergies was not an independent factor for the persistence of egg allergy.

86 Baked Egg Food Challenges - Clinical Outcomes And Determination Of Negative And Positive Predictive Values For Skin Test To Baked Egg And Ovomucoid

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RATIONALE: Many children with IgE mediated egg allergy (EA) may be able to tolerate products containing baked egg. Aside from formal food challenge, no test currently exists to determine the likelihood that an egg allergic individual will be able to tolerate baked egg.

METHODS: EA children who were strictly avoiding all egg in their diet, with either a convincing clinical history of EA and positive IgE to egg (SPT/ ss IgE) or positive SPT/ ss IgE >95% PPV for EA were invited to undergo an open observed standardised baked egg (muffin) challenge. SPT to egg white, ovomucoid and fresh muffin were performed prior to challenge.

RESULTS: 134 patients underwent baked egg challenge. 66% of EA children were able to tolerate baked muffin. Of the 45 positive challenges, 9 children had systemic reactions including respiratory and/or cardiovascular symptoms.

The mean SPT diameter in positive challenges compared with negative challenges was; egg white-9.5mm/7.7mm, baked muffin-6.5mm/4.4mm and ovomucoid 7.5mm/5.9mm respectively. Receiver operating characteristic (ROC) curves were generated for SPT to baked egg and ovomucoid. The area under the curve was 0.823 for baked egg, and 0.794 for ovomucoid. A SPT of <2mm to muffin had a negative predictive value of 94%.

CONCLUSIONS: Many children with IgE mediated EA can tolerate baked egg in the form of muffin on OFC. A SPT of <2mm to baked muffin has a high negative predictive value. This may be useful in selecting EA patients for abbreviated challenge protocols or for challenge in a non tertiary setting.
Development of a National Guideline for the Diagnosis of Cow’s Milk Allergy (CMA) in The Netherlands

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RATIONALE: In the Netherlands, CMA is diagnosed in primary care (Baby Health Clinics or General Medical Practices), secondary and tertiary care. However, no integrated national guideline for the diagnosis of CMA was available.

Aim: To develop an evidence-based national guideline for the diagnosis of CMA for use in primary, secondary, and tertiary care.

METHODS: Questionnaires were distributed to investigate difficulties in the diagnostic process of CMA. A guideline development group studied recent leading guidelines on food allergy and CMA, such as the DRACMA, NICE, and NIHAD guidelines. A complementary literature search was performed. The quality of the literature was assessed using GRADE. Clinical questions were formulated and recommendations were developed and piloted. Ready-to-use test foods based on extensively hydrolysed (EHF) and amino acid infant formulas (AA) for use in DBPCFC are under development by the manufacturer.

RESULTS: In the Dutch national guideline, the DBPCFC is promoted for diagnosis in secondary and tertiary care, and, if feasible, in primary care in low-risk infants. The use of the open cow’s milk challenge is restricted to reject the diagnosis of CMA. The widespread use of DBPCFCs is facilitated by the use of the ready-to-use test foods and standardized protocols. EHF is recommended for the diagnosis of CMA, while AA is recommended when EHF is not effective (Low quality evidence).

CONCLUSIONS: In the Netherlands a national guideline is developed using DBPCFCs for definite diagnosis of CMA. By the time of presentation the results of the pilot study regarding the feasibility of the recommendations will be available.

Predicting Positive Food Challenges at the Introduction of Nuts in Sensitised Children

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RATIONALE: Children with atopic diseases in early life are frequently found with positive IgE tests to nuts, without a history of previous ingestion. We aimed to identify risk factors for reactions to nuts at their first introduction.

METHODS: A detailed retrospective case note and database analysis was performed. Inclusion criteria were: patients aged 3 to 16 years who had had a standardized food challenge to peanut and/or tree nuts due to primary sensitisation to the nut (positive specific IgE or SPT). A detailed assessment was performed of factors relating to food challenge outcome with univariate and multivariate logistic regression analysis.

RESULTS: There were 98 food challenges (48% peanut, 52% tree nut) with 29 positive, 67 negative and 2 inconclusive challenges. A positive maternal history and a specific IgE > 2 kU/L were strongly associated with a significantly increased risk of a positive food challenge (OR 3.54; 95% CI 1.28 to 9.81; and OR 4.82; 95% CI 1.57 to 14.86; respectively). There was no significant association between the type of nut, age, presence of other food allergies, paternal or sibling atopic history, other atopic conditions or severity of previous reaction to other foods.

CONCLUSIONS: We have demonstrated an association between the presence of a maternal atopic history and a specific IgE > 2 kU/L, and a significant increase in the likelihood of a positive food challenge in children with primary sensitisation to nuts. Although requiring further prospective validation we suggest these easily identifiable components should be considered when deciding the need for a nut challenge.

The Prevalence of Clinical Cross-reactivity of Non-peanut Legumes to Peanut in Patients with Persistent Peanut Allergy

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RATIONALE: To describe the prevalence of non-peanut legume (NPL) allergy in patients with persistent peanut allergy.

METHODS: Records of peanut allergic patients seen in a referral clinic were reviewed. Patients with a clear reaction history and positive skin test or IgE to a NPL were included.

RESULTS: Of 793 patients (median age of initial observation 1.4 years, range 0-16) with persistent peanut allergy, 75 (9.5%) were considered NPL allergic (median age of diagnosis 1 year, range 0.1-15.6), including: soy 48, pea 19, lentil 7, chickpea 4, green bean 3; 69 reacted to 1 NPL, 5 to 2, 1 to 3. 106 NPL reactions were reported: soy 69, pea 21, lentil 7, chickpea 5, green bean 3, with symptoms including urticaria/angioedema, 40.6%, gastrointestinal, 30.2%, atopic dermatitis (AD), 23.6%, oral erythema/pruritus, 13.2%, lower respiratory, 6.6%, cardiovascular, 1.9%, 82.7% had AD, 64% asthma, and 62.7% allergic rhinitis. AD was more common in NPL allergic than peanut-only allergic patients (82.7% versus 69.1%, p=0.02). Median peak NPL-IgEs were: soy 30 kU/L, lentil 15.4, chickpea 21.9, pea 15.6, green bean 9.3. Median peanut-IgEs were greater in NPL allergic patients (initial 69.7 kU/L versus 25.8, p=0.002; peak >100 versus 59.5, p=0.002). Median peak NPL-IgE/peanut-IgE ratios were: soy 0.6, lentil 1, chickpea 2, pea 0.6, and green bean 1.9.

CONCLUSIONS: In this referral population, the clinical cross-reactivity of NPL to peanut was 9.5%. In NPL allergic patients, the median peak NPL-IgE to peanut-IgE ratio was consistently >0.5. NPL allergic patients were also more likely to have AD and higher peanut-IgE levels.

The Prevalence of Clinical Cross-reactivity of Non-peanut Legumes to Peanut in Patients with Persistent Peanut Allergy

D. L. Neuman-Sunshine 1, J. A. Eckman 2, C. A. Keet 3, E. C. Matsui 4, R. D. Peng 5, P. J. Lenehan 5, R. A. Wood 5, 1The Johns Hopkins University School of Medicine, Department of Medicine, Division of Allergy and Immunology, Baltimore, MD, 2University of Cincinnati, Department of Internal Medicine, Division of Immunology, Cincinnati, OH, 3The Johns Hopkins University School of Medicine, Department of Pediatrics, Division of Allergy and Immunology, Baltimore, MD, 4The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

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CONCLUSIONS: In this referral population, the clinical cross-reactivity of NPL to peanut was 9.5%. In NPL allergic patients, the median peak NPL-IgE to peanut-IgE ratio was consistently >0.5. NPL allergic patients were also more likely to have AD and higher peanut-IgE levels.
Evaluation of Oral Food Challenges With Hazelnut And Actual Reintroduction Of Hazelnut In The Diet After Negative OFC

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RATIONALE: Hazelnut (Hn) allergy is quite common in Europe. This study aimed to evaluate the outcome of Oral Food Challenges (OFC) in patients with a suspected Hn allergy, and to assess the actual reintroduction of hazelnuts in the diet of patients with a negative OFC.

METHODS: A retrospective study was performed in the period dec. 2009- dec.2010 at the outpatient allergy clinic “Kinderhaven” and the Department of Allergology, ErasmusMC in Rotterdam, the Netherlands. Patients, with negative Hn OFC, were contacted by telephone (> 5 months later) for additional information about the reintroduction of Hn in the diet after a negative OFC.

RESULTS: In total 801 patients with a suspected Hn allergy underwent a SPT with Hn extract. 375/801 had a positive SPT with Hn extract. 47 patients underwent an additional OFC with Hn. The outcome of the OFC was negative for 40/47 patients and no severe reactions were reported during the OFCs. Follow up by telephone resulted in 7/40 (17.5%) patients who still avoided hazelnuts: 5 for reasons like fear and aversion and 2 did not trust the outcome of the OFC. 27/40 patients stated to eat only hazelnut products and 5 tried pure hazelnuts.

CONCLUSIONS: No severe reactions were reported during and after the OFC, which indicates the safety of the OFC procedures. Secondly, Hn reintroduction failed for a substantial amount of patients, which emphasizes the need for post OFC consultations, supported with psychological and dietary advices.

Milk And Egg Allergy In Adulthood


RATIONALE: Milk and egg allergy in the adulthood is very rare. Yet, the allergy workup confirms only a small percentage of the suspected patients.

METHODS: An observational, retrospective study was carried out in patients older than 15 years old attended in our Allergy Service during 2010. A total of 7211 patients were attended, 1133 referred food related symptoms. Among them, 19 and 22 reactions were associated to egg or milk, respectively.

RESULTS: Allergic diagnosis, based on anamnesis, prick-test, sIgE or oral challenge, was confirmed in 68.4% of egg (46.1% bird-egg syndrome) and 22.7% of the milk cases. Mean age of symptom’s onset was lower in allergic groups [37.8±14.1 years (egg), 24.6±6.1 (milk)] than in non-allergic patients [45.3±13.2 and 36.1±15.6 years, respectively]. Cutaneous symptoms were mainly reffered by both allergic groups (53.8% of egg and 60% of milk cases) compared to non-allergic groups (16.7% and 17.6%, respectively). Gastrointestinal symptoms were more frequently present in non-allergic patients (83.5% and 76%, respectively), compared to allergic group (61% of egg and 60% of milk cases).

ATopic dermatitis was observed in 38.5% of egg group. Other antecedents: food allergies, rhinoconjunctivitis-asthma, urticaria or drug allergy, were similar in all groups.

CONCLUSIONS: Milk and egg allergy in adulthood is rare and symp-toms of the allergic population do not differ significantly from the non-allergic groups. The presence of egg allergy is frequently associated with atopic dermatitis.

Cellular and Serologic Profiling of Adults with Peanut Allergen Sensitization


RATIONALE: We compared the expression of basophil and serologic measures in peanut-allergen (PA) sensitized adults with varying clinical manifestations.

METHODS: Adult subjects were evaluated by history, total IgE, peanut-specific IgE, IgE anti-Ara-h 1,2,3, and 8 (ISAC and ImmunoCAP), basophil spontaneous histamine release (SHR), PA-mediated basophil histamine release (BHR), and baseline basophil CD203c expression.

RESULTS: Adults with confirmed peanut allergy (Group B, n=14) were compared to peanut-sensitized, but tolerant, adults (Group C, n=4). Peanut-specific IgE (median 15.2 vs. 1.03 kUA/L, p = 0.099) and specific total IgE ratios (7.11% vs. 0.39%, p = 0.022) were increased in Group B compared to Group C, but not total IgE levels. Group B subjects demonstrated broader IgE reactivity to A. h 1,2,3, and 8 by ISAC (28.11 ISU vs 0 ISU, p = 0.05) and ImmunoCAP (34.11 vs. 0.34 kUA/L, p = 0.019). Group B had markedly increased Ara-h 2 (15.4 vs. 0.055 kUA/L, p = 0.007) and similar Ara-h 8 ImmunoCAP reactivity (p=0.95). Basophil SHR was higher in Group B (6.3% vs. 2.65%, p = 0.15), but not baseline surface CD203c expression. PA concentrations eliciting threshold and maximum BHR were several logs lower in Group B (p <0.01).

CONCLUSIONS: Adults with peanut allergy were found to have broader PA-specific IgE expression and heightened measures of basophil reactivity compared to subjects with asymptomatic sensitization.

The Effects of CCR9+ and CD103+ Dendritic Cells on Regulatory T-cells in Food Allergy

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RATIONALE: We have previously implicated CD103+ DCs (dendritic cells) and CCR9+ pDCs (plasmacytoid DCs) in food allergy. We coinoculated dendritic cells with T-cells in food allergy and healthy patients and measured the degree to which regulatory T-cells (Tregs) could be induced. We hypothesized that food allergy patients, having fewer numbers of tolerogenic DCs, would have reduced induction of Tregs.

METHODS: To model the gut environment, PBMCs (peripheral blood mononuclear cells) from healthy (HC) or food allergic (FA) subjects were coinoculated with the Caco2 epithelial gut cells, ATCC cell line for 18 hours either with or without stimulation by the allergen. To model the periphery, DCs and T-cells isolated from whole blood were coinoculated with the Caco2 epithelial gut cells, ATCC cell line for 18 hours either with or without stimulation by the allergen. To model the periphery, DCs and T-cells isolated from whole blood were coinoculated with the Caco2 epithelial gut cells, ATCC cell line for 18 hours either with or without stimulation by the allergen. To model the periphery, DCs and T-cells isolated from whole blood were coinoculated with the Caco2 epithelial gut cells, ATCC cell line for 18 hours either with or without stimulation by the allergen. To model the periphery, DCs and T-cells isolated from whole blood were coinoculated with the Caco2 epithelial gut cells, ATCC cell line for 18 hours either with or without stimulation by the allergen.

RESULTS: In the gut model studies, we saw an increase of Tregs in HC compared to FA subjects (-1.3 vs. -1.0%, n=3, p<0.05), following incubation with Caco2 cell after food allergen stim-ulation. In the peripheral blood incubations, Tregs increased the most after 24 hours when adding both anti-CCR9 and IAT*. *inhibitor of indoleamine 2,3-deoxyxygenase (IDO) in DCs.

CONCLUSIONS: These findings are the first to implicate CD103 gut DCs with human food allergy. Additionally, we have shown that DCs can be altered in their Treg induction ability when blocking specific chemokine receptors (CCR9) and tolerogenic enzymes (IDO) in DCs. Continuing to identify important dendritic cell biomarkers will better enable us to diagnose and treat food allergy patients in the future.
94 Relationship of Cytokine and Regulatory Gene Expression to the Outcomes of Milk and Egg Allergy in an Atopic Cohort (COCFAR2)
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RATIONALE: To determine lymphocyte activation biomarkers associated with milk/egg allergy over time.

METHODS: Children aged 3-15 months with likely milk/egg allergy and no diagnosed peanut allergy were followed at baseline, 6, 12 and 24 months and categorized as egg/milk allergic or tolerant by predefined criteria. Mononuclear cell allergen stimulation screening for CD25, CISH, FOXP3, GATA3, IL-10, IL-4, IFN-gamma and TBET expression using casein and egg white as stimulants were performed at each of the time points. Repeated measures analyses with generalized linear modeling were constructed to determine if gene expression was predictive of the allergy state (allergic or tolerant).

RESULTS: The number of children with a confirmed/convincing milk allergy and number tolerant in parentheses among those tested at baseline (n=492), 6 (n=432), 12 (n=420) and 24 (n=367) months were: 237(105), 197(137), 197(163) and 145(190), respectively. The corresponding results for egg were: 139(23), 132(35), 129(55) and 99 (117), respectively. The significant (p < 0.05) predictors of allergy based upon current gene expression values were: increased IL4 expression (milk) and decreased GATA3 (egg). Considering change in activation from baseline, risk was associated with a decrease in TBET (milk) and increased IL4 (egg). Tolerance to egg was associated with current decreased CISH, and decreased TBET compared to baseline. However, the predictive capacity of the findings was minimal, with only a few percentage points of risk being predicted by large changes in expression.

CONCLUSIONS: Allergen-stimulated lymphocyte gene expression failed to significantly reflect allergy states over time.

95 Oral Immunotherapy for Egg Allergy Clinical and Immunologic Results
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RATIONALE: Allergy to hen’s egg represents an important issue in medical practice: The majority of children with IgE mediated food allergy achieving spontaneous tolerance to egg. However, some children have persistent symptoms beyond the age of 5; for these children we performed oral immunotherapy (OIT) with egg.

METHODS: Twenty - eight children aged 5 to 16 years were equally randomized to desensitization with egg white (EW) or placebo. We used dried capsules containing EW (ovalbumin, ovomucoid, ovotransferrin) and talcum powder as excipient. The placebo ‘s capsules containing only talcum powder (Lofarma - Milan, Italy). Specific IgE, and IgG4 levels to EW were measured at baseline and at the end of the study. Double Blind Placebo Controlled Food Challenge (DBPCFC) was carried out at the beginning and at the end of the trial.

RESULTS: Full tolerance to egg (200mg) was achieved in 12 active patients (86%). One patient stopped desensitization after experiencing: urticaria, rhinitis, wheezing. One patient achieved partial tolerance, tolerating the dose of 50 mg. egg. No reactions occurred in controls, whose sensitivity to EW remained unchanged. Specific IgG4 increased (median EW IgG4 level) from 1.80 mg/mL at baseline to 24.18 mg/mL, P=0.02. Only a slight decrease of both EW and ovomucoid Specific IgE was observed (median EW IgE level decreased from 24.32 KU/L at baseline to 18.12 KU/L, P=0.06). No significant changes occurred in placebo group.

CONCLUSION: This updosing desensitization for egg IgE mediated food allergy performed under medical supervision was effective, rather safe and induced immunologic changes.

96 Milk Oral Immunotherapy: A Single-Center Pilot Study of Safety and Efficacy
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RATIONALE: Cow’s milk allergy (CMA) is the most common food allergy among children. While many outgrow, 20% have persistent CMA. Given its prevalence and ubiquitous nature in food, we investigated the safety and efficacy of CMA oral immunotherapy (OIT).

METHODS: Children (6-17 years) with confirmed CMA underwent OIT, starting with one day rush initial desensitization (reaching 15 mL, as tolerated). Weekly doses to reach maintenance (eight ounces) were administered in the hospital. Subsequent daily doses were given at home. After a month at maintenance, diets were liberalized to include additional dairy products. Cetirizine was administered daily during the study.

RESULTS: Average enrollment age was 9.3 years (62% male, 38% female). 92% had additional food allergies, 92% had seasonal allergies, 77% had asthma, and 62% had atopic dermatitis. Milk-specific IgE ranged from 1.71 kU/L to >100 kU/L. Average final milk dose evoking clinical reaction during initial food challenge was 18.3 milliliters. Twelve of 13 subjects reached daily maintenance dosing without significant incident. One subject experienced two separate respiratory reactions during build-up (required epinephrine) and did not continue OIT. With intercurrent illness, maintenance dosing was reduced to avoid potential reactions. With seasonal allergies, a few subjects experienced mild urticaria (controlled with diphenhydramine).

At study completion, subjects continued to have positive milk skin prick testing and milk-specific IgE. However, both were decreased when compared with prior results.

CONCLUSIONS: Milk OIT is safe and effective when performed in a graded fashion in the hospital. Most subjects achieve desensitization without significant adverse effect and experience improved quality of life.
97  **Milk Oral Desensitization: Role of Invariant Natural Killer T Cells (iNKTs)**

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**RATIONALE:** Milk derived lipid sphingomyelin (milk-SM) specifically activates peripheral blood (PB)iNKTs via their T-cell receptor (TCR) to produce TH2-cytokines. This effect is greater in children with IgE-mediated milk allergy (FA-MA). Therefore, we tested the hypothesis that milk oral desensitization (MOD) may induce quantitative and qualitative differences in the iNKT population.

**METHODS:** PB mononuclear cells (PBMC) from 10 FA-MA (6-17 years), obtained before (T0) and 3 months after completing (T2) a MOD (maintenance dose eight grams cow’s milk protein), were analyzed via flow cytometry for iNKT percentages and absolute numbers per ml of blood (#/ml) and their intracellular cytokine production ex-vivo and after culture with α-galactosylceramide (αGal) or vehicle dymethylsulfoxide (DMSO). Total (t-iNKTs) and milk-specific iNKT (m-iNKTs) were measured using anti-CD3, human-CD1d-tetramers loaded with milk-SM (milk-SM-hCD1d+) (m-iNKTs) or PBS57 (PBS57-hCD1d+) (t-iNKTs).

**RESULTS:** Ex-vivo, there was no difference between T0 and T2 in t-iNKTs or m-iNKTs. After eight day culture with αGal, we observed a higher percentage and #/ml of m-iNKTs (0.01±0.01 vs 0.1±0.05 and 9±6 vs 106±21 p<0.03), but not of t-iNKT (30±5 vs 46±8 and 1973±7787 vs 20847±4730). Moreover, we observed an increased ratio INFγ+iNKTs/IL-13+iNKT (5±2 vs 36±8; p<0.02) in the αGal expanded t-iNKT stimulated with PMA and Ionomycin.

**CONCLUSIONS:** This data shows for the first time that iNKT may play a relevant role in MOD. Indeed, in FA-MA children undergoing MOD we observed an increase in iNKTs that specifically recognize milk derived lipid via their TCR and a shift of the iNKT-derived cytokine expression towards a Th1-profile.

98  **Clinical Follow-Up After An Oral Induction Tolerance Protocol with Cow Milk: 4 Years Later**

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**RATIONALE:** To study the clinical follow-up, after 4 years, of a group of patients who underwent with success a protocol of oral induction tolerance (OIT) to cow’s milk, and the incidence of new of allergic diseases.

**METHODS:** 21 patients were evaluated by clinical history, skin prick tests and determination of specific IgE to a set of different foods, including cow’s milk allergy, and allergens (dust mites, pollens, moulds and animals dander).

**RESULTS:** After 4 years, 21 children (12 girls, 9 boys) with a mean age of 7.6 years, performed the study. Out of the 21 patients, 20 are still tolerating milk, and 1 has stopped its intake because of several adverse effects. 95% of the patients (20/21) have rhinitis and/or asthma. 15 out of that 20 have milk, and 1 has stopped its intake because of several adverse effects. 95% of the patients (20/21) have rhinitis and/or asthma.

**CONCLUSIONS:** The majority of the patients tolerate milk 4 years after the OIT protocol. The percentage of rhinitis and/or asthma observed in this group, is much higher than in general pediatric population.

99  **Rush Oral Immunotherapy For Wheat-induced Anaphylaxis In Japan**

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**RATIONALE:** There are few reports on oral immunotherapy (OIT) in children with anaphylactic type of persistent wheat allergy.

**METHODS:** Eighteen wheat anaphylactic subjects, who had been confirmed by DBPCFC, underwent rush oral immunotherapy (ROIT). Each patient received OIT under premedication of oral antihistamine (Loratadine) and leukotriene receptor antagonist (LTRA: Montelkast). They took Udon noodle (Japanese style of wheat noodle) twice a day in our hospital for 5 days, increasing up to 200 g (maintenance dose) according to the severity of their adverse reactions. Afterwards they took Udon noodle or a piece of bread as maintenance therapy once a day at home. Subjects, who had taken maintenance doses without any symptoms for at least 4 weeks, underwent oral food challenge after 2 weeks’ wheat avoidance to confirm tolerance acquisition (Final OFC).

**RESULTS:** The median age of 18 subjects was 8.6±2.5 (mean±SD) years. Average wheat specific IgE was 73.2±35.5 Ua/ml. The threshold eliciting anaphylaxis without premedication was 23.1±14.5 g of Udon noodle. Two subjects dropped out due to difficulty in taking wheat products. At the final day of ROIT, 15 subjects could take 200 g of Udon noodle. Until now, 7 subjects could undergo the final OFC. Three subjects were confirmed achieving tolerance acquisition by the final OFC, 4 subjects developed symptoms by the final FC.

**CONCLUSIONS:** OIT for wheat anaphylaxis seemed to be effective to reduce the risk of anaphylaxis. Premedication with antihistamine and LTRA during ROIT seem to be effective regarding the suppression of respiratory symptoms.

100  **Baked Proteins In The Management Of Cow’s Milk And Egg Allergic Children, Less Than 2 Years Of Age: Are Infants At Increased Risk Of Breakthrough Reactions?**

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**RATIONALE:** The use of baked proteins (BP) in food hypersensitivity, has been investigated mostly in older children. We are describing our initial experience with the use of BP in infants with immediate reactions to cow’s milk or egg proteins.

**METHODS:** Children less than 2 years of age with a well documented, clinical reaction to egg or CMP and evidence of IgE mediated protein specific sensitization, were offered a graduated open challenge in the clinic with a baked cookie, containing a predefined quantity of the culprit protein. If well tolerated, parents were advised to continue consumption of the same recipe daily, and return periodically for follow up, skin testing and further challenges as needed.

**RESULTS:** 4 infants tolerated well the BP challenge and continued exposure at home. Two children exhibited continued tolerance of the heated proteins with resolution of sensitization to the native protein achieved within approximately one year. After varying periods of uneventful BP consumption at home, the other two infants developed an episode of immediate type hypersensitivity within the context of a mild upper respiratory episode or a course of antibiotics. Both reactions were milder than the initial clinical presentation and both patients were subsequently re-challenged successfully.

**CONCLUSIONS:** Although treatment with baked protein products, offers a viable solution to many food allergic children, it should still be regarded as a true “immunomodulatory” intervention and as such may not be without it’s pitfalls or risks, especially in the younger aged children.
101 Induction of Regulatory T Cells After Peanut Sublingual Immunotherapy
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RATIONALE: Prior work with peanut sublingual immunotherapy (SLIT) in allergic children has demonstrated increased reaction thresholds with immunoglobulin and cytokine changes suggesting desensitization. The effect of peanut SLIT on regulatory T cells (Tregs) remains unclear.

METHODS: Sixteen peanut-allergic children underwent a 6 month build up and 6 month maintenance course of peanut SLIT followed by a double-blind, placebo-controlled food challenge (DBPCFC) to 2500 mg of peanut protein. At baseline and at the DBPCFC, peripheral blood mononuclear cells were isolated and incubated with crude peanut extract (200 mcg/ml) for 7 days. These were then surface stained for CD4 and CD25 and intracellularly stained for the transcription factor forkhead box protein 3 (FoxP3). Tregs were reported as the percentage of CD4+ cells that were also CD25+ and FoxP3+.

RESULTS: The percentage of Tregs increased significantly after 12 months of peanut SLIT from a median of 3.11% to 7.93% (p<0.004). The median DBPCFC result for the group was 1710 mg of peanut protein for the median challenge (DBPCFC, maximum 1 gm peanut protein), subjects were randomized to receive 10mcg to 3063 mcg, followed by 3 biweekly doses of 3063 mcg. After initial escalation, SLIT/OIT doses were increased biweekly to weekly to 3696 mcg/day SLIT and 2000 mcg/day OIT. A 10 gm DBPCFC was administered after 6 months of maintenance.

CONCLUSIONS: In this small cohort, 12 months of peanut SLIT resulted in a significant increase in the percentage of CD4+CD25+FoxP3+ Tregs. These results indicate a likely role for Tregs in the effects of SLIT and further studies should be undertaken to elucidate the exact mechanism.

102 A Randomized, Double-Blind, Placebo-Controlled Pilot Study of Sublingual versus Oral Immunotherapy for the Treatment of Peanut Allergy
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RATIONALE: To compare safety and efficacy of oral versus sublingual immunotherapy (OIT and SLIT) for the treatment of peanut allergy.

METHODS: After a baseline double-blind placebo-controlled food challenge (DBPCFC, maximum 1gm peanut protein), subjects were randomized to active SLIT/placebo OIT or active OIT/placebo SLIT. After initial escalation, SLIT/OIT doses were increased biweekly to weekly to 3696 mcg/day SLIT and 2000 mcg/day OIT. A 10 gm DBPCFC was administered after 6 months of maintenance.

RESULTS: 21 subjects, 7-13yrs (median 11.1; male 52%) were enrolled. Baseline characteristics included median total IgE 667 kUA/L (range 170-1557), peanut IgE 169 kUA/L (35.1-814), peanut IgG4 1.12 mg A/L (0.19-5.89), 5-log muscle end point skin titration (PEST) mean wheal 5.5 mm (1.3-8.9), and DBPCFC threshold 21 mg (1-146). Three subjects withdrew (1 systemic reaction, 1 GI symptoms, 1 unrelated to study). There were significant increases at maintenance in total IgE (median 844, p<0.001), and peanut IgG4 (5.79, 0.47-47.2; p<0.001). Of the 9 subjects completing the 6 month DBPCFC, median challenge threshold increased to 246 mg (21-4996; p<0.05) and PEST mean wheal decreased to 1.8 mm (range 0.7-7.2; p<0.05). Significant increases in total and peanut IgE remained (p<0.05). Symptoms elicited by the 4772 home doses included: 15.4% oropharyngeal, 10.2% GI, 3.8% lower respiratory, 2.4% skin, and 0.9% upper respiratory. Treatment included antihistamines (43.2% of doses), beta2-agonists (1.5%), and epinephrine (1 dose).

CONCLUSIONS: Preliminary results demonstrate peanut SLIT and OIT are effective in changing challenge threshold, serologic markers, and skin reactivity with infrequent systemic symptoms. SLIT versus OIT will further be compared after unblinding following the 12 month DBPCFC.

103 Early Intervention with Oral Immunotherapy is a Promising Strategy for the Treatment of Peanut Allergy
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RATIONALE: Peanut oral immunotherapy (OIT) causes desensitization, but long-term tolerance induction has not been shown and safety and adherence remain important concerns. We designed a clinical trial to test the hypothesis that early intervention (EI) improves the efficacy of peanut OIT without adversely affecting safety or adherence.

METHODS: Forty peanut-sensitized children aged 9-36 months were enrolled within six months of their initial reaction, or if the peanut-specific IgE (pIgE) exceeded 5 kUA/L in the absence of exposure. All subjects underwent baseline oral peanut challenge and were randomized to receive low- or high-dose OIT. Peanut-specific immune responses were serially analyzed. We utilized our research database to compare demographic, safety, adherence, and immunologic data between the EI cohort and an ongoing, previously reported trial of OIT in older children (PMIT).

RESULTS: Compared to the PMIT group, at enrollment subjects receiving EI had lower median age (30 mo vs. 58 mo) and pIgE levels (15 kUA/L vs. 87 kUA/L) [both p<0.0001]. EI subjects were approximately half as likely as PMIT subjects to have an allergic side effect (ASE) deemed “likely” or “possibly” related to OIT [RR 0.56 (95%CI 0.50-0.62), p<0.0001]. Study termination due to ASE occurred in 3/26 (11.5%) PMIT compared to 1/40 (2.5%) EI [p=0.29]. After one year, peanut skin test size decreased similarly in both groups, and pIgE levels remained stable.

CONCLUSIONS: Early intervention with OIT may enhance safety and targets young peanut-allergic children for treatment while their pIgE levels are low. This may lead to improved long term outcomes.

104 A Phase 1 Study of Heat/Phenol-Killed, E. coli-Encapsulated, Recombinant Modified Peanut Proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) for the Treatment of Peanut Allergy
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RATIONALE: To investigate the safety and immunologic effects of a vaccine containing modified peanut proteins.

METHODS: This was a Phase 1, two-center, non-randomized, open label trial of EMP-123, a rectally administered solution of recombinant Ara h 1, Ara h 2 and Ara h 3, modified by amino acid substitutions at major IgE binding epitopes, encapsulated in heat/phenol killed E. coli. EMP123 was administered to 5 healthy adults for 4 weekly escalating doses and then to 10 peanut allergic adults by weekly dose escalation over 10 weeks from 10 mcg to 3063 mcg, followed by 3 biweekly doses of 3063 mcg.

RESULTS: There were no significant adverse effects in the healthy volunteers. Of the 10 peanut allergic subjects [4 with intermittent asthma, median age 24yrs (range, 19-35), peanut IgE 33.3 kUA/L (7.2-120.2), peanut prick skin test wheal 11.3 mm (6.5-18)], 4 experienced no adverse reactions, one had mild rectal symptoms, 3 were discontinued per protocol with mild-moderate allergic reactions, and 2 were discontinued with severe allergic reactions at doses of 875 mcg and 3063 mcg. Median baseline peanut IgE was significantly higher in the 5 reactive subjects (82.4 versus 17.2 kUA/L, p=0.032), as was baseline anti-Ara h2 IgE (43.3 versus 8.3, p=0.036). Peanut skin titration was significantly reduced from baseline (p=0.02) but no significant changes were detected for total IgE, peanut IgE, peanut IgG4, or basophil activation (CD63+ and CD203+).
105 Peanut Oral Immunotherapy and Omalizumab Treatment for Peanut Allergy

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RATIONALE: We hypothesize that treatment with omalizumab prior to starting oral immunotherapy (OIT) will reduce symptoms, allowing acceleration of the build-up phase and achievement of maintenance dosing more quickly.

METHODS: Peanut-allergic patients aged 12 years and older were enrolled in this study. Omalizumab was given for 4 months prior to initiation of OIT, followed by a modified rush day(s), a build-up period, and a daily home maintenance phase with a final dose of 4000 mg of peanut protein. Omalizumab was continued for one month after reaching maintenance dosing.

RESULTS: 6 patients had evaluable safety data. The median peanut-specific IgE in this group was 68.7 kU/L. The median total IgE was 486.5 kU/L. 68% of patients experienced symptoms on the rush desensitization days with 2021 symptoms graded as mild, primarily respiratory in nature, comparable to previously published safety data for rush desensitization without omalizumab. The median peanut starting dose after rush desensitization with omalizumab was 300 mg (range 100-400), higher than that seen without omalizumab pretreatment. On dose escalation days, 9.5% of doses (6 of 63) resulted in symptoms in the omalizumab group, in contrast to 43.3% of doses (123 of 284) resulting in symptoms in previous studies. CONCLUSION: These results, although limited by small sample sizes, suggest that omalizumab therapy has the potential to reduce side effects during dose escalation and allow a higher starting dose of peanut. This may enhance safety and allow patients to reach maintenance dosing sooner than in previous peanut OIT trials.

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106 Measurement of the Eliciting Dose Threshold at Baseline is Useful for Establishing the Starting Dose in Peanut Oral Immunotherapy (OIT)

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RATIONALE: The majority of peanut OIT trials to date have initiated therapy with a standard dose of peanut flour. We hypothesized that by initiating therapy at a dose proportional to the eliciting threshold dose measured at entry challenge, subjects would be able to safely start at an accelerated dose.

METHODS: Subjects with ImmunoCAP peanut-specific IgE ≥ 7 kU/L and peanut skin prick test (SPT) wheal diameter ≥ 5mm underwent a double-blind placebo-controlled food challenge (DBPCFC) to peanut. Those who reacted during the DBPCFC returned to receive peanut OIT with lightly roasted peanut flour. For subjects tolerating a cumulative dose of >75mg of protein, the starting dose of therapy was initiated at 2 dosing increments below the highest single tolerated dose based on the build-up protocol. Subjects returned for dose escalation bi-weekly.

RESULTS: Eleven of twelve subjects (ages 5 - 16 years) reacted to peanut during the DBPCFC, and 9/12 subjects tolerated >75mg of protein. For the remaining 8 subjects, reaction-eliciting doses ranged from 75 to 250mg (median 112.5mg) of protein. Therapy was initiated between 12.5 and 150mg of protein (median 37.5mg). During dose escalation, 1 subject had 2 severe reactions precipitated by: (1) taking a hot shower within 2 hours of the dose, and (2) taking dose on an empty stomach. All other subjects complained of only mild symptoms, similar to reports from previous trials.

CONCLUSION: Consideration for an individualized starting dose based on the eliciting dose threshold appears to safely accelerate the dosing schedule for subjects receiving peanut OIT.

107 A Prospective, Randomized, Case Controlled Pilot Study to Evaluate the Effect of Ketotifen on the Adverse Events Associated with Peanut Desensitization in Children with Peanut Allergies

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RATIONALE: To decrease allergic reactions during oral peanut desensitization we studied the effect of premedication with ketotifen, an anti-allergic used to treat ocular allergy and allergic asthma.

METHODS: 6 Patients (4M:2F) with prior histories of peanut anaphylaxis (grade 2 and specific IgE > 100) were enrolled in a single blind placebo controlled study, with escalating doses of ketotifen pretreatment of up to 4mg. A rush oral peanut desensitization protocol was used according to previous published studies, up to 50mg peanut flour (≈ 25mg protein) on day-1, followed by a biweekly schedule of escalating doses. Ketotifen continued after day-1.

RESULTS: All moderate to severe reactions (10) occurred in placebo patients and were gastrointestinal (abdominal cramps, nausea, vomiting, diarrhea). The overall day-1 reaction rate was 18.5% and all reactions occurred between the 3mg and 50mg doses. The overall reaction rate for the following 2 weeks was 38% (26% in treatment and 72% in placebo). 3 anaphylactic reactions occurred: day-1 placebo patient at 50mg dose (gastrointestinal, cutaneous); day-5 treatment patient at 50mg dose, occurred post exercise, (gastrointestinal, cutaneous, respiratory); day-6 placebo patient at 12mg dose, associated with fever, (gastrointestinal, cutaneous, respiratory), epinephrine was given and patient withdrew from study. Eosinophil counts were elevated for all patients upon 2nd visit, and decreased over time.

CONCLUSIONS: Pretreatment with ketotifen 4mg greatly decreases side effects associated with the desensitization procedure, most of which were gastrointestinal. Eosinophilia was observed in all patients after day-1. We suspect that anti-inflammatory gastrointestinal cytoprotection may be the mechanism of action of ketotifen.

108 Oral Food Immunotherapy: Protective Doses Reached Within Two Months

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RATIONALE: Oral food immunotherapy (OFI) has been shown to be efficacious. Protocols vary as to the amount of time needed to reach doses that may be protective of an accidental product ingestion.

METHODS: The clinical records of 252 patients enrolled in an active OFI program for milk, peanut and egg protein were reviewed. This individualized dose escalation protocol includes a 4 day induction-desensitization phase performed in a hospital based out-patient clinic where a maximal individualized tolerated dose (MITD) is determined. The MITD is consumed twice daily at home for 24 days and the cycle repeated.

RESULTS: Patients ranged in age from 4-27 years (average 8.5 years). 152 (60%) were male. 125/252 (49.6%) had a history of asthma. Treatment groups included 235 milk, 12 peanut, and 5 egg patients. After completing at least three dose escalation sessions over two months, 178/208 (85%) of milk allergy patients were able to consume 180 mg or more, an amount considered to be protective for an accidental dairy contaminated product ingestion. After three sessions 6/8 (75%) of peanut patients were able to consume 300 mg or more, the protein equivalent of one peanut, and 2/4 (50%) of egg patients were able to consume 1500 mg or more, the protein equivalent of 1/4 of an egg. Treatment failures totaled 24/235 (9.5%).

CONCLUSIONS: Using the current oral food immunotherapy protocol, the majority of milk, peanut and egg allergic patients are able to achieve a protective dose of allergenic protein within two months of treatment.
**AB29 SATURDAY**

**109** Classification, Prevalence and Outcomes of Non-IgE Mediated Reactions to Oral Food Immunotherapy

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**RATIONALE:** Effective oral food immunotherapy (OIT) is sometimes discontinued due to non-IgE mediated reactions (nIgEmr). We aim to characterize, classify and discuss the management of nIgEmr related to OIT.

**METHODS:** nIgEmr were defined as non-inflammation reactions, either due to gastrointestinal (GI) symptoms, asymptomatic eosinophilia (>1400 cells/mm3) or psychological/food aversion to OIT. All patients enrolled (n=247, >4 years), were without previous known GI disorders. A daily report on the child status was obtained.

**RESULTS:** 45/247 individuals presented nIgEmr, all but one to milk OIT (485 days follow-up). GI complaints with blood eosinophilia (>1400 cells/mm3) were observed in 18 (7.3%) patients, including 3 who were diagnosed with eosinophilic esophagitis (EoE, >24 eosinophils/µl). 14/18 of these patients continued OIT following either a transient stop, reduction or slowing of the incremental protocol. One reached full (7200mg) and 13 partial (450-4050mg) milk tolerance. 4/18 stopped OIT. Two out of the three patients with EoE improved and one stopped treatment. GI complaints without blood eosinophilia were found in 14 (5.7%) individuals, 11 became asymptomatic (7 with and 4 without treatment changes). Three patients had asymptomatic eosinophilia, and they continued treatment and reached milk tolerance (7200mg). Ten (4%) individuals had psychological/food aversion (6 stopped treatment, 4 reached partial tolerance (150-1000mg) 2 continuing OIT.

**CONCLUSIONS:** While symptomatic blood eosinophilia (including EoE) was observed in 7%, it is reversible and most patients can continue OIT with a modified protocol. Food aversion appears to be the most difficult nIgEmr to treat. Overall, nIgEmr were observed in 18.2% of the individuals during OIT.

**110** Participation in Peanut Oral Immunotherapy Improves Quality of Life

**J. S. Kamilaris, P. H. Steele, M. D. Kulis, A. H. Edie, B. P. Vickery, A. W. Burks; Duke University Medical Center, Durham, NC.

**RATIONALE:** Although food allergy is well known to adversely impact health-related quality of life (QOL), it is unknown whether food allergy treatment can improve QOL. We hypothesized that experimental treatment with peanut oral immunotherapy (OIT) would increase the perceived quality of life of young children and their families.

**METHODS:** Parents of 16 peanut allergic children, 9 to 36 months of age, who are enrolled into an OIT study, were given a validated quality of life questionnaire to complete at enrollment. This survey was then re-administered after approximately 100 weeks into the study (completion of the build-up phase and one year of study maintenance). The change in parent’s perception related to their quality of life was then measured to assess the impact of OIT.

**RESULTS:** Compared to baseline after participation in peanut OIT, parents report an increase in the areas of emotional, physical, and social well-being. They feel less anxious about their child participating in activities in which they are not present and less anxious about their child becoming more independent in food selection. Issues relating to finding time to take the daily dose and remembering to take the daily dose were areas of stress reported by the parents.

**CONCLUSION:** Participation in peanut OIT improves the quality of life for families of young children with peanut allergy.

**111** Food Allergy Quality of Life (FAQOL) Is Improved For Food Oral Immunotherapy (OIT) Treated Patients and Their Families

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**RATIONALE:** Food OIT is an alternative to the avoidance management strategy (AMS). Food allergy (FA) patients treated with AMS have decreased FAQOL. Food OIT FAQOL outcomes will influence treatment decisions.

**METHODS:** The validated FAQOL Questionnaire* contains 17 questions, scored 0-6, and assesses family life limitations in: family/social activities, school, time for meal preparation, health concerns and emotional issues. (0= not limited, 6= extremely limited) We reviewed FAQOL in 24 families of patients who had been on maintenance OIT (egg, milk, or peanut) for ≥6 months and had no other food allergies. Two of the 24 patients completed OIT for two foods. The results were compared to the responses of 352 families of children with FA treated with AMS*. Average total scores and average per question scores were reported for both AMS and OIT patients.

**RESULTS:** The average total score for families of all children on OIT maintenance was 0.21. The average total score of all AMS subjects was 2.8. Average per question scores were all lower for OIT patients compared to AMS patients. Percent difference between individual question scores between AMS and OIT patients ranged from 82.9% to 98.3%.

**CONCLUSIONS:** This study suggests that OIT markedly improves FAQOL. Because AMS and OIT treated families come from different areas and were queried at different times, this is a tentative conclusion that must be verified by a prospective controlled study.


**112** The Effects of Peanut Oral Immunotherapy on Food Allergy Related Quality of Life


**RATIONALE:** Food allergies have an impact on quality of life (QOL). Peanut (PN) oral immunotherapy (OIT) is promising treatment and data on QOL is lacking.

**METHODS:** Patients with PN allergy were enrolled based on reaction history, SPT size, and ImmunoCAP level. A previously described OIT protocol was adopted; the initial dose of 0.1 mg PN protein was escalated every 2 weeks to a maintenance dose (MD) of approximately 450 mg daily. Patients and/or their parents completed age-specific validated food allergy-related QOL surveys upon entry and upon reaching the MD. The surveys use a 7 point Likert scale with higher values reflecting greater impact on QOL. The minimal important difference (MID) between measurements is 0.5.

**RESULTS:** In this ongoing study, 57 patients (mean age ± SD, 9.4 ± 3.4 years, range 5-17) have enrolled. Four children (5-12 yrs) and 6 adolescents (13-17 yrs) reached the MD. Parents’ assessment of children’s QOL showed clinically significant improvement in 7/8 (87.5%) questions in the domain of Food-Related Anxiety; 5/13 (61.5%) in Emotional Impact, and 8/9 (88.9%) in Social and Dietary Limitations. Overall, mean change exceeded MID in 26/30 (86.7%) questions. Among adolescents, mean change exceeded MID in 18/23 (78.3%) questions. Two of 10 questions in the Allergen Avoidance and Dietary Restriction domain were statistically improved (p<0.04); 3 others were 0.05< p<0.09. Three of 6 questions (50%) in the Risk of Accidental Exposure domain were significantly improved (p<0.02).

**CONCLUSION:** PN OIT improves food allergy related QOL. Parents’ assessments of their children’s QOL are about equal to those of adolescents.
113 Patient and Parent Perspectives on Quality of Life during Participation in a Study of Rapid Oral Desensitization with Omalizumab Therapy in Patients with Milk Allergy

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RATIONALE: With the development of new oral immunotherapy/desensitization protocols for individuals with food allergies, it is important to better understand the impact of participation on quality of life (QoL).

METHODS: Following completion of a protocol evaluating rapid oral desensitization with omalizumab therapy for milk allergy, 9 children ages 9 to 18 years and 8 of their parents completed interviews about the impact of participation on QoL.

RESULTS: All children were ingesting milk products on a daily basis. In child interviews, the most commonly reported changes in QoL following desensitization were increased dietary options (reported by 44% of participants), inclusion in social situations (parties/cafeteria, 44%), and decreased anxiety about reactions (33%). Older children were more likely to identify decreased anxiety as an outcome of desensitization (p<.05). 78% of children identified omalizumab injections and blood draws as the hardest parts of the study, and 56% reported worry about possible reactions during desensitization or challenges. Reported coping strategies included focusing on potential positive study outcomes (56%), use of numbering cream for injections (33%), and staff explanations of how reactions would be managed (33%). In parent interviews, the most commonly reported changes in QoL following desensitization were reduced anxiety about allergic reactions (63%), the child’s inclusion in social activities (50%), ability to eat at restaurants (50%), and decreased planning/increased spontaneity around food-related events (38%).

CONCLUSIONS: Findings suggest improvements in QoL following children’s participation in rapid oral desensitization for milk allergy, as well as areas for increased support to families participating in such protocols.

114 Effect of A Reaction During Oral Food Challenges (OFC) on Food-specific IgE levels (sIgE)

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RATIONALE: To determine the impact of a positive (failed) OFC on sIgE.

METHODS: Retrospective chart review of children undergoing outpatient oral food challenges for clinical purposes from 2008-10 with inclusion of those having sIgE measured (ImmunoCAP, kIU/mL) to the challenged food within a year prior and at least once in the 36 months following OFC. Post-OFC sIgE was compared with pre-OFC by non-parametric paired t-tests. Data from individual patients with multiple sIgE determinations were tested independently by time post-OFC, grouped as 0-12 mo, 12-24 mo, and 24-36 mo.

RESULTS: Of 142 positive OFCs (20% positive rate from among 701 OFCs to 14 foods/food groups), 70 (49%) had qualifying tests performed (4 had 3 post-OFC sIgE; 30 had 2; and 36 had 1 test). Data pooled from all challenges showed that sIgE were elevated in the 1st year post-OFC (mean [median], post-OFC vs baseline): 5.86 [1.34] vs 2.91 [0.91]; p=0.01, n=51), approached pre-challenge level in the 2nd year (4.92 [1.00] vs 2.49 [1.07]; p=0.21, n=45), and continued to decline in the 3rd year (1.31 [0.58] vs 1.33 [0.85]; p=0.36, n=11).

CONCLUSIONS: Food specific IgE levels were transiently elevated in the year following a positive OFC, but fell toward baseline afterwards. Prospective study with controls is required to validate and quantify the impact of this observation.

115 Biphasic Reactions in Children Undergoing Oral Food Challenges


RATIONALE: Numerous studies have reported biphasic anaphylactic reactions in adults, but little is known about how frequently biphasic reactions occur in children and which interventions help decrease their likelihood.

METHODS: We conducted a retrospective chart review of all oral food challenges (OFC) performed in children with suspected IgE-mediated food allergies over a 3.75-year period. Of 1771 OFC reviewed, 1688 were completed conclusively and used for final analysis. Main outcomes examined were OFC result (positive vs negative), presence of biphasic reaction, and effect of initial treatment with steroids or epinephrine on likelihood of biphasic reaction. Reactions were considered biphasic if any symptoms recurred after a symptom-free period of 2 hours.

RESULTS: Average age at time of OFC was 5.1 years (range 0.4 - 20 years). Sixty-six percent were male. Six-hundred and twelve OFC were positive (36%), with various symptoms. Ten resulted in a biphasic reaction (1.6% of positive OFC, 0.6% of all OFC). The biphasic reactions were to egg (n=4), peanut (n=3), milk (n=2), and wheat (n=1). Children with positive OFC who were treated with steroids were as likely as those who were not to have a biphasic reaction (OR 3.42, 95% CI: 0.91-12.88, p=0.067). Similarly, children with positive OFC treated with epinephrine were as likely as those who were not to have a biphasic reaction (OR 2.71, 95% CI: 0.67-10.96, p=.182).

CONCLUSIONS: Biphasic reactions in children with food allergies appear to be rare. Furthermore, treatment with steroids or epinephrine at initial reaction does not appear to significantly affect likelihood of a biphasic reaction.

116 Clinical Protocols For Allergen Threshold Studies: Does One Stand Above The Rest?

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RATIONALE: Different dosing schemes are used by various clinical investigators when attempting to determine low dose thresholds for food-allergic subjects. We explored various dosing schemes statistically to determine the approach that might yield the maximum information on thresholds.

METHODS: Estimated population thresholds for egg, peanut, and soy flour were created after screening clinical publications for DBPCFC thresholds and combining data with unpublished clinical results. Simulated individual allergen thresholds were pulled from their respective distributions, fit to a selected dose scheme’s NOAEL and LOAEL values, and analyzed using Interval-Censoring Survival Analysis in SAS. One recommended protocol starts at 10 μg protein and progresses by log-scale to 100 mg protein. Other dosing schemes used in the simulations included variations that started lower than 10 μg or ended between 1 and 10 μg. The curve fits from each protocol were compared to the unaltered thresholds fit to examine the robustness of the dose scheme.

RESULTS: While ED01, ED05, and ED10 estimates were similar in most dosing schemes, the overall fit of the curve and ED50 estimates were significantly different in many cases. Select schemes fit well across egg, peanut, and soy flour, while other schemes fared poorly with shifts to higher or lower thresholds. Schemes with consistent dosing intervals of 3, 5, or 10-fold increases were the most consistent and produced comparable results.

CONCLUSIONS: The dose scheme chosen for a DBPCFC is critical for providing good, usable data on the topic of allergen thresholds. A scientifically sound clinical dose scheme will benefit everyone involved with food challenges.
117 Hen’s Egg White Hypersensitivity among a group of Egyptian Allergic Children
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**RATIONALE:** Egg allergy is potentially life-threatening. The prevalence of egg allergy in Egypt is still unclear. This study is to evaluate the prevalence of egg hypersensitivity in a group of Egyptian allergic children.

**METHODS:** Eighty allergic children were enrolled, each is subjected to clinical evaluation, skin prick testing (SPT) using a commercial egg white extract, and serum egg white specific IgE (SpIgE) estimation. Six patients with suspected egg allergy consent to perform open oral egg challenge.

**RESULTS:** Twenty-eight patients had history of exacerbation of their allergic diseases upon exposure to egg white. Of these, 8 had negative SPT and serum egg white SpIgE. SPT was positive in 25 (31.2%) patients. Of these patients, 3 (4%) were +3, 22 (28%) were +2, of whom 5 patients tolerate eggs without adverse effects. Serum egg white SpIgE was positive in 19 (24%) patients with a mean of 0.81 kUA/L (range: 0.35-4.52 kUA/L). While egg white SpIgE did not correlate with the ages, positive SPT was significantly more frequent among younger patients (t= 1.7, p=0.02). Open oral egg challenge was positive in one patient with positive history but negative tests giving an overall prevalence of egg allergy of 30% (n=24). Egg sensitization and allergy did not affect the severity of asthma.

**CONCLUSIONS:** Although positive SPT/serum specific IgE to eggs are good tools for diagnosis, oral food challenge remains the gold standard in suspected cases. Further wide-scale studies are needed to outline the real prevalence of egg allergy in Egypt.

118 Outcomes Of Pediatric Oral Food Challenges In A Singapore Hospital
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**RATIONALE:** Many patients presenting with allergic symptoms attribute their reactions to food triggers without confirmatory tests, thus leading to unnecessary dietary restrictions. Oral food challenge is the gold standard in the diagnosis of food allergies. The aim of this study is to evaluate the outcomes of oral food challenges performed in the centre.

**METHODS:** This is a retrospective study to review oral food challenge outcomes in association to skin prick test (SPT) results, serum IgE levels, demographic information from records of all pediatric patients referred for evaluation of food allergies to National University Hospital, Singapore from 2008-2010.

**RESULTS:** A total of 184 challenges were performed in 63 patients (1-16 years old). Of these, 60% were male. Sixty-five percent presented with at least one atopic co-morbidity such as asthma, eczema or allergic rhinoconjunctivitis. Hives was reported by 57% and anaphylaxis in 5% at presentation. Sixteen patients were tested on 1 type of food while 47 patients were tested on 2 types or more. The most common food tested were almonds (28/184), eggs (20/184) and peanuts (19/184). Ten patients reacted during the challenges but reactions were mostly cutaneous (urticaria and angioedema). No episodes of anaphylaxis or epinephrine required. Challenge positive subjects had either positive SPT (wheat > 3mm) or raised serum IgE levels to the specific food that they reacted to during the challenges.

**CONCLUSIONS:** This study demonstrated that SPT is a good test in evaluating patients suitable for food challenges. When performed in an experienced tertiary centre, food challenges are safe with low complication rates.

119 Oral Food Challenge Outcomes in a Tertiary Care Allergy Center
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**RATIONALE:** Open oral food challenges are the usual clinical standard for food tolerance. However, clinicians continually search for better predictive approaches.

**METHODS:** A retrospective chart review of all food challenges in children between 2008 and 2010 was performed.

**RESULTS:** Using available predictive approaches 313 challenges were performed (105 peanut, 71 egg, 41 milk, 29 tree nut, 67 other) in children 8 months–18 years old. There were 104 failures (33%); 82 objective, and 22 subjective. Among objective challenge failures, 45/105 (43%) peanut, 10/29 (34%) tree nut, 9/41 (22%) milk, and 12/71 (17%) egg failed. Most (73%) failed peanut/tree nut challenges occurred at doses <1.0g while 71% egg/milk challenges failed at >3g/mL (57% at 10-20g/10-30mL). Sixteen children required epinephrine (9 peanut, 6 tree nut, 1 milk). No egg reactions required epinephrine. There were 5/8(63%) failed cashew challenges, of which 4/5(80%) required epinephrine; 3/5(60%) of whom had no known prior exposure to cashew (skin tested 2nd nut allergy). Reactions to challenge were different from presenting reactions. Age was similar between failed and passed challenges. Atopic dermatitis (79% vs 61%), and asthma (74% vs 47%) were common to failed challenges.

**CONCLUSIONS:** Most challenge failures were to peanut and most severe reactions were to peanut and tree nut. Failures to peanut and tree nut occurred at low doses while most egg and milk reactions occurred at high doses. Those who failed a challenge had more atopic disease. Cashew challenges commonly caused anaphylaxis even in children with no known prior exposure to cashew.

120 Bullying of Food-Allergic Youth: Results from a Parent and Child Survey
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**RATIONALE:** Parents’ reports suggest that bullying of food-allergic (FA) youth is prevalent (Lieberman et al., 2010), but child reports are lacking.

**METHODS:** Following an IRB-approved consent, we separately surveyed patients (children and adolescents), and their parents, for bullying, teasing and harassment. We examined bullying both generally and specific to FA, using questions from the Olweus bullying questionnaire. All families with children ages 8 to 17 were invited to participate during outpatient allergy clinic visits.

**RESULTS:** A planned interim analysis of 111 cases found that 28.8% of children said that they were “bullied, harassed or teased” due to FA, and 43.8% reported having had those experiences for any reason. Among respondents in the age range of 6th-10th graders, 32.6% reported having been bullied, harassed, or teased due to FA while 48.8% reported having had those experiences for any reason (compared with 17% of bullying in 6th-10th graders in a national survey; Nansel et al., 2001). Parents did not know about 32% of the child-reported FA-related cases. In a subgroup of 11 children who reported being bullied at least 2-3 times per month due to FA, parents were unaware that the child was bullied in 64% of the cases. Offenses were most likely to occur at school.

**CONCLUSIONS:** Children with FA are vulnerable to bullying, harassment, and teasing. At least one third of the incidents do not come to parents’ attention. Practitioners should consider specifically asking about bullying in this vulnerable population and provide anticipatory guidance about it even if it is not initially disclosed.
ORS: Of 576 patients with egg allergy who received the influenza vaccine, 56 patients with history of egg anaphylaxis were found. Forty-three (77%) were male and 40 (71%) had asthma. Mean age at time of vaccination was 6.7 years (range: 7 mo - 13 y 9 mo). Within this group there were 120 cases of influenza vaccination. One hundred and thirteen of these vaccinations were preceded by influenza vaccine SPT of which 14 (12%) cases had wheal size of 3 mm or more. There were two reported vaccine reactions: (1) isolated hive at a non-injection site, and (2) eczema flare and local hives. Both resolved with oral antihistamine. The remaining 118 doses of influenza vaccination were tolerated without any symptoms including cutaneous or respiratory reactions.

CONCLUSIONS: Influenza vaccine SPT results in a high number of false positive results. Children with egg anaphylaxis can undergo influenza vaccination without issue.

Parental Perceptions of Anaphylaxis in Children with Egg Allergy
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RATIONALE: Accurate diagnosis of food-related anaphylaxis is important. We compared responses of parent’s perceptions of anaphylaxis to established diagnostic criteria defining anaphylaxis to assess if parents could accurately recognize anaphylaxis in their own child.

METHODS: Parents of egg allergic children receiving the influenza or H1N1 vaccines at the University of Michigan Allergy and Immunology Clinic between 2009-2011 completed a questionnaire assessing symptoms of their child’s most severe reaction to egg, and rated if they felt anaphylaxis had occurred. The investigators reviewed the questionnaire, verified symptom presentation through chart review, scored for symptoms constituting anaphylaxis according to FAAN/NIAID criteria, and compared the results.

RESULTS: Among 108 parents, 78% (84/108) correctly identified symptoms of anaphylaxis to egg, and 22% (24/108) of parents did not. Among those failing to correctly identify anaphylaxis, 25% (6/24) falsely believed anaphylaxis had occurred when it had not. Conversely, 75% (18/24) of parents with a child actually suffering an anaphylactic episode were not identified as such. Symptom patterns among those overestimating anaphylaxis included 4 with isolated skin findings, 1 with isolated GI symptoms, and 1 with a large ImmunoCAP test only. Symptoms most underestimated included 14/18 with skin/GI (77.7%), 9/18 (50%) with skin/respiratory, and 5/18 (27.8%) with 3 organ system involvement.

CONCLUSIONS: Among this population of egg allergic individuals, symptoms of anaphylaxis were underestimated. These findings suggest additional educational efforts in parents are needed to improve identification of anaphylaxis, and identify risk factors that may pre-dispose misidentification.
124 Geographical Variability In The Ltp Recognition In A Large Sample Of Rosaceae Fruit Allergic Patients

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BACKGROUND: Plant lipid transfer proteins (LTPs) have been widely studied as allergens involved in cross-reactivity among foods and food/pollen. Thus, dietary recommendations for patients sensitized to foods of this family are tangled. Studies including both an extensive and representative panel of LTPs and a large number of patients could help us better understanding of cross-reactions mediated by LTPs.

METHODS: Fourteen representative LTPs, from the most frequent plant food allergens in Spain, were purified from different sources and printed in an epoxy-array. Likewise, 212 patients were recruited on the basis of their Rosaceae fruit allergy from seven different pollinic regions and individual sera were tested with the LTP array.

RESULTS: Pru p 3, the peach LTP, was the most frequently recognized by fruit allergic patients, as expected, followed by Mal d 3, from apple, Sin a 3, from mustard, and nut tree nuts LTPs such as, Cas s 8 and Jug r 3. These results reflect the most common cross-reactions observed in the clinical context. Patients with fruit allergy showed polysensitization to LTPs, this being particularly pronounced in subjects without pollen sensitization. This behavior was more frequent in areas such as Barcelona and the Canary Islands, where sensitisation to pollen LTPs related to Pru p 3, Pla a 3 and Art v 3 have been described.

CONCLUSION: LTPs are clearly associated with fruit allergy and are responsible for many cross-reactivities. The local pollen profile modulates LTP recognition, this being stronger in the presence of mugwort and plane pollen.

125 In Vivo And In Vitro Studies On The Sensitisation To A Panel Of Allergens In A Large Rosacea Allergic Group Of Patients

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RATIONALE: Allergy to peach and apple is a frequent problem in the Mediterranean area. Both fruits share allergens between them and with those from other plants and pollens. Component resolved diagnosis assays(CRD) enable to detect IgE antibodies to a wide panel of allergens. A detailed clinical evaluation plus CRD permit a precise analysis of sensitizations to many allergens. Our aim was to analyse sensitisation to fruit and pollen allergens by in vivo/in vitro methods in patients allergic to peach and/or apple.

METHODS: We included 107 patients. A detailed history, including questions related with response or tolerance to different fruits and plants, skin prick test(SPT) with a large panel of representative allergens in our area, and specific IgE antibodies using a CRD platform(ISAC, Phadiat).

RESULTS: Sixty-six cases(61.68%) had symptoms with peel peach, 46(42.99%) with pulp peach and 21(19.62%) with apple. SPT with Pru p 3 was positive in 53(49.53%), and to Mal d 1 in 38(35.51%). CRD was positive to Pru p 3 in 45(42.05%), to Pru p1 in 9(8.41%) and to Mal d 1 in 6(5.6%). From the total group, 27(25.23%) tolerated peach pollen, 47(43.92%) pulp peach and 76(71.02%) apple. Patients had skin test and CRD positive to allergens from fruits that they tolerated. Furthermore, different degree of clinical response and sensitization was obtained with all the other allergens evaluated.

CONCLUSIONS: In vivo and in vitro evaluations with an extensive panel of allergens enable to make a precise diagnosis of allergic patients to fruits. However, discrepancies exist between clinical response and sensitization. Further studies are in progress for understanding these findings.

126 Co-sensitization Between Specific IgE to nAct d 2 and rAlt a 1

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RATIONALE: To verify the existence of an association between specific Ig E (sIgE) sensitization to nAct d 2 (a kiwi allergen with thaumatin function) and rAlt a 1 (Alternaria tenuis) measured by MIA-ISAC in a sample of allergic patients in Spain.

METHODS: Retrospective review of 387 determinations of sIgE (MIA-ISAC 103 allergens version, Phadia, Uppsala, Sweden) performed in La Paz University Hospital’s Immunology laboratory from November 2009 to February 2011. Statistical analysis was carried out with SPSS v9. Geometric mean, standard deviation, and rank of sIgE were calculated in sera positive to nAct d 2 and/or rAlt a 1. In the complete sample Pearson correlation coefficient and kappa concordance coefficient were calculated.

RESULTS: sIgE antibodies to nAct d 2 and/or rAlt a 1 were detected in 98 sera. 71 showed sIgE to nAct d 2 (geometric mean 3.36 ISU), whereas 90 had IgE to rAlt a 1 (geometric mean 6.72 ISU). sIgE to rAlt a 1 was higher than to nAct d 2 in 62(63.26%) determinations. Pearson correlation coefficient was 0.61 (p <0.001) and Kappa concordance coefficient was 0.72 (p< 0.001).

CONCLUSIONS: Correlation between sIgE to rAlt a 1 and nAct d 2 was proven in our environment. This association could be explained through the existence of cross allergenicity. Alt a 1 function is unknown, it would be necessary to study if it belongs to the thaumatin family.
**AB34 Abstracts**

**127 Assessing Specialists’ Opinion Related to Prescribing Auto-injectable Epinephrine In An Unusual Case of Peanut Sensitivity with Oral Allergy**

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**RATIONALE:** To determine if the majority of allergist/immunologists would prescribe epinephrine to a patient with an unusual form of peanut sensitivity, we hypothesized that the majority of specialists would prescribe auto-injectable epinephrine although this treatment may not be necessary.

**METHODS:** At the annual Florida Allergy, Asthma, and Immunology meeting attendees were given a theoretical patient case based upon a clinical experience of the authors and asked to answer a question. The clinical vignette described a patient with tingling and itching of his lips and tongue after eating peanuts but without systemic symptoms. Erythematous skin testing was strongly positive to soy, trees, grasses, peanut and dust mites. The patient also reported regularly eating peanut butter sandwiches without adverse effect. Individual specialists were asked if they would prescribed an auto-injectable epinephrine syringe.

**RESULTS:** 95 questionnaires were distributed and 67 questionnaires were returned for a response rate of 74.4%. 45 physicians or 67.1% stated they would prescribe auto-injectable epinephrine to the patient. 22 physicians or 33.2% replied in the negative.

**CONCLUSIONS:** The majority of allergy/immunology specialists recommend epinephrine auto-injector for peanut oral allergy without evidence of systemic hypersensitivity. NIAID Food Allergy Guidelines recommend the use of antihistamine medications for mild forms of allergic reactions, such as flushing, urticaria, or symptoms of OAS.

**128 Reaction To Soymilk But Not Other Soy Products In Gly m 4 Sensitized Birch Pollen-Allergic Patients**

**R. Q. Chaudhry1, R. Bigelsen2, A. Wolf1; 1University of Medicine and Dentistry of New Jersey, Newark, NJ, 2Tulane University, New Orleans, LA.**

**RATIONALE:** Cases of soy allergy in Gly m 4 sensitized birch pollen-allergic patients with oral allergy syndrome are now being recognized. We report 7 cases of specific reactions to soymilk in birch pollen-allergic patients who tolerate other soy products.

**METHODS:** After ingesting soymilk, all cases experienced severe oral pharyngeal reactions which included throat tightening, hoarseness, lip swelling and raised hives on the tongue. Three patients also experienced systemic symptoms including hives, wheeze and nasal congestion. All tolerated other soy products including tofu and edamame. All have a history of birch pollen allergy and mild oral allergy symptoms to birch tree related fresh fruit.

**RESULTS:** All patients had positive skin (n=4) or RAST (n=3) tests to soy. Four patients had component allergen testing positive to the Gly m 4 component of soy (average= 13 ISU, individual results =0.4, 1.8, 1.8, 4.8) and many other PR-10 allergens, but negative to other soy components.

**CONCLUSION:** Reactions to soy protein drinks have been reported in patients with birch tree pollenosis. The Gly m 4 component of soy protein is cross-reactive with birch tree Bet v1/ PR-10 allergens. Avoiding all soy products is extremely difficult. When a diagnosis of birch pollen related, Gly m 4 soy allergy is confirmed, patients may only need to avoid soy drinks rather than all soy products. Patients with oral allergy to birch related fruits should be warned of this potential reaction.

**129 Gastrointestinal food allergies in children with Ehlers Danlos type 3 syndrome**


**RATIONALE:** Ehlers Danlos Syndrome Type 3 (EDSIII) is a benign connective tissue disorder affecting joints. It leads to joint pain and hypermobility and has been associated with gastrointestinal (GI) symptoms in adults. However, no such association has been described in paediatrics. We therefore set out to describe a group of children with EDS III and concomitant GI food allergies.

**METHODS:** This retrospective descriptive study was performed at Great Ormond Street Hospital for Children. Children with a confirmed allergy by elimination diet, followed by challenge resulting in reproducible deterioration and confirmed EDS III using the Brighton score were included. Data collection included: demographics, gastrointestinal symptoms, comorbidities, food elimination, feeding routes and number of health professionals involved.

**RESULTS:** Nineteen children (81 - 156 months) were recruited (11 boys; 8 girls). The mean age was 102 months (SD 39). The most common food allergies were: eosinophilic colitis (n= 7, 36.8%) and allergic dysmotility (n=6, 31.6%). The most common GI symptoms included constipation in 39.5% and abdominal pain in 89.5%. Extra-intestinal manifestations included: night sweating in 10% and headaches 10%. All required food exclusion, with the most common exclusion being milk, egg, wheat & soy (73.7%). Thirteen children required artificial nutrition: 42.1% via gastrostomy and 26.3% received parental nutrition. All of the children saw more than one additional allied healthcare professional.

**CONCLUSION:** We describe a group of children with food induced GI allergies that also have EDS III. They commonly have multiple food allergies, eosinophilic colitis and require both enteral and parental nutritional support.

**130 Food Protein-induced Enterocolitis Syndrome (FPIES): Our Experience**

**M. Ruiz Garcia; Fundacion Jimenez Diaz, Madrid, SPAIN.**

**RATIONALE:** Food protein-induced enterocolitis syndrome (FPIES) is an uncommon, pediatric, non-immunoglobulin E (IgE)-mediated disorder triggered by the ingestion of food proteins.

**METHODS:** Retrospective study over the past 12 years. 16 children, 10 boys/6 girls (age: 11 months-12 years) diagnosed with FPIES by clinical history and/or oral challenge. Ulterior tolerance done by oral challenge.

**RESULTS:** Skin prick test and specific serum IgE against the triggering food were negative. 50% of patients were atopic. Causative foods for the 16 children and the mean age of presentation were: milk (n=7) 4.7 months (all tolerate soja), fish (n=5) 14 months, gluten free cereals (n=1) 6 months, cereals containing gluten (n=1) 7 months, soja milk (n=1) 6 months, legumes (n=1) 20 months, chicken (n=1) 7 months. All presented symptoms after the first ingestion of the causative food except one.

Mean episodes before diagnosis was 2.4. 5/16 patients (31%) had symptoms with more than one food. Mean time for symptoms to appear after ingestion was 2 hours. The most common clinical features were: vomiting (81%), diarrhea (56%), lethargy (19%), irritability (19%), pallor (13%) and underweight (13%). 6/16 (37.5%) have good tolerance, demonstrated by oral challenge, after food avoidance diet: 3/7 tolerate milk (mean age tolerance MAT: 2.6 years), 2/5 tolerate fish (MAT: 7 years), 1/1 tolerates legumes (MAT: 7.3 years).

**CONCLUSIONS:** FPIES is a severe illness debuting in the first 2 years of life. Clinical suspicion is essential for an early diagnose. In our study milk and fish were the most common triggering foods. The mean age of resolution in our study is higher than that reported by other authors.
131 The Acquisition of Food Allergy in Children after Liver Transplantation

RATIONALE: The acquisition of food allergy (FA) has been described mainly after liver transplantation in children. However, the precise pathogenesis remains uncertain. This study sought to identify the incidence and risk factors of food allergy in post liver-transplanted children.

METHODS: This study was a retrospective analysis of pediatric liver transplant recipients in our hospital. We reviewed the medical records of all patients who underwent liver transplantation during study period. Data collected including age at liver transplantation, immunosuppressive drugs, causal allergens, type of allergic manifestations, preceding-hepatic diseases and etc.

RESULTS: Between November 2005 and May 2010, 106 children received liver transplantation. The most common indication for liver transplantation was biliary atresia. Fifteen patients (10 female and 5 male) developed new-onset FA (14.2%). The average age at transplantation was 10 months and FA has been developed within 2 years. All patients received immunosuppressive therapy based on tacrolimus regimen. Allergic manifestations were as follows: urticaria and angioedema (80%) or gastrointestinal symptoms (50%). Non-IgE-mediated gastrointestinal allergy was suspected in two patients. Most common allergen was egg (50%). Eleven patients with BA (23.4%) and 4 patients with the other conditions (6.8%) developed new-onset FA (P = 0.023).

CONCLUSIONS: This study suggested that new-onset food allergy after liver transplantation is clinically important problem, especially during infancy and early childhood. We observed a trend toward an excess of FA in patients with biliary atresia compared to patients with other indications for liver transplantation. The risk management is needed to prevent life-threatening events in this population.

132 Atopic Dermatitis And Assessment Of Food Tolerance By Oral Food Challenges
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RATIONALE: Patients with food allergy often have concomitant atopic conditions such as atopic dermatitis. It is unclear if the presence of atopic dermatitis (AD) affects achievement of tolerance by oral food challenges (OFC) in individuals with clinical food allergy.

METHODS: 58 subjects (average age 63.7 m, n = 30 male) underwent OFC between January and July 2011 who had a history of clinical food allergy (as diagnosed by symptoms on exposure and positive percutaneous test (PST) or elevated serum specific IgE (sIgE). Subjects with history of clinical food allergy and AD (n = 40) and without AD (n = 18) who underwent OFC were compared with respect to pass rate and time from initial diagnosis.

RESULTS: 30/40 (75%) of subjects with AD and 16/18 (89%) without AD passed OFC. In those who failed OFC, 6/12 with AD and 2/2 without AD had cutaneous manifestations. Tolerance was seen to occur within the first 2 years of diagnosis in 14/30 (47%) subjects with AD compared to 9/16 (56%) without AD.

CONCLUSIONS: Atopic dermatitis is common in individuals with food allergies, and does not predict time to tolerance. Cutaneous manifestations in failed OFC are present in individuals with and without AD.

133 Frequency of Sensitization to Egg and Cow’s Milk in Children with Atopic Dermatitis and Associated Factors
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RATIONALE: Several studies have documented the association between food allergy and atopic dermatitis. Our objective was to evaluate the frequency of sensitization to egg and cow’s milk in children with atopic dermatitis (AD) and related factors.

METHODS: We evaluated pediatric patients with AD who had clinical history suggestive of food allergy. Sensitization to food allergens was investigated by IgE specific for casein, alpha-lactalbumin, beta-lactoglobulin and egg white (ImmuNoCAP). The factors associated with risk of food sensitization investigated were: age at onset, the severity of AD (SCORAD), the period of breastfeeding and timing of introduction of solid foods.

RESULTS: We evaluated 30 patients, aged between 1 and 15 years. The frequency of sensitization was: egg white - 40%, casein - 33%, beta-lactoglobulin - 30% and alpha-lactalbumin - 26%. Among patients with severe disease, 60% showed sensitization to milk and/or egg, while among those with mild/moderate this percentage was 30% (p = 0.0003). The frequency of sensitization was lower in the group where weaning occurred between 3 and 6 months of life (16%) compared to patients who weaned at an age < 3 months (50%) or > 6 months (57%) [p = 0.0001]. There was no significant difference in the analysis of the other factors.

CONCLUSIONS: We observed a high frequency of sensitization to egg proteins and cow milk, with a rate two times higher in patients with severe DA. The lowest percentage of sensitization to milk and egg in patients whose weaning occurred between 3 and 6 months of life supports the hypothesis of the “window of immunological tolerance”.

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RATIONALE: Allergic contact dermatitis (ACD) results from skin contact with allergens and Patch Testing (PT) remains the gold standard in its diagnosis. Studies in dermatologic literature suggest that only one-third of patients are fully evaluated by use of a limited PT screening process. Our aim was to investigate the specific allergens which would be commonly missed by using a limited PT panel such as the True Test® (TT) alone.

METHODS: A retrospective chart review of PT within the last 5 years was conducted at Winthrop University Hospital, Hackensack University Medical Center, and Weill Cornell Medical Center.

RESULTS: 427 patients (mean age = 49.8 years) underwent PT. 82% were female. 54% reported an atopic history. The most common occupation was health care worker. The top 23 allergens were identified. Of those, 9 allergens were not included in the TT: Glutaraldehyde (4.4%), iodopropynyl butylcarbamate-0.5 (2.6%), disperse blue 106 (2.3%), dithiomorpholine (2.1%), sodium thiosulfate (1.6%), cinnamic aldehyde (1.5%), 4-aminoazobenzene (1.0%), cocamidopropyl betaine (0.8%), and N,N-diphenylguanidine (0.8%), accounting for a total of 17% of missed reactions.

CONCLUSIONS: Our study suggests that limited PT with the TT will miss key allergens responsible for ACD. Glutaraldehyde, our seventh most common allergen that is found in sterilizing products, is a relevant allergen for health care workers. Cocamidopropyl betaine is the second most common allergen found in shampoos and is not included in the TT. Although cinnamic aldehyde is a component of Fragrance Mix in the TT, its use as a flavoring agent warrants its testing as a separate allergen.
AB36 Abstracts

135 Skin Immunity Is Regulated By Histamine Receptors Through Dendritic Cell Number And Function
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RATIONALE: Histamine exerts immunomodulatory effects beyond acute allergic inflammation, including modulating dendritic cell function. Recently, mast cells have been shown to regulate contact hypersensitivity (CHS) but the critical mediators involved are not known. Since mast cells are resident in the skin and are a potent source of histamine, we hypothesized that histamine might regulate immunity via the skin.

METHODS: Oxazolone was used to study CHS responses in histamine receptor knockout mice. Adoptive transfer of splenocytes was performed to explore sensitization versus elicitation. Histiation, gene expression and ELISA were used to determine differences. Also, bone marrow-derived dendritic cells (BMDC) were investigated.

RESULTS: While both H1R and H2R-/- mice had diminished CHS responses, double H1R/H2R-/- mice were dramatically reduced, but had normal irritant responses to croton oil. This was partially due to a defect in sensitization, since their splenocytes could not transfer responses into WT donors. Additionally, WT splenocytes failed to elicit responses in H1R/H2R-/- donors, suggesting a concomitant defect in elicitation. Skin from H1R/H2R-/- had significantly impaired expression of CCL5 and CCL17, both critical for CHS. In vitro, BMDC from H1R/H2R-/- mice were quantitatively fewer in number than WT and, upon stimulation, produced significantly less CCL17. Similarly, treatment with both pyrilamine and ranitidine blocked WT BMDC expansion and CCL17 expression.

CONCLUSIONS: Histamine receptors regulate skin immunity via both H1R and H2R, likely by regulating dendritic cell numbers and/or function. Clinically, this suggests that combination therapy targeting both H1R and H2R may provide benefit for skin inflammation.

136 Role of Mast Cells in the Development of Atopic Dermatitis induced by IL-13
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RATIONALE: Mast cells, expressing c-kit, are crucial effector cells in allergic and anaphylactic responses. Increased mast cell counts are found in atopic dermatitis (AD) skin lesions. However, the role of mast cells in AD has not been investigated sufficiently. Inducible selective-expression of IL-13 in mouse skin caused AD-like phenotypes including increased mast cells and mediators. We hypothesized that mast cells play an important role in IL-13-induced AD and tested this using skin-specific IL-13 Tg mice with mast cell deficiency.

METHODS: Tg/c-kit-/- mice obtained from crossing breed IL-13 Tg mice with c-kit deficient Kit W-sh/Kit W-sh mice were compared with IL-13 Tg mice carrying wild type c-kit (Tg/c-kit+/+). Skin mast cells in Kit W-sh/Kit W-sh mice usually disappear by the age of 13 weeks. The IL-13 transgene in the skin was activated at age of 15 weeks by withdrawal of doxycycline from drinking water. AD clinical scores, scratching numbers were recorded. Skin samples were obtained and analyzed by H&E staining, toluidine blue staining, and ELISA.

RESULTS: All Tg/c-kit+/+ mice developed severe AD-like phenotypes by the age of 5 months. Remarkably, only 30% of Tg+c-kit-/- mice developed mild AD symptoms, which was significantly delayed in onset by 3 months (AD and itching scores). In addition, Tg+/-/- mice showed significantly decreased eosinophils, decreased levels of TSLP and IL-4 but comparable levels of IFN-γ in the skin compared to Tg+c-kit+/+ mice.

CONCLUSION: This study suggests that mast cells are important in initiating and maintaining skin inflammation of AD induced by IL-13 probably through induction of TSLP and IL-4.

137 Involvement of Human Histamine N-methyltransferase Gene Polymorphisms in Susceptibility to Atopic Dermatitis in Korean Children
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RATIONALE: Histamine N-methyltransferase (HNMT) catalyzes one of two major metabolic pathways for histamine. Histamine is one of the mediators for pruritus of atopic dermatitis. The aim of this study was to evaluate the role of HNMT polymorphisms in children with atopic dermatitis.

METHODS: We genotyped 763 children for allelic determinants at four polymorphic sites, which were -465T>C, -413C>T, 314C>T and 939A>G in the HNMT gene, and the functional effect of the 939A>G polymorphism was analyzed. The genotyping was screened using the TaqMan fluorogenic 5’ nuclease assay (ABI, Foster City, CA, USA).

RESULTS: Among these 763 children, 520 had eczema and 542 had atopy. Distributions of the genotype and allele frequencies of HNMT 314C>T polymorphism were significantly associated with non-atopic eczema (P = .004) and those of HNMT 939A>G polymorphism were significantly associated with eczema in atopy groups (P = .048). However, those of HNMT 654T>C and -413C>T polymorphisms were not. In addition, subjects with the homozygous AA or heterozygous AG of the 939A>G polymorphism showed significantly higher IgE levels than those with the homozygous GG genotype (P = .009). In U937 cells, the variant genotype reporter construct showed significantly higher mRNA stability (P < .001) and HNMT enzyme activity (P < .001) than the common genotype.

CONCLUSIONS: Polymorphisms in the HNMT gene appear to confer susceptibility to atopic dermatitis in Korean children.

138 The Association of Polymorphisms in the Interleukin 33 (IL33) and Interleukin 1 Receptor-like 1 (IL1RL1) Genes with Risk of Atopic Dermatitis
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RATIONALE: Atopic dermatitis (AD) is an inflammatory skin disorder often associated with elevated serum total IgE (tIgE) levels and propensity to development of asthma. We hypothesized that genes encoding IL33 and its receptor, IL1RL1, for which we have also observed associations with asthma and tIgE levels, may represent genetic risk factors for AD.

METHODS: Single nucleotide polymorphisms (SNPs) in IL33 (N=7) and IL1RL1 (N=6) genes with prior evidence for association with asthma were genotyped in two independent populations recruited through the NIAID-supported Atopic Dermatitis Research Network (ADRN). 474 European American (EA) patients (313 AD cases and 163 non-AD controls) and 375 African American (AA) patients (111 and 164, respectively). Individual-SNP trend tests for association were performed with permutation-based P-values.

RESULTS: In the EA population, four markers in IL33 (rs1342326, rs992969, rs928413 and rs17498196) were associated with risk of AD phenotype (P=0.011-0.037). Given the correlation between AD and asthma/IgE as well as the association with IL33/IL1RL1 and asthma/tIgE, both asthma status and tIgE levels were included as covariates in these models, after which two SNPs (rs1342326 and rs17498196) remained significant (P=0.008 and 0.018, respectively). SNP rs10173081 in IL1RL1 was also associated with tIgE levels (P=0.004).

CONCLUSIONS: These results suggest that variants in IL33 and IL1RL1 previously associated with IgE mediated phenotypes such as asthma likely also play a role in AD phenotype. This may be in part mediated by tIgE levels. Sequencing of these genes is underway to test for associations at each observed variant in place of a tagging approach used here.
139 TLR4 Deficiency Exacerbates Allergen-Induced Atopic Dermatitis
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RATIONALE: Despite its presence on resident skin cells, the role of TLR4 in skin diseases remains poorly understood. We aim to establish the contribution of TLR4 to atopic dermatitis (AD).
METHODS: TLR4-deficient and wild type mice were epicutaneously exposed to Aspergillus fumigatus (Asp). Skin barrier function was assessed by trans-epidermal water loss (TEWL). Inflammation was determined by immunohistochemistry and RT-PCR. Gene expression data of RNA samples derived from acutely exacerbated AD skin (publically available on NCBI Gene Expression Omnibus) were analyzed.
RESULTS: The absence of TLR4 resulted in enhanced TEWL, epidermal thickness, AD symptom scores, epicutaneous sensitization, and decreased skin filaggrin expression. However, TLR4 deficiency had no impact on nascent Th2-driven skin inflammation (eosinophil, mast cell and IL-4 mRNA skin levels). IL-17A and TNFα skin levels were increased, and IFNγ levels decreased in Asp-exposed TLR4 deficient mice, but their levels did not correlate with TEWL. Levels of S100A8/A9 (calprotectin, an inflammatory mediator) did not correlate with TEWL. Similarly, acutely exacerbated skin samples from AD patients revealed decreased TLR4 skin levels associated with increased S100A8/A9 and impaired skin barrier.
CONCLUSIONS: Signaling through TLR4 limits allergen-induced skin barrier dysfunction and AD severity. TLR4 deficiency is associated with increased skin expression of S100A8/A9 in an experimental AD model and in human AD patients. S100A8/A9 skin levels correlate with skin barrier dysfunction suggesting that calprotectin may be a useful biomarker of AD disease severity.

140 In Silico Analyses Reveal Putative Regulatory Elements Upstream of the Thymic Stromal Lymphopoietin Receptor Gene
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RATIONALE: We previously reported the unique propensity for dendritic cells from patients with atopic dermatitis (AD) to upregulate thymic stromal lymphopoietin receptor (TSLPR) following allergen stimulation. The objective was to interrogate DNA sequences upstream of the TSLPR gene for transcription factor binding sites that could provide candidate regulatory elements relevant to AD.
METHODS: Bioinformatics approaches were used to identify candidate DNA sequences located 5’ to the TSLPR gene coding region that may contain transcription factor binding sites. Databases including MatrixCatch, UCSC’s Genome Browser, TRANSFAC and NCBI Entrez were queried using either the TSLPR gene name (CRLF2), accession number (AB052639), or DNA sequence obtained from the NCBI human genome database.
RESULTS: Clustering of transcription factor binding sites for NF-AT, NFκB, PU.1 and IRF proteins was identified in a 5,000 nucleotide span immediately upstream of the TSLPR gene transcription start site. This region contained NF-AT, PU.1 and IRF sites proximal to (and NFκB sites distal to) the transcription start site. MatrixCatch uniquely identified the presence of several possible PU.1/IRF composite sites which are known to influence the transcriptional regulation of an array of immunomodulatory genes including CIITA, which controls MHCI expression during dendritic cell maturation. Molecular cloning of this putative promoter region into a luciferase reporter vector was successful using genomic DNA isolated from AD patients.
CONCLUSION: In silico analyses identified a putative TSLPR gene promoter containing regulatory elements that could yield new insight into the genetic mechanisms underlying allergen-responsive ness in dendritic cells from AD subjects.

141 Filaggrin Deficiency Impairs Viral Containment in Mice Cutaneously Inoculated with Vaccinia Virus (VV)
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RATIONALE: Eczema Vaccinatum (EV) is a life threatening complication of exposure to smallpox vaccination in patients with atopic dermatitis (AD) characterized by dissemination of VV in skin and internal organs. Filaggrin deficiency is present in up to 48% of patients with AD, and filaggrin null mutations are associated with eczema herpeticum. We examined the effects of filaggrin deficiency on the response of mice to cutaneous VV inoculation.
METHODS: Unsensitized or ovalbumin (OVA)-sensitized shaved unstriped skin of filaggrin-deficient ft/ft mice on BALB/c background or WT controls was inoculated with 10^7 plaque-forming units of VV by scarification. Viral load and cytokine mRNA expression were assessed by quantitative PCR, and skin histology was examined by H&E staining.
RESULTS: VV inoculation in unsensitized skin resulted in significantly increased size of primary lesions, number of satellite lesions, viral loads in skin and internal organs, dermal cellular infiltration and mRNA expression of IL-17, IL-4, IL-13, and IFN-γ in ft/ft mice compared to WT controls, which showed minimal response to VV inoculation. Inoculation of VV at sites of OVA application to unstriped skin accentuated all the aforementioned changes in ft/ft mice, but had no detectable effect in WT controls. The number of satellite lesions and the viral loads in internal organs were significantly decreased in ft/ft mice bred onto the IL-17 null background after VV inoculation in unsensitized skin, as well as in OVA-sensitized skin.
CONCLUSIONS: Filaggrin deficiency may predispone to EV, particularly if VV is introduced at sites of cutaneous antigen sensitization. Blocking IL-17 may attenuate EV in filaggrin deficiency.

142 Sequencing Of The Fig2 Gene In Patients With Atopic Dermatitis And Eczema Herpeticum In A Population Of European Descent.
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RATIONALE: Atopic dermatitis (AD) is characterized by disseminated viral skin infections, particularly eczema herpeticum (ADEH). Previously, we genotyped 8 common (>1% minor allele frequency) non-synonymous coding single nucleotide polymorphisms in FLG2, a gene on the epidermal differentiation complex locus associated with ADEH, at the conclusion of the 2011 AAAAI meeting. Currently we are sequencing the entire gene to discover additional rare and novel variants that may be associated with ADEH.
METHODS: We are currently sequencing 15kb of the FLG2 gene using Agilent’s targeted deep-sequencing platform on 112 ADEH- and 125 ADEH+ European ancestry patients. To date a subset (100 ADEH-, 100 ADEH+) has been sequenced on exons 2 and 600 base pairs of exon 3 nearest exon 2, using Sanger sequencing. Genetic associations for risk of ADEH and associated traits were determined by the Cochran-Armitage trend test.
RESULTS: Sequencing so far has uncovered three previously known SNPs, and two novel variants in exon 3 not previously documented in dbSNP or the Thousand Genomes Project. Tests for association on single SNPs and a collapsed test on all rare variants did not reveal any associations with ADEH. We also performed analyses adjusting for FLG mutations RS01X and 2282del4, but did not observe significant associations with ADEH.
CONCLUSIONS: Interim sequence data on FLG2 has not yielded novel associations with ADEH; however, we anticipate additional novel variants to be identified by targeted deep-sequencing of the entire gene, and will perform additional burden tests collapsing on all newly identified rare functional variants previously un-captured by any tagging genotyping strategy.
143 Relevance of Patch Test Results to the Clinical Diagnosis of Allergic Contact Dermatitis - Need for Standardization

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RATIONALE: The clinical relevance of positive patch testing results varies widely because there is no standardization of assessing relevance to the patients’ clinical diagnosis.

METHODS: 7 years of patch test results were retrospectively evaluated for clinical relevance to the final diagnosis of Allergic Contact Dermatitis (ACD). Patch Testing was performed utilizing the allergens in the Thin-Layer Rapid-Use Epicutaneous (T.R.U.E.) Test and the North American Contact Dermatitis (NACD) Panel. Patients were not on immunosuppressive therapy. Patients with positive patch test(s) had physician assigned relevance as 0-none, 1-definite, 2-probable, and 3-possible relevance. All negative tests were assigned 0. ACD was considered a primary relevant diagnosis and all other diagnosis’ were considered negative. A 2x2 Chi Square test was performed using SAS 9.2.

RESULTS: Data was obtained from 181 patients (female 76%) and 61.3% of patients had a positive patch test at least one allergen. Of those, 48.6% had definite relevance, 17.1% probable, 24.3% possible and 9.9% none. Alternatively, 43.6% of the total 181 patients had no relevance to their testing results and 30.4% had definite relevance. This study demonstrates sensitivity of clinical relevance to be 65.9% and specificity to be 93.7%. Positive predictive value of the clinical relevance was 89.2% and negative predictive value was 78.1%.

CONCLUSIONS: Patch testing results were less sensitive to patients’ diagnosis, but demonstrate positive predictive value. Standardization of how to assess clinical relevance needs to be implemented.

144 Systemic Tolerability of Intermittent Topical Corticosteroid Therapy Using Salivary Cortisol Measurements in Infants with Atopic Dermatitis

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RATIONALE: Little is known about the safety of intermittent use of topical steroids on hypothalamic-pituitary-adrenal (HPA) axis function in infants with atopic dermatitis (AD). This study evaluates the morning salivary cortisol levels in children using maintenance treatment with topical corticosteroids with and without proactive approach.

METHOD: Patients with moderate-to-severe AD were prospectively randomized to proactive treatment group or reactive treatment group. In the proactive treatment group, hydrocortisone 1% ointment was applied twice daily on every-3-days or less to all previously identified affected areas. Salivary samples for the analysis of cortisol and dehydroepiandrosterone levels were collected at 0, 3 and 6 months and adrenocorticotropic hormone stimulation test were performed at 3 months of the study period. Morning salivary samples were collected at home on three consecutive days.

RESULT: Eleven patients were eligible for this study. Median morning salivary cortisol was lower around the first visit compared with at 3 months, which was similar at 6 months of the study period. In both groups, serum cortisol responses after adrenocorticotropic hormone stimulation test at 3 months were normal. The morning salivary cortisol levels of the children with proactive treatment group and the reactive treatment group were comparable, but significantly fluctuated during three consecutive days, irrespective of the day of topical steroid or emollient.

CONCLUSION: This study demonstrates the low risk of developing adrenal suppression secondary to the proactive topical corticosteroid therapy.

145 Factors Determining The Effectiveness Of Oral Cyclosporine In Children With Severe Atopic Dermatitis (AD)

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RATIONALE: Oral cyclosporine is a systemic immunosuppressive drug used for the treatment of AD unresponsive to topical corticosteroids and calcineurin inhibitors. There is little or no information as to which patients, particularly children, are most likely to respond to this drug.

METHODS: Clinical features and outcome of 35 children (20 (57%) male; 22 (63%) Caucasian) receiving oral cyclosporine (starting dose 5mg/kg/day) for severe AD (median age at initiation of therapy 6 (3 - 10 years)) at a single tertiary paediatric eczema clinic were surveyed retrospectively.

RESULTS: All children had severe generalized AD and disrupted sleep due to unremitting pruritus; 33 (91%) were on regular moderate - potent topical corticosteroids. Median duration of treatment with cyclosporine was 19 weeks (range 2 to 93 weeks) and plasma trough level was 47 mcg/L. 16 (46%) had a marked improvement / clearing of their eczema. No patients had any abnormalities in renal function (U&Es) checked on a monthly basis. One patient developed eczema herpeticum. The only factor that determined outcome was whether or not the AD was infection driven based on clinical features (2/19 (10%) of patients without secondary infection improved compared to 10/16 (63%) with secondary infection; P < 0.001). This improvement was significant even after controlling for concomitant antibiotic use by multivariate analysis.

CONCLUSIONS: Oral cyclosporine is most effective in severe infection-driven AD once infection has been treated with appropriate antibiotic. Further work is required to determine if the beneficial effect is specific due to inhibition of bacterial superantigen activated T-lymphocytes.

146 Omalizumab - One Year Experience In The Treatment Of Severe Atopic Dermatitis

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RATIONALE: Omalizumab is a humanized monoclonal anti-IgE antibody that interrupts the allergic cascade which suggests that it can be effective in the treatment of severe allergic conditions, including atopic dermatitis (AD). We describe one year follow-up of 4 patients with severe AD and high levels of total IgE, successfully treated with omalizumab.

METHODS: Four patients (2 male, 2 female; 24-year-old average) with severe AD, allergic respiratory disease and high levels of IgE (> 2000 UI/ L) not responding to conventional therapies, were proposed for omalizumab treatment. They were evaluated for SCORAD index and daily/rescue medication before, during and after treatment. Omalizumab was administered subcutaneously at 375 mg every 2 weeks for a year.

RESULTS: Before treatment all patients were medicated with anti-H1 and H2 antihistamine (maximum daily dose), montelukast 10 mg/day, topical and oral steroids (average dose 20mg/day) and topical primacrolimus. Three patients were also medicated with cyclosporine and one of them was previously medicated with intravenous immunoglobulin G (1000mg/kg/month) with no response. After one year all patients improved, although the onset for initial improvement was variable. The daily/rescue medication decreased in both dose and number of drugs. Systemic steroids were stopped with no relapse of symptoms. SCORAD index was 66.7 average at the beginning and 24.4 after one year treatment. The patients did not experienced adverse side effects.

CONCLUSIONS: Our data suggests that Omalizumab may be a safe and effective alternative in the treatment of severe atopic dermatitis in allergic patients.
A Case Series Of Severe Atopic Dermatitis Treated By Anti-IgE Therapy: A Paediatric Perspective

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RATIONALE: Atopic dermatitis (AD) is a common skin disease of childhood. AD is usually associated with presence of high circulating IgE levels. Omalizumab is a humanized monoclonal anti-IgE antibody that has revealed some potential in the treatment of severe and recalcitrant AD. Here, we present 7 patients who have been treated with omalizumab.

METHODS: We reviewed the files of all patients with severe AD treated with omalizumab since 2005. Clinical and biological data were collected in order to characterize and compare their profile before and after treatment with omalizumab.

RESULTS: The seven patients presented severe atopic dermatitis before 2 years old of age and all had associated asthma, allergic rhinitis and food allergies. Before the anti-IgE therapy was started, most of them were treated for at least one year with oral prednisone, cyclosporine or azathioprine without any satisfying results. At treatment initiation of anti-IgE, the mean age of the patients was 10.3 years old and their mean IgE level was 16,007 U/mL. The mean duration of treatment was 23.4 months and after a few months of treatment all of them shown a significant drop of the IgE levels and an improvement of their SCORAD index. Since the beginning of their treatment, their follow-up has been almost free of any remarkable event and treatment with omalizumab has been well tolerated.

CONCLUSIONS: Omalizumab appears to be a safe and well tolerated treatment and should be considered as a potential treatment in cases of severe AD resistant to classical therapy in the paediatric population.

MRSA Skin Colonization Is Associated With Higher Total IgE in Young Children With Atopic Dermatitis


RATIONALE: The objective of this study was to determine whether laboratory parameters correlate with either colonization with MRSA or low vitamin D level in pediatric atopic dermatitis (AD).

METHODS: The National Jewish Health Research Database was queried for children aged below or equal to 12 years with the diagnosis of AD. Staphylococcus aureus culture data, vitamin D levels, and total serum IgE from March 2008 until March 2011.

RESULTS: 205 patients were identified: 41 (20%) Methicillin resistant S. aureus (MRSA), 133 (65%) Methicillin sensitive S. aureus (MSSA), and 31 (15%) without S. aureus. Patients colonized with MRSA had significantly higher total IgE levels than both patients with MSSA (3.6 vs. 3.3 [log Ku/l], p=0.04) and no staph (3.6 vs 3.1 [log Ku/l], p=0.02). The difference between MRSA and MSSA remained significant in subjects less than 6 years old, but not if greater than 6 years old (3.5 vs. 3.0 [log Ku/ l], p=0.04; 3.8 vs. 3.7 [log Ku/l], p=0.87). 50% of subjects had vitamin D insufficiency (< 30 ng/ml). Vitamin D level less than 20 ng/ml (n=19), but not less than 30 ng/ml, was associated with higher IgE levels (3.8 vs. 3.3 [log Ku/l], p=0.006). The mean vitamin D levels between compared S. aureus groups were not different.

CONCLUSIONS: For young children with AD, MRSA skin colonization was associated with higher total serum IgE than with MSSA colonization. Vitamin D levels in MRSA colonization were not different than other groups suggesting that the elevated IgE in MRSA colonized AD was not due to vitamin D status.

Relationship of Serum 25-hydroxyvitamin D Levels with the Severity of Atopic Dermatitis in Children

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RATIONALE: Atopic dermatitis (AD) is a chronic inflammatory relapsing skin disorder. Vitamin D, well known for its role in calcium homeostasis, is found to have additional role on the immune system, especially on the development of allergic diseases. The aim of our study is to determine whether 25-hydroxyvitamin D [25(OH)D] level is associated with the atopic sensitization and the severity of AD.

METHODS: We enrolled 103 children with AD, and 20 control subjects without history and symptoms of allergic diseases. Blood was drawn to evaluate complete blood cell count, total eosinophil count (TEC), total IgE, specific IgE to common allergens, 25(OH)D, and IL-31. Serum 25(OH)D was measured using high-performance liquid chromatography and SCORAD index was used for evaluating the severity of AD (severe, >50; moderate, 25-50; mild, <25). Vitamin D status was categorized according to 25(OH)D levels (deficient, <20 ng/ml; insufficient, 20-30 ng/ml; sufficient, >30 ng/ml).

RESULTS: Mean value of 25(OH)D was significantly lower in AD group compared to control group and significantly decreased in moderate AD compared to mild AD. Children with atopic sensitization showed significantly lower 25(OH)D levels than non-atopic children. Serum 25(OH)D level was inversely correlated with SCORAD index and total IgE levels. Children with severe AD had significantly higher TEC and total IgE levels than children with mild AD. Vitamin D-deficient group showed higher SCORAD score than vitamin D-sufficient group. Serum IL-31 level was not related to AD, SCORAD index, or 25(OH)D levels.

CONCLUSIONS: Serum 25(OH)D level is associated with the atopic sensitization and severity of AD in children.
152 Dietary Intervention With Specific Non-digestible Oligosaccharide Mixtures Is Effective During Induction Of Murine Cow’s Milk Allergy But Not Suitable As Treatment In The Same Allergic Disease Model

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RATIONALE: In this study, the effects of dietary supplementation with specific non-digestible oligosaccharides (prebiotics) either as single components or in mixtures on the outcome of the murine allergic response when provided before and during oral sensitization with whey were investigated. In addition, the feasibility to treat allergic mice with these prebiotics was addressed.

METHODS: Mice were fed diets containing 1% single prebiotics, combinations of scGOS (short chain galacto-oligosaccharides), LcFOS (long chain fructo- oligosaccharides) and/or pAOS (pectin-derived acidic oligosaccharides) or a control diet. The diets were provided two weeks prior to and during oral whey-sensitization (prevention) or for four weeks starting one week after the last sensitization (treatment). The acute allergic skin response (ITH) and antigen-induced anaphylaxis (AiA) were determined one hour after intradermal whey-challenge. Whey-IgE/G1/G2a and mouse mast cell protease-1 (mMCP-1) were determined one hour after intradermal whey-challenge. Whey-IgE/G1/G2a and mouse mast cell protease-1 (mMCP-1) were determined one hour after intradermal whey-challenge. Whey-IgE/G1/G2a and mouse mast cell protease-1 (mMCP-1) were determined one hour after intradermal whey-challenge.

RESULTS: Children with AD compared to control group presented with lower height for age z-score (P=0.007), lower BMC at lumbar spine [16.5(6.4) vs. 19.8(8.3), P=0.027] and total femur [12.2(4.0) vs. 14.2(5.0) g, P=0.029]. Bone resorption marker (CTX) was lower in children with AD compared to healthy controls [1.36(0.59) vs. 1.67(0.79)ng/mL, P=0.026] and a tendency of lower bone formation marker (alkaline phosphatase) was also observed [228(75.3) vs. 255(70.7)ng/mL, P=0.074]. Children with AD presented inferior levels of serum cortisol in comparison to healthy group [9.06(4.8) vs. 10.57(4.9), P=0.061].

CONCLUSIONS: Lower lumbar and total femur BMC and lower bone turnover found in children with AD is probably due to a chronic GC use.

151 Nutritional Status and Bone Metabolism in Children With Atopic Dermatitis

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RATIONALE: Atopic dermatitis (AD) is an inflammatory allergic skin disease that often requires topical glucocorticoid therapy (GC). To determine the impact of AD on nutritional status and bone parameters in children with moderate/severe AD compared to control group.

METHODS: Forty-nine children with AD (4-12 years) and 48 healthy controls were evaluated regarding nutritional status (height/age z-score, weight/age z-score and BMI z-score), disease activity and severity, topical GC use, and bone parameters. Bone mineral content (BMC), bone mineral density and z-score were measured at lumbar spine and total femur by DXA. Laboratory parameters analyzed were calcium, alkaline phosphatase, CTX, 25OHD and cortisol.

RESULTS: Children with AD compared to control group presented with lower height for age z-score (P=0.007), lower BMC at lumbar spine [16.5(6.4) vs. 19.8(8.3), P=0.027] and total femur [12.2(4.0) vs. 14.2(5.0) g, P=0.029]. Bone resorption marker (CTX) was lower in children with AD compared to healthy controls [1.36(0.59) vs. 1.67(0.79)ng/mL, P=0.026] and a tendency of lower bone formation marker (alkaline phosphatase) was also observed [228(75.3) vs. 255(70.7)ng/mL, P=0.074]. Children with AD presented inferior levels of serum cortisol in comparison to healthy group [9.06(4.8) vs. 10.57(4.9), P=0.061].

CONCLUSIONS: Lower lumbar and total femur BMC and lower bone turnover found in children with AD is probably due to a chronic GC use.

153 Different Timings of Prenatal or Postnatal Tobacco Smoke Exposure Have Different Effects on The Development of Atopic Eczema/Dermatitis Syndrome (AEDS) during Infancy

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RATIONALE: Tobacco smoke is an important environmental factor for allergic diseases. Prenatal tobacco smoke exposure affects both fetal lung and immune function with increased subsequent risk of asthma and respiratory infection in infancy. About the skin, tobacco smoke increases the prevalence of wrinkling, skin cancer, psoriasis and systemic lupus erythematosus. The influence of tobacco smoke for the development of atopic eczema/dermatitis syndrome (AEDS) is controversial and when is the critical period unclear. Therefore, we investigated whether the different periods of maternal tobacco smoke exposure have different impacts on the development of AEDS, or not.

METHOD: A total of 1,436 infants (age 2.0–18.0 months) was enrolled in this cross-sectional questionnaire survey. Family history of allergic diseases, number of older siblings, prenatal and postnatal maternal tobacco smoke exposure, development of physician-diagnosed AEDS and so forth were asked by a self-writing questionnaire. An adjusted logistic regression model was analyzed by STATA software.

RESULTS: The recovery rate was 97.2% (1436/1476). The prevalence of AEDS was significantly increased in the infants with tobacco smoke exposure during third trimester pregnancy (aOR, 6.146; 95% CI, 1.282 - 29.453) than those without tobacco smoke exposure. The prevalence of AEDS was no significant differences between the infants with tobacco smoke exposure during first trimester pregnancy, during the first 6 months of life and after 6 months after birth than those without tobacco smoke exposure, respectively.

CONCLUSIONS: Maternal tobacco smoke exposure during third trimester pregnancy might have the strongest impact effect on increasing the development of AEDS in their offspring.
Correlates of Asthma Deaths at a Children’s hospital with a Multidisciplinary Team (MDT) Asthma Program for High Risk Asthmatics

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RATIONALE: To correlate the impact of the initiation of a multidisciplinary team clinic (MDTC) “CHAMP initiative” (Children’s Hospital Asthma Management Program) and asthma related deaths.

METHODS: A 10 year retrospective chart review of pediatric patients from 1 to 18 years of age who died from asthma 3 years before and 7 years following the start of the multidisciplinary team clinic.

RESULTS: A total of 14 children died from asthma in a ten year time period at a children’s hospital. Of the 14 children who died only 3 were in care at the asthma clinic and 11 the children were never followed in the asthma clinic despite prior hospitalizations for asthma. During the first three years of the MDTC for high risk asthmatics with the availability of a liaison with social service, there were no deaths of children in care.

CONCLUSION: 79% (11/14) of the deceased children’s records indicated a lack of involvement in outpatient specialty asthma follow-up, despite prior hospitalizations and appointments on discharge. Deaths of high risk asthmatics were decreased by a combination of MDTC as well as the use of a social service liaison for increased family support. This further emphasizes that barriers to care and clinic attendance necessities further attention.

Reducing Health Disparities For Asthma With a School Based Asthma Education Program

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RATIONALE: Asthma education programs, such as the American Lung Association’s Open Airway for Schools (OAS), improve asthma control and prevent exacerbations but are often not readily available to populations at risk for health disparities.

METHODS: This randomized, controlled study enrolled 8 urban public elementary schools at-risk for health disparities and high asthma prevalence to evaluate implementation of OAS. Asthmatic students (49 intervention, 41 control) in grades 3-6 completed pre- and post-assessments of asthma knowledge scores (17-20), asthma related complications, yet there is limited epidemiologic data related to asthma patients’ risk of the novel 2009 H1N1 influenza virus infection. At present, no population-based study has been conducted to address this question.

METHODS: We selected a fluticasone propionate MDI, 125 microgram and a fluticasone propionate/salmeterol MDI, 125/5 microgram (GlaxoSmithKline) as the studied medications. Five canisters each of both medications were used. The weight change per ten actuations of each canister was recorded in units of grams. Data between canister weight and the number of actuations were plotted and used to develop regression equations to predict the number of actuations. To confirm the accuracy of the equation, we used another five canisters of each medication as test sets. Each canister was weighted after 30, 60, 90 and 120 actuations. We placed each canister weight in the regression equation and compared the predicted value with the actual number of actuations.

RESULTS: The mean canister weight before actuation was 18.89 ± 0.05 grams for a fluticasone propionate MDI and 20.78 ± 0.06 grams for a fluticasone propionate/salmeterol MDI. The regression equation for a fluticasone propionate MDI was: number of actuations = 276.16 - (14.62 x canister weight) and for a fluticasone propionate/salmeterol MDI: number of actuations = 303.93 - (14.63 x canister weight). Comparing between 30, 60, 90 and 120 actual actuations, the ranges of predicted values were 29-31, 59-61, 88-92 and 118-122 actuations for fluticasone propionate MDI and 27-31, 57-61, 86-92 and 116-123 actuations for fluticasone propionate/salmeterol MDI, respectively.

CONCLUSIONS: The canister weight is a reliable factor for estimating the number of actuations from a MDI.
159 Prevalence Of The Correct Technique Of Using An Inhaler Among Asthmatic Patients Reporting In Tertiary Care Hospitals Of Rawalpindi, Pakistan.


RATIONALE: To assess the technique of using an inhaler among asthmatic patients reporting in the Out Patient Department of tertiary care hospitals of Rawalpindi.

METHODS: We carried out a cross-sectional study, in which previously diagnosed Asthmatic patients reporting in the out patient department of three major tertiary care hospitals of the city were asked to perform a practical demonstration of using the inhaler which had been prescribed to them before. The inhalation technique was observed and each step was marked on a standardized performa containing 11 steps. All known Asthmatic patients who had been prescribed an inhaler were included in the study. Statistical analysis was performed using the Statistical Package for Social Sciences version 14.

RESULTS: Only 1 patient was observed to make no inhalation errors when evaluated (P < 0.05). 37% patients did not perform step 4 (Tilting head back and expiring the air out) correctly. 45% patients did not perform step 9 (Waiting about 1 minute and repeating steps 2-8, if more than one puff required) correctly and 58% patients performed step 11 (Gurgling and rinse mouth with steroid-type inhalers) incorrectly.

CONCLUSION: The technique of using the inhaler can be substantially improved with good instructions by the treating physician and it should be kept in mind that technique instruction is an essential ingredient of patient’s management.

160 Risk of Hospitalization in Thai Children with Acute Asthma

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RATIONALE: Acute asthma is a leading cause of hospitalization in childhood. The aim of this study was to describe the characteristics of Thai children admitted to the hospital with acute asthma and to identify risk factors that may prevent future hospital admissions.

METHODS: We conducted a case-control study from a hospital database of all patients with asthma aged 6 to 15 years who presented at the Emergency Department (ED) from January 1, 2010 to May 31, 2011 (n = 239). All children admitted to the pediatric ward were defined as cases. We randomly selected children who were successfully discharged from ED as controls with a 1:1 ratio.

RESULTS: Thirty cases and thirty controls were identified. In both cases and controls, patients had similar characteristics regarding mean age, sex, mean body mass index and previous history of asthma. Compared to patients discharged, the admitted group had a longer duration of asthma and more severe exacerbations but had similar characteristics regarding mean age, sex, mean body mass index and previous history of asthma. Compared to patients discharged, the admitted group had a longer duration of asthma attack (10.5 hours vs. 3.5 hours, p<0.01), more children received bronchodilator at home (55% vs. 16.6%, p<0.01), more severe exacerbation patients (70% vs. 3%, p<0.01), higher respiratory rate (39.7±12.8/min vs. 30.9±6.5/min, p<0.01) and less oxygen saturation (92.6±3.8% vs. 96.6±2.3%, p<0.01). Regarding ED treatments, the admitted group received less frequent nebulized β2-agonist as the controls (interval between dosing schedule 32.1±17.7 minutes vs. 17.5±5.0 minutes, p<0.01).

CONCLUSIONS: Admitted children had more severe exacerbations but received less frequent bronchodilator treatment than the group discharged. Appropriate asthma management at the ED is needed to prevent further hospitalization.
163 Mast Cells with a Unique Phenotype Are Highly Elevated in Chronic Rhinosinusitis with Nasal Polyps
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METHODS: We collected nasal tissue and nasal lavage fluid from patients with CRS and control subjects. We analyzed mRNA for the mast cell proteases tryptase, chymase and carboxypeptidase A3 (CPA3), using real-time PCR, and assessed the presence of mast cell proteases using ELISA and immunohistochemistry. We also performed immunofluorescence to observe the pattern of colocalization of the mast cell proteases.
RESULTS: Tryptase mRNA and protein were significantly increased in nasal polyps from patients with CRSwNP compared with uncinate tissue from patients with CRS or healthy subjects. We made the striking observation that there were abundant mast cells localized within glands of nasal polyps and that these mast cells expressed all three proteases. We also observed increased numbers of mast cells in epithelium but not elsewhere within the lamina propria in nasal polyps. The mast cells detected in epithelium in nasal polyps were characterized by expression of tryptase and CPA3 but not chymase.
CONCLUSIONS: Herein we demonstrate increased mast cells in epithelium and glands of nasal polyposis tissue, and show that nasal polyp mast cells have unique phenotypes that vary by tissue location. These diverse subsets of mast cells may contribute to the pathogenesis of CRSwNP.

164 Age-related Reduction Of S100A8/A9 In Chronic Rhinosinusitis Is Associated With Increased Production Of Soluble gp130
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RATIONALE: Calprotectin, S100A8/A9 heterocomplex, plays an important role in innate immunity and epithelial barrier function. We previously reported that levels of epithelial cell-derived S100A8/A9 were significantly diminished in chronic rhinosinusitis (CRS) and inversely correlated with increasing age. In this study, we further investigate the mechanism of this age-related reduction of S100A8/A9 in patients with CRS.
METHODS: IL-6 and STAT3 signaling is known to be important in the production of S100 proteins. Using ELISA and western blot analysis with nasal lavage fluids and tissue extracts from patients with CRS and normal controls, we first examined age-related production of IL-6, phosphorylated STAT3 (pSTAT3), and soluble gp130 (sgp130) - sgp130 has an antagonistic activity to the IL-6 response in epithelial cells. We further treated normal human bronchial epithelial cells (NHBE) with IL-6 and measured gene expression of S100A8 and S100A9 by real time PCR to see if IL-6 induces production of S100A8/A9 in human airway epithelial cells.
RESULTS: Although there was no correlation between age and levels of IL-6 or pSTAT3, sgp130 levels increased significantly with aging in patients with CRS (r = -0.76, p < .02). The gene expression of S100A8 and S100A9, induced by IL-6, increased almost 4-fold in a dose dependent manner in NHBE cells. The level of induction was further augmented by adding TNF-alpha.
CONCLUSIONS: This study suggests that age-related reduction of S100A8/A9 generation from epithelial cells in patients with CRS may be due to increased sgp130 which in turn inhibits IL-6 induced production of S100A8 and S100A9.

165 Elevated Expression of mRNA for CCL2, CCL19, CCR7 and CXCR3 in Chronic Rhinosinusitis with Nasal Polyposis
RATIONALE: Chronic rhinosinusitis with nasal polyposis (CRSwNP) is a condition characterized by chronic inflammation in the nasal mucosa. We postulate that chemokines and chemokine receptors play an important role in this inflammatory process and contribute to polyp formation characteristic of CRSwNP.
METHODS: Nasal polyp tissue was obtained from individuals with CRSwNP and uncinate tissue (UT) was obtained from CRS patients and normal control subjects. Two different assays, Affymetrix Microarray (AF - based on hybridization) and Taqman Gene Expression Assay (TQ - based on real-time PCR), were used to characterize the expression of various chemokines and chemokine receptors.
RESULTS: The level of mRNA expression for several genes was significantly elevated in polyp tissue from individuals with CRSwNP compared to UT from normal subjects in both assays. These include the chemokines CCL2 (5 fold in AF [p<0.01], 3.9 fold in TQ [p<0.05]) and CCL19 (4.2 fold in AF [p<0.05], 3.8 fold in TQ [p<0.05]), and the chemokine receptors CCR7 (3.4 fold in AF [p<0.05], 22.8 fold in TQ [p<0.01]) and CXCR3 (2.2 fold in AF [p<0.05], 5 fold in TQ [p<0.05]).
CONCLUSIONS: These findings demonstrate confirmed elevation in nasal polyp tissue of mRNA for the chemokines CCL2 (MCP-1) and CCL19, and the chemokine receptors CXCR3 (traditionally associated with recruitment of Th1 cells) and CCR7 (receptor for CCL19). This may contribute to the chronic inflammatory state of the nasal mucosa characteristic of CRSwNP. Further studies will be required to confirm these findings at the protein level and establish their relevance to the pathogenesis of CRSwNP.
Vitamin D Modulates Immune Defense Molecules in Patients with Chronic Rhinosinusitis

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RATIONALE: The dysregulated mucosal inflammation in chronic rhinosinusitis (CRS) is often difficult to control with medication. We sought to determine whether the immunomodulatory properties of vitamin D might be beneficial in this disease.

METHODS: We performed a randomized, placebo controlled trial in adult subjects meeting research criteria for CRS, low serum vitamin D levels (<40 ng/mL), and no contraindications to vitamin D therapy. Subjects were randomized to receive vitamin D (n=12) or placebo (n=12) for 12 weeks in addition to their standard regimen. PBMCs and nasal epithelial cells were collected at the beginning and end of the trial and assessed for the upregulation of molecules involved in mucosal immunity by RT-PCR. We employed the SNOT-22 and the SF-36 to measure clinical response.

RESULTS: Serum vitamin D levels were elevated in the vitamin D group (P<0.05) and unchanged in the placebo group (P>0.05) after treatment. Cathelicidin, human β-defensin 2, and the autophagy-related protein light chain 3 alpha (LC3A) were upregulated after vitamin D administration in PBMCs (P<0.05, all) with similar results in nasal epithelial cells (P<0.05). There were no significant changes in these mediators in the placebo group.

CONCLUSIONS: These results suggest that vitamin D might modulate mediators involved in immune responses in CRS. Further study, with larger numbers of patients, will be needed to clarify the clinical efficacy of vitamin D in this disorder.

Efficacy of Mometasone Furoate Nasal Spray in Relieving Congestion in Patients With Nasal Polyposis, Regardless of Baseline Eosinophil Counts

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RATIONALE: Chronic rhinosinusitis with nasal polyposis, regardless of baseline eosinophil counts.

METHODS: We performed a randomized, placebo controlled trial in adult subjects meeting research criteria for CRS, low serum vitamin D levels (<40 ng/mL), and no contraindications to vitamin D therapy. Subjects were randomized to receive vitamin D (n=12) or placebo (n=12) for 12 weeks in addition to their standard regimen. PBMCs and nasal epithelial cells were collected at the beginning and end of the trial and assessed for the upregulation of molecules involved in mucosal immunity by RT-PCR. We employed the SNOT-22 and the SF-36 to measure clinical response.

RESULTS: Serum vitamin D levels were elevated in the vitamin D group (P<0.05) and unchanged in the placebo group (P>0.05) after treatment. Cathelicidin, human β-defensin 2, and the autophagy-related protein light chain 3 alpha (LC3A) were upregulated after vitamin D administration in PBMCs (P<0.05, all) with similar results in nasal epithelial cells (P<0.05). There were no significant changes in these mediators in the placebo group.

CONCLUSIONS: These results suggest that vitamin D might modulate mediators involved in immune responses in CRS. Further study, with larger numbers of patients, will be needed to clarify the clinical efficacy of vitamin D in this disorder.

Efficacy Of Gentamicin Nasal Irrigation In Chronic Rhinosinusitis In Children

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RATIONALE: Chronic rhinosinusitis is a common condition in patients with allergy or antibody deficiency. The mainstay of treatment includes both medical and surgical treatment. Topical antibiotic therapy for refractory sinusitis in adults has been shown to improve symptoms and quality of life. The data of topical antibiotic therapy in children is lacking.

METHODS: Children with chronic rhinosinusitis who received gentamicin nasal irrigation from January 2005 through February 2011 were evaluated. The clinical symptoms, frequency of sinusitis, hospitalization and antibiotic treatment due to rhinosinusitis between pre- and post-treatment of gentamicin nasal irrigation were compared.

RESULTS: Forty-five patients (27 males) with a mean age of 12.7±1.1 years were recruited. The most common comorbidities were allergic rhinitis (22/45) and antibody deficiency (22/45). A comparison was performed between pre- and post-gentamicin nasal irrigation. After the initiation of gentamicin nasal irrigation, there were significantly improve-ment in nasal congestion, rhinorrhea, itching, sneezing, post nasal drip, anosmia, purulent nasal discharge, halitosis and chronic cough (P<0.05). The frequency of sinusitis, hospitalization and the frequency of antibiotic treatment were significantly decreased after the treatment (P<0.05). In a subgroup of patients (22/45) who received the treatment more than 12 months, the frequency of sinusitis was decreased in 15 patients (68.1%). No complications were reported.

CONCLUSIONS: This study shows that gentamicin nasal irrigation is useful in the reduction of symptoms and hospitalization due to chronic rhinosinusitis in children.

Impacts of Sinus Surgery on Lower Airway Disease

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RATIONALE: Upper respiratory illness can adversely impact on the lower airway. This study aimed to evaluate the relationship between clinical background and improvement in the lower airway after surgery for treatment of chronic rhinosinusitis.

METHODS: A total of 33 patients with chronic rhinosinusitis surgically treated between January 2010 and May 2011 were included in the study. The presence or absence of asthma and peripheral blood eosinophils count were examined for all patients in addition to preoperative spirometry and postoperative tests. Spirometry was conducted and fractional exhaled nitric oxide (NO) was measured at the preoperative, 1-month postoperative, and 3-month postoperative visits.

RESULTS: The average level of NO in the exhaled air at the preoperative, 1-month postoperative, and 3-month postoperative visits was 51.2 ppb, 42.4 ppb, and 40.1 ppb, respectively. Although the average results of spirometry didn’t show any improvement, forced expiratory volume in 1 second percentage improved in 4 of 9 cases with obstructive ventilatory disturbance. There were 13 improved cases, which were defined as those showing more than 1.5-fold difference in the ratio of preoperative NO level to the 3-month postoperative one. The remaining 20 cases, including 8 of 11 cases with asthma, 11 of 13 cases with eosinophilia, and 4 of 5 cases with animal dander allergy were unimproved cases.

CONCLUSIONS: Chronic rhinosinusitis can cause latent lower respiratory disease. Endoscopic sinus surgery can improve the status of the lower airway. However, the effectiveness of surgery is significantly less in cases of eosinophilia.
The Examination of the Association Between the Chronic Rhinosinusitis and the Inflammation of the Lower Respiratory Airway by Using the Exhaled Nitric Oxide (NO) and the Respiratory Function Test

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RATIONALE: It is said that the chronic rhinosinusitis influences the lower respiratory airway and there is a report that a tendency to increase the eosinophilic rhinosinusitis later, but they are still unknown.

METHOD: S We divided the chronic rhinosinusitis patients with the adaptation of the operation into four groups and exacted whether there was a difference in the respiratory function, exhaled NO, and the number of the blood eosinophil.

CONCLUSION: Objects 50 patients with chronic rhinosinusitis, who planned the endonasal sinus surgery.

RESULTS: By a comparison with four groups, it was the worst on the respiratory function (one second rate) in the group with nasal polyps and without nasal allergy. It was recognized the connection between the respiratory function and the presence of the nasal polyps. It was possible that the expiration NO became the index to the lower airway inflammation.

CONCLUSION: We classified the chronic rhinosinusitis and evaluated the lower airway function. It helped the pathogenetic understanding of the chronic rhinosinusitis. And it led to understand of the influence on the lower airway inflammation.

Absence Of IgE Neosensitization In House Dust Mite Allergic Patients Following Sublingual Immunotherapy

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RATIONALE: The impact of sublingual immunotherapy (SLIT) on allergen-specific IgE and IgG responses, as well as the risk of neosensitization remain to be evaluated in large cohorts of patients.

METHODS: Antibody responses were assessed in sera from 509 European HDM-allergic patients before and after one year of daily sublingual immunotherapy, using tablets containing either Dermatophagoides pteronyssinus plus D. farinae extracts or placebo, with an additional year of follow-up after treatment. IgE and IgG4 antibodies specific for group 1, 2 and 10 allergens were assessed using ImmunoSolid-Phase AllergenChip (ISAC).

RESULTS: After one year of SLIT, both mite specific IgE and IgG4 titers increased by 1.5 to 4 fold in the active group, but not in the placebo group. IgG4 induction occurred in a subgroup of “immunoreactive” patients, without any correlation with improvement in the rhinoconjunctivitis symptom score. Preexisting IgE levels to mite allergens were usually boosted during immunotherapy, but no de novo IgE responses were induced during SLIT in patients who were not sensitized prior to immunotherapy. Similarly, no neosensitization to wheat germ or yeast components used in the mite culture medium was observed, consistent with the lack of detection of these proteins in mite extracts.

CONCLUSIONS: We document on a large cohort of patients over a two year period that the induction of seric mite-specific IgG4 is not a biomarker for SLIT clinical efficacy. SLIT does not induce IgE neo sensitization to allergens contained in the vaccine, including group 1,2 as well as the food-related group 10 allergens.

The Adenoid Inflammation of Children with Rhinosinusitis and Alternaria Hypersensitivity

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RATIONALE: Although SCF, c-kit, CHI3L1, IL-32, ECP, and tryptase are known to play roles in both innate and adaptive immunity, major aspects about the nature of these mediators and their roles in various inflammations remain unclear.

METHODS: We measured the levels of SCF, c-kit, CHI3L1, IL-32, ECP, and tryptase in pediatric adenoid tissues, and compare them among the various inflammatory conditions, including allergic rhinitis, local Alternaria-hypersensitivity, and CRS.

RESULTS: SCF, c-kit, CHI3L1, IL-32, ECP, and tryptase levels were increased in relation with the mucosal IgE to Alternaria in adenoid mucosal inflammation from some children with CRS.

CONCLUSIONS: Mucosal IgE to Alternaria, mast cells and eosinophils might play a significant role in adenoid mucosal inflammation from some children with CRS.

Eleven Year Follow Up Of an African-American Adolescent with Destructive Sinus Disease and Intermittent Asthma Diagnosed with Allergic Fungal Sinusitis (AFS) and Incidentally Found to have Allergic Bronchopulmonary Mycosis (ABPM)

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RATIONALE: AFS is a noninvasive fungal sinusitis in individuals with fungal-specific IgE, intractable sinusitis, and nasal polyposis. ABPM is suggested to be lower airway manifestation of similar process. In rare cases, these diseases have occurred concomitantly. To our knowledge, no cases of AFS and ABPM in the same patient have been reported for this length of time.

METHODS: Spirometry, CT sinuses and chest, total IgE, endoscopic sinus surgery, bronchoscopy.

RESULTS: Initial presentation of this 17yo African-American male has been reported (Chest 2002;121:1670-6). After initial diagnosis in 2000, he received frequent prednisone bursts for exacerbations. Beginning in 2004, he required chronic prednisone for progression of bronchiectasis. IgE peaked at 8222 IU/mL before chronic steroids initiated, decreasing to 2000s-4000s while asymptomatic and in response to steroid bursts, and spiking to 6000s during exacerbations. Spirometry demonstrated improvement from moderate-severe obstruction to mild obstruction on prednisone. Six years following initial sinus surgery he had recurrence of nasal polyps with impacted allergic mucin and bony destruction requiring further surgical intervention. While off prednisone he had recurrence of nasal polyps with impacted allergic mucin and bony destruction requiring further surgical intervention. While off prednisone he had recurrence of nasal polyps with impacted allergic mucin and bony destruction requiring further surgical intervention. While off prednisone he had recurrence of nasal polyps with impacted allergic mucin and bony destruction requiring further surgical intervention.

CONCLUSIONS: His clinical course highlights challenges associated attempts at weaning prednisone.
Improvement In Quality Of Life With Administration Of A 300 IR Sublingual Tablet Of 5-grass Pollen Allergen Extract In Adults With Grass Pollen-induced Allergic Rhinoconjunctivitis

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RATIONALE: In single season studies, pre- and co-seasonal administration of a 300 IR sublingual tablet of 5-grass pollen allergen extract improves quality of life (QoL). Here we report the Year 1 to Year 4 QoL results from an ongoing 5 year study.

METHODS: 633 adults were randomized to placebo or 300 IR pre- and co-seasonally for three grass pollen seasons. Active treatment was initiated 4 months [4M] or 2 months [2M] before each season and continued for its duration. Patients were followed during the subsequent, treatment-free, grass pollen season. Each year, the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ, responses to 28 questions, 7-point Likert scale with low score = better QoL) was completed before and midway through the pollen season. An ANCOVA model was used to analyze differences vs. placebo.

RESULTS: The LS mean changes from baseline in the placebo, 4M and 2M groups were 1.67, 1.36 and 1.21 in Year 1, 1.45, 1.06 and 1.01 in Year 2, 1.36, 0.94 and 0.95 in Year 3 and 1.41, 0.95 and 0.88 in treatment-free, Y4. The relative LS mean QoL improvement in the 4M and 2M groups (vs. placebo) was 18.5% and 27.4% in Year 1, 27.0% and 30.6% in Year 2, 30.4% and 29.8% in Year 3 and 32.8% and 37.6% in Year 4, respectively (p<0.002).

CONCLUSIONS: Discontinuous treatment with a 300 IR sublingual tablet of 5-grass pollen allergen extract resulted in significant improvement in QoL vs. placebo in each of three treatment seasons and the first pollen season post-treatment.

Characteristics of Systemic Reactions to Inhalant Allergen Immunotherapy (SRIT) in the University of Michigan Health System (UMHS)

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RATIONALE: All SRIT to SCIT are systematically recorded at UMHS. We hypothesized that identifiable characteristics of SRIT could lead to safer SCIT prescribing practices.

METHODS: Retrospective review of all reported SRIT from July 1, 2009-June 30, 2011.

RESULTS: 104 SRIT were recorded in 90,723 individual injections during 38,548 patient SCIT visits (0.27% of SCIT visits and 0.11% of injections). Females accounted for 60.5% of all SCIT visits and 73% of all SRIT. 58% of SRIT occurred in asthmatics. 2.8% SRIT were on ACE inhibitors and none on beta blockers.

SRIT WHO grade was available for 96 of 104 patients: 32.3% grade 1; 56.3% grade 2; 4.2% grade 3; 5.2% grade 4. 88% of grade 3 or 4 SRIT occurred in men.

Onset of SRIT was available for 101 patients (range 1-2160 min): ≤20 min in 52%; <30 min in 69.3% and <60 min in 89.3%. Of late (>30 min) SRIT 39% were grade 1; 57% grade 2; and 4% grade 4. 67% of SRIT occurred while building to maintenance (BTM): 43.2% in concentrate (Red), 31.3% in 1:10 (Yellow), 10.4% in 1:100 (Blue), 11.9% in 1:1000 (Green) and 2.9% in 1:10,000 (Silver). SRIT at maintenance dosing accounted for 33% of all SRIT and all were in concentrate.

CONCLUSIONS: SRIT occur more in asthmatics and females. More severe SRIT occur in males. Most SRIT occur while BTM in concentrate or 1:10. Further studies are needed to determine if any relationship exists between SCIT extract characteristics and grade or onset of SRIT.

A Us Study Of 5-grass Pollen Allergen Extract In Adults With Grass Pollen-induced Allergic Rhinoconjunctivitis - Results Of Secondary Efficacy Assessments

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RATIONALE: The efficacy and safety of treatment with a 300 IR sublingual tablet of 5-grass pollen allergen extract have been demonstrated. Here, we present the results of secondary efficacy assessments.

METHODS: 473 adults were randomized to either 300 IR or placebo. Treatment started 4 months before the pollen season and continued for its duration. The primary efficacy endpoint was the daily Combined Score (CS, scale 0-3). Secondary efficacy variables included daily Rhinoconjunctivitis Total Symptom Score (RTSS, the sum of the six rhinoconjunctivitis symptoms each scored from 0=absent to 3=severe), daily Rescue Medication Score (0=No rescue medication, 1=antihistamine, 2=oral corticosteroid, 3=oral corticosteroid), and change from baseline to Visit 5 in overall Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) score. Daily CS, RTSS, and RMS were analyzed using a repeated measures ANCOVA model and overall RQLQ score by an ANCOVA model.

RESULTS: During the pollen period, compared to placebo, statistically significant improvements were observed in daily CS [relative LS Mean difference of -22.9% (p=0.0036)], daily RTSS [relative LS Mean difference of -28.2% (p=0.0003)], daily RMS [relative LS Mean difference of -46.5% (p=0.0095)] and overall RQLQ score (p=0.0042).

CONCLUSIONS: The positive results on secondary efficacy endpoints, including symptom scores, rescue medications scores and patient quality of life assessment were consistent with the results of the primary endpoint, confirming the efficacy and associated patient benefits of treatment with 300 IR 5-grass pollen sublingual allergen extract tablet in US adults with grass-pollen allergic rhinoconjunctivitis.
177 Post-treatment, Long-term Efficacy Of A 300IR Sublingual Tablet Of 5-grass Pollen Allergen Extract In Adults With Grass Pollen-induced Allergic Rhinoconjunctivitis: The Relationship With Disease Severity

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RATIONALE: The sustained efficacy (i.e., after 3 treatment years) of discontinuous treatment with 300IR sublingual tablet of 5-grass pollen allergen extract has been demonstrated in an ongoing, 5-year study. Here we report the relationship between disease severity and clinical efficacy over four consecutive grass pollen seasons.

METHODS: 663 adults were randomized to placebo or 300IR pre- and co-seasonally for three grass pollen seasons starting 4 months [4M] or 2 months [2M] prior to the season each year and followed up during the subsequent, treatment-free, season. Average Adjusted Symptom Score (AASS, adjusting Rhinoconjunctivitis Total Symptom Score for rescue medication usage, range 0-18) was the primary efficacy endpoint. To evaluate the relationship with disease severity, pooled centers were ranked from lowest to highest mean AASS in the placebo group, and divided in three tertiles. As post hoc subgroup analyses, AASSs during the pollen period for each of the first 4 study years were analyzed by tertile using an ANCOVA model.

RESULTS: For each study year, relative mean differences in AASS for active groups compared to Placebo in tertiles with medium and high mean AASS in placebo group were statistically significant while no significant differences were observed in the low tertile. For example, in year 4, relative mean differences in 4M vs. placebo in high, medium, and low disease severity tertiles were -34.0%, [[Unsupported Character - Codename -]] 28.3% and +4.1%, respectively.

CONCLUSIONS: In each of three treatment seasons and the post-treatment season, the 300IR 5-grass-pollen tablet was most effective in populations with higher symptom severity.

178 Comparative Efficacy and Tolerability of Subcutaneous and Sublingual Allergen Specific Immunotherapy

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RATIONALE: Allergen specific immunotherapy (ASIT) has been used for 100 years with efficacy confirmed of Subcutaneous Allergen Immunotherapy (SCIT) seen in numerous double-blind placebo controlled studies but recent evidence notes efficacy of sublingual immunotherapy (SLIT) which may be better tolerated than SCIT. This study assesses the efficacy and tolerability of both SCIT and SLIT in clinical practice.

METHODS: 22 patients (13 females, 9 males) with allergic rhinitis or rhinoconjunctivitis (6 with mild asthma) were studied. Mean patient age was 32±6 years. Duration of ASIT was 2±0.96 years. SCIT was used in 12 patients while SLIT was used in 10 patients (Stallergenes extracts). All patients were asked about symptom changes during ASIT (3 point system: worse, no change, better) and about adverse events.

RESULTS: Nasal secretions and nasal obstruction decreased in 96% of patients, while sneezing and nasal itching decreased in 91% of patients. Itching and tearing of eyes decreased in 82%, and eye redness in 77% of patients. Asthma symptoms were less in 67 % of patients. Total symptom scores improved similarly in both SCIT and SLIT patients. I patient from the SCIT group had local reaction after allergen extract injection and 1 patient from SLIT group had slight tongue edema after allergen extract administration in the initial phase of ASIT.

CONCLUSIONS: Both SCIT and SLIT methods are effective and well tolerated in the treatment of allergic rhinoconjunctivitis and mild asthma.
181 Controlled Allergen Exposure in the Environmental Exposure Chamber (EEC) Results in a Late Phase Inflammatory Response Evidenced by Increased Eosinophils in the Upper Airway of Allergic Patients

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Rationale: An increase in eosinophils is an important inflammatory biomarker in the respiratory airways of allergic patients upon allergen exposure. Historically, nasal lavage results have proved difficult to interpret due to high eosinophil cell count variability. Therefore, the ability of controlled and extended allergen exposure in the EEC to induce a detectable increase in eosinophils in the upper airway of allergic patients was investigated.

Methods: Patients with a positive SPT to ragweed and a history of seasonal allergic rhinitis during the last two ragweed seasons were exposed to aerosolized ragweed allergen (3500±500 grains/m³) for 8 hours in the EEC. Nasal lavage samples were collected from 40 subjects pre- and post-EEC as part of a larger study. Samples were processed and cytospin slides were prepared. Eosinophils were counted and expressed as a percentage of leukocytes in the sample.

Results: A significant increase (p = 0.005) was observed in the mean percentage of eosinophils in the nasal lavage sample after an 8 hour allergen EEC exposure (12.37±2.98%) compared to 0.85±0.432% prior to EEC entry. Thirteen of the 40 subjects showed a >10% increase in percentage of eosinophils with a mean value of 32.90±6.261% and a coefficient of variation of 68.61%.

Conclusion: Extended allergen exposure in the EEC elicited a significant inflammatory response, characterized by an increase in eosinophils in the nasal cavity of allergic patients. The EEC provides an effective screening tool for identifying patients in late phase allergy, thereby reducing variability when using eosinophil counts as an end point and providing further mechanistic insights for drug action.

182 Mold and House Dust Mite Allergens Activate and Sensitize Sensory Neurons Innervating Airways

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Rationale: The peripheral nervous system can contribute to the initiation and maintenance of chronic airway inflammation, a hallmark of several pulmonary diseases. We hypothesize that certain mold and house dust mite (HDM) allergens belonging to the protease family activate and sensitize sensory neurons innervating airways. This actions on sensory neurons lead to the release of neuropeptides and could initiate of neurogenic inflammation in airways.

Methods: Neuronal cultures were generated from rat T1-T3 dorsal root (DRG) and jugular-nodeose ganglia (NG). Activation and sensitization of neurons were measured using Ca2+ imaging and patch-clamp electrophysiology. Neuropeptide release from airways was assessed using radio-immuno assay. Sensory neurons innervating airways were identified by back labeling from airways. Data were analyzed with one or two way ANOVA.

Results: Asthma-inducing HDM and mold allergens DerP3&9, PenC13 and DerP1 belonging to the protease family generate Ca2+ influx and currents in distinct subsets of T1-T3 DRG and NG neurons. A set of responsive sensory neurons innervate airways. These allergens also sensitize the action of acrolein - an irritant from cigarette smoke and an environmental pollutant. Importantly, the allergens are capable of inducing and sensitizing the release of the vasoactive calcitonin-gene related peptide (CGRP) from bronchi preparations.

Conclusion: HDM and mold allergens attributed to the induction of asthma and belonging to the protease family activate and sensitize sensory neurons innervating airways. The activation of these sensory neurons leads to the release of CGRP, possibly contributing to the initiation of airway inflammation.

183 Peripheral Blood Mononuclear Cells from Patients with Bronchial Asthma show Impaired Regulatory Responses to Rhinovirus in vitro

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Rationale: Asthmatic subjects tend to have impaired antiviral immune responses to rhinovirus (RV) whereas they are likely to exhibit more severe and prolonged symptoms upon RV infection. To clarify the mechanisms how inflammatory reactions in asthmatic subjects are exaggerated, cytokine and chemokine secretion profiles in PBMCs after exposure to RV were examined.

Methods: Thirty-five currently asthmatic subjects (BA) and 36 age-matched control subjects (nBA) were recruited. PBMCs obtained from BA without clinical exacerbation within 2 weeks before the phlebotomy and from nBA were stimulated with RV-14 for 72h. The concentrations of interferon-gamma-inducible protein-10 (IP-10), IFN-gamma, IL-4, IL-13, and IL-10 in the supernatants were measured by ELISA.

Results: The levels of IFN-gamma and IP-10 were correlated positively (r = 0.34, p = 0.004). The concentrations of IL-10 were significantly higher in nBA (221.7 ± 37.1 vs. 670.8 ± 95.3 pg/ml, p < 0.0001), but there were no statistically significant differences in the levels of IFN-gamma and IP-10 between BA and nBA (229.9 ± 38.1 vs. 417.2 ± 96.3 pg/ml, p = 0.07; 5151 ± 2994 vs. 616.6 ± 461.6 pg/ml, p = 0.4, respectively). Concentrations of IL-4 and IL-13 were below the detection limits. The ratio of IFN-gamma/IL-10 and IL-10/IL-12 were significantly higher in BA than nBA (p = 0.009 and p = 0.01, respectively).

Conclusions: Our results suggest that asthmatic subjects are likely to have comparable ability to attract CXC-1R3-positive cells with normal subjects, whereas they have impaired regulatory response which presumably leads to exhibit more severe and prolonged symptoms upon RV infection.

184 Pharmacological Characterization of TASP0412098, A Selective CRTH2 (Chemoattractant Receptor-Homologous Molecule Expressed on Th2 Cells) Antagonist

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Rationale: CRTH2 plays important roles in allergic diseases, and CRTH2 and PGD2 might have important role(s) in allergic diseases such as bronchial asthma, allergic rhinitis and atopic dermatitis. We succeeded in optimization of a novel CRTH2 antagonist, the isoquinoline derivative TASP0412098, and examined biological activities and pharmacological efficacies on allergic responses.

Methods: Receptor binding assay was performed by CRTH2 transfected cells. Antagonistic activity was determined by intracellular Ca2+ influx using CRTH2 transfected cells, migration assay and IL-13 production assay performed by Th2 cells derived from healthy adult volunteer. Eosinophil shape change assay was performed by eosinophil derived from peripheral blood cells of guinea pig. In vivo efficacy were examined using guinea pig asthma model and murine contact allergic dermatitis model. In cellular studies, TASP0412098 also inhibited PGD2-induced intracellular Ca2+ influx, Th2 cells migration and IL-13 production from Th2 cells.

Results: TASP0412098 exhibited strong inhibitory effects on CRTH2-PGD2 interaction (IC50: 2.1 nM) in highly specific manner. In cellular studies, TASP0412098 also inhibited PGD2-induced intracellular Ca2+ influx, Th2 cells migration and IL-13 production from Th2 cells.

Conclusions: CRTH2 plays important roles in allergic diseases, and TASP0412098 is a useful compound for the treatment of allergic diseases.
185 Pro-fibrotic Effect Of Dexamethasone In Human Airway Fibroblasts
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RATIONALE: Airway remodelling in asthma is characterized by deposition of extracellular matrix proteins (ECMs). Collagen is the most abundant ECM protein in the airways and its cross-linking by lysyl oxidase (LOX) influences its strength and stability. Inhaled corticosteroids are the standard treatment for chronic asthma. We hypothesized that corticosteroids may have a role in regulating the production of LOX by fibroblasts from asthmatic airways.

METHODS: Bronchial fibroblasts from normal or asthmatic subjects were treated with dexamethasone in the presence and absence of TGFβ. LOX and collagen I mRNA expression was measured using qRT-pCR. The level of LOX in culture supernatants was measured using western blot analysis.

RESULTS: TGFβ2 significantly up-regulated collagen I mRNA expression. In contrast, dexamethasone had no effect on collagen I mRNA expression. TGFβ2 also up-regulated LOX mRNA expression in fibroblasts from both healthy and asthmatic subjects (p=0.002). In fibroblasts from asthmatic donors only, dexamethasone up-regulated LOX mRNA expression and had an additive effect with TGFβ2 (p=0.02). Both TGFβ2 and dexamethasone enhanced the production of pro-LOX and active LOX in fibroblasts from normal or asthmatic donors (p=0.03).

CONCLUSIONS: TGFβ2 and dexamethasone up-regulated LOX expression by bronchial fibroblasts with the potential for increased cross-linking of collagen in the airways. These findings suggest that apart from their immediate anti-inflammatory effects, corticosteroids may have a long term detrimental effect on collagen cross-linking, affecting airway wall mechanics. Should such an action be evident in vivo this could have a detrimental effect on decline in lung function and disease persistence in asthma.

186 Characterization of a Novel, Potent and Selective Small Molecule Spleen Tyrosine Kinase (SYK) Inhibitor in In Vitro and In Vivo Models of Asthma.
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RATIONALE: Spleen Tyrosine Kinase (SYK) is a key activator of signaling pathways downstream of multiple surface receptors implicated in asthma. SYK function has been extensively studied in mast cells downstream of the high affinity IgE-receptor (FcεR1). Most studies evaluating SYK function in preclinical models have relied on poorly selective compounds, anti-sense oligonucleotides or SYK knockout mice. Here we describe the characterization of MRK-A, a highly selective, potent small molecule SYK inhibitor in preclinical models of asthma.

METHODS: Enzyme potency and selectivity of MRK-A were evaluated. Functional inhibition of FcεRI signaling was evaluated in primary human mast cell lines and in a rat airway passive sensitization model. Attenuation of allergic airway responses was evaluated in rat and sheep inhaled allergen challenge models.

RESULTS: MRK-A inhibits SYK enzyme activity with an IC50 of 0.9 μM and is >100 fold selective against all other kinases tested in biochemical assays. MRK-A inhibited FcεRI-mediated mast cell degranulation with an EC50 of 27 nM, and dose-dependently blocked IgE-mediated tracheal extravasation in rat. In rat ovalbumin-sensitized airway challenge model, oral dosing of MRK-A led to a dose-dependent attenuation of airway inflammation. Intravenous dosing of MRK-A was able to significantly inhibit both early and late allergen induced changes in airway resistance in an acarasis-sensitive sheep allergen challenge model.

CONCLUSIONS: MRK-A is a potent and selective SYK inhibitor that can attenuate the endpoints in several in vivo models of allergic airways.

This compound provides an important selective tool to interrogate the role of SYK signaling in disease biology.

187 Increased Expression of Importin α3 (KPN4) and Decreased VDR in the Lung of OVA-Sensitized and Challenged Mice
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RATIONALE: The nuclear import of p65 and p50 subunits of activated NF-kappaB is dependent on importin α3 (KPN4) and importin α4 (KPN3). We previously reported that pro-inflammatory cytokines increase the expression of importin α3 and decrease the expression of VDR in human bronchial smooth muscle cells (HBSMCs). Our results also demonstrate that co-stimulation with calcitriol attenuated the increased expression of importin α3 thereby leading to decrease in the activated NF-κB in the nucleus through a mechanism mediated by VDR. In this study, we evaluated the mRNA and protein expression of VDR and importin α3 in the lungs of OVA-sensitized and challenged mice as compared to control mice.

METHODS: BALB/c mice were sensitized and challenged with OVA and AHR to methacholine was established. Protein and mRNA expressions of VDR, and importin α3 in whole lung tissue were analyzed using immunofluorescence and qPCR, respectively.

RESULTS: There was significantly higher mRNA and protein expression of importin α3 and decreased expression of VDR in the lung tissue of OVA-sensitized and challenged mice as compared to PBS mice.

CONCLUSIONS: The decrease in the expression of VDR in OVA sensitized mice suggest the role of vitamin D in allergic asthma. An increase in importin α3 is likely due to a decrease in VDR expression in lungs of OVA-sensitized mice. We, therefore, conclude that Vitamin D supplementation in asthmatic mice can decrease the AHR and the expression of importin α3 and thus inflammation. Thus, Vitamin D might be helpful in alleviating allergic airway inflammation and airway hyperresponsiveness in allergic asthma.

188 Similarities And Differences Between Lung Ligands For Mouse Siglec-f And Human Siglec-8
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RATIONALE: Siglec-F and Siglec-8 are functional paralog inhibitory receptors on mouse and human eosinophils respectively. Both siglec-Fs are known to preferentially recognize the ligand 6'-sulfated sialyl Lewis X (6'-su-sLex), but their natural tissue ligands are unknown.

METHODS: The Consortium for Functional Glycomics glycan microarray (version 4.2) was used to identify glycans that bind to Siglec-F and Siglec-8. Distribution of Siglec-F and Siglec-8 ligands was studied on mouse and human lung tissue via immunohistochemistry by staining with Siglec-F- Ig or Siglec-8-Ig fusion proteins. A novel polyclonal anti-6'-su-sLex IgY was generated by immunizing chickens. Western blotting was performed to determine whether Siglec-F-Ig and Siglec-8-Ig recognized the same molecules in mouse and human lung cell-derived material.

RESULTS: Among ~450 glycans tested, Siglec-F-Ig and Siglec-8-Ig recognized 6'-su-sLex as expected but also its non-fucosylated form. The anti-6'-su-sLex IgY blocked binding of Siglec-F-Ig to these two glycans. Using immunohistochemistry, Siglec-F-Ig bound mouse lung epithelium and the staining was blocked by anti-6'-su-sLex IgY pre-incubation. In contrast, Siglec-8-Ig did not bind human lung epithelium but instead bound human airway glands and was poorly blocked by the IgY. Western blotting using primary mouse tracheal epithelial cell (mTEC) lysates, human bronchial explant supernatants, Siglec-F-Ig and Siglec-8-Ig revealed that both reagents detected sialidase-sensitive high molecular weight (~400 kDa) material from both species. A lower molecular weight (~225 kDa) band was detected but only in the mTEC lysate using Siglec-F-Ig.

CONCLUSIONS: Lung ligands for Siglec-F and Siglec-8 appear to be different in their location of expression and glycan composition.
Relationship Between Leptin, Bronchial Hyperresponsiveness To Mannotil And Urinary Leukotriene E4/exhaled Nitric Oxide Ratio In Asthmatic Children

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RATIONALE: Effects of leptin on airway inflammation and bronchial hyperresponsiveness (BHR) have not yet been demonstrated in the human airway. We speculated that leptin might be more closely related with cysteinyl leukotriene inflammation [as measured by urinary leukotriene E4 (LTE4)] than with cosinophilic inflammation [as measured by fractional exhaled nitric oxide (FE(NO))]. We also speculated that leptin might be related with BHR to indirect stimuli (mannitol). The aim of this study was to address the relationship between leptin, BHR to mannitol and LTE4/FE(NO) ratio in asthmatic children.

METHODS: Sixty-one prepubertal children between the ages of 6 and 10 years were included and comprised asthmatic (n=40) and healthy (n=21). We measured FE(NO) and serum leptin levels. We performed mannitol provocation challenges. The urinary concentrations of LTE4 and the 92-111PGF2α were measured at baseline and 30 min after mannitol challenge. The response to mannitol was expressed as a provocative dose causing a 15% fall in FEV1 (PD15) and the response-dose ratio (RDR) (% fall in FEV1/cumulative dose).

RESULTS: The urinary excretion of 111PGF2α (79.8 ± 17.8 vs. 33 ± 16.6 ng/mmol creatinine1), peak versus baseline) and LTE4 (48.3 ± 16.5 vs. 36.2 ± 12.7) increased significantly 30 min after mannitol challenge. There was a significant correlation between serum leptin levels and RDR mannitol (r=0.468, P=0.021). The serum leptin levels were positively correlated with LTE4/FE(NO) ratios (r=0.347, P=0.038).

CONCLUSIONS: Levels of the leptin are correlated with BHR to mannitol and LTE4/FE(NO) ratios. This suggests that an increased role of mast cells or leukotrienes may exist in the relationship between leptin and BHR to mannitol.

Silymarin Reduces OVA-induced Allergic Airway Inflammation

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RATIONALE: Inexpensive, readily available drugs are needed for prevention and treatment of asthma. Oxidative stress, or an imbalance of reactive oxygen species (ROS) and antioxidants, occurs in asthma with infiltration of inflammatory cells, such as eosinophils, into the airways resulting in inflammation and tissue damage. Silymarin, a flavonoid complex from Silybum marianum (milk thistle plant), reduces ROS by increasing antioxidant enzymes, such as catalase, in human hepatotoxicity and cancer. However, the effect of silymarin on allergic airway inflammation and asthma is unknown. We hypothesized silymarin decreases ovalbumin (OVA)-induced airway inflammation in BALB/c mice by increasing catalase levels.

METHODS: BALB/c female mice were sensitized to OVA conjugated with aluminum hydroxide on day -14. Two weeks later, mice were challenged with 1% OVA aerosol daily on days 0 to 3. One hour prior to each OVA challenge mice were orally given silymarin (50-200mg/kg body weight) or vehicle (0.4% methylcellulose). On day 4, blood, bronchoalveolar lavage (BAL) fluid and lungs were harvested. Serum IgE levels and IL-13, a Th2 cytokine upregulated with allergic airway inflammation, were determined by ELISA. Infiltration of inflammatory cells was examined in BAL fluid. Catalase mRNA expression was examined by real-time PCR.

RESULTS: Silymarin significantly decreased lymphocytes and eosinophils in the BAL fluid (n=11-12, p<0.05) and decreased BAL fluid levels of IL-13. IgE serum levels were not altered with silymarin (p=0.013). Silymarin increased mRNA expression levels of catalase (n=11-12, p<0.05).

CONCLUSIONS: Silymarin attenuated OVA-induced allergic airway inflammation, but had no effect on IgE levels. Therefore, silymarin may be a potential therapeutic for asthma.

Does the Immunological Status Influence in Behavior of Albini Mice with Experimental Asthma Model?

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RATIONALE: Depression is prevalent in asthma and implicated in acute asthma exacerbations. Objective was to examine under controlled laboratory conditions, by which we performed experimental asthma model in mice after C tala immunization and developed experiments to determine whether the immune system response may alter the behavior of mice in a classical depression test.

METHODS: We studied 30 Albino Swiss mice, one day old, divided in: (a) asthma induced by C tala injection, 1 ug of glycoprotein intra-peritoneal weekly for 3 weeks (n: 20), and control group injected with PBS (n: 10). All animals were controlled in behavior weekly, from immunization to 21 day later by a method of forced swimming, and finally challenged with aerosolized tala 3 days. All mice were euthanized and lung harvested. Histopathology examination of the lungs was performed. Blood and sera samples were collected and studied to specific IgE to C tala by ELISA

RESULTS: The asthmatic presented IgE positive to tala in 20/20, the control group was IgE (-) to C tala, p <0.0001. The tala immunized animals presented asthmatic responses to challenge with tala. The histopathology presented classical asthmatic responses. The behavior was different in the 2 groups. The group (a) presented in first control climbing trends 20/20, at 14 day (swimming) 16/20 and day 21 (floating) 13/20. The (c) group presented only climbing trends in all weekly determinations (p=0.0085).

CONCLUSIONS: The results suggest that immunological status induced depression in animals with asthmatic responses and these findings probably explain the depression observed in this type of patients.

The Effects of Omalizumab on the Late-Phase Response to Nasal and Skin Allergen Challenge

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RATIONALE: Using omalizumab as a mechanistic tool, we examined the contribution of basophil versus mast cell activation in allergic airway challenge, skin tests and nasal eosinophil recruitment during the late-phase response.

METHODS: Cat-allergic adults underwent a randomized, double-blind, placebo-controlled trial of omalizumab. At baseline, anti-IgE and cat allergen-induced BHR (basophil histamine release), cat IST (intradermal skin test) at 15 min (early-phase) and at 24 hours (late-phase), NAC (nasal allergen challenge) with sneezes measured within 45 min (early-phase) and 4-24 hours (late-phase) (recorded based on recollection), pre-NAC nasal lavage eosinophils and 24-hour post-NAC nasal brush for eosinophils were performed. BHR studies were repeated biweekly. Baseline procedures were repeated when cat allergen-induced BHR <80% or on day 56 (NAC-2).

RESULTS: Subjects were divided into presumptive treatment (TRE, n=7) and placebo groups (PLA, n=7) based on the shift of anti-IgE BHR relative to baseline. Average day of NAC-2 was 46 for TRE and 59 for PLA. Early and 24-hour late-phase IST size decreased more in TRE than in PLA (37% vs. 7% and 76% vs. 5%). Nasal lavage eosinophil presence pre-NAC decreased more in the TRE compared to PLA (44% vs. -52%) as well as 24-hour post-NAC nasal brush eosinophils (57% vs. 25%). Early-phase sneezes decreased more in the TRE than in the PLA (51% vs. -11%), but not in the late-phase (83% vs. 71%).

CONCLUSION: Basophil hyporesponsiveness secondary to omalizumab is associated with a reduction in acute and late-phase skin test size, basal and NAC induced nasal eosinophil recruitment, and acute nasal challenge sneezes.
Preventive Effects of **Lactobacillus rhamnosus** (Lcr35) through the suppression of inflammatory cytokines in Mouse Model with Atopic Dermatitis

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**RATIONALE:** Probiotics have been regarded to induce immune regulation or tolerance in allergic diseases. This study was to investigate the protective effects on atopic dermatitis (AD) by oral administration of **Lactobacillus rhamnosus** (Lcr35) in AD mouse model.

**METHODS:** To develop the mouse model of AD, ovalbumin (OVA) was sensitized through epicutaneous for 1 week at three times followed by sensitization in hairless mice. To evaluate the preventive effects of Lcr35, 1 x 109 CFU of it was administrated orally everyday from 1-week before first sensitization to the end of the study. Evaluation of clinical signs (itchy frequency and erythema), transepidermal water loss (TEWL), histopathology (H&E stain) and immunohistochemistry of inflammatory cytokines on skin were performed. In addition, serum total IgE and OVA-specific IgE were measured to confirm systemic immunization.

**RESULTS:** The administration of Lcr35 attenuated the phenotype of AD in mouse model: clinical signs, TEWL on skin, inflammation of skin on histopathology compared to positive control. And Lcr35 treated group showed decrease of the production of serum total and OVA-specific IgE. In addition, the expression of IL-4 and thymic stromal lymphopoietin (TSLP) on immunohistochemistry of skin were suppressed in Lcr35 treated group.

**CONCLUSION:** Oral application of Lcr35 attenuates the development of major characteristics of AD and suppresses the expression of inflammatory cytokines of IL-4 and TSLP on skin in AD mouse model. These findings suggest that probiotics inhibit AD through suppression of inflammatory cytokine in the skin.

Expression of Vitamin D Receptor and CYP24A1 Enzyme in Airway Epithelium in Allergic Asthma

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**RATIONALE:** Vitamin D deficiency is associated with increased AHR and decreased pulmonary function. However, its role in the initiation of inflammation and development of airway remodeling is poorly understood. In this study, we examined the expression pattern of Vitamin D receptor (VDR) and CYP24A1 and their correlation with the degree of inflammation and airway remodeling in the lungs of ovalbumin (OVA)-sensitized and challenged (OVA-treated) mice.

**METHODS:** Lung tissues were isolated from four groups of Balb/c mice: PBS and OVA-treated mice on days 33, 45 and 80. H&E, PAS, and trichrome stainings were used to show the severity of lung histopathology. The expression patterns of VDR and CYP24A1 in the airways were examined using immunofluorescence. BEAB-2b human airway epithelial cells were stimulated with TGF-β1, TGF-β2, IL-4 and IFN-γ alone and in combination. Stimulated cells were analyzed for mRNA transcripts of VDR and CYP24A1.

**RESULTS:** VDR was constitutively expressed, while CYP24A1 was barely detectable in airway epithelium in PBS-treated mice. However, expression of both VDR and CYP24A1 was significantly increased in the airway epithelium of OVA-treated mice. There was differential VDR expression within the inflammatory cell population in OVA-treated mice. Expression of both VDR and CYP24A1 was increased in BEAB-2b cells following TGF-β1 stimulation.

**CONCLUSIONS:** Pro-inflammatory cytokines, including TGF-β1, significantly affect the expression of both VDR and CYP24A1, the catabolizing enzyme for active vitamin D, in airway epithelial cells. It is, however, unclear whether such an increase in VDR and CYP24A1 is a defensive/compensatory mechanism to protect airway epithelium against the detrimental effects of inflammatory cytokines.

Challenge With Ragweed Pollen Extract (RWPE) In Allergic Rhinitis Induces Rapid Increase In 8-hydroxydeoxyguanosine (8-OhDg), IL-10 and G-CSF

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**RATIONALE:** RWPE contains NADPH oxidases that rapidly induce oxidative stress in the airways that is critical for allergic inflammation in sensitized mice. However, response to oxidative stress is also dependent on host-specific factors. To identify these host factors, we performed saline or RWPE challenge in subjects with allergic rhinitis, and quantified 8-OHdG levels and 48 cytokines as markers of innate oxidative stress and host response respectively.

**METHODS:** Six RWPE allergic subjects were challenged intranasally with saline and RWPE, and symptom scores were recorded. Small strips of filter paper were applied to the nose 30 min post-challenge and stored. Cytokines were recovered from these filter strips, and 8-OHdG in these fluids was measured by ELISA.

**RESULTS:** RWPE nasal challenge increased the patient symptom score at 30 min post-challenge (2.3 ± 0.8 saline, 6.2 ± 0.7 RWPE, p<0.05). In nasal lavage fluid, RWPE challenge increased 8-OHdG levels (0.2 ± 0.1 saline, 0.3 ± 0.1 RWPE, mg/ml, p<0.05). In nasal filter paper eluate, RWPE challenge increased levels of IL-10 (40 ± 40 saline, 72 ± 39 RWPE, pg/ml, p<0.05) and G-CSF (61 ± 39 saline, 154 ± 105 RWPE pg/ml, p<0.05).

**CONCLUSIONS:** RWPE nasal challenge in allergic rhinitis patients increased symptoms, and levels of 8-OHdG, IL-10, and G-CSF. These observations suggest that RWPE rapidly induces ROS and DNA damage, but simultaneously increases anti-inflammatory cytokines IL-10 and G-CSF that may suppress host response to allergens.
**AB52 Abstracts**

**ABS 5.1.0 DTD**

**197** Phosphorylation of N-Terminal Serine Residues of Glucocorticoid Receptor (GR) and Anti-inflammatory Response by Resolvin E1 (RvE1)

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**RATIONALE:** RvE1 (a lipid mediator derived from omega-3 eicosapentaenoic acid) and dexamethasone (Dex) both resolve inflammation. However, little is known about the role of RvE1 in the resolution of allergic inflammation. We hypothesized that RvE1 effects are mediated by phosphorylation of regulatory N-terminal serine residues (s203, s211 and s226) in the GR.

**METHODS:** Phosphorylation of GR at s203, s211 and s226 was determined by Western blot analysis in primary human bronchial smooth muscle cells treated with 100 nM RvE1 and/or Dex for 0, 1, 2, 4, 6, 12 and 24 hr. Cytokines were measured by multiplex ELISA.

**RESULTS:** RvE1-treated cells showed ~4 fold increased phosphorylation at s211 at 24 hr as compared to control, and greater than phosphorylation at s203 and s226. Phosphorylation at s211 (~8 fold) and s226 (~14 fold) are consequences of Dex-treatment, and reflect canonical GR activation. Human GM-CSF, EOTAXIN and IL-10 decreased with both treatments at 24 hrs, and VEGF and IP-10 were also reduced by both treatments at 12 hr. The inhibitory effects of Dex and RvE1 were at least additive for GM-CSF, EOTAXIN, IP-10 and IL-10.

**CONCLUSIONS:** Our findings suggest that both compounds significantly decrease cytokine production and differentially regulate GR phosphorylation. GR phosphorylation at s211 represents an activation signal; such activation of RvE1 in the absence of canonical ligand suggests that there may be novel strategies to overcome glucocorticoid resistance. Further studies are needed to understand the mechanism of regulation of GR signaling by RvE1 and other non-canonical ligands.

**198** IL-8 And Neutrophils In Bronchoalveolar Lavage Fluids Distinguish Mild Asthma From Moderate To Severe Asthma

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**RATIONALE:** In this study we sought to identify cells and cytokines in BAL fluids (BALF) that distinguish asthma from healthy controls subjects, and those that distinguish mild asthma from moderate and severe asthma.

**METHODS:** After obtaining informed consent, 39 human subjects were recruited in Kings College, London, UK for this study. These included 12 healthy control subjects (FEV1 = 102%, 89-110), 17 subjects with mild asthma (FEV1 98%, 81-113) and 10 subjects with moderate or severe asthma (FEV1 64%, 48-76). BALF were obtained from all subjects. The number of eosinophils, neutrophils, lymphocytes and monocyte/macrophages were quantified in BALF. In addition, 48 cytokines and 8-hydroxydeoxyguanosine were measured in these fluids using Bio-Plex cytokine array and ELISA, respectively. Statistical analysis was performed by ANOVA, and significance was set at p<0.05.

**RESULTS:** Compared to healthy control subjects, patients with asthma had significantly more eosinophils (17-fold higher %) neutrophils (2-fold higher %) IL-1R2 and IL-12 (1.7 fold each), IL-5 (1.6 fold), IL-6 (2.3 fold), IL-8 (1.4 fold), and RANTES (1.7 fold) in their BALF. Surprisingly, the only biomarkers that distinguished moderate/severe asthma from mild asthma were more neutrophils (1.5 fold higher %) and IL-8 (1.4 fold).

**CONCLUSIONS:** Eight biomarkers (eosinophils, neutrophils, IL-1R2, IL-12, IL-6, IL-8, and RANTES) distinguished healthy control subjects from subjects with asthma. In contrast, only neutrophils and IL-8, but not eosinophils, Th2 or other cytokines, distinguished mild asthma from moderate/severe asthma. Future larger studies should validate these observations, and elucidate the role of these biomarkers in the pathophysiology of asthma.

**199** Histamine And Endogenously Produced Spasmogenic Prostaglandins Increase The Strength Of Airway Smooth Muscle

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**RATIONALE:** Airway hyperresponsiveness (AHR) is a characteristic feature of asthma and could be due to increased force-generating capacity (strength) of airway smooth muscle (ASM). We have recently shown that increasing ASM-tone with acetycholine increases muscle strength over time, a process dubbed “force adaptation”. We hypothesize that the increased airway tone observed in asthmatics due to the release of inflammation-derived spasmogens contributes to AHR by fostering force adaptation. Here we sought to determine whether force adaptation occurs in response to: 1) the inflammatory spasmogen histamine; and 2) the intrinsic tone that prevailed in some ovine ASM preparations due to the endogenous production of spasmogenic prostaglandins.

**METHODS:** Ovine ASM strength (in response to electrical field stimulation) was assessed at 5-min intervals before, during and after histamine administration or after the addition of the cyclooxygenase inhibitor indomethacin (the later was only used with ASM strips showing an intrinsic tone caused by the endogenous production of prostaglandins).

**RESULTS:** Histamine-induced tone enhanced the ability of ASM to generate force (16±6% p<0.001). On the other hand, indomethacin not only abrogated the intrinsic tone but also reduced ASM strength over time.

**CONCLUSIONS:** These results suggest that ASM tone induced either spontaneously due to the release of endogenous spasmogenic prostaglandins or artificially by adding histamine (a spasmogen released in asthmatic airways following allergen bronchoprovocation) increase ASM strength. This gain in ASM force caused by force adaptation (i.e., incremental force-generating capacity due to tone caused by spasmogen exposure) may be relevant to the understanding of AHR.

**200** A Model for the Investigation of Allergic Sinus Congestion and Treatment

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**RATIONALE:** Sinus congestion and increased difficulty breathing are prominent symptoms of allergic upper airway inflammation in humans. In order to test the efficacy of putative drugs that might ameliorate these symptoms, we developed a model of sinus congestion in the guinea pig (GP), which has relevant histamine-driven allergic similarities to humans.

**METHODS:** GP’s were administered ragweed pollen (RWP; 0-50ug) intranasally once per day, for 5 days, to determine both the dose-response relationship of sinus airway inflammation, and the maximal effect dose against which to test intranasal anti-inflammatory steroids, dexamethasone, mometasone, and fluticasone. After administration of RWP with and without steroid treatment, sinus cavity filling was measured as an index of allergic sinus congestion, and compared across treatments by ANOVA.

**RESULTS:** Significant concentration-dependent increments in sinus congestion were observed with increasing concentrations of RWP, to levels that indicated up to 80% sinus occlusion at the highest concentrations of RWP tested (P<0.05). Significant concentration-dependent reductions of sinus congestion were produced by treatment with all three steroids, such that all steroids tested were able to completely abolish sinus congestion, to levels similar to RWP-vehicle-treated controls (n.s.).

**CONCLUSIONS:** These results indicate proof-of-concept for this GP sinus congestion model, as being reliable for both measurement of the induction of allergic sinus congestion associated with upper airway inflammation, and its reduction with anti-inflammatory compounds typically used to treat nasal congestion both therapeutically (fluticasone, mometasone), and experimentally (dexamethasone). We conclude that this model should be suitable for evaluating novel compounds for developed for the treatment of allergic sinus inflammation and congestion.
201 Influence of Maternal Allergen Exposure in the Development of Allergic Airway Disease in the Offspring: Effects of Antigen and Timing of Exposure


**RATIONALE:** The role of maternal allergen exposure in the development of allergic airway disease in the offspring is poorly understood. We examined whether the development of allergic airway disease is determined during the peri-natal phase and is dependent on the type of allergen and timing of maternal exposure.

**METHODS:** Female mice were sensitized or not to ovalbumin (OVA) or house dust mite (HDM) before mating. At parturition, the offspring were switched between sensitized mothers and naïve mothers. Sensitized mothers were exposed to allergen during pregnancy or during lactation. At 6 weeks of age, the offspring were sensitized and challenged with OVA or HDM, followed by assessment of allergic airway responses.

**RESULTS:** Compared with mice breastfed by unexposed naïve mothers, mice breastfed by mothers exposed to OVA during lactation, but not during pregnancy, were protected against the development of allergic airway disease. OVA-specific Th2 cytokine (IL-4, IL-5, IL-13, IL-10) production was inhibited; serum levels of total IgE and OVA-specific IgG1 were attenuated; airway inflammation was reduced; Treg numbers were increased. By contrast, mice breastfed by mothers exposed to HDM during pregnancy or lactation were not protected against the development of allergic airway disease. No significant changes were detected in HDM-specific Th2 cytokine production, specific IgG1 and total IgE levels, lung inflammation and Treg numbers.

**CONCLUSIONS:** The susceptibility to develop allergic airway disease may be determined by breastfeeding and is dependent on the type of allergen and timing of maternal exposure.

202 Effect of Prenatal Cigarette Smoke Exposure on the Development of Allergic Airway Disease in Mice


**RATIONALE:** Prenatal cigarette smoke exposure (CS) is associated with adverse respiratory health in children. However, the effects of prenatal smoke exposure on allergen sensitization are not well defined. This study was carried out in a mouse model to define the effects of passive prenatal smoke exposure on the development of allergic airway disease.

**METHODS:** Pregnant female mice were exposed during pregnancy to CS or filtered air and house dust mite (HDM) extract or saline. The offspring were sensitized twice and intranasally challenged three times with HDM. Airway inflammation and antibody responses were assessed in the offspring after the last intranasal allergen challenge. The results were compared between the different groups and related to maternal allergy and prenatal CS exposure.

**RESULTS:** Compared to the offspring of non-allergic mothers, the offspring of HDM-exposed allergic mothers developed increased airway responses to HDM following prenatal CS exposure. By contrast, in absence of maternal allergy, prenatal CS exposure was associated with reduced allergic airway responses to HDM in the offspring. However, although prenatal CS exposure was also associated with attenuated allergic airway responses in the offspring of HDM-allergic mothers, the latter developed enhanced allergic airway responses to HDM compared to the offspring of non-allergic mothers in absence CS exposure.

**CONCLUSIONS:** These findings suggest that prenatal CS exposure may increase the risk of allergic airway disease in the offspring of allergic mothers. On the other hand, prenatal CS exposure may also attenuate the development of allergic airway responses when the mothers are not allergic.

203 The Single Nucleotide Polymorphism, CRTh2-6373G>A, is Associated with Allergic Asthma and Increased Expression of CRTh2

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**RATIONALE:** CRTh2 (chemoattractant-receptor homologous molecule expressed on Th2 cells) is expressed by Th2 cells and other cells involved in allergic inflammation. Single nucleotide polymorphisms in CRTh2 (rs11571288, 545659, 634681) have been associated with phenotypes of allergic disease in ethnically distinct populations, but association of other CRTh2 SNPs with allergic disorders has not been observed.

**METHODS:** CRTh2-6373G>A (rs533116) was genotyped in a large ethnically diverse population (n=1282). The proportion of circulating peripheral blood cells expressing CRTh2 was determined in subjects with allergic airways disease and controls as well as in vitro differentiated Th2 cells. Receptor function was assessed by responsiveness of Th2 cells to the CRTh2-specific agonist 13,14-dihydro-15-keto-PGD2 (DK-PGD2) and intracellular staining for IL-4 and IL-13.

**RESULTS:** CRTh2-6373G>A was associated with allergic asthma in Caucasians (OR 2.67 [1.09 - 6.51]) and expression of CRTh2 was higher in subjects with allergic airways disease compared to controls. Amongst allergic individuals, -6373G>A was associated with significantly more eosinophils and higher expression of CRTh2 by both CD4+ T cells and eosinophils. In vitro, the A allele coincided with a higher percentage of CD4+ T cells expressing CRTh2 under Th2 differentiating conditions and the percentage of IL-4 and IL-13 positive cells following DK-PGD2 stimulation.

**CONCLUSIONS:** These findings show an association between CRTh2-6373G>A and allergic asthma and suggest this may be mediated by higher numbers of circulating eosinophils and elevated expression of CRTh2, leading to heightened responsiveness to PGD2 and production of Th2 cytokines.

204 Transglutaminase 2 Knock-out Protects Against Airway Inflammation And Tissue Remodeling In Ova-specific Allergic Asthma In Mice

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**RATIONALE:** CRTh2 (TGase 2) is over-expressed in a variety of inflammatory diseases including allergic asthma. TGase 2 inhibitors block TGase 2 over-expression. This study aimed to investigate whether TGase 2−/− protects against airway inflammation and tissue remodeling associated with OVA-induced allergic asthma in mice. C57BL/6 or TGase 2−/− mice were sensitized and challenged with OVA to induce asthma. OVA-specific serum IgE and leukotrienes (LTs) levels were measured by ELISA, and the recruitment of inflammatory cells into BAL fluid or lung tissues was stained with Diff-Quik and H & E, goblet cell hyperplasia by PAS. AHR was determined in a whole body plethysmographic chamber. Expression of TGase 2, eosinophil major basic protein (EMBP), VCAM-1, Muc5ac, PLA2 protein was measured by Western blotting while mRNA levels of Muc5ac, cytokines, MMPs, and TIMPs were quantified by RT-PCR. NF-B levels were evaluated by EMSA. TGase 2−/− protected against OVA-specific IgE production, the recruitment of total inflammatory cells, macrophages, neutrophils, lymphocytes and eosinophils in BAL fluid. TGase 2−/− reduced the number of goblet cells, AHR; expression of EMBP, Muc5ac, VCAM-1, CD40/CD40L, mRNA levels of several cytokines and chemokines, NF-B activity, PLA2 expression, and LTs levels in BAL cells and lung tissues. Expression of TIMP1/2 was recovered with TGase 2−/−.

Our data suggest that TGase 2−/− protects against the expression of numerous molecules associated with airway inflammation and remodeling by suppressing NF-B activation, and that TGase 2 may be a potential therapeutic target for treating allergic asthma.
205 Diesel Exhaust Alters Nasal Innate Immune Mechanisms in Allergic Rhinitics
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RATIONALE: We have previously demonstrated that diesel exhaust (DE) exposure enhances virally-induced exacerbation of allergic airway inflammation. We have also shown that natural killer (NK) cells are important immune cells present in nasal lavages, yet the role of NK cells and innate immunity in the inflammatory effects of DE are not known.

METHODS: We conducted a double-blind, randomized, placebo-controlled study of nasal responses to viral infection in the setting of diesel exhaust exposure. 22 human subjects with allergic rhinitis were randomized to clean air or DE (100 µg/m³) exposure and subsequently nasally inoculated with live attenuated influenza vaccine (LAIV). Nasal lavage was performed prior to inoculation/exposure (day 0) and on days 1, 2, and 7. Cytokine protein analysis and flow cytometry were performed to evaluate alterations in mediator profiles and NK cell surface markers, respectively.

RESULTS: Compared to baseline, LAIV inoculation increased CCL26 (eotaxin-3) and decreased CCL22 (MDC) levels in DE-exposed subjects. CCL26 is the ligand for CX3CR1, which is expressed on CD16+ NK cells, a subset of NK cells known for cytolysis. The DE-exposed group also had a higher percentage of CD16+ NK cells, as compared to air-exposed subjects. CCL26 is the ligand for CX3CR1, which appears to be particularly true regarding mechanisms of innate immunity in the inflammatory effects of DE are not known.

CONCLUSIONS: In allergic rhinitics, DE exposure alters markers of nasal innate immunity and NK cell activity following viral exposure. This appears to be particularly true regarding mechanisms of innate immunity associated with exaggerated inflammatory response.

206 Silica Crystals Cause Cellular Injury in TLR3-Activated Human Bronchial Epithelial Cells
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RATIONALE: Exposure to fine-particulate air pollution has the possibility to cause serious health problems including aggravation of asthma symptoms. Silica crystal (Silica) is the main mineral component of yellow sand dust, and activates the inflammasome to induce IL-1β secretion in macrophages. In this study, we examined the effects of Silica on cultured normal human bronchial epithelial cells (NHBE).

METHODS: NHBE were treated with TNF-α, LPS and PolyIC in the presence or absence of various concentrations of Silica (20-200 µg/ml). Cell viability was assessed by MTT assay and by measuring release of LDH activity and HMGB1 protein. Cell supernatants were analyzed for IL-1β secretion by ELISA and Western blotting. Activation of caspase-1 was examined by Western blotting.

RESULTS: PolyIC-, but not TNF-α-, nor LPS-activated Silica showed significant decrease in MTT activity by simultaneous treatment with Silica in a dose-dependent manner. Coincident with the decrease of cell viability, LDH activity, HMGB1 and IL-1β protein in the supernatants of PolyIC-activated NHBE were significantly increased by simultaneous treatment with Silica. Furthermore, IL-1β protein detected in the supernatants was found to be the precursor form (31 kDa) but not the mature form (17 kDa) by Western blotting. Caspase-1 in NHBE was not activated by a combination treatment with PolyIC and Silica treatment.

CONCLUSIONS: Unlike macrophages, Silica did not activate the inflammasome in NHBE. Instead, TLR3-activated NHBE were injured by exposure to Silica, probably through induction of necrosis. Our findings suggest that inhalation of yellow sand dust during viral infection may cause severe epithelial damage.

207 Alternaria Induces Stat-6 Dependent Acute Airway Eosinophilia And Epithelial FIZZ1 Expression That Promotes Airway Fibrosis And Epithelial Thickness
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RATIONALE: The fungal allergen, Alternaria, is specifically associated with severe asthma, including life-threatening exacerbations. To better understand the acute innate airway response to Alternaria, inflammatory and epithelial changes were investigated after naïve mice were exposed to a single airway challenge of Alternaria.

METHODS: Naïve WT C57/B6 mice were administered a single intranasal challenge with Alternaria, Candida, or Aspergillus extracts and BAL/ lung analyzed 24 hours later. RNA was extracted from airway epithelial cells after bronchial brushing and processed for gene microarray analysis. Immunofluorescent staining of lung sections was performed. Single cell suspensions from lungs were incubated with rFIZZ1, stained for cell type, and analyzed by FACS. Finally, mice were given rFIZZ1 repetitively and lung sections and BAL cell counts analyzed.

RESULTS: Naïve WT mice developed significant BAL eosinophilia following Alternaria challenge when analyzed 24 hours later but not after Aspergillus or Candida challenges. Gene microarray analysis of airway epithelial cell brushings demonstrated that Alternaria-challenged WT mice had an over 20-fold increase in level of expression of ‘‘Found in Inflammatory Zone 1’’ (FIZZ1/Retnla) confirmed by qPCR and immunofluorescence. Epithelial FIZZ1 expression as well as BAL eosinophils were significantly reduced in STAT6-deficient, but not PAR-2-deficient mice. rFIZZ1 displayed binding to CD45+CD11c+ (macrophages and dendritic cells) as well as collagen-1 producing CD45 negative cells (fibroblasts). Direct administration of recombinant FIZZ1 to naïve WT mice led to airway eosinophilia, peribronchial fibrosis, and increased thickness of the airway epithelium.

CONCLUSION: Alternaria induces STAT-6 dependent acute airway eosinophilia and epithelial FIZZ1 expression that promotes airway fibrosis and epithelial thickness.

208 Chemokines, Soluble Receptors and Mediators of Cord Blood Mononuclear Cells and Atopic Sensitization at 2 Years of Age in At Risk Infants Participating in a Probiotic Supplementation Clinical Trial
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RATIONALE: From a birth cohort of at risk infants (first degree family with atopic disease), we evaluated the influence of intrinsic immunologic risk factors for atopic sensitization at age 2 years to dietary and inhalant allergens.

METHODS: Cord blood samples were collected from 162 subjects of a birth cohort of 253 subjects participating in a double-blind placebo randomized trial on probiotic (Lactobacillus rhamnosus GG and Bifidobacteria longum, birth to 6mths) supplementation. Chemokines, soluble receptors and mediators of lipopolysaccharide stimulated cord blood mononuclear cells were analyzed using the Bio-Plex multiplex assay.

RESULTS: At 2 years, 44 subjects developed atopic sensitization and 118 remained non-sensitized. Soluble factors, CXCL6 (Granulocytes chemotactic protein-2), IL-2Ra, and MCSF (Macrophage colony-stimulating factor) were significantly increased in subjects who developed atopic sensitization compared to non-atopics (adj p values: 0.015, 0.005 and 0.008, respectively) after multivariate adjustment for wheeze, birth order, and maternal asthma. A multivariate logistic regression analysis that included all clinical and soluble factors showed that IL-2Ra was the only factor that remained significantly increased (OR 3.3; p = 0.002). Probiotic supplementation did not affect the outcome of atopic sensitization in this study.

CONCLUSION: In infants at genetic risk of atopy, an intrinsic hyperresponsive profile of soluble inflammatory factors is associated with atopic sensitization at age 2 years.
209 Gene-Environment Interaction Between Early Life Exposure and CD14, TLR4, IL13 in Development of Allergic Diseases or Atopy

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RATIONALE: Intestinal microbiota is altered by early life factors and influences the immune response and long-term effect. We studied the factors like delivery mode, feeding type and antibiotics which might affect intestinal microbiota in development of the allergic disease and the interaction between these factors and the polymorphism of cluster of differentiation 14(CD14-159C/T rs2569190), Toll-like receptor factors like delivery mode, feeding type and antibiotics which might affect intestinal microbiota in development of the allergic disease and the interaction between these factors and the polymorphism of cluster of differentiation 14(CD14-159C/T rs2569190), Toll-like receptor 4(TLR4-8595C/T rs1927991), Toll-like receptor 2(TLR2-1087A/G rs20541).

RESULTS: Skin prick test for 16 allergens was performed and polymorphisms were genotyped using TaqMan assay. Formula-fed subjects showed increased adjusted odd ratio(aOR) on atopy. Antibiotic use during infancy increased aOR in asthma, allergic rhinitis and atopic dermatitis. The rate of atopy was significantly higher in subjects who were taken antibiotic and born by cesarean delivery or formula-fed. The subjects of CT+TT genotypes in TLR4, especially with formula feeding and antibiotic use during infancy, were associated with asthma. AG+AA genotype in IL-13 polymorphism was related with asthma, allergic rhinitis, but not related with atopy.

CONCLUSIONS: Delivery mode, feeding type and antibiotic use during infancy influence the development of allergic disease. And these factors interact with CD14, TLR4 and IL13 polymorphism to develop the allergic diseases. These results indicate gene-environment interaction may work in the development of allergic diseases or atopy.

210 Exogenous Interferons Reduce Rhinovirus Replication in Human Bronchial Epithelial Cells

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RATIONALE: Human rhinoviruses (HRV) are the most common cause of asthma exacerbations. Reduced production of interferons (IFNs) by bronchial epithelial cells (BECs) may increase susceptibility to HRV infection and illness severity. Therefore, supplementing this attenuated innate immune response is a plausible therapeutic approach for HRV-induced asthma exacerbations. Thus, we investigated the effects of exogenous IFNs on HRV replication in BECs.

METHODS: Frozen stocks of human BECs from tracheal explants of six lung transplant donors were cultured in monolayers in 75cm2 flasks and then passaged into 12-well plates. BECs were grown to 65%-75% confluence and pre-treated with either 0.1 ng/mL, 1 ng/mL, or 10 ng/mL doses of IFN-α, IFN-β, IFN-λ1 or IFN-λ2 (PeproTech, Rocky Hill, NJ). After 24 hours, BECs were infected with either a high (5x10^6 pfu/mL) or low (5x10^5 pfu/mL) dose of HRV1a. Cells were lysed 24 hours post-infection, supernatants collected, and viral RNA was quantified using real-time PCR.

RESULTS: Compared to control (no IFN treatment), exogenous IFN-α, IFN-β, and IFN-λ1 (0.1 ng/mL), all significantly reduced HRV replication in both low (p<0.0001) and high (p<0.0005) HRV dose cultures. In contrast, IFN-λ2 did not significantly reduce HRV replication at a concentration of 0.1 ng/mL. However, all IFNs at dose of 1 ng/mL and 10 ng/mL significantly decreased HRV replication after infection with both low (p<0.0001) and high (p<0.0002) HRV dose.

CONCLUSIONS: Exogenous interferons significantly reduce HRV replication in BECs. IFN-α, IFN-β, and IFN-λ1 had similar efficacy and warrant further study as a potential therapy for virus-induced asthma exacerbations.

211 Transforming Growth Factor-β Regulates the Expression of Toll-Like Receptors in Human Bronchial Epithelial Cells

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RATIONALE: The airway epithelium is important in defending the body against airborne microorganisms and allergens. Toll-like receptors (TLRs) on airway epithelium are an important component of the immunoregulatory mechanisms in allergic and non-allergic inflammation in the lung. This is correlated with increased TGF-β1, which has been shown to be responsible for damaging the epithelium. TLRs are involved in the pathophysiology of allergic asthma, but little is known about how TGF-β1 and TLR signals interact. Here, we examined the effect of TGF-β1 and TGF-β2 on the mRNA expression of TLR 2, 4, and 9 on bronchial epithelial cells.

METHODS: Human bronchial epithelial cells (BEAS-2B) were stimulated with TGF-β1 (10ng/ml), TGF-β2 (10ng/ml), IL-4 (20ng/ml), or IL-13 (10ng/ml) for 24 hours. Following stimulation, RNA was extracted and mRNA transcripts for TLR 2, 4, and 9 were analyzed by qPCR.

RESULTS: TGF-β1, in a dose-dependent manner, decreased TLR2 and TLR9 mRNA transcripts in human bronchial epithelial cells. However, TLR2 decreased with the combination of TGF-β1 and TGF-β2. There was no effect of IL-4 or IL-13 on TLR2, TLR4, and TLR9 mRNA levels. TGF-β2 did not have any effect on TLR4 mRNA transcripts, but significantly decreased TLR2 and TLR9 mRNA transcripts in human bronchial epithelial cells.

CONCLUSIONS: These data suggest that both TGF-β1 and TGF-β2 can regulate TLR expression in human airway epithelium, suggesting that bronchial airway epithelial cells responds to various agents, including lipopolysaccharides, viral antigens and CpG on the exacerbation or resolution of airway inflammation could depend on the amount of TGF-β1 or TGF-β2 in chronic airway inflammation.
AB56 Abstracts

212 Enhancer of Zeste Homolog 2 induces Pulmonary Artery Smooth Muscle Cell Proliferation and Migration
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INTRODUCTION: Pulmonary Arterial Hypertension (PAH) is a progressively devastating disease characterized by excessive proliferation of the Pulmonary Arterial Smooth Muscle Cells (PASMCS). Studies suggest that PAH and cancers share an apoptosis-resistant state featuring excessive cell proliferation. The proliferation of cancer cells is mediated by increased expression of Enhancer of Zeste Homolog 2 (EZH2), a mammalian histone methyltransferase that contributes to the epigenetic silencing of target gene. In this study, it hypothesized that EZH2 could play a role in the proliferation of PASMCS.

METHODS: The expression patterns of EZH2 were investigated in normal and hypertensive mouse PASMCS. The effects of EZH2 overexpression on the proliferation of human PASMCS were tested. PASMCS were transfected with EZH2 or GFP using Lonza 4D nucleofector system. The proliferation and cell cycle analysis were performed using flow cytometry; the state of apoptosis of the PASMCS was determined using annexin V staining; and cell migration tested by wound-healing assay.

RESULTS: EZH2 protein expression levels were correlated with an increase in right ventricular systolic pressure and Right Ventricular Hypertrophy (RVH). The overexpression of EZH2 in PASMCS enhances proliferation, migration, and decreases the state of apoptosis when compared to GFP-transfected cells. In the G2/M phase of the EZH2 transfected cells, a 3.5-fold increase in proliferation and a 1.5-fold increase in the percentage of cells were observed, while there was a significant decrease in rate of apoptosis of the PASMCS.

CONCLUSION: EZH2 could play a role in development of PAH and serve as a potential target for new therapies for PAH.

213 Differential recruitment of CD49d+ Neutrophils by Toll-like Receptor Agonists
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RATIONALE: Polymorphonuclear neutrophils (PMN) are important innate immune cells. Using the Sendai virus (SeV) model, we have shown that CD49d+ PMNs are critical for the development of post-viral atopic disease. SeV infection, but not LPS (TLR4 agonist) administration was shown to recruit these CD49d+ PMNs to the airways. We undertook the current study to determine if any other toll-like receptors (TLR) were capable of recruiting CD49d+ PMNs to the lung.

METHODS: C57BL/6 mice were inoculated intranasally with PBS, SeV, or various TLR agonists. One day later, bone marrow, peripheral blood, lung tissue, and bronchoalveolar lavage (BAL) fluid were isolated and examined for CD49d+ expressing PMN by flow cytometry.

RESULTS: Compared to control mice, SeV and a TLR7 agonist reduced the frequency of CD49d+ PMN in the peripheral blood (reduced by 4.6±1.7%, p<0.08; and 6.9±0.8%, p<0.01, compared to PBS; n=3), while TLR3 and 9 agonists increased the percentage of these cells (increased by 18.2±5.6%, p<0.05; and 28.6±1.0%, p<0.0001; n=3). In the lungs, a TLR9 agonist increased the percentage of CD49d+ PMNs (48.8±2.1% TLR9 treated versus 23.7±4.8% for control, p<0.05, n=3). As we previously showed, TLR4 stimulation failed to increase CD49d+ PMN, while markedly increasing the CD49d- fraction. SeV was the only agonist to increase the percentage of BAL PMN expressing CD49d (71.8±1.6% SeV versus 4.1±4.1% PBS, p<0.001, n=3).

CONCLUSIONS: Virally associated TLRs appear to increase CD49d+ PMN mobilization from the bone marrow and/or recruitment into the lungs. However, entry into the alveolar space depends upon currently undefined signals associated with viral infection.

214 Participant Survey Results From the Starting Hizentra Administration With Resources and Education (SHARE) Program
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RATIONALE: Collaboration between patients and nurse educators is key to successful self-administration with subcutaneous immunoglobulin (SCIG). The Starting Hizentra Administration with Resources and Education (SHARE) program is designed to optimize nurses’ education and training techniques. Nurses were surveyed following participation in the SHARE program to evaluate perceptions about Hizentra and SCIG self-administration.

METHODS: Nurses from diverse care settings attended SHARE programs across the United States. The survey topics included: experience treating or administering SCIG to primary immunodeficiency disease (PIDD) patients; most frequent questions about Hizentra (infusion regimen, physical/chemical properties, safety, efficacy, general SCIG challenges); benefits of SCIG attributes (IgG steady-state levels, patient independence/convenience, improved systemic tolerability over intravenous immunoglobulin); and future educational/outreach interests.

RESULTS: Nurses represented practices with SCIG and PIDD experience ranging from none to extensive; 66% had ≤ 5 patients using SCIG. The most frequent questions on Hizentra were SCIG-specific challenges (30% of 223 interpretable responses), infusion regimen (21%) and safety (18%); 23% of nurses had no questions. 51% of respondents (155 interpretable responses) considered all SCIG attributes beneficial; 31% found the combination of IgG steady-state levels and patient independence/convenience to be most beneficial. Nurses were most interested in future educational programs on diseases outside of PIDD (25% of 106 interpretable responses), nursing outreach/educational topics (23%), and more information or updates on Hizentra (19%).

CONCLUSIONS: Although the benefits of SCIG are generally appreciated, 30% of nurses desire additional information regarding SCIG self-administration. Nurses are interested in additional educational/outreach, particularly on non-PIDDs and Hizentra.

215 Factors Associated with Daily Stress and Asthma Stress in Caregivers of Children with Asthma
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RATIONALE: To determine if caregiver report of daily or asthma daily stress scores are associated with frequency of symptom days in children with persistent asthma.

METHODS: As part of an inner-city asthma feedback study, caregivers of the 300 children enrolled were surveyed to include demographic, environmental tobacco exposure, daily and asthma stress levels (0-10 scale) and child asthma characteristics. Level of stress was primary outcome. Chi-square test was used for comparison of categorical variables and ANOVA for continuous variables by stress scores.

RESULTS: Children were primarily male (60%), African American (96%), young (mean 5.7 years) and Medicaid insured (92%). Caregivers were biological mother (92%), ≥ high school educated (70%) and low-income <$20,000 (72%). Mean baseline caregiver stress was daily stress 5.66 (SD 2.5) and daily asthma stress 4.29 (SD 2.8). At 2 months, daily stress was significantly associated with having a sibling, visitor or relative as smoker in household (p=0.05). A trend was noted for mean daily stress and increased symptom days/14 days scores: 0-2 days: 5.34; 3-5 days: 5.90 and 6+ days: 6.10 (p=0.07). At 2 months, mean daily asthma stress was associated with younger child age (3-5 years: 4.58 versus 6-10 years: 3.92; p=0.04) and increased symptom days: 0-2 days, 3.75; 3-5 days, 4.74; and 6+ days, 4.87; p=0.004.

CONCLUSIONS: Having a young child with asthma was associated with higher daily asthma stress scores suggesting newly diagnosed or newly managed children with asthma may be more stressful for caregivers. Asthma management plans need to address underlying caregiver stress to be effective.
216 Increase in Food Protein consumed during Milk and Egg Open Food Challenges are not associated with increase in failures. S. A. Lowe1, J. E. Conner1, L. A. Crandall1, C. M. Lee1, M. B. Ho1, M. B. Feuling2, C. L. Soo1, M. Vasudev1; 1Medical College of Wisconsin, Milwaukee, WI, 2Children’s Hospital of Wisconsin, Milwaukee, WI.

Rationale: Oral food challenges (OFC) are an integral part of evaluating patients with suspected food allergy or to establish tolerance. Indications for food challenge are not standardized and the decision to proceed to OFC is based on likelihood of achieving tolerance. We compared the outcomes of OFC after increasing the amount of protein consumed.

Methods: 63 who underwent milk and egg OFC (1-10 years, 60% male) between January 2006 and December 2007 were compared to 38 subjects (1.4-15 years, 68% male) who underwent OFC to milk and egg between January and July 2011. Total protein ingested was 0.54 g cow’s milk and 6.1 g egg in the first group versus 6.8 g and 13.6 g respectively in the second group. Pass rate defined as lack of immediate or delayed symptoms following OFC was determined.

Results: Similar milk OFC failure rates occurred in both groups, 20% (n = 6/30) vs. 18% (n = 3/22). A reduction in egg OFC failure rates from 14% (n = 6/33) to 0% (n = 0/16) was seen despite increase in protein content. Reactions observed or reported were similar in both groups including pruritus (n = 7), emesis (n = 1), urticaria and angioedema (n = 13), cough and wheeze (n = 2). Failed OFC were treated successfully with cetirizine in all but one patient. One patient after milk OFC required epinephrine and prednisone for anaphylaxis without further sequelae.

Conclusion: Increasing the amount of protein content in milk and egg OFC was safely tolerated.

217 Churg-Strauss syndrome: The Clinical Features and Long-term Prognosis of 47 Patients C. Lee1, B. Lee1, J. Lee2, D. Choi1; 1Samsung medical center, Sungkyunkwan University School of Medicine, Seoul, REPUBLIC OF KOREA, 2Center for Health Promotion, Samsung Medical Center, Seoul, REPUBLIC OF KOREA.

Rationale: Churg-Strauss syndrome (CSS) is a necrotizing systemic vasculitis which affects the small to medium sized blood vessels. Few large single center studies have been published due to the rarity of the disease. Therefore, CSS treatment remains an unresolved problem.

Methods: We retrospectively analyzed clinical manifestations and long-term prognosis of 47 patients who diagnosed with CSS at Samsung Medical Center from 1995 to 2011. For patients with high risk (heart involvement, Gastrointestinal disease, Renal insufficiency or CNS involvement) or peripheral motor neuropathy, IV pulse methylprednisolone followed by oral daily corticosteroids(CS) and monthly IV cyclophosphamide(CPM) was administered. For patients without high risk and peripheral motor neuropathy, they were treated with IV pulse methylprednisolone followed by only daily oral CS.

Results: Subjects included 25 men and 22 women with a mean age of 42.9 (18-80) years. The mean follow-up period was 48.6 (3-152) months. Commonly involved organs were peripheral nervous system (66%), lung (55%), skin (45%), and heart (15%). IV pulse CPM and daily oral CS was given only daily oral CS and 9(64%) achieved remission and 5 patients developed treatment failure or relapse. Three patients expired during follow up period. One patient died of heart failure due to uncontrolled vasculitis, and two died of pancreatic cancer and unknown etiology.

Conclusions: We have introduced IV pulse CPM treatment not only for major organ involvement, but also for peripheral motor neuropathy, and it was effective and safe.

218 Food Allergy Educational Needs of Allergy Dietitians in the UK C. Venter1, R. Meyer2, L. Reeves3; 1The David Hide Asthma and Allergy Research Center, Isle of Wight, UNITED KINGDOM, 2Great Ormond Street Hospital, London, UNITED KINGDOM, 3Oxford Health NHS Foundation Trust, Oxford, UNITED KINGDOM.

Rationale: The NIAID (US)(1) published food allergy guidelines in 2010 followed by NICE guidelines (UK)(2), highlighting the importance of a dietetic consultation. Recently, the CoFar research group(3) conducted a survey to establish the knowledge and needs of UK dietitians dealing with food allergy. Their survey indicated that many paediatric dietitians felt their proficiency was overall moderate. The objective of our survey was to determine the food allergy knowledge and needs of UK based dietitians and how this compares to US based dietitians.

Methods: A survey based on Groetch et al.(4) was posted on the BDA website.

Results: 350 UK paediatric dietitians completed this survey vs. 311 US dietitians. 40% of the UK dietitians were mainly working in outpatient settings, similar to the 46% from the US. Dietitians in the US felt more knowledgeable about the definitions of food llergies and intolerances (59% and 42% scored high in the US vs. 34% and 33% scored high in the UK). However, UK based dietitians indicated more confidence in designing food challenge protocols (13.5% UK vs. 8% US scored high) although both these scores indicate some uncertainty in designing food challenge protocols, with 16% in the UK and 19% in the US indicating no proficiency at all. Interestingly, dietitians from both countries indicated that their most immediate need was standardisation patient handouts/diet sheets.

Conclusions: There is a huge need amongst dietitians dealing with paediatric patients, from both these countries, to increase their knowledge regarding a wide range of food allergy related subjects to improve patient care.

219 Role of Preventive Anti-histamine Medications for Local Reactions with Conventional Aeroallergen Subcutaneous Immunotherapy (SCIT). S. Golubski, T. Gobel, R. Gutta, L. Pien; Cleveland Clinic, Cleveland, OH.

Rationale: The use of oral anti-histamines 2 hours prior to venom immunotherapy (IT) has shown to decrease the rate of large local reactions. Our goal was to review the role of oral anti-histamines for patients who developed large local reactions with inhalant IT.

Methods: We performed a retrospective chart review study of 57 patients who were on conventional aeroallergen SCIT at our institute. We collected data on 46 patients who had large local reactions with our SCIT regimen.

Results: A total of 12,244 SCIT injections were administered to 21 (37%) males and 36 (63%) females. 463 injections lead to large local reactions (>3cm). Our rate of large local reactions was 3.78/100 injections. 37/46 patients had recurrence of large local reactions (> 3 times). 35/46 patients had delayed large local reactions. 8/46 patients had systemic reactions. Out of 37 patients with recurrent large local reactions, 31 patients had delayed local reactions and 7 patients had systemic reactions. 39/46 patients were on oral anti-histamines prior to IT, only 1 patient took anti-histamine 2 hours prior to SCIT. SCIT dose was decreased in 44/46 patients, without any decrease in recurrence of large local reactions (36/37), delayed large local reactions (33/35) and systemic reactions (8/8).

Conclusions: Based on the review of effects with dose adjustments on these patients, we plan to change our IT practice for localized reactions. Additional studies are essential to clarify the preventive role of taking oral antihistamines two hours prior to inhalant IT.
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SATURDAY

220 Response to State Epinephrine IM (epi IM) Dose vs. BMI and WAO Systemic Reaction Grading in the Incidence of Systemic Reactions (SRs) to Prick (P) and Intradermal (ID) Skin Tests

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RATIONALE: To determine incidence of SRs to P and ID tests, response to stat epi IM, dosage of epi IM vs. BMI, and World Allergy Organization (WAO) Grading of SRs.

METHODS: From 07/2010 to 06/2011 SRs were recorded for combination of pollen, animal emanation, mold and Hymenoptera to P and ID testing in 1,332 subjects. Stat epi IM (1:1000 v/v), 0.2 mg was administered by medical staff for signs/symptoms (SS) of SRs, including, not limited to, itchy eyes, nose, pharynx, palms; rhinorrhea, nasal congestion, sneezing; generalized erythema, pruritus, or urticaria. Total epi IM dose and SS utilizing the WAO Grade system were recorded. BMI were obtained from WHO criteria.

RESULTS: 2%, 31 subjects, had SRs: 77% (24) female, 23% (7) male; 16% (5) pediatric, 84% (26) adult. 84% (26) Grade I, 16% (5) Grade II; no Grade III, IV, V. Occurred. During P 42% (13) experienced SS. During ID or at completion of P and ID 58% (18) experienced SS. All received stat epi IM. 24 SRs, mean BMI 28.5 (overweight range 25.0-29.9) received epi IM 0.2 mg; one BMI 20.4 (normal 18.5-24.9) received two epi IM (total 0.3 mg). No underweight (<18.5) or obese (>30.0) subjects had SS.

CONCLUSIONS: There was no relationship of epi IM dose to BMI. 2% (31) of 1,332 tested subjects had SRs to P and/or ID tests; 30 received one stat epi IM dose (0.2 mg); 1 received two doses (total 0.3 mg). All but one were WAO Grade I reactions. Stat epi IM may prevent serious SRs.

221 Temporal Changes In Lung Function In A Mouse Worker Population

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RATIONALE: Whether mouse workers have greater than expected declines in lung function over time is unclear.

METHODS: Newly employed workers (18-74y), at the Jackson Laboratory, were enrolled. Participants had skin testing at baseline and screening pre-lung transplant patients for LTBI.

RESULTS: 205 workers had at least one visit with both MA exposure and lung function measurement. 91% were white, 57% female, 54% atopic, and 42% had ever smoked. Median follow-up time was 12 months. Lung function declined with time, independent of age and gender. FEV1 declined by 33mL/year (95% CI [-56 to -9], p=0.006); FVC declined by 17mL/year (95% CI [-39 to 5], p=0.13); and FEV1 expressed as % of FVC (FEV1/FVC) declined by 0.39%/year (95% CI [-0.75 to -0.02], p=0.044). MA did not modify the rate of decline of lung function (interaction term p-values; FEV1, p=0.91; FVC, p=0.52; FEV1/FVC, p=0.84). Atopy did not modify the rate of decline of lung function either, but smoking history did.

CONCLUSIONS: Workers at The Jackson Laboratory have similar declines in lung function as the general population, suggesting that their occupational mouse allergen exposure is not contributing to greater than expected lung function decline.

222 Clinical Utility of Anergy Panel Testing in Conjunction with Purified Protein Derivative (PPD) Tuberculin Skin Testing (TST) for Detection of Latent Tuberculosis Infection (LTBI) in Pre-lung Transplant Patients

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RATIONALE: American Transplant Society recommends screening of all pre-lung transplant patients utilizing anergy panel with PPD-TST for LTBI. Our institute’s anergy panel consists of PPD-TST, Candida, and Tetanus. American Thoracic Society does not recommend using anergy panel screening for LTBI. Our aim was to review the clinical utility of anergy panel testing in these patients, considering the difference of opinion.

METHODS: We did a retrospective chart review of 100 pre-lung transplant patients receiving anergy panel testing at our institute from August 2010 to January 2011.

RESULTS: Patients include 56% males and 44% females. 85% of the patients were Caucasians, 13% were African Americans, and 2% were Hispanics. Mean age was 56.1 years. One patient had prior history of BCG vaccination. 51% of our patients were on oral steroid medications; 8% were on immunosuppressive drugs. Delayed Type Hypersensitivity (DTH) response was present in 59% of patients and absent in 41% of patients. PPD was negative in 56/59 of patients with intact DTH, positive in 3/59 patients with intact DTH and could not be interpreted in 41/41 patients with absent DTH response. Only 11/41 of patients with absent DTH response had further in-vitro and microbiological testing, all were negative for LTBI. One patient with history of BCG vaccination had intact DTH response and negative PPD test.

CONCLUSIONS: Anergy panel testing prompted change in clinical management in only a minority of patients. Further studies are essential to enumerate the role of QuantiFERON-TB test versus anergy panel for screening pre-lung transplant patients for LTBI.

223 Development of Standards of Care for Immunoglobulin Replacement Therapy

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RATIONALE: Immunoglobulin is a biological modifier with complex administration. Immunoglobulin replacement therapy is prescribed for those patients who have primary immunodeficiency (PID) characterized by hypogammaglobulinemia and/or the inability to make antibodies in response to exposure to a pathogen. Infusions can be given intravenously or subcutaneously. Dosing is based on the patient’s weight, serum immunoglobulin and antibody levels, and the clinical response to therapy. Multiple variables for dosing, expansion of delivery methods, and new indications for use contribute to the complexity of administration. A need was identified to develop standards of care for practitioners unfamiliar with administration. Patient safety and prevention of medical errors at the site of care (infusion center, home therapy) was the motivating factor to develop the standards of care. Allied Health providers were the target population.

METHODS: A workgroup comprised of members of the Nurse Advisory Committee of the Immune Deficiency Foundation (IDF-NAC) performed a review of the current literature and critiqued administration of practice sites both clinic and home health based. Standards addressed product specifics, patient status, and adverse event management.

CONCLUSION: Standards were completed in 2011, will be published, and will be available as a reference on the IDF website, www.primaryimmune.org. Availability of standards that are web based provides ready access to Allied Health members.
224 The Newport News Healthy Homes Initiative: Targeting A Hard To Reach Low Income Asthmatic Population

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RATIONALE: NNHHI, provided PFTS, SPTs, and home remediation supplies for indoor allergens to a low-income, hard-to-reach pediatric asthmatic population. The participants were mostly Black (91%) males (55.9%) with a median age of 9.5 years and the primary caregiver was their mother (94%). A third of the children had an emergency room (ER) visit for asthma in the 3 months prior to baseline and 43.1% had been prescribed oral steroids.

METHOD: Randomized Controlled Trial.

RESULTS: Caregivers reported symptoms of asthma in the last 14 days/ nights. Pulmonary function tests on 89 of the 101 program participants were reviewed and a severity classification assigned using EPR 3. Between 41%- 61% were not taking inhaled corticosteroids, based on severity of disease and therapeutic recommendations, suggesting a level of unnecessary morbidity exists in this population.

Caregiver quality of life is highly correlated with asthma morbidity of their children and was measured at baseline, 6-months and 12-months. The Pediatric Asthma Caregivers Quality of Life questionnaire measures overall quality-of-life and includes an activity and emotional coping domain. A clinically important (and statistically significant) change was noted in the group which received the remediation supplies in emotional function and activities domain as well as the overall quality of life score between baseline and 6-month follow-up, and baseline and 12-month follow-up (p<0.05).

CONCLUSIONS: The data shows that caregiver quality of life increased at both a clinically and statistically significant level in a hard to reach patients living in Southeastern Virginia.

225 Indoor Allergens in the Rocky Mountains: Dust Collection and Analysis in the Arid North

E. C. Weiler; University of Montana, Missoula, MT.

RATIONALE: With asthma being prevalent in the northern Rocky Mountains, indoor dust and the allergens it contains can pose a problem. Little research in this geographical area has been conducted to determine what allergens are present and in what quantity they exist in indoor dust. It has been assumed as well for many years that Montana’s elevation and climate are not conducive to dust mite survival and thus many allergists don’t consider them as a potential problem for their patients.

METHODS: Using 40 homes located around rural western Montana and eastern Idaho, dust samples were collected using three methods along with collecting humidity data during winter months from 2008-2010. Dust samples were analyzed using new extraction techniques and a pre-mixed multiplex array for indoor allergens to determine presence and concentration of these five allergens: Canine, Feline, European dust mite, American dust mite, and a secondary mite group (Can f 1, Fel d 1, Der p 1, Der f 1, Der p 2/Der f 2, respectively). Skin prick tests were performed on subjects less than 18 years old in each home.

RESULTS: Preliminary data shows evidence of canine and feline in the majority of house dust samples. Allergen sensitivity to these two allergens was also very prevalent. Two homes show presence of dust mite allergen, with two different subjects showing allergen sensitivity to dust mite.

CONCLUSIONS: Additional analyses are being performed to verify positive allergen results in house dust, and to determine accurate concentrations of each allergen.

226 Effects of Asian Dust and Spherical Particles Exposure on Human Health and Allergic Symptom, Fukuoka, Japan
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RATIONALE: Asian dust arise in arid areas of China and spread over large areas diminishing the particle size on East China, Korea and Japan. People Asia region have been exposed to the particles which contained various chemical species and suffered from the potentially hazardous effect on respiratory symptoms. Moreover spherical particles including SO42- and NO3- are transported too. We have investigated into effects of Asian dust and spherical particles on human health especially allergic symptom.

METHODS: The voluntary, informed consented 20-Y-O average nurse students with or without rhinoconjunctivitis who have healthy campus life are object every year from 2008 to 2010. They have kept symptoms score diary February to May, detected allergy by self-report and questionnaire.

We detected the days of Asian dust and spherical particles coming to our region by Light Detecting and Ranging data, SPM(μg/m3), O3(ppb) and the weather report. The conifer pollen count is monitored by Fukuoka Medical Society network. We analyzed the symptoms score before, during and after the events day every year using Wilcoxon’s test.

RESULTS: Every year 176, 221 and 218 students have performed their diary. The students with rhinoconjunctivitis, about 40% of all, have mild but higher symptom score than that without allergy. Just during the events they have become more increased nose and prolonged pharyngeal symptoms than before significantly at not only Asian dust but also the higher sulfate mist and ozone days.

CONCLUSIONS: We thought exposure to the transported SPM from Asia-continent associated with the increased nose and pharyngeal symptoms score of healthy but allergic young adults, Fukuoka.

227 Recent Increase in Aeroallergen Indices in Texas Panhandle and Use of Nano Air Purifier to Alleviate Allergic Rhinitis and Asthma


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RATIONALE: We have been analyzing the aeroallergen indices of Texas Panhandle area for a decade to determine if that have any effect on allergic rhinitis cases. Nano, a filter less air purifier that uses UV-PCO technology was used to improve the air quality.

METHODS: We determine the daily aeroallergen index by microscopic observation on the prepared slides from stained Melinex tape collected from a Burkard Volumetric Spore Trap. We recorded the daily temperature, rainfall and wind speed with the aeroallergen counts. The aeroallergen indices were correlated with the meteorological data and the number of cases of asthma and allergic rhinitis recorded at the Allergy ARTS Clinic.

Nano, filter-less air purifier contains a PCO Cell coated with nano Nickel HCT catalyst bonded to the PCO cell. When photons from the germicidal UVC lamp excite water and oxygen molecules on the catalyst surface, redundant oxidizers, including hydroxyl radicals, are formed. Using a Nano air purifiers improved the indoor air quality by reducing the aeroallergen and VOCs and thereby reducing the breathing ailments.

RESULTS: We found a direct correlation between the increased aeroallergen indices and early flowering with the increased cases of breathing ailments including allergy and asthma. Prepared slides from exposed double sticky tape and GC-MS analysis showed a gradual reduction in the indoor allergen and VOCs when a Nano air purifier was used.

CONCLUSIONS: Increased aeroallergen indices and early flowering caused the increased cases of allergy and asthma. Usage of Nano air purifiers improved the indoor air quality to alleviate the breathing ailments.
228  
Research Of The Allergenicity Evaluation System Of Recombinant Human Lactoferrin (rhLF) With BN Rats  
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RATIONALE: BN rats were used to study the potential allergenicity of Recombinant human Lactoferrin (rhLF). The results were compared with that observed from bioinformatics and simulated gastrointestinal fluid digestion experiments to analyze the current allergenicity evaluation system of transgenic animal food.

METHODS: BN rats were divided into 8 groups and treated with rhLF, Bovine Lactoferrin (blfF), positive control OVA and negative control saline respectively through oral gavage and intraperitoneal injection. Sera and blood were collected on day 0, 14, 28 and 42 for measurement of specific IgG, IgG2a, IgE and eosinophils. On day 49, all groups were challenged with corresponding proteins and their blood pressure was measured. Then animals were sacrificed and their heart, liver, spleen, lung, kidney and thymus were harvested for histology and organ index.

RESULTS: OVA, rhLF and blfF can cause BN rats’ same immunological reaction when they were given animals by either oral gavage or intraperitoneal injection. Specific IgE and eosinophils levels were significantly increased (P<0.05) in the OVA sensitized rats. And rats’ blood pressures in OVA groups were reduced. Specific IgE levels were significantly increased (P<0.05) in rats treated with blfF. The rhLF groups didn’t perform significant sensitization.

CONCLUSIONS: The rhLF almost can’t cause BN rats’ allergy, this is contrast to the results obtained from its bioinformatics. This prompted that the current allergenicity evaluation system of transgenic animal food need to be improved and maybe the animal trials are necessary to refine the evaluation.

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Predictive Value Of Caregiver Report And Expert Assessment Of Home Mouse Allergen Levels  
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RATIONALE: Mouse allergen exposure is associated with asthma morbidity in urban children. It is unclear if caregiver report or expert assessment of mouse infestation better predicts mouse allergen levels.

METHODS: Questionnaires were administered to caregivers of 150 Baltimore City children (5-17y) with moderate to severe asthma. Trained study staff assessed homes for mouse infestation. Settled dust samples were obtained for allergen analysis. Positive (PPV) and negative (NPV) predictive values were calculated.

RESULTS: 57% of children were male, 91% black, and 55% reported an annual income of <US$25K. Prevalence of caregiver reported infestation was 66%. Prevalence of staff assessment of infestation was 62%. Median Mus m 1 levels for bedroom and bed were 2.3mcg/g (IQR: 0.6-12.2) and 1.2mcg/g (IQR: 0.3-3.9) respectively. Caregiver report of infestation predicted staff observation of infestation (PPV 72%, NPV 60%). Neither caregiver report nor study staff observation of mice was a reliable predictor of low allergen levels; but caregiver report of mice predicted potentially harmful allergen levels in bedroom (≥0.5 mcg/g) (PPV 87%, NPV 46%) and bed (≥0.4 mcg/g) (PPV 83%, NPV 53%). Study staff observation of infestation also predicted potentially harmful allergen levels in the bedroom (PPV 85%, NPV 38%) and bed (PPV 79%, NPV 45%).

CONCLUSIONS: Caregiver report of infestation performed as well as staff observation in predicting high mouse allergen levels in this population. Home inspection, when caregiver history is available and direct measurement of mouse allergen is not feasible, does not aid in identifying children exposed to high mouse allergen levels.
Real-world Effectiveness Of Asthma Step-up Options: Matched Comparison Of Extrafine Hydrofluoroalkane-beclometasone AND Inhaled Corticosteroid / Long-Acting Beta-Agonist

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RATIONALE: If inhaled corticosteroid (ICS) therapy fails to provide asthma control, guideline recommendations include increasing ICS dose or adding long-acting beta2-agonist (LABA). Extrafine hydrofluoroalkane-beclometasone dipropionate (EF HFA-BDP) has been shown to be highly effective in achieving asthma control.

METHODS: This retrospective study using the UK General Practice Research Database compared real-world effectiveness of step-up therapy from ICS (budesonide, beclometasone, fluticasone) to (i) higher dose ICS (≥50% increase) as EF HFA-BDP (n=1065) or (ii) ICS/LABA (no change in ICS dose or drug) (n=1065). Patients aged 4–80 years were matched (1:1) on key demographic and disease characteristics over 1 year pre-step-up: age, sex, asthma control status, reliever medication use, baseline ICS dose, and routine asthma consultations. Co-primary outcomes over 1 year were severe exacerbations (ATS/ERS definition) and asthma control (absence of: severe exacerbations; out-of-hours care; outpatient department attendance; antibiotics for lower respiratory infections). Secondary outcomes included treatment success (asthma control plus no additional therapy) and short-acting-beta2-agonist (SABA) usage.

RESULTS: There were no statistically significant differences in odds of achieving asthma control: as compared with ICS/LABA Odds Ratio (OR)(95%CI) for EF HFA-BDP, 1.16(0.94-1.45) or in exacerbation rates, RR(95%CI), 0.88(0.69-1.11). Odds of achieving treatment success were significantly higher for EF HFA-BDP: OR(95%CI), 1.50(1.27-1.85). SABA daily dose range was marginally, but insignificantly higher for EF HFA-BDP (median[IQR], 164.4[82.2-328.8] mcg vs 164.4[54.8-328.8] mcg; p=0.112).

CONCLUSIONS: These real-world data suggest stepping-up therapy with an increased dose with EF HFA-BDP provides overall asthma control as effective as adding a LABA, indicating the importance of treating small with an increased dose with EF HFA-BDP provides overall asthma control:

CONCLUSIONS: Altairnaria sensitization was associated with increased airway hyperresponsiveness and sinusitis in children with severe asthma and may account for the increased symptom burden in this population. Routine evaluation of Altairnaria sensitization may be useful in the clinical management of children with severe asthma.

Alternaria Sensitization is Associated with Increased Airway Hyperresponsiveness and Sinusitis in Children with Severe Asthma

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RATIONALE: Although aeroallergen sensitization is a distinguishing feature of children with severe asthma, the specific role of aeroallergens in the modulation of asthma severity is not entirely understood. Given increased reports of Alternaria sensitization and asthma symptoms in adults and children, we determined whether Alternaria sensitization and associated clinical features differed between children with severe and mild-to-moderate asthma enrolled in the NHLBI Severe Asthma Research Program (SARP).

METHODS: 187 children 6-17 years of age with physician diagnosed mild-to-moderate (n=97) and severe (n=90) asthma were included in this analysis. Alternaria sensitization was determined by skin prick testing (SPT).

RESULTS: Overall, 28% (n=53) of all children with asthma enrolled in SARP had a positive SPT to Alternaria. However, Alternaria sensitization was more prevalent in children with severe (37%, n=33) versus mild-to-moderate (21%, n=20) asthma (p<0.01, OR 2.2, 95% CI [1.2,4.3]). Within the group of children with severe asthma, lung function (FEV1% predicted), lung volumes, blood eosinophils, serum IgE, medication requirements, and healthcare utilization did not differ according to Alternaria sensitization. However, children with severe asthma with Alternaria sensitization had increased airway hyperresponsiveness (0.75 ± 0.91 PC20 mg/mL vs 3.3 ± 4.8 PC20 mg/mL; p<0.01) and were more likely to report a history of acute/chronic sinusitis requiring antibiotic treatment (58% vs 42%, p<0.05).

CONCLUSION: Alternaria sensitization is associated with increased airway hyperresponsiveness and sinusitis in children with severe asthma and may be useful in the clinical management of children with severe asthma.

Disconnect Between Sputum Neutrophilia and Indices of Mucosal Inflammation in Severe Asthma

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RATIONALE: Phenotypic heterogeneity in severe asthma has been described in terms of the nature and intensity of granulocytic inflammation in the airways, but few studies have directly compared various methods of airway sampling. We sought to examine the relationships between mediators of inflammation in endobronchial biopsies, induced sputum, and peripheral blood in a large cohort of severe asthmatics.

METHODS: Severe asthmatics (N=67), defined by ACQ≥1.5 despite treatment with high dose inhaled corticosteroids (≥1000 micrograms/day fluticasone equivalent), were assessed in an observational study in which induced sputum, endobronchial biopsies, and peripheral blood were collected. Cell counts and inflammatory cytokines were measured by immunohistochemistry.

RESULTS: In induced sputum, there was a negative correlation between the proportions of eosinophils and neutrophils. However, in endobronchial biopsy tissue there was a strong positive correlation between eosinophil and neutrophil counts. Eosinophil and neutrophil counts and multiple other markers of inflammation in biopsies as determined by immunohistochemistry were significantly intercorrelated (p<0.05), including TSLP, IL25, IL17A and IL17F. While sputum and tissue eosinophils were both positively correlated with serum periostin, neutrophil percentage was negatively correlated while tissue neutrophil counts showed a trend toward positive correlation with serum periostin. None of these measures was significantly correlated with lung function or asthma control within this severe asthma cohort.

CONCLUSIONS: Of multiple indices of asthmatic airway inflammation, most metrics were positively intercorrelated with the exception of sputum neutrophil percentage. Definitions of subphenotypes of severe asthma as determined solely by sputum granulocyte proportions may misrepresent the pathophysiology present in bronchial tissue.
**Co-associations Between IL10 Genetic Variants, IL 10 Production And Helminth Infection In A Tropical Population Of Brazil With High Prevalence Of Asthma**

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**RATIONAL:E** Helm int infections have been associated with protection against immune-mediated diseases, such as allergies and autoimmunity. It is postulated that induction of IL-10, suppression of IgE and increased IgG4 are some of the possible mechanisms related to that protection. We hypothesized that IL10 polymorphisms are associated with helminth infection, asthma and allergy.

**METHODS:** We genotyped 12 IL10 SNPs in 1,353 children (303 asthmatic/1050 non-asthmatic) aged 4-11 years living in a poor urban area in Salvador, Brazil, using TaqMan™ probe based, 5’ nuclease assay minor groove binder chemistry. We determined asthma/wheeze status, IL-10 production in peripheral blood leukocytes stimulated with Ascaris lumbricoides extract, serum total IgE, specific IgE against Ascaris lumbricoides and skin prick test (SPT) to aeroallergens, IgG4 anti-Ascaris lumbricoides, as well as Ascaris lumbricoides and Trichuris trichiura infection in the total sample. Association tests were performed by logistic regression including sex, age and helminth infection as covariates when indicated using PLINK.

**RESULTS:** Allele C of SNP rs3024498 was associated with both increased IL10 production (OR=1.70; p=0.02) and specific IgE for Ascaris lumbricoides (OR=1.44; p=0.006). The same allele was associated with protection of Ascaris lumbricoides (OR=0.57; p=0.03) and Trichuris trichiura (OR=0.25; p=0.0005) chronic infection as well as specific IgG4 against Ascaris lumbricoides (OR=0.59; p=0.002). No associations were found between rs3024498 variant and SPT or wheezing.

**CONCLUSIONS:** Our findings suggest that IL10 SNP rs3024948 plays a role in the production of IL-10, and the immune response mediated by IgE against helminths.

**Glucocorticoid Receptor Translational Isoforms Contribute to Distinct Glucocorticoid Responses of Neutrophils and Eosinophils**

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**RATIONAL:E** Neutrophils, but not eosinophils, have been suggested to mediate steroid resistance in asthma and COPD, the mechanisms of which are unknown. We determined whether the recently described glucocorticoid receptor (GR) translational isoforms underlie the distinct steroid sensitivity of neutrophils and eosinophils.

**METHODS:** Neutrophils and eosinophils from deidentified human blood samples were isolated to 97% purity by gradient centrifugation, immunomagnetic column separation, and fluorescence-activated cell sorting. Sensitivity of isolated cells to glucocorticoids was examined by measuring apoptotic markers such as annexin-V and caspase 3 activation. GR translational isoforms were determined using Western blot analyses.

**RESULTS:** The pro-apoptotic GR-A isoform was five-fold higher in eosinophils than in neutrophils, whereas the non-apoptotic GR-D isoform was five-fold higher in neutrophils compared to eosinophils (n=9, Student’s t-test, p<0.05). The ratio of GR-A/D in eosinophils was more than ten-fold higher than that in neutrophils (p<0.05). Dexamethasone (1 μM, 16 hr) increased eosinophil apoptosis by almost 50%; in contrast, neutrophil apoptosis decreased by approximately 40% in the presence of dexamethasone (p<0.05 for both). These distinct steroid responses in eosinophils and neutrophils were blocked by RU486 (1 μM, 16 hr), a selective GR antagonist, indicating that selective GR isoforms play a role in the different steroid sensitivities of eosinophils and neutrophils. GR[A], a splice variant of GR previously implicated in steroid resistance, was undetectable in either cell type.

**CONCLUSIONS:** Neutrophils and eosinophils have distinct GR translational isoforms. The GR-D isoform in neutrophils likely mediates their resistance to steroids and may underlie steroid-resistant diseases characterized by neutrophil-predominant inflammation.

**Epigenetic Biomarkers of Established Allergic Disease in Peripheral Blood Mononuclear Cells**

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**RATIONAL:E** A significant proportion of the North American population suffers from seasonal allergic rhinitis. However, genomic DNA sequence can not explain why certain individuals develop allergic disease and others do not. Epigenetic modifications, such as DNA methylation, have begun to play a key role in explaining gene-environment interactions. We hypothesized that a genome-wide comparison of DNA methylation in atopic and controls would identify epigenetic modifications relevant to the allergic phenotype.

**METHODS:** Peripheral blood mononuclear cells (PBMCs) from 15 patients with established seasonal allergic rhinitis and a positive skin prick test to rye grass and 8 non-atopic controls were interrogated for genome-wide differences in DNA methylation using the Infinium Methylation 450K BeadArray (Illumina). Quality control and initial normalization was performed using GenomeStudio (Illumina). False Discovery Rate (FDR) was estimated using Bonferroni correction. Linear regression and non-parametric t-tests were performed on quantile-normalized and filtered data using Matlab software (v.R2011a, The MathWorks).

**RESULTS:** After correcting for age and gender, 77 sites exhibited greater than a 5% difference in mean methylation between controls and atotics. Hypermethylated genes included those involved in complement/immune signaling and ion channels. Hypomethylated genes included proteases, cell adhesion molecules, transporters and eosinophil peroxidase.

**CONCLUSIONS:** Epigenetic modifications may mediate gene-environment interactions relevant to the allergic phenotype.
238 Genetic Polymorphisms of Transforming Growth Factor-β Signaling Pathway and Kawasaki Disease in the Taiwanese population

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RATIONALE: Kawasaki disease is a systemic vasculitis associated with cardiovascular symptom. A previous study in the European descent has indicated that genetic variants of the transforming growth factor-β (TGF-β) signaling pathway are involved in the KD susceptibility and clinical status. This study was conducted to investigate if polymorphisms in transforming growth factor-β signaling pathway are associated with KD susceptibility, coronary artery lesion formation or intravenous immunoglobulin (IVIG) treatment responses.

METHODS: A total of 907 subjects (381 KD patients and 526 controls) were investigated to identify 12 single nucleotide polymorphisms in transforming growth factor-β signaling pathway (TGFβ2: rs2796817, rs10482751, rs2027567, rs1209576; TGFβ2R: rs11466480; SMAD3: rs12901071, rs7162912, rs1438386, rs6494633, rs12910698, rs4776309) by using the TaqMan Allelic Discrimination assay.

RESULTS: rs1438386, one of the 12 single nucleotide polymorphisms in the TGF-β signaling pathway is significantly associated with Kawasaki disease susceptibility. However, no significant associations between 12 single nucleotide polymorphisms and coronary artery lesion (CAL) formation or intravenous immunoglobulin (IVIG) treatment response were observed. Haplootype analysis did not yield any significant result.

CONCLUSIONS: This study showed that genetic polymorphism rs1438386 associated with KD susceptibility, but not CAL formation, or IVIG treatment response in the Taiwanese population.

239 The German Mouse Clinic (gmc): A Systemic Phenotyping Platform To Uncover New Models For Allergic Diseases

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RATIONALE: A series of new candidate genes for allergic diseases has been uncovered by the use of a comprehensive systemic primary screen which covers, amongst others, the areas of immunology and allergy in the German Mouse Clinic (GMC). High-throughput strategies for the detection of allergy-prone mutant mice have been applied to identify phenotypic alterations in genetically modified mice with the aim to find new strategies for allergy diagnosis and anti-allergic therapy.

METHODS: Total IgE levels in murine plasma are used as the first-line allergy-screening parameter. Mutant lines showing an interesting phenotype are subjected to a more in-depth assessment. This contains a well-established challenge-screen that includes a model of allergic sensitization and aerosol challenge. The subsequent analysis includes high-throughput quantification of immunoglobulins (bead-array technology), classification of cells from bronchoalveolar-lavage (flow-cytometry), immune phenotyping of lymphocytes, and quantification of multiple cytokines.

RESULTS: This study has emerged new and interesting mouse mutant lines with particular allergy-phenotypes. Amongst the unexpected findings were sex-hormone-dependent levels of IgE, distinct T cell patterns after allergen-challenge in mice with a defect in intracellular vesicle transport, and a skewed airway inflammatory response in mutants with a defect in LPS-induced apoptosis caused by a gene of the SET domain family, known to be involved in methyl transferase activity.

CONCLUSIONS: We have successfully revealed distinct gene functions in mutant mouse lines that enhance or reduce allergic disease in the murine model. These high-throughput technologies provide important advances linking new and previously unexpected genes to the pathophysiology, diagnosis, and potential therapy of allergic disorders. (support: BMBF-NFENplus-01GS0868)

240 Exposure to Indoor Allergens in Urban Elementary Schools and Homes of Children with Asthma

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RATIONALE: Most studies of indoor allergens have focused on home environment. However, schools may be an important site of allergen exposure for children with asthma.

METHODS: Settled dust and airborne samples from classrooms, gymnasiums, and cafeterias in 12 inner-city schools were analyzed for indoor allergens by MARIA technology. School samples were linked to students with asthma enrolled in the School Inner-City Asthma Study to determine the presence of indoor allergens in their school environment. Bedroom settled dust samples of students were analyzed for indoor allergens in the same manner.

RESULTS: From schools, 229 settled dust and 197 airborne samples were obtained. From homes, 118 settled dust samples were obtained. Generalized linear models using logit links showed significantly higher school settled dust levels of mouse (OR = 6.45, 95% CI: 3.60, 11.56, P < 0.0001), dog (OR = 2.44, 95% CI: 1.47, 4.04, P < 0.0008), and cat (OR = 2.98, 95% CI: 1.46, 6.09, P < 0.0032) as compared to homes. Less than 25% of enrolled students had pets at home. Settled dust mouse allergen levels in classrooms were moderately correlated with airborne levels (r = 0.48, P < 0.0001).

CONCLUSIONS: Inner-city children with asthma were exposed to higher levels of mouse, dog and cat allergens in their schools as compared to their homes, with Mus m 1 being the highest. There was a significant correlation between classroom settled dust and airborne Mus m 1 levels only. Further studies are needed to evaluate the role of school indoor allergen exposure and its effect on asthma morbidity in students with asthma.

241 Reduction in Dust Allergen Exposure through Healthy Homes Education

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RATIONALE: Asthma is typically related to exposure to protein allergen triggers found in house dust and indoor environments. To examine reductions in dust borne allergen triggers after institution of a Healthy Homes program the following studies were conducted.

METHODS: Families with at least one asthmatic child were enrolled into a healthy homes program. Homes were evaluated for indoor and outdoor environmental conditions initially and subsequently to 6 months participation. Residents received education on maintaining a safe and healthy home and assistance in remediating unsafe or unhealthy conditions. Dust collections were taken in the asthmatic subject’s bedroom using a HUD developed protocol. Dust collections were analyzed for Fel d1, Can f1, Der f1, Der p1, Mus m1 and Bla g2 as well as fungal antigens from Alternaria, Aspergillus, Cladosporium and Penicillium.

RESULTS: Initial mean dust measurements for allergens from the first 12 homes to fully complete the study varied from 7.6 mcg/g dust for Penicillium to 3.0 mcg/g dust for Aspergillus. Mean specific allergen values varied from 1.1 mcg/g for Mus m1 to 0.16 U/g for Bla g2. After at least 6 months participation dust borne allergen levels were reduced at least 50% for Fel d1, Penicillium, Bla g2 and Mus m1. Significant reductions were seen for Bla g2. Measured allergen levels remained unchanged or increased for Can f1, Der p1 and Aspergillus.

CONCLUSIONS: Healthy homes education along with remediation can result in reduction in dust borne allergen levels. More research is needed to understand specific factors related to the removal of dust borne allergenic material.
Neither Dust Mite nor Cat Allergen Exposure Is Associated with Lung Function or Asthma Morbidity in Sensitized Baltimore City Children

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RATIONALE: Although pest allergen exposures are associated with asthma morbidity in sensitized Baltimore City children, the effects of dust mite (DM) and cat allergen (Ct) exposures on asthma in this population are unclear.

METHODS: 144 children (5-17y) with asthma were skin prick tested (SPT) at baseline. Lung function, health care utilization data, and bedroom dust samples were collected at baseline, 3, 6, 9, and 12 months. Der f 1 and Fel d 1 content in dust samples was quantified by ELISA. +SPT was defined as a net wheal >3mm. Exposure was defined as bedroom DM>2 µg/g and bedroom Ct> 8 µg/g. Analyses were adjusted for age, gender, type of insurance, total IgE, and sensitization to exposure and mouse and cockroach.

RESULTS: Participants were predominantly African American (91%), had public health insurance (85%), and had ≥1 +SPT (90%). FEV1/FVC% did not significantly differ between cat sensitized and exposed (S+E+) and non-cat-sensitized or non-cat-exposed (S-E-) participants (predicted% [95% CI]: (81.3[78.8-83.7]) and 80.2[79.4-82.0], respectively; p=0.61). This was also true for DM (S+E+: 81.8[78.8-84.8]; S-E-: 80.8[79.5-82.1], p=0.5). Acute asthma visits were similar for the Ct S+E+ and Ct S-E-groups (OR[95%CI]: 1.13[0.61-2.12]). The same trend held for DM S+E+ vs DM S-E- participants (0.41[0.16-1.03]). These findings were similar across various exposure cutpoints and with or without adjustment for pest allergen exposure and sensitization.

CONCLUSIONS: DM and Ct allergen exposures are not major contributors to asthma morbidity in Baltimore City children. Public health initiatives in Baltimore should focus on pest allergens, which are known to contribute to asthma morbidity.

Indoor Airborne Spore Levels Before and After Healthy Homes Education and Remediation

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RATIONALE: Asthmatic children are often sensitization to airborne fungi yet many live in homes containing poor indoor air quality (IAQ). To examine the reduction in airborne spores at the beginning and after 6 months enrolment in a Healthy Homes program the following studies were conducted.

METHODS: Homes enrolled in this project contained at least one child with asthma. Homes were evaluated for indoor and outdoor environmental conditions related to the maintenance of IAQ at the time of enrolment and after 6 months of participation in a healthy homes program. After enrolment, residents of these homes received education on maintaining a safe and healthy home along with assistance in remediating unsafe or unhealthy IAQ conditions in the home. Air collections were taken using a BioAire™ Model B520 spore trap both before and after education/remediation at two locations outside the home and at 5 locations inside the home. Air collections were evaluated microscopically for commonly identified spores.

RESULTS: Results from the first 10 homes to fully complete the study indicate the major fungal spores present include Cladosporium (before 97% of collections; after 90% of collections); Aspergillus/Penicillium (87%/75%); Alternaria (35%/45%) and Stachybotrys (24%/6%). Overall spore reductions in the child’s bedroom for these 10 homes included Stachybotrys (100%), Aspergillus/Penicillium (55%). Mean total indoor spore levels were reduced from 24% of outdoor ambient levels before the healthy homes program to 16% of outdoor ambient levels at completion.

CONCLUSIONS: Healthy homes education along with remediation for unsafe or unhealthy conditions resulted in substantial reduction in airborne fungal spore exposure.

Among Middle-income Children In NYC, Neighborhood Reports Of Mouse Sightings Were Associated With Sensitization To Mouse


RATIONALE: Previously, we observed that in middle-income NYC homes, mouse allergen in bed dust correlated with neighborhood asthma prevalence (NAP), but not with mouse sensitization among 7-8 year-old children. We then hypothesized that mouse sensitization would be associated with reported mouse sightings in a child’s home and neighborhood.

METHODS: The NYC Neighborhood Asthma and Allergy case-control study recruited 7-8 year-old children covered by a single health insurance plan through a parent’s employment from communities with a range in NAP (3-19%). Children with IgE >0.35 IU/ml were considered seroatopic. Parents were queried on recent mouse sightings in their home. The neighborhood level frequency of rodent sightings in residential buildings was obtained from a NYC Department of Health survey and matched to a child’s zip code.

RESULTS: Of 313 children, 38 (12.1%) were mouse seroatopic. Children living in homes where mice were sighted at least weekly (8.6% of children) were more likely to be mouse seroatopic than those who were living in a home with less frequent sightings (10.5% vs. 29.6% seroatopic, P=0.009).

CONCLUSION: Given the potential of mouse allergen for passive transfer, both community and individual residential mouse exposure may contribute to sensitization.
245 A Randomized, Double-Blind, Placebo-Controlled, Multicenter Trial of Egg Oral Immunotherapy in Children: An Analysis of Clinical Tolerance

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RATIONALE: Oral immunotherapy (OIT) has been used to induce clinical desensitization, but no OIT protocols have been evaluated in well-controlled trials to assess tolerance induction.

METHODS: Egg-allergic children (5-18 y/o) received daily OIT with egg white (n = 40) or placebo (n = 15). Initial escalation, build-up, and maintenance (2000 mg) phases were followed by an oral food challenge (OFC) to egg white at 10, 22, and 24 months. Immune mechanisms were evaluated.

RESULTS: Fifty-five subjects enrolled; 6/40 (15%) egg OIT and 2/15 (13.3%) placebo subjects withdrew before 24 months. After 10 months of therapy, 0/15 (0%) placebo and 22/40 (55%) egg OIT subjects were desensitized; after 22 months, 30/40 (75%) were desensitized. Egg OIT was then stopped for 6-8 weeks and subjects underwent another OFC; 11/22 (50%) egg OIT subjects were desensitized. Egg OIT treatment led to the following mechanistic changes: basophil activation declined from baseline to 22 months (p < 0.001); prick skin test size declined (p = 0.02); egg-specific IgG4 increased (p < 0.001); egg-specific IgE declined (N.S.). Among egg OIT subjects, smaller PST size at 22 months was correlated with desensitization (p = 0.009) and tolerance (p = 0.005), as was change from baseline to 22 months (p = 0.01).

CONCLUSIONS: These results establish that egg OIT can induce desensitization in most patients and clinical tolerance in some. Reductions in basophil and mast cell activation and increases in IgG4 antibody indicate effective immunomodulation and may predict desensitization and tolerance.

246 Peanut Challenge Outcomes Following Sublingual Immunotherapy (SLIT) Correlate With Increased Peanut-Specific Salivary IgA

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RATIONALE: Our group has recently demonstrated that SLIT for peanut allergy causes desensitization in a double-blind, placebo-controlled trial. However, there is variation in the amount of peanut protein during double-blind, placebo-controlled food challenge (DBPCFC) that elicits allergic symptoms in subjects receiving SLIT. We investigated whether peanut-specific IgA levels in saliva and serum would be indicative of peanut challenge outcomes.

METHODS: Saliva and serum were collected at baseline and at time of DBPCFC approximately 12 months into the study. Peanut-specific IgA and secretory-IgA were measured by ELISA. Peanut-specific IgA in serum was measured by ImmunoCAP. Significance between matched-pairs was determined by the Wilcoxon Signed-Rank test; Correlations were determined by linear-regression analysis.

RESULTS: Subjects receiving SLIT (n = 10) for 12 months had an increase in peanut-specific salivary IgA (p < 0.05) and peanut-specific secretory-IgA (p < 0.01), whereas subjects on placebo (n = 7) did not change over 12 months. Peanut-specific serum IgA also increased for the SLIT group (p < 0.05) but not the placebo group. There was a strong correlation between mg of peanut protein ingested on DBPCFC and change in peanut-specific salivary IgA (R² = 0.52; p < 0.001). A significant, but more modest correlation was found between DBPCFC outcome and peanut-specific secretory-IgA (R² = 0.35; p < 0.013). No correlation was found between peanut-specific IgA in serum and DBPCFC outcome.

CONCLUSIONS: Increased peanut-specific IgA in saliva from subjects undergoing peanut SLIT are associated with an ability to ingest larger amounts of peanut during DBPCFC following SLIT. Changes in salivary peanut-IgA may represent a biomarker to monitor efficacy of SLIT for peanut allergy.

247 Plasma from Subjects on Peanut Oral Immunotherapy (OIT) Suppresses ex vivo Basophil Activation in Peanut-Allergic Subjects

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RATIONALE: We have previously reported that peanut-allergic subjects on peanut OIT show a decrease in basophil responses to ex vivo peanut stimulation during the course of treatment. We investigated the possible involvement of a plasma factor in this decrease.

METHODS: Plasma was removed from whole blood samples from peanut-allergic subjects (n = 10, median peanut-specific IgE 82.3 kU/L) and replaced with plasma from subjects receiving peanut (n = 7) or placebo (n = 5) OIT. After stimulating the samples with peanut, basophil activation was assessed by measuring up-regulation of CD63. Plasma samples from 0 and 12 months on OIT were used. Statistical analyses were performed with Prism software.

RESULTS: Peanut-allergic donor samples receiving plasma from subjects on peanut OIT for 12 months showed a significant decrease in basophil activation when compared to samples receiving plasma from subjects on peanut OIT for 0 months (n = 28, p < 0.0001) and when compared to samples receiving plasma from subjects on placebo OIT for 12 months (n = 21, p < 0.0001). Basophil activation between samples receiving plasma from 0 and 12 months on placebo OIT (n = 21) was not significantly different. For the transferred plasma, there was an increase in peanut-specific IgG between 0 and 12 months on peanut OIT (median increase 18 mg/L to 108 mg/L; p < 0.01), and no increase for placebo.

CONCLUSIONS: A factor in the plasma of subjects on peanut OIT suppressed ex vivo basophil activation in peanut-allergic subjects. We hypothesize that the factor is peanut-specific IgA, although further studies are needed to definitively identify it and determine its mechanism of action.
Sublingual Immunotherapy for Peanut Allergy: A Randomized, Double-Blind, Placebo-Controlled Multicenter Trial (CoFAR) 

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RATIONALE: To investigate the safety, efficacy, and immunologic effects of peanut sublingual immunotherapy (SLIT).

METHODS: After a baseline oral food challenge (OFC) of up to 2gm (median tolerated dose 46mg), 40 subjects, aged 12-17 (median 15) years, were randomized 1:1 across 5 sites to receive daily peanut (escalating from .00017mcg to 1386mcg) or placebo SLIT. A 5gm OFC was performed after 44 weeks. Placebo subjects then crossed-over to peanut SLIT to a maximum of 3696mcg, followed by a week-44 5gm OFC. Week-44 OFC was compared to baseline OFC: subjects successfully consuming 5gm or at least 10-fold more peanut than baseline OFC threshold were considered “responders.”

RESULTS: 14/20 (70%) subjects receiving peanut SLIT were responders compared to 3/20 (15%) receiving placebo (p<0.001). In peanut-SLIT responders, median successfully consumed dose increased from 3.5mg to 496mg; none tolerated the entire 5gm OFC. Of 10,701 peanut doses through week-44 OFCs, 61.6% were symptom-free; excluding oral/pharyngeal symptoms, 95.2% were symptom-free. Basophil activation did not decline significantly from baseline to 44 weeks; median peanut-IgE levels significantly declined from weeks 29 to 44 in the peanut group compared to placebo (p=0.01). 6/15 crossover subjects with week-44 OFC assessment to date were responders; median successfully consumed dose increased from 11mg to 496mg. Three subjects (2 peanut, 1 placebo) withdrew during the blinded phase prior to week-44 OFCs; 9 subsequently withdrew (3 peanut, 6 original placebo).

CONCLUSIONS: Initial results demonstrate that peanut SLIT can safely and efficaciously induce clinical tolerance, particularly for women and young adults, are needed.

Development of Clinical Tolerance after Peanut OIT

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RATIONALE: This study aims to identify immunologic changes accompanying development of clinical tolerance in subjects completing a peanut OIT protocol.

METHODS: Nineteen subjects with peanut allergy completed an OIT protocol in 3 phases: initial desensitization, build-up, maintenance. All subjects underwent an oral food challenge (OFC) 4 weeks after stopping OIT to evaluate clinical tolerance. Skin prick testing (SPT), peanut-specific IgE (Pn-IgE) and IgG4 (Pn-IgG4) were performed throughout the study.

RESULTS: After 33-70 months on peanut OIT, 11/19 (58%) subjects (median age 11.2 years) meeting criteria passed a peanut OFC 4 weeks after stopping OIT and are considered clinically tolerant with regular dietary consumption of peanut. During OFC, 19 subjects had increased consumption of peanut (median of 5000 mg), when compared to initial day consumption of <50 mg that produced symptoms. Median baseline Pn-IgE was 84.1 kU/L (range 9.1-401 kU/L). While on OIT, Pn-IgE levels declined significantly from baseline to tolerance OFC (median decrease 57.4 kU/L, p=0.0001). Pn-IgG4 levels increased with treatment (median increase 9.4 mg/L, p=0.0001). The Pn-IgE/IgG4 ratio decreased with treatment (p=0.0001). SPT size decreased over time (median decrease 7.5 mm, p=0.0011). The 11 subjects who were tolerant had lower baseline Pn-IgE (p=0.0057), lower final Pn-IgE (p=0.0015), and lower baseline SPT (p=0.0229) compared to subjects who did not pass the OFC off therapy.

CONCLUSIONS: 58% of peanut allergic subjects were able to discontinue peanut OIT after a median 44 months of treatment and regularly consume peanuts in their diet. Mechanistic immune studies support the development of clinical tolerance.

Differences in Asthma Controller Medication Adherence by Age and Gender

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RATIONALE: Adherence to asthma controller medication is thought to improve clinical outcomes; however, literature examining the relationship between age and gender and adherence is limited. This study investigated the relationship between controller medication adherence and age and gender in an administrative claims database.

METHODS: Asthmatics were identified from MarketScan medical claims from January 2006-December 2008. Medication Possession Ratios (MPR) were calculated for controller medications: mast cell-stabilizing agents (MCSA), inhaled corticosteroids (ICS), long-acting beta-agonists (LABA), ICS-LABA combinations (ICS-LABA), leukotriene modifiers (LM), chronic oral steroids (COS) (continuous use ≥3 months), monoclonal antibodies (MA), and theophylline. Adherence was classified as low, medium, and high based on MPR tertile for each medication category. Age was categorized as 18-34, 35-49, and 50-65. Linear associations between age and adherence were calculated using the Mantel-Haenszel Chi-Squared test, and Chi-squared tests were conducted for gender and adherence categories.

RESULTS: The study included 53,532 men and 92,418 women. Average MPR ranged from 0.20 (women, MCSA) through 0.76 (men and women, COS). There was a significant difference (p < 0.0001) positive linear association between age and adherence for all drug categories, except for COS. Men were more adherent with MCSA (p=0.014), ICS (p<0.0001), LABA (p<0.0001), and ICS-LABA (p<0.0001). Women demonstrated better adherence with LM (p < 0.0001).

CONCLUSIONS: Overall, compliance with controller therapy was poor in this study. Older and male asthmatics are more likely to be adherent to most controller medications. Further studies examining barriers to adherence, particularly for women and young adults, are needed.

Survey of Asthma Management and Referral Preferences by Primary Care Pediatricians at a Pediatric Training Hospital

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RATIONALE: Since publication of the updated NAEPP Expert Panel Report 3 (EPR 3) asthma guidelines in 2007, there has been minimal literature exploring the practice and referral habits of primary care physicians regarding asthma management in the pediatric population.

METHODS: An electronic survey consisting of 23 questions was distributed to 80 primary care pediatrics at the authors’ institution. Questions focused on current asthma management in specific situations, use of the EPR 3 guidelines and referral preferences to sub-specialists.

RESULTS: There was a 32.5%(26/80) response rate. We found that 12/26(46.2%) of physicians “always” utilized the EPR 3 guidelines, and 10/26(38.5%) responded they didn’t so because they felt the guidelines were too cumbersome. In persistent asthmatics, 16/26(61.5%) reported they initiate inhaled corticosteroids. From those physicians who do not, 9/11(81.8%)reported patient non-compliance as the major barrier. After initiating controller medication, 12/24(50%) never step down therapy or wait until after one year. The main reason for sub-specialist consultation was difficult to manage cases 23/24(95.8%), followed by request for additional testing 12/24(50%) and assistance with diagnosis 10/24(41.7%). 0/24(0%) referred patients with asthma to an allergist alone, while 17/24(70.8%) preferred referral to a pulmonologist.

CONCLUSIONS: Perceptions of asthma treatment practices amongst primary care pediatricians are varied. Our survey is unique in that we specifically polled pediatrics involved in the education of residents and medical students. Allergists can be helpful in educating primary care pediatricians, especially within their own teaching institutions, about current management strategies and the role of the allergist in the care of asthmatic patients.
252 The Comparison of Asthma Control Judgment Based on Japanese Guidelines for Asthma, GINA, EPR3, and ACT


RATIONALE AND METHODS: The criteria for assessment of asthma control status was newly defined in Japanese Guidelines for Asthma (JGL) 2009. In JGL 2009, “well-controlled” status is defined as having no symptoms, use of reliever, and the criteria is stricter than international guidelines Global Initiative for Asthma (GINA) and Expert Panel Report 3 (EPR3). We compared the difference in judgment of asthma control status of 70 patients based on JGL, GINA, EPR3 and Asthma Control Test (ACT).

RESULTS: The ratio of “well-controlled”, “insufficiently-controlled” and “poorly-controlled” patient was 50%, 35.7%, 14.3% when judged by JGL, 58.6%, 30%, 11.4% by GINA, and 62.9%, 27.1%, 10% by EPR3, respectively, suggesting that the JGL criteria was stricter than others. When judged by ACT, 75.7% of patients were classified as totally or well-controlled (score ≥ 20 points) and 24.3% as not-well controlled (score < 19). Although 35 patients were judged as insufficiently or poorly-controlled by JGL, GINA and EPR3 judged 6 and 9 out of 35 patients as well-controlled, respectively. The difference in judgment was mainly based on the allowance of symptoms or use of reliever 2 times per week. None was judged as “well-controlled” by JGL if patients showed ACT score ≥ 21 points.

CONCLUSIONS: The assessment criteria for control status in JGL was stricter than GINA, EPR3 and ACT and “well-controlled” judgment in JGL required ACT score ≥ 22 points. The significance of treatment step-up in patients judged as “insufficiently-controlled” only by JGL should be further clarified in view of pulmonary function, symptom, cost, and QOL.

253 Combined Corticosteroid Use in Patients with Asthma, Allergic Rhinitis, and Atopic Dermatitis

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RATIONALE: Asthma, allergic rhinitis, and atopic dermatitis, termed “allergic triad”, are global health concerns that affect millions of children worldwide and are often managed by different corticosteroid preparations. Few studies have examined the prevalence of combined corticosteroid use in patients with the allergic triad.

METHODS: This study is a retrospective review of medical records of patients ages 1 to 18 years, followed in the Allergy and Immunology division at Cohen Children’s Medical Center from 9/1/2000-9/1/2010 with at least two ICD-9 diagnoses of asthma (493.493.1, 493.9), allergic rhinitis (470, 477.2, 477.9), and/or atopic dermatitis (691.8).

RESULTS: Out of 197 charts, 48% of patients were identified with three combined diagnoses of asthma, allergic rhinitis, and atopic dermatitis and 40% of these patients were treated with corticosteroids for all three diagnoses. Remainder of the patients were diagnosed with at least two conditions, subdivided into Group 1 (asthma and allergic rhinitis), Group 2 (asthma and atopic dermatitis), and Group 3 (allergic rhinitis and atopic dermatitis). More than 50% of patients in Group 1 and 2 were treated with corticosteroids for both diagnoses simultaneously. Overall, corticosteroids were being prescribed by at least four physicians of different disciplines for each diagnosis.

CONCLUSIONS: Patients with allergic triad are treated with different types of corticosteroid preparations that are prescribed by multiple physicians to control their symptoms. These findings identify a unique subset of population who are potentially at risk for substantial corticosteroid exposure and reinforce the need for improved communication and coordination of care among physicians.
**255 Differential Regional Expression Of Innate Immune Antimicrobial Proteins In Sinonasal Mucosa**

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**RATIONALE:** Nasal mucosal innate host defense is essential for suppressing growth of pathogenic organisms and proper functioning of the upper airways. It has been recognized for long that sinonasal tissues express antimicrobial proteins important in host defense; however the regional distribution of these antimicrobial proteins in upper airways has not been studied in detail. In this study we analyzed the expression of selected antimicrobial proteins in sinonasal tissues at the front (inferior turbinate-IT) and rear (uncinate tissue - UT) of the anterior chamber of the nose in control subjects.

**METHODS:** Tissue samples were collected and analyzed by ELISA for expression of the antimicrobial proteins beta defensin-2 (hBD2), S100A7, SPLUNC1, lactoferrin, and pentraxin-3. Immunohistochemical analysis (IHC) was performed to further characterize the localization of two proteins (S100A7 and SPLUNC1) in sinonasal tissues samples.

**RESULTS:** We observed a 4-21 fold regional differential expression of antimicrobial proteins in the sinonasal mucosa. S100A7 and hBD2 were more highly expressed in IT compared to UT, whereas lactoferrin and SPLUNC1 were more highly expressed in UT compared to IT. Interestingly, expression levels of pentraxin-3 were similar in IT and UT. We confirmed the differential localization of S100A7 and SPLUNC1 by IHC analysis. S100A7 was primarily expressed in mucosal epithelium and glands in IT. SPLUNC1 was highly expressed primarily in UT and was mostly glandular with minimal staining in mucosal epithelium.

**CONCLUSIONS:** Our findings indicate the existence of marked regional variability in the expression of antimicrobial proteins in sinonasal tissues suggesting specialized regional functions of these proteins within the sinonasal cavity.

**256 STAT3 and NF-kB Regulate S100A7 Expression in Human Bronchial Epithelial Cells**

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**RATIONALE:** Previously, we have shown that S100A7 is decreased in nasal lavage fluids from patients with chronic rhinosinusitis (CRS). Loss of expression of this host defense molecule may contribute to CRS pathogenesis. Elucidating the mechanisms responsible for S100A7 expression may provide insight into the complex pathogenesis of CRS.

**METHODS:** Normal human bronchial epithelial cells (NHBEs) were stimulated with various cytokines with or without inhibitors of STAT3 or NF-kB. Expression of S100A7 mRNA and protein was assessed by qRT-PCR and ELISA. STAT3 and NF-kB activation was assessed by western blot. Nasal polyp (NP) and uncinate tissues (UT) from patients with CRS with or without polyps (CRSsNP and CRSwNP), and normals were extracted and analyzed by western blot or ELISA.

**RESULTS:** Basal levels of pSTAT3 were significantly reduced in NP, as well as UT from patients with CRSsNP and CRSwNP, compared to UT from normals (p<0.0001). In vitro, epithelial S100A7 gene expression was poorly induced by STAT3 activators (IL-22; 6 fold increase over media alone), but was synergistically induced by a combination of IL-22 with either TNF or IL-17 (71 fold and 212 fold increase respectively). Inhibition of either STAT3 or NF-kB activation reduced S100A7 expression to basal levels. Interestingly, while TNF rapidly induced activation of NF-kB, IL-17 stimulation did not, and these cytokines may function through different mechanisms.

**CONCLUSIONS:** STAT3 and NF-kB were required for robust induction of S100A7 in NHBEs. The profound decrease in pSTAT3 seen in CRS tissues may account for the reduction of S100A7 in these patients and contribute to disease pathogenesis.

**257 Clinical Characteristics of Patients with Chronic Rhinosinusitis and Specific Antibody Deficiency**

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**RATIONALE:** Specific antibody deficiency (SAD) is characterized by an inadequate polysaccharide vaccine response and is considered in patients with chronic rhinosinusitis (CRS). There is limited data on patients with CRS and SAD. We assessed the clinical characteristics, comorbidities and outcomes of these patients.

**METHODS:** We reviewed the electronic database records of patients with CRS who were evaluated for immunodeficiency with quantitative immunoglobulins or pneumococcal antibody titers checked pre- and post-Pneumovax® between 2002 and 2011. The subjects’ clinical and laboratory results were assessed.

**RESULTS:** Of 528 subjects with CRS, 149 (28%) had CRS with nasal polyps (CRSsNP) and 379 (72%) had CRS without nasal polyps (CRSsNP). Of the 247 subjects evaluated for SAD, 50 (20%) had an inadequate response to the vaccine (10 of 68 [15%] in the CRSsNP group and 40 of 179 [22%] in the CRSwNP group). Nonresponders had significantly lower IgG levels, although within normal range (>700 mg/dL), compared with responder and normal baseline subjects (874.0 ± 308.2 vs. 956.7 ± 212.4 vs. 1022.5 ± 281.0, p<0.01). Nonresponders received more antibiotics courses relative to responders in the two years following Pneumovax® (3.12 ± 2.52 vs. 2.30 ± 2.42, p<0.05). CRSsNP asthmatics had significantly lower post-immunization titers compared to CRSsNP non-asthmatics (9.17 ± 3.28 vs. 7.49 ± 3.585, p<0.05). Eleven nonresponders (22%) received immunoglobulin (Ig) replacement therapy.

**CONCLUSIONS:** Of 247 CRS patients evaluated for immunodeficiency, 20% had SAD. On average, those with SAD received more courses of antibiotics than those without. Eleven patients with SAD (22%) received Ig therapy over an eight-year time period, suggesting that a majority of these patients do not need Ig therapy.
**Omalizumab Is Effective In Allergic And non-allergic Patients With Nasal Polyps And Asthma**

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**RATIONALE:** Adult patients with nasal polyps often suffer from comorbid asthma, adding to the serious impact on quality of life of these patients. Nasal polyps and asthma may represent a therapeutic challenge. Omalizumab is a human monoclonal anti-IgE antibody with proven efficacy in severe allergic asthma. The goal of this study was to investigate the clinical efficacy of omalizumab in patients with nasal polyps and comorbid asthma.

**METHODS:** A randomized double-blind, placebo-controlled study of 24 patients with nasal polyps and comorbid asthma was conducted. Subjects received 4 to 8 (subcutaneous) doses of Omalizumab (n=16) or placebo (n=8) depending on serum IgE concentrations (30-700kU/l) and body weight. The primary endpoint was the reduction of the total nasal endoscopic polyp score after 16 weeks. Secondary endpoints included a change in sinus CT-scan, nasal and asthma symptoms, validated questionnaires (SF-36, RSOM-31 and AQLQ) and serum/nasal secretion biomarkers.

**RESULTS:** There was a significant decrease in total nasal endoscopic polyp score after 16 weeks in the omalizumab-treated group compared to placebo (−2.67; P=0.001), which was confirmed by CT-scan (Lund-Mackay score). Omalizumab had a beneficial effect on airway symptoms (nasal congestion, anterior rhinorrhea, loss of sense of smell, wheezing and dyspnea) and on the quality of life scores, irrespective of the presence of allergy.

**CONCLUSIONS:** Omalizumab demonstrated clinical efficacy in the treatment of allergic and non-allergic patients with nasal polyps and asthma, supporting the importance and functionality of local IgE formation in the airways.

**IL-21 Is Increased in Nasal Polyposis and after Stimulation with Staphylococcus Aureus Enterotoxins**

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**RATIONALE:** Chronic rhinosinusitis with nasal polyposis (CRSwNP) is a T-helper 2 mediated inflammatory response associated with eosinophilic infiltration and local IgE production. This local hyperglobulinemia is associated with germinal center like structures. T-follicular helper (Tfh) cells and their main effector cytokine interleukin-21 (IL-21) play an important role in germinal center B-cell proliferation, survival, and maturation.

**METHODS:** The expression of IL-21 in nasal tissue was determined by qPCR in three groups of patients: control, chronic rhinosinusitis without nasal polyposis (CRSsNP) and with nasal polyposis (CRSwNP). Moreover the expression of IL-21 mRNA was measured in nasal polyp tissue after stimulation with 0.5 Ig/ml staphylococcus enterotoxine B (SEB) during 24 hours. By FACS we analyzed presence of CD4+ T cells producing IL-21 in nasal polyp tissue. Furthermore single cell suspension of nasal polyp tissue was incubated with SEB and a FACS analysis was performed.

**RESULTS:** IL-21 mRNA expression was increased in nasal tissue of patients with CRSsNP (p=0.04) and CRSwNP (p=0.03) versus control tissue. Furthermore SEB was able to increase IL-21 significantly (p=0.0008) in nasal polyps. After FACS analysis IL-21+CD4+ T cells were identified in polyp tissue namely IL-21+IFNγ- cells and IL-21+IL-17- cells. After incubation with SEB, an upregulation of IL-21+IFNγ- cells (4.8% to 6.9%) and IL-21+IL-17- cells (6.1% to 9%) was seen.

**CONCLUSIONS:** IL-21 is increased in CRSwNP and an increase of IL-21 is measured after SEB stimulation. Therefore, we speculate that T-follicular helper cell produced IL-21 plays a role in the pathophysiology of nasal polyposis by stimulating local hyperglobulinemia and germinal center formation.

**The Interaction Between Ara h 1 and TSLP Selectively Expands Skin-Homing Th2 Cells in Peanut-Sensitized Children**

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**RATIONALE:** The Th2-promoting cytokine, TSLP, is expressed by inflamed skin and has been implicated in the pathogenesis of atopic dermatitis (AD) and food allergy. We aimed to explore whether TSLP and Ara h 1 interact to amplify Th2 responses.

**METHODS:** PBMCs from peanut-sensitized children with a history of AD in early life (ages 3-19, geometric mean (gm) total IgE=339IU/ml) were stimulated with Ara h 1 ± TSLP. The phenotype of responder CD4+ T cells was assessed using CFSE dilution and flow cytometry analysis (day 8).

**RESULTS:** Co-stimulation with Ara h 1+TSLP induced a higher frequency of activated CFSE responder CD4+ T cells (gm=46%) than either Ara h 1 or TSLP alone (gm=30% and 3% respectively, p<0.05). Surprisingly, IL-4+ T cells were rare among TSLP-induced responder cells (<1%) indicating weak Th2 stimulatory capacity. While Ara h 1 induced a higher proportion of IL-4+ responder cells (18%), these cells were markedly amplified by TSLP (37% IL-4+ cells, p<0.01). The magnitude of Th2 amplification induced by TSLP was not related to anti-peanut IgE antibodies or total IgE titre. Notably, TSLP had no effect on Ara h 1-induced IFN-γ cells. Analysis of tissue-homing markers showed that TSLP preferentially enhanced Ara h 1-induced responder cells with skin-homing, but not those with lung- or gut-homing characteristics.

**CONCLUSIONS:** Our findings support an interaction between Ara h 1 and TSLP which drives the selective expansion of skin-homing Th2 cells and are consistent with the view that, in AD, cutaneous exposure to peanut allergen in the presence of TSLP promotes Th2-driven inflammation in vivo.

**Allergen Induces Dual Upregulation of TSLP Receptor on Circulating Basophils and Dendritic Cells in Atopic Dermatitis**

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**RATIONALE:** We previously reported a link between the high affinity IgG receptor, FcεRI, and thymic stromal lymphopoietin (TSLP) pathways in monocyte-derived dendritic cells from patients with atopic dermatitis (AD). Our findings indicated a propensity for dendritic cells (DCs) from atopic donors to upregulate TSLP receptor (TSLPR). We aimed to rigorously assess the capacity of diverse circulating immune cell types from AD patients to upregulate TSLPR in response to allergens and elucidate the mechanism involved.

**METHODS:** PBMCs isolated from AD and non-AD subjects were cultured with and without allergens, or cross-linking anti-IgE antibody (24 hours), and phenotyped by multi-color flow cytometry.

**RESULTS:** Stimulation with diverse allergens (Fel d 1, Der p 1 and Der p 2) induced TSLPR+ cells that were highly enriched within a lineage-negative population (CD3-CD14-CD16-CD20-CDS) and this phenomenon was restricted to AD patients. While TSLPR+ cells in the absence of any stimulus were exclusively CD11c+ myeloid DCs, allergen-induced TSLPR+ cells comprised predominantly of basophils (FceRI+HLA-DR CD123+). Wright-Giemsa staining of flow cytometry sorted TSLPR+ basophils confirmed their characteristic nuclear morphology and cytoplasmic granular content. Lesser TSLPR+ populations included a heterogeneous population of FceRI+HLA-DR+ myeloid DCs which expressed variable levels of CD11c, CD11c, CD123 and CD141, as well as rare CD34+ cells that were consistent with DC precursors. Cross-linking FceRI with anti-IgE antibody produced a TSLPR+ cellular profile comparable to that induced by allergen.

**CONCLUSIONS:** Our findings demonstrate allergen-triggered IgE-dependent upregulation of TSLPR on circulating basophils and DCs in AD, implying a dual role for these cells in promoting TSLP-driven Th2 responses.
262 IL-33 Mediates Both Innate and Adaptive Th2-type Responses Induced by Proteases in the Airway
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RATIONAL: Various airborne allergens have cytokine protease activities. When administered subcutaneously, a cytokine protease, papain, induces strong Th2-type responses. However, our knowledge regarding the roles of cytokine proteases in the airways is limited.
METHODS: Naive Balb/c mice or mice deficient in ST2 (i.e. IL-33 receptor) (ST2−/−), TLR4 (TLR4−/−), or Rag-1 (Rag-1−/−) were exposed intranasally to papain or bromelain. The adjuvant effects of these proteases were examined by exposing animals to ovalbumin (OVA) with or without proteases.
RESULTS: Airway exposure of naive mice to bromelain resulted in increased lung levels of IL-33 and TSLP within 3 h, followed by increases in IL-5 and IL-13 within 12 h. The responses were abolished by a cytokine protease inhibitor, E64, but were not affected in Rag-1−/− mice, suggesting involvement of protease activity and innate Th2 responses. Airway exposure of naive mice to OVA alone did not sensitize animals. In contrast, when OVA was administered with bromelain, mice produced anti-OVA IgE and IgG1, suggesting development of adaptive Th2 responses. These animals demonstrated robust airway Th2 cytokines, airway eosinophilia, bronchial hypertrophy and airway hyperreactivity when subsequently re-exposed to OVA alone. The innate and adaptive Th2-type immune responses in the airways induced by bromelain were nearly abolished in ST2−/− mice, but were enhanced in TLR4−/− mice.
CONCLUSIONS: Cysteine proteases, which are commonly found in airborne allergens, trigger robust Th2-type immune responses in the airways, and IL-33 is likely involved in this process. Cysteine proteases and IL-33 may play important roles in allergic airway diseases.

263 Roles for IL-33 and TSLP in Chronic Eosinophilic Airway Inflammation Induced by Airborne Allergen Exposure
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RATIONAL: A majority of experimental mouse models of asthma reflect acute airway inflammation induced by specific antigen [e.g. ovalbumin (OVA)] in sensitized animals. Because chronic airway inflammation is a distinct feature of human asthma, we sought to develop a mouse model to mimic the pathology and elucidate the immunological mechanisms.
METHODS: Non-sensitized naïve BALB/c mice were exposed to a mixture of natural allergen extracts, including Alternaria, Aspergillus, and house dust mite, and a model antigen, OVA (OAAH). Allergen exposure was carried out 3 times a week for up to 4 weeks, followed by examination of mice 24 hours after the last exposure. Mice deficient in ST2 (i.e. IL-33 receptor, ST2−/−) and TSLP receptor (TSLPR−/−) were also used.
RESULTS: A single exposure of naive mice to OAAH induced increased production of IL-33 and TSLP in the lungs in 3 or 6 hours. When mice were exposed to OAAH for 4 weeks, they developed pathological features of human asthma, including peribronchial infiltration of T cells and B cells, airway eosinophilia, mucus hypersecretion, and production of IL-4, IL-5 and IL-13. Mice also developed IgE and IgG1 antibodies to OVA. These pathological changes were abolished in Rag-1-deficient mice, suggesting that adaptive immunity is involved. Importantly, airway eosinophilia and production of Th2 cytokines and IgE antibody were significantly reduced in ST2−/− and TSLPR−/− mice by 20−70%, depending on the parameters examined.
CONCLUSIONS: Exposure to airborne allergens in naïve mice induces chronic airway inflammation resembling human asthma; IL-33 and TSLP likely play pivotal roles in this model.

264 Role of Thymic Stromal Lymphopoietin (TSLP) in Chronic Rhinosinusitis
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RATIONAL: Chronic rhinosinusitis with nasal polyposis (CRSwNP) is associated with Th2-dominant inflammation. Thymic Stromal lymphopoietin (TSLP) is a cytokine that triggers dendritic cell-mediated Th2 inflammatory responses and that enhances IL-1-dependent Th2 cytokine production in mast cells. Although elevated levels of TSLP mRNA have been found in nasal polypos (NP), expression of TSLP protein and its function in CRS have not been fully explored.
METHODS: We investigated the presence and stability of TSLP protein in NPs by ELISA and western blot, and the function of TSLP in nasal tissue homogenates using activation of human peripheral blood-derived mast cells.
RESULTS: Although TSLP mRNA was significantly increased in NP tissue from patients with CRSwNP (p<0.05) compared to uncinate tissue from patients with CRS or control subjects, TSLP protein was significantly decreased in NP tissue (p<0.05) reading by the commercial ELISA kit. We found that recombinant TSLP was time-dependently degraded by NP homogenates and this degradation was completely inhibited by a protease inhibitor cocktail, suggesting that TSLP is sensitive to tissue proteases. Interestingly, NP homogenate-treated TSLP had higher activity in mast cells, although the amount of full length TSLP was reduced by more than 50%. NP homogenates significantly enhanced IL-1β-dependent IL-5 production in mast cells compared with uncinate tissue homogenates from controls, and responses were significantly inhibited by anti-TSLP (p<0.05), suggesting that NP contained sufficient levels of TSLP and/or metabolic products of TSLP to activate mast cells.
CONCLUSIONS: TSLP and its metabolic products may play an important role in the inflammation in CRSwNP.

265 Influenza Vaccine Administration in Egg Allergic Children
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RATIONAL: Assess the safety of administering influenza vaccine to egg allergic children.
METHODS: 660 egg allergic children were evaluated for tolerance to influenza vaccine. Egg allergy was based on clinical history and positive egg skin test and/or positive serum egg IgE. Children were stratified based on tolerance to egg in baked goods and level of egg specific IgE. All patients were influenza skin test prior to administration of either full dose or split dose influenza vaccine and then observed for 30 minutes post vaccination.
RESULTS: 643 children with egg allergy ages 6 months to 13 years (mean age 5 years) received influenza vaccine. 3/643 (0.5%) children reacted adversely to the vaccine. Only local redness and swelling was observed. Of those with adverse reactions two received full dose and one split dose vaccine; skin test wheal to egg varied from <10mm to >20mm; and none had known tolerance to egg in baked goods. Tolerance to egg in baked goods was predictive of tolerance to influenza vaccine. 17 children did not receive influenza vaccine due to parental concerns or time constraints.
CONCLUSIONS: Our study suggests that it is safe to administer influenza vaccine to children with a wide spectrum of egg allergy. Reaction rates were low and only local reactions observed. All children with tolerance to egg in baked goods tolerated the influenza vaccine. Neither size of egg skin test nor level of serum egg specific IgE correlated with the likelihood of reaction to influenza vaccination. Splitting the dose did not show any benefit.
266 Experience With Carboplatin Desensitization: A Case Series A. Updegraff, D. Bestul, D. Doshi; 1William Beaumont Beaumont Hospital, Department of Internal Medicine and Pediatrics, Royal Oak, MI. 2William Beaumont Beaumont Hospital, Department of Pharmacology, Royal Oak, MI. 3William Beaumont Beaumont Hospital, Department of Internal Medicine and Pediatrics, Division of Allergy and Immunology, Oakland University William Beaumont School of Medicine, Royal Oak, MI.

RATIONALE: Carboplatin is a platinum base chemotherapy agent utilized in multiple malignancies. Many patients have experienced either true hypersensitivity reactions or severe adverse reactions to carboplatin. Given the necessity to receive therapy, desensitization is clearly indicated in this group of patients.

METHODS: This is a retrospective analysis of patients who underwent desensitization to carboplatin for clinical evidence of hypersensitivity or severe adverse reactions (non-IgE) to carboplatin from August 2005-January 2011 in our institution. A multidisciplinary team developed an institutional desensitization protocol utilizing appropriate pre-medications and an accelerating, graded increase in drug concentration. All patients were admitted to the ICU for desensitization with continuous monitoring, with a board certified allergist available.

RESULTS: Twenty patients with a previously identified reaction to carboplatin underwent 38 separate desensitization procedures. Of these, 35/38 (92%) procedures were completed successfully. There were no reported symptoms in 28/38 (74%) procedures. Minor symptoms were reported in 7/38 (18%) procedures which were relieved with a single dose of diphenhydramine allowing successful completion of therapy. Only 3/38 (8%) procedures were discontinued due to adverse reactions including hypotension, generalized pruritus with shortness of breath, and a reaction to another medication prior to initiating carboplatin desensitization. There were no major events of anaphylaxis or severe reactions necessitating epinephrine use or resuscitation.

CONCLUSIONS: Careful and selected desensitization in appropriate patients using a rigid protocol provides a safe opportunity for patients with documented hypersensitivity and/or severe adverse reactions to receive carboplatin. This allows completion of necessary chemotherapy at required doses without compromising cancer treatment.

267 Nasal Inflammatory Mediators In Non-stereoidal Anti-inflammatory Drugs (nsaids) Cross-intolerant Subjects After Lysine Nasal Challenge I. Dona1, P. Campo1, M. Sanak2, J. Cornejo2, A. Correa2, N. Blanca-Lopez2, M. Salas1, S. Sanchez2, G. Canto2, M. Blanca1; 1Allergy Department, Hospital Carlos Haya, Malaga, SPAIN. 2Molecular Biology, Dept. of Medicine, UJ CM, Krakow, POLAND. 3Allergy Laboratory, F. IMABIS, Malaga, SPAIN. 4Allergy Department, Hospital Infantia Leonor, Madrid, SPAIN.

RATIONALE: Cross-intolerant reactions to NSAIDs show differences in clinical and tissue response (respiratory/cutaneous), although it is believed that share a common mechanism by inhibition of COX-1 enzyme. The aim of the study was evaluating possible differences in mediators release in subjects with hypersensitivity reactions to NSAIDs with respiratory vs. cutaneous involvement.

METHODS: Subjects with confirmed history of hypersensitivity to NSAIDs (≥2 episodes with at least 2 different NSAIDs or positive drug provocation) and tolerant controls were recruited. Nasal lavage at times 0, 15, 60 and 120 minutes after lysine-aspirin challenge was analyzed for ECP, tryptase, PGE2, PGD2, LTD4 and LTE4 release.

RESULTS: Twenty cutaneous, 20 respiratory subjects and 10 controls were recruited. Subjects with respiratory involvement had higher levels of ECP at baseline (51.09 μg/mL), and higher increase at 15, 60 and 120 minutes after 1 challenge (73.72, 88.16 and 23.8 μg/mL) compared to cutaneous involvement (2.59, 3.48, 2.9 and 2.76 μg/mL) and controls. Similar results were obtained for tryptase levels (respiratory: 2.08, 1.15, 10.56 and 1.19 μg/mL) when compared to cutaneous (1.1, 1.1 and 1 μg/mL) and controls. Respiratory subjects showed higher baseline PGE2 levels (74.5 pg/ml) that decreased at 15 (55.72 pg/ml) and 30 minutes (23.18 pg/ml) after challenge as compared to cutaneous (25.7, 18.6 and 9.8 pg/ml), PGD2, LTD4 and LTE4 were increased at 60 minutes in respiratory subjects vs. cutaneous and controls.

CONCLUSIONS: Subjects with hypersensitivity to NSAIDs manifested by respiratory involvement showed higher levels of mediators release in nasal lavage compared to those with cutaneous involvement.

268 Anaphylaxis as a Potential Cause of Death in Heroin Users X. Zhou1, M. White1, L. Lau1, S. Williams1, A. C. Bateman2, E. Abu2, A. F. Walls3; 1University of Southampton, Southampton, UNITED KINGDOM. 2Southampton University Hospitals NHS Trust, Southampton, UNITED KINGDOM.

RATIONALE: Fatalities associated with heroin abuse are common, but the processes that lead to death are frequently unclear. Reports of high levels of tryptase in the blood have raised the possibility of an anaphylactic reaction having occurred. There is a need to investigate other markers of mast cell activation and to examine the potential for allergic sensitivity.

METHODS: Blood samples were collected post mortem from 20 cases of heroin-associated death (aged 27–65; median 36; 20M/0F) and 20 of non-drug-related death (aged 18–86; median 45; 13M/7F). Concentrations of mast cell carboxypeptidase, chymase and tryptase were measured by specific ELISA. The presence of heroin-specific IgE levels was determined by indirect ELISA and by measurement of β-hexosaminidase release following addition of heroin to cells of the LAD-2 mast cell line which had been passively sensitized with the serum samples.

RESULTS: Concentrations of mast cell carboxypeptidase were greater in blood from heroin-associated cases than in the non-drug related deaths (p=0.01). Levels of chymase were also greater (p=0.03), though the difference in tryptase levels did not reach significance. Addition of heroin to LAD-2 cells provoked non-cytotoxic β-hexosaminidase release (up to 20% net) at a concentration of 10 and 100 μM, but this did not appear to be mediated through IgE. However, by ELISA, heroin-specific IgE levels were greater in the blood of heroin-associated than non-drug related deaths (p=0.03).

CONCLUSIONS: The evidence for extensive mast cell activation and the presence of heroin-specific IgE in the circulation calls attention to the potential for anaphylactic mechanisms underlying sudden death in heroin users.

269 Beta Blocker Pretreatment before Coronary CT Angiography does not Increase the Rate of Contrast Reactions C. B. Lauter, G. L. Raff, K. M. Chinnayani, A. Abidov; William Beaumont Hospital, Royal Oak, MI.

RATIONALE: Coronary computed tomography angiography (CCTA) is an accurate noninvasive test for coronary artery disease. Beta blocker premedication is required in most patients. Previous studies suggest atopic patients are at higher risk for contrast allergy after beta blocker administration.

METHODS: This retrospective study reviewed 9083 CCTA patient records between April, 2009 and July, 2010 from the Advanced Cardiovascular Imaging Consortium (ACIC), a Blue Cross Blue Shield of Michigan registry. Data was examined for prior history of contrast allergy, premedication with antihistamine and/or steroids, beta blocker administration, and allergic complications post-procedure, including anaphylaxis, respiratory distress or rash/hives.

RESULTS: A total of 378 patients had history of contrast allergy; 268 patients by their history and additional 110 patients pre-mediated for presumed allergy; whereas 8705 had no evidence of prior contrast reaction. Overall, 318/378 (84%) received allergy prophylaxis. Among allergic patients, 327/378 (87%) received beta blockers; their overall rate of allergic complications was 3/327 (0.9%); whereas, 51 received no beta blockers and their rate of allergic reactions was 1/51 (2.0%) (P=0.44).

Among patients without presumed contrast allergy, 6780/8705 (78%) received beta blockers, and their incidence of allergic reactions was 15/6780 (0.2%); whereas 1925 did not receive beta blockers and their incidence of allergic reactions was 9/1925 (0.5%) (P=1.00).

CONCLUSIONS: Among patients with or without history of contrast allergy, there was no difference in the rate of allergic complications if beta blockers were administered prior to coronary CT angiography.