Perinatal Outcomes in Women Pregnant Women Exposed to Omalizumab: Interim Results from a Prospective, Observational Study

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3 Omalizumab Therapy in Patient Suffering from Severe Asthma and Concomitant Ulcerative Colitis

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RATIONALE: Asthma and ulcerative colitis (UC) are both chronic inflammatory diseases with acute exacerbations. Though asthma is Th2-dependent condition and UC Th1-driven disease, they may sometimes coexist.

METHODS: We present a case of a 61 year-old man suffering from severe allergic asthma with concomitant UC. His asthma was severe since its beginning in 1998. The patient did not respond to standard therapy, including high doses of oral corticosteroids (OCS). He had frequent severe asthma exacerbations and hospitalizations. In 2004 patient was hospitalized once again due to asthma exacerbation but this time after standard treatment of exacerbation severe abdominal pain appeared and paralytic intestinal obstruction developed. Patient underwent surgical intervention and temporary artificial anus was made. Based on clinical course and histopathology examination ulcerative colitis was diagnosed. Sulfasalazine therapy was introduced and OCS were continued also for this indication. As asthma further deteriorated, in 2006 the patient was qualified for omalizumab (OMA) treatment. The dose was calculated according to manufacturer’s dosing table (150 mg/4 weeks).

RESULTS: Asthma control improved within 3 months after OMA introduction (ACQ from 5.9 to 3.9 pts), frequency of exacerbations and hospitalizations/year decreased. After 1 year the demand for OCS decreased from 40 mg to 10 mg/day, and later OCS were withdrawn. The course of UC during OMA therapy did not deteriorate even after OCS withdrawal. UC relapses have been mild to moderate and occurred rarely.

CONCLUSIONS: Omalizumab was highly effective for severe asthma treatment in patient with concomitant CU, without negative impact on CU course.
AB2 Abstracts

4 Pulse Corticosteroids As an Omalizumab Enabling IgE Reduction Modality in an Asthmatic without Allergic Bronchopulmonary Aspergillosis

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RATIONALE: The effects of corticosteroid therapy on IgE levels in asthmatics without allergic bronchopulmonary aspergillosis (ABPA) has not been well described. IgE level reduction may be desirable when considering omalizumab treatment in asthmatics with very elevated IgE levels, as omalizumab treatment using clinically approved doses will not provide adequate IgE binding in these patients and insurers will typically not authorize reimbursement for this treatment.

METHODS: The clinical course of a patient with asthma is described with respect to IgE levels and treatment.

RESULTS: A 74-year-old male presented with adult onset severe steroid-dependent asthma. He had an FEV1 of 27% predicted. His IgE was 1142 kU/L and allergen specific IgE was elevated for several aeroallergens but not for molds, including aspergillus fumigatus. There was no bronchectasis. Eight years earlier, he had normal pulmonary function tests and his IgE level was 809 kU/L. He had prior sinus surgery for polyposis/chronic sinusitis. After 60 mg/day prednisone treatment for 10 days, an IgE level was 989 kU/L. After 20 mg/day prednisone treatment for 1 month, 1000 mg/day methylprednisolone was administered intravenously for 2 days. A repeat IgE level was 460 kU/L. Omalizumab was initiated. Two months later the patient had discontinued prednisone and his FEV1 was 63% predicted.

CONCLUSIONS: Even in the absence of effects from high dose oral prednisone, pulse corticosteroid treatment can result in significant reductions in IgE in non-ABPA asthma, allowing for omalizumab treatment to be given under current clinical indications.

5 Omalizumab: A Review of Efficacy in a Real-Life Pediatric Asthma Clinic Population

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RATIONALE: Omalizumab is FDA approved for treatment of moderate to severe persistent allergic asthma in patients >12 years of age who are not controlled by inhaled corticosteroids. There is limited data on its use in patients <12 years of age. This is a review of efficacy and safety of omalizumab in a real-life pediatric patient population, including patients <12 years of age.

METHODS: We carried out a retrospective chart review of subjects 6-17 years of age who received omalizumab at an academic children’s hospital from January 2008 to June 2014. ACT (Asthma Control Test) scores and number of asthma exacerbations were evaluated at 52 weeks post-initiation of omalizumab.

RESULTS: The mean age of subjects was 11.3 (± 3.1) years. Fifty-six percent (18/32) of subjects were <12 years old and 50% (16/32) were male subjects. The mean baseline IgE level was 956.5 (± 917, range 26-4320) IU/mL. ACT scores improved by 8 (± 3.9) points at 52 weeks (p<0.0001). All subjects experienced a decrease in emergency room visits by 71%, number of hospitalizations by 76%, and number of oral steroid courses by 58% at week 52 of therapy (p<0.0001). Subjects <12 years of age had a decrease in emergency room visits by 72%, number of hospitalizations by 80% and number of oral steroid courses by 66% (p<0.0001). There was an adverse event rate of 0.68% (3/444) among administered injections.

CONCLUSIONS: Omalizumab dramatically improved asthma control and is well tolerated in a pediatric patient population where the majority of children were <12 years of age.

6 Measuring Total Immunoglobulin E Is Useful in Detecting Exacerbations and Monitoring Treatment in Patients with Allergic Bronchopulmonary Aspergillosis Treated with Omalizumab

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RATIONALE: The increase in total immunoglobulin-E (IgE) is a key marker of diagnosis and exacerbations of allergic bronchopulmonary aspergillosis (ABPA). Omalizumab was reported to successfully treat ABPA, but the ability to assess adequate reductions in IgE, or to detect further exacerbations afterwards, is unknown.

METHODS: Describe the serial measured IgE levels in two adult patients with severe asthma (ATS criteria) and bilateral bronchiectasis, while receiving omalizumab.

RESULTS: Patient 1 (male, 44 years old) diagnosed with ABPA. In 2010 started omalizumab 600 mg/2 weeks (mean baseline IgE1000 KU/L), with clinical stability and decrease of total IgE to 315 KU/L within the first year. In 2013 clinical and spirometrical decline, blood eosinophilia (1393 mm3), high specific IgE and IgG to Aspergillus fumigatusand increase in total IgE (from 400 to 3788 IU/mL), that progressively decreased after initiating treatment with oral corticosteroids and itraconazole (2347 and 475, one and 6 months later, respectively), with clinical improvement. Patient 2 (female, 50 years old), with aspirin-exacerbated respiratory disease. Omalizumab was started in 2008 (375 mg/2 weeks, baseline total IgE 500 KU/L), achieving clinical and spirometrical stability. Six years later severe asthma exacerbations. Blood work: total IgE 2402 KU/L, specific Aspergillus IgG 66 mg/L, rAsp f 4 2,12 KU/L. Total IgE decreased to 2000 and 1500 KU/L, respectively, and one two months after starting oral steroids and voriconazole in July 2014.

CONCLUSIONS: Our data suggest that the pattern of measured IgE levels can still be useful in diagnosing ABPA exacerbations and their response to treatment in patients already receiving omalizumab.

7 Omalizumab Enrollment in a Tertiary Care Allergy and Asthma Clinic in Canada

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RATIONALE: Omalizumab has been approved in Canada since 2004 for the treatment of moderate to severe persistent allergic asthma in patients 12 years of age. Severe persistent allergic asthma can lead to the possibility of prolonged use of high doses of inhaled and oral corticosteroids, frequent emergency room (ER) visits with possible hospitalizations and frequent absences from work/school and social activities. The use of Omalizumab can decrease the use of corticosteroids, ER visits and improve the overall quality of life (QoL) for patients.

METHODS: A retrospective chart review of our database at our large tertiary care clinic from 2004 to 2014 was performed. Data was collected regarding asthma exacerbation, ER visits and hospitalization, as well as oral and inhaled corticosteroids use. QoL questionnaires completed upon enrollment and at specific intervals during treatment with omalizumab were analyzed.

RESULTS: A steady number of patients were enrolled each year since 2004, showing its greatest increase in enrollment numbers since 2012. Our data indicates that the majority of patients improved with significantly less asthma exacerbation, less ER visit and hospitalization, reduction in the use of oral and inhaled corticosteroids and better QoL.

CONCLUSIONS: Omalizumab is effective in the treatment of moderate and severe allergic asthma. It improves QoL and reduces asthma exacerbation, ER visit and hospitalizations, and significantly reduced the use of oral corticosteroids and inhaled corticosteroids.
8 Omalizumab Is Effective in the Treatment of Difficult-to-Treat Chronic Spontaneous Urticaria
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RATIONALE: Chronic spontaneous urticaria (CSU) is a condition, lasting at least 6 months, where patients experience frequent episodes of red, itchy hives and/or angioedema with no apparent external trigger. For approximately 30-50% of patients this condition can resolve spontaneously but has been known to persist for years. CSU can have a major impact on a patient’s quality of life as it can affect daily activities, sleep, emotional wellbeing and social interactions. Omalizumab was approved in the U.S., Europe and eight other countries in March 2014, and most recently in Canada in August 2014, for the treatment of CSU in patients with inadequate response to H1-antihistamines and oral prednisone, patients completed a quality of life (QoL) questionnaire prior to beginning treatment with Omalizumab and every two weeks throughout the treatment. In addition, these patients were also monitored closely for clinical response.

RESULTS: All the patients who started on omalizumab for CSU were evaluated. The majority of patients were able to decrease or stop the use of H1-antihistamines after the 3rd dose of omalizumab. The results of the questionnaires indicated a 15% improvement in QoL with an accompanying 18% decrease in the symptom score.

CONCLUSIONS: Omalizumab is an effective therapy in difficult-to-treat CSU in our tertiary community based allergy and asthma clinic.

9 Omalizumab Treatment of Moderate to Severe Asthma in the Adolescent and Pediatric Population
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RATIONALE: In Canada and the US, omalizumab is indicated for adults and adolescents (≥12 years of age) with moderate to severe persistent allergic asthma. In the EU, omalizumab has been approved for children (age 6 – 11 years) since 2009. The pediatric population within Canada and the United States has very few treatment options available for severe asthma. Oral corticosteroids can lead to other health concerns such as adrenal insufficiency and osteoporosis. These cases demonstrate that early treatment of moderate to severe asthma with omalizumab is effective and can help to prevent long term use of other treatment options.

METHODS: A retrospective chart review of our database was performed and patients < 17 years of age receiving omalizumab treatment were evaluated. Data was collected on FEV\(_1\), inhaler corticosteroid (ICS) and oral corticosteroid (OCS) use.

RESULTS: 12 patients were identified as adolescent/pediatric at the start of treatment with omalizumab. After the first 6 months of treatment, all 12 patients showed an increase in FEV\(_1\) results and a decrease in ICS dose and OCS use, both for those patients taking daily dose as well as those requiring periodic bursts to control exacerbations.

CONCLUSIONS: Early treatment of moderate to severe asthma with omalizumab in adolescent/pediatric patients may improve quality of life and help prevent health concerns associated with side effects and/or long term use of ICS and OCS in growing children. Regular re-evaluation of the treatment regime to ensure the use of the lowest effective dose of corticosteroids and consideration of other treatments would also be beneficial.

10 Effects of Serum Vitamin D Levels on Allergic Diseases in Korean Children and Adolescents: The Korea National Health and Nutrition Examinations Survey (KNHANES)
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RATIONALE: There are limited reports demonstrating the relation between serum vitamin D status and allergic diseases in children and adolescents based on population-based studies. The aim of this study was to evaluate the association between serum vitamin D concentrations and allergic diseases in Korean children and adolescents.

METHODS: A cross-sectional study was performed by using collected from 4,447 individuals from 10 to 18 years who participated in Korean National Health and Nutrition Examination Survey(KNHANES) for 5 years (2008 – 2012).

RESULTS: The serum concentration of 25(OH)D was higher in boys and subjects residing in rural area. Serum vitamin D level tended to be lower in subjects with wheezing during the recent 1 year. After adjusted for sex, body mass index for age and sex, rural residence and socioeconomic status, the vitamin D level was significantly associated with the wheezing episode in the past year. This relationship was not observed in participants with physician-diagnosed asthma, allergic rhinitis and atopic dermatitis.

CONCLUSIONS: Korean children and adolescents with the low serum 25(OH)D concentration have an increased likelihood of recent wheezing episodes.

11 The in Vivo Profile of CT133, a Potent, Well Tolerated, and Selective CRTH2 Antagonist for the Treatment of Allergic Asthma and Rhinitis
Dan Guo; CSPC Pharmaceutical Group, Princeton and Liyun Liu, CSPC Pharmaceutical Group, Hebei Province, China.

RATIONALE: CT133 is a novel selective receptor antagonist of CRTH2, a potential target for treatment of asthma, allergic rhinitis and obstructive pulmonary diseases.

METHODS: Preclinical studies using albumin-induced allergic asthma and rhinitis mice models were performed.

RESULTS: Serum results demonstrated that CT133 significantly inhibits the concentration of serum total IL-5, anti-OVA-IgE, and therefore improves the slow respiration symptom of allergic rhinitis, heightens respiratory frequency, and reduces the frequency of sneeze. After a single oral administration, the bioavailability in mice, rat and dog is 89.6%, 71.6% and 85.4%, respectively. There is no significant difference in T1/2, Cmax, Cmin and AUCinf between the first day and the seventh day after oral administration, the bioavailability in mice, rat and dog is 89.6%, 71.6% and 85.4%, respectively. This relationship was not observed in participants with physician-diagnosed asthma, allergic rhinitis and atopic dermatitis.

CONCLUSIONS: Korean children and adolescents with the low serum 25(OH)D concentration have an increased likelihood of recent wheezing episodes.
12 Inhaled Salmeterol Induces Salivary Alpha Amylase Activity in Healthy Subjects
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RATIONALE: Salivary alpha-amylase (sAA) secretion is an indirect method of measuring beta-receptor activation. Increases in sAA are seen in isoproterenol-infused mice (attenuated by beta-receptor antagonists) and in humans under physical or mental stress. We previously demonstrated that albuterol given by inhalation increases sAA (peaking at 15 minutes) in healthy volunteers and patients with asthma. We hypothesized that inhaled salmeterol, but not the anticholinergic ipratropium, will induce sAA secretion.

METHODS: Five healthy volunteers were enrolled. Spirometry was performed; and sAA, blood pressure and heart rate were collected at baseline and at 15, 30, 60 and 120 minutes after inhalation of fluticasone-salmeterol HFA (90 mcg/42 mcg). Two weeks later, two of the subjects repeated the protocol after inhaling ipratropium (34 mcg). Three additional subjects performed spirometry maneuvers alone to confirm stability of sAA during forced expiratory maneuvers.

RESULTS: Baseline sAA was 143 ± 51 (U/mL ± SEM), which increased after salmeterol inhalation to 190 ± 57, 230 ± 68, 276 ± 100, and 239 ± 70 at 15, 30, 60, and 120 minutes, respectively; peaking at 60 minutes (p<0.05 at 60 and 120 minutes; ANOVA). sAA did not change after ipratropium inhalation or spirometry alone. FEV1 did not change from baseline after inhalation of salmeterol or ipratropium in these healthy volunteers. No significant changes in blood pressure and heart rate were observed in any group.

CONCLUSIONS: Salmeterol induces sAA in healthy subjects, but peaks later than albuterol, consistent with its pharmacokinetic properties. These findings reinforce the concept that sAA is a surrogate marker for beta-2-receptor activation and may be useful in assessing tachyphylaxis or unresponsiveness to beta-2-agonists.

13 A New Look at an Old Drug
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RATIONALE: To study the efficacy of subcutaneous terbutaline in asthmatics who have already used inhaled beta agonists 3-10 times prior to their visit and to survey physician use of subcutaneous terbutaline in asthmatics who have an exacerbation.

METHODS: The asthmatics enrolled in this study were on daily medications including a inhaled steroid LABA combination. In addition, they used a beta agonist by inhalation at least three times as well as oral corticosteroids during the previous 24 hours. Twenty seven patients met these requirements. They were treated with subcutaneous terbutaline and an albuterol aerosol. The survey asked physicians to respond anonymously to the following: If a patient presents with an exacerbation of asthma you: ALWAYS, SOMETIMES, RARELY, or NEVER use subcutaneous terbutaline.

RESULTS: Twenty seven patients responded, at times dramatically to terbutaline, followed by an albuterol aerosol; with the addition of prednisone the symptoms resolved within 3-14 days. After the subcutaneous terbutaline and an albuterol aerosol, pulmonary function tests revealed an average increase in the FVC of 9%, FEV1 of 7%, FEF25-75% of 4%, and Peak Flow of 11%. Two patients (7%) required hospitalization. The survey was sent to 838 physicians; 372 responded (44%), 0% ALWAYS, 2% SOMETIMES, 12% RARELY, and 88% NEVER use subcutaneous terbutaline.

CONCLUSIONS: Subcutaneous terbutaline is clearly efficacious for those who have not responded to multiple beta agonist treatments. Subcutaneous terbutaline should be considered in patients who are receiving maximum pharmacological therapy.
Effect of Fixed Airflow Obstruction (FAO) Status on Lung Function, Asthma Control Days (ACD), and Asthma Symptom Score (AS) Responses to Budesonide/Formoterol (BUD/FM) Treatment in Patients with Moderate-to-Severe Asthma

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RATIONAL: Assess FAO status effect on FEV1, and asthma symptoms in response to BUD/FM in moderate-to-severe asthma patients.

METHODS: This post-hoc analysis assessed patients randomized to bid BUD/FM pMDI 320/μg, BUD pMDI 320μg, FM DPI 9μg, or placebo (PBO) (NCT00652002) for FAO status postbronchodilator at screening and post-study-drug at weeks 2, 6, and 12, via FEV1/FVC < LLN (FAO+), or ≥LLN (FAO–), excluding withdrawals before week 2. FAO variable patients had inconsistent FAO status among visits. Mean AS response, % patients with mean change from baseline in FEV1, ≥100mL and % ACD are reported.

RESULTS: Percentages of patients with changes in predose FEV1, ≥100mL for BUD/FM vs BUD, FM, and PBO were: FAO+ (75% [n=32] vs 40.7% [n=34], 32% [n=37], and 23.5% [n=34]), FAO variable (57.1% [n=28] vs 46.9% [n=32], 29.7% [n=37], and 16.7% [n=30]), FAO variable (59.6% [n=47] vs 47.1% [n=34], 70.4% [n=27], and 48.1% [n=27]). AS change was greater for BUD/FM vs BUD, FM, and PBO patients for FAO+ (−0.21 vs −0.14, −0.08, and −0.07), FAO variable (−0.30 vs −0.13, −0.10, and 0.01), and FAO– (−0.33 vs −0.19, −0.21, and −0.07). Percentage ACD improved most with BUD/FM vs BUD, FM, and PBO patients for FAO+ (17.0% vs 2.2%, 6.5%, and 1.7%), FAO variable (14.5% vs 3.1%, 7.5%, and 3.3%), and FAO– (21.8% vs 14.5%, 2.8%, and 3.7%).

CONCLUSIONS: BUD/FM appeared to show greater lung function improvement compared with BUD and FM for FAO+ and FAO variable patients and greater symptom improvements compared with BUD and FM for FAO+ and FAO variable (FAO–) (PBO) (NCT00652002) for FAO status postbronchodilator at screening and post-study-drug at weeks 2, 6, and 12, via FEV1/FVC < LLN (FAO+), or ≥LLN (FAO–), excluding withdrawals before week 2. FAO variable patients had inconsistent FAO status among visits. Mean AS response, % patients with mean change from baseline in FEV1, ≥100mL and % ACD are reported.

RESULTS: Percentages of patients with changes in predose FEV1, ≥100mL for BUD/FM vs BUD, FM, and PBO were: FAO+ (75% [n=32] vs 40.7% [n=34], 32% [n=37], and 23.5% [n=34]), FAO variable (57.1% [n=28] vs 46.9% [n=32], 29.7% [n=37], and 16.7% [n=30]), FAO variable (59.6% [n=47] vs 47.1% [n=34], 70.4% [n=27], and 48.1% [n=27]). AS change was greater for BUD/FM vs BUD, FM, and PBO patients for FAO+ (−0.21 vs −0.14, −0.08, and −0.07), FAO variable (−0.30 vs −0.13, −0.10, and 0.01), and FAO– (−0.33 vs −0.19, −0.21, and −0.07). Percentage ACD improved most with BUD/FM vs BUD, FM, and PBO patients for FAO+ (17.0% vs 2.2%, 6.5%, and 1.7%), FAO variable (14.5% vs 3.1%, 7.5%, and 3.3%), and FAO– (21.8% vs 14.5%, 2.8%, and 3.7%).

CONCLUSIONS: BUD/FM appeared to show greater lung function improvement compared with BUD and FM for FAO+ and FAO variable patients and greater symptom improvements compared with BUD and FM regardless of FAO status.

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Evaluation of Efficacy of Flunisolide HFA (AEROSPAN) in Children 4 to 11 Years of Age: A Sub-Group Efficacy Analysis By Baseline Asthma Medication Use

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RATIONAL: A 12-week, multicenter, placebo- and active-controlled trial in pediatric patients 4 to 11 years (n=513) with mild-to-moderate asthma was conducted, where 80 mcg and 160 mcg BID doses of flunisolide HFA were compared to 250 mcg and 500 mcg BID doses of flunisolide CFC. Post-hoc analyses were performed to evaluate the effect of flunisolide HFA by baseline asthma medication, either an inhaled corticosteroid [ICS] or antileukotriene agents.

METHODS: Sub-groups were analyzed for the primary endpoint (change from baseline to 12 weeks of treatment in % predicted FEV1). The most commonly used ICSs prior to study entry were: beclomethasone (n=129; mean daily dose of 236.7 mcg), fluticasone (n=69; mean daily dose of 325.3 mcg) and triamicinolone (n=52; mean daily dose of 445.5 mcg). Antileukotrienes were montelukast and zafirlukast (n=38).

RESULTS: Patients treated with flunisolide HFA, following a 2-week run-in with flunisolide CFC 500mcg BID, had % predicted FEV1 values that improved over their previous ICS. Respective mean improvements in % predicted FEV1 were: 7.6% (160 mcg) to 4.8% (80 mcg) for the beclomethasone subgroup; 2.9% (80 mcg) to 6.3% (160 mcg) for the fluticasone subgroup; and 13.6% (80 mcg) to 8.0% (160 mcg) for the triamicinolone subgroup. In addition, patients treated with antileukotrienes had a 7.7% (80 mcg) to 14.7% (160 mcg) improvement in % predicted FEV1.

CONCLUSIONS: After 12 weeks of treatment, pediatric patients 4 to 11 years treated with flunisolide HFA (80 and 160 mcg BID) had meaningful improvements in efficacy as assessed by % predicted FEV1, regardless of their previous ICS.
18 Once-daily Tiotropium Respimat® Add-on to at Least ICS Maintenance Therapy Reduces Airflow Obstruction in Patients with Symptomatic Asthma, Independent of Allergic Status

**Kevin R. Murphy, MD**
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**RATIONALE:** A significant proportion of patients with asthma remain symptomatic despite treatment with ICS LABA maintenance therapy. We investigated whether the efficacy of once-daily tiotropium Respimat® (tioR) add-on to at least ICS was influenced by allergic status in adult patients with varying severities of symptomatic asthma.

**METHODS:** Five Phase III double-blind, placebo-controlled, parallel-group trials: PrimoTinA-asthma9 (NCT00776984/NCT00722538; n=907), tioR 34μg or placebo Respimat® (pboR) add-on to high-dose ICS-LABA; MezzoTinA-asthma10 (NCT04112808/NCT04172821; n=1546) and GraziaTinA-asthma11 (NCT04136830; n=464) tioR 5μg, 2.5μg, or pboR add-on to medium-dose and low-dose ICS, respectively. Patients had symptomatic asthma requiring treatment with at least ICS for 24 weeks before screening; COPD was excluded. Pre-planned analyses of peak FEV1(0-3h) and trough FEV1 were performed according to allergic status as per total serum IgE (< 2430μg/L [equivalent to 179.2 IU/L]), blood eosinophils (< 0.26x10^9/L), or investigator judgment (No/Yes).

**RESULTS:** Peak FEV1(0-3h) and trough FEV1 significantly improved with tioR versus pboR in all trials, independent of IgE (interaction p-values for peak FEV1(0-3h) = 0.74, 0.97, 0.16 and trough FEV1 = 0.62, 0.84, 0.63 for PrimoTinA-asthma9, MezzoTinA-asthma10, and GraziaTinA-asthma11, respectively), eosinophil count (interaction p-values for peak FEV1(0-3h) = 0.70, 0.24, 0.38 and trough FEV1 = 0.75, 0.51, 0.36, respectively), and investigator judgment of allergic status (interaction p-values for peak FEV1(0-3h) = 0.21, 0.62, 0.60 and trough FEV1 = 0.41, 0.67, 0.87, respectively).

**CONCLUSIONS:** Once-daily tiotropium Respimat® add-on to at least ICS maintenance therapy reduces airflow obstruction in patients with mild to severe symptomatic asthma, independent of allergic status defined by IgE, eosinophil count, or investigator judgment.

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19 Dose-Ranging Study to Evaluate the Efficacy and Safety of Four Doses of Fluticasone Propionate/Salmeterol Multidose Dry Powder Inhaler (FS MDPI) Compared with Fluticasone Propionate (Fp) Mdpi and FS DPI in Subjects with Persistent Asthma

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**RATIONALE:** Dose-response efficacy/safety study of 4 single-dose regimens of fluticasone/salmeterol multidose dry powder inhaler (FS MDPI) versus fluticasone propionate (Fp) MDPI and FS dry powder inhaler (DPI) in subjects with asthma.

**METHODS:** Multicenter, randomized, double-blind, single-dose, 6-period crossover, dose-ranging study in subjects aged ≥12 years with persistent asthma and predose maximum FEV1 of 40%–85% of predicted normal. Seventy-two subjects were randomized to a treatment sequence and received one dose of each treatment: FS MDPI 100/6.25μg, 100/12.5μg, 100/25μg, or 100/50μg; Fp MDPI 100μg; or FS DPI 100/50μg (GlaxoSmithKline, Research Triangle Park, NC). Efficacy was evaluated by measuring baseline adjusted FEV1 area under the curve over 12 hours postdose (AUC0-12). Pharmacokinetics over 12 hours postdose and tolerability were assessed.

**RESULTS:** Baseline adjusted FS MDPI 100/50μg FEV1 AUC0-12 was significantly higher than FS DPI (LS mean 57.88; P=0.0017). FS MDPI 100/25μg trended toward higher efficacy (LS mean 34.14; P=0.0624). FS MDPI 100/12.5μg was comparable (LS mean 3.42; P=0.8503), and FS MDPI 100/6.25 μg was significantly lower than FS DPI (LS mean -41.7 mL; P<0.0229). Fp MDPI pharmacokinetics demonstrated lower salmeterol AUC0-4 for FS MDPI 100/6.25μg, 100/12.5μg, and 100/25μg versus FS DPI; AUC0-4 for FS MDPI 100/50μg was higher. All FS MDPI doses were well tolerated.

**CONCLUSIONS:** For FEV1 AUC0-12, all FS MDPI doses were superior to Fp MDPI at the same Fp dose, demonstrating the value of adding salmeterol to Fp MDPI for asthma treatment. FS MDPI 100/12.5μg demonstrated similar bronchodilation to FS DPI 100/50μg with lower systemic exposure to salmeterol. Study supported by Teva.

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20 Characteristics of Complementary and Alternative Medicine (CAM) Use Among Older Adults with Asthma

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**RATIONALE:** A growing number of patients are using CAM. Prior studies have not focused on CAM use among the older adult asthma population.

**METHODS:** The Behavioral Risk Factor Surveillance Survey (BFRSS) is a national telephone survey. The 2011 Asthma Call-Back survey (ACBS) is a survey conducted among the BFRSS individuals reporting asthma. The study population consisted of 7,479 individuals aged 55 years or older with current asthma. The primary outcome was self-reported CAM use. The relationship of CAM use with multiple variables was analyzed using logistic regression.

**RESULTS:** CAM use was reported in 39% (2,913). Females were more likely to use CAM (OR 1.15, p<0.03). As age increased, there was a trend toward decreased odds of CAM use (p=0.01). An inverse relationship was noted between income and CAM use (p<0.001). Adults with a cost barrier to healthcare (OR 1.63, p<0.001) or an ER visit within 12 months (OR 1.41, p<0.001) had increased odds of using CAM. Those with poor asthma control, as defined by symptoms affecting sleep and symptoms limiting activities, were more likely to use CAM. Flu vaccine recipients had decreased odds of CAM use (OR 0.88, p<0.02), whereas those with a history of depression had increased odds of CAM use (OR 1.37, p<0.01).

**CONCLUSIONS:** The proportion of older adults with asthma who use CAM is similar to the general adult population, although there was an inverse relationship between CAM use and increasing age. Characteristics associated with increased CAM use included lower socioeconomic status, poor asthma control, lack of flu vaccine, and depression.
21 Changes in DNA Methylation from Age 18 to Early Pregnancy Suggest a Th2 Bias
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RATIONALE: Pregnancy is an immunological state with a bias towards T helper 2 (Th2) responses, yet little is known about how these immunological changes are established. We explored whether DNA methylation (DNA-M) is associated with Th2 bias during pregnancy.

METHODS: DNA-M was measured in peripheral blood from 18-year-old girls (n=245) from the Isle of Wight (UK) birth cohort, using the Illumina HumanMethylation450 beadchip. Blood samples were also collected from 39 of these women early in pregnancy (weeks 1-21) and 34 later in pregnancy (weeks 22-38). We focused on cytosine-phosphate-guanine (CpG) sites in genes in four immune pathways: Th1 (157 CpGs in 19 genes), Th2 (68 CpGs in 12 genes), Th17 (110 CpGs in 15 genes), Treg (10 CpGs in 2 genes), and a random sample of 427 CpGs. DNA-M at age 18 years (non-pregnant state) was compared with DNA-M in early and late pregnancy using mixed linear models, to find immune gene CpGs with statistically significant changes (p<0.05).

RESULTS: More CpGs within Th2 genes (29.4%) and Treg genes (40%) than within the random sample (15.5%) changed significantly in DNA-M between age 18 and early pregnancy (p=0.005; 0.02, respectively). Among the 20 CpGs in Th2 that are significantly differentially methylated with pregnancy, six belong to IL1RL, five to GATA3 and two to IL1R1. No significant changes were found for late pregnancy.

CONCLUSIONS: Significant more changes in DNA-M in Th2 and Treg pathways suggest an involvement of DNA-M in the gestational Th2 bias. Future studies should observe whether these changes are paralleled by allergic manifestations and are also reflected in cord blood.

22 Evaluation of Two Different Activation Markers in the Basophil Activation Test to Quinolones
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RATIONALE: Quinolones are well-tolerated antibiotics increasingly prescribed. Hypersensitivity reactions as immediate urticaria and anaphylaxis have been reported. These reactions can be evaluated in vitro by basophil activation tests (BAT) although with not optimal sensitivity. Many factors could influence the results among them the election of basophil activation markers. The objective of this study was to evaluate the influence of two different activations markers, CD63 and CD203c, in the sensitivity of BAT for the evaluation of immediate allergic reactions to quinolones.

METHODS: We studied 20 patients with immediate allergic reactions to quinolones. BAT was performed with moxifloxacin and ciprofloxacin at 2 different concentrations (2 and 0.2mg/ml) using CD193 (CCR3) for basophil selection and CD203c or CD63 as activation markers.

RESULTS: In general, the percentage of activation was lower when using moxifloxacin than ciprofloxacin. Comparing both activations markers, CD63 or CD203c, we found a higher percentage of positive cases using CD63 (64.3%) than using CD203c (28.6%) when ciprofloxacin was used in the test (p=0.025). No differences were found when moxifloxacin was used in BAT (14.3% for both markers). Similar results were found when patients were separated according to the culprit drug in the reaction.

CONCLUSIONS: These results indicate that the use of CD63 as basophil activation marker in the evaluation of immediate reactions to quinolones shows a higher or equal sensitivity than the other widely used activation marker, CD203c.

23 Pattern of Sensitization of Tomato Seed Lipid Transfer Protein
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RATIONALE: Food allergy is an increasing health problem with many proteins involved that belong mainly to a limited number of families. In the Mediterranean area, the most prevalent food allergens are those of vegetal origin. Although tomato (Solanum lycopersicum L.) is one of the implicated foods, studies on the identification and relevance of their allergens have not been carried out in detail. This could be particularly relevant for tomato seeds as happen in other fruits like kiwi. The aim was to analyse the sensitisation pattern to tomato seeds in patients from two hospitals integrated in the RIRAFF.

METHODS: A large group of tomato-sensitized patients (N=96) was recruited. We included patients who suffered at least two episodes with tomato and/or having a positive skin prick test (SPT). Raw tomato seed extract was prepared and the protein profile characterized by SDS-PAGE. Patient sera were used for determining recognition profiles by western blotting. The band most frequently recognised was sequenced by mass spectrometry.

RESULTS: Data from western blotting showed different patterns of IgE recognition. From all the bands those approx. of 10 kDa was the most frequently recognised in 46% of the patients. This band specifically appears in 100% of serum from patients with anaphylaxis, 83% with urticaria, 0% with angioedema and 9% with OAS. Data from sequencing revealed that this protein belongs to the lipid transfer protein family (LTP).

CONCLUSIONS: These preliminary results show that tomato seed LTP could be a relevant allergen. Whether this is predictive of systemic reactions is being evaluated.
Allergen Specific IgE Response Is Similar in HIV-1 Seropositive and Seronegative Adults: Implications for HAART Induced Th2 to Th1 Switching

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RESULTS: Advanced HIV disease has been associated with Th2 mediated IgE responses, including IgE anti-HIV, and decreased atopy. The effect of HAART (highly active antiretroviral therapy) on Th2 mediated allergic IgE responses has not been determined.

METHODS: Retrospective chart review was performed for adults seen in Allergy clinic at University Hospital of Brooklyn during 7/2000 to 5/2014. Subjects included HIV-1 patients on HAART and a control group consisting of HIV-1 seronegative patients (age and sex matched with a clinical visit in the same month). Total and allergen specific serum IgE was determined by ImmunoCAP assay; Skin prick test (SPT; Dermagik) for aeroallergens was performed using the Dermagik method. A mixed linear model was constructed for each dependent variable (IgE, no. of positive allergy results). Fixed factors were HIV status and gender; age was introduced as a linear covariate; matched pair ID was introduced as a random factor.

RESULTS: Seventy-four subjects were analyzed with equal number of subjects in each group. HIV-1 seropositive subjects on HAART and controls had similar total serum IgE of 90.95 IU/ml and 144.54 IU/ml respectively (p = 0.23) and similar numbers of positive allergen specific IgE responses of 3.34 and 4.06, respectively (p = 0.28).

CONCLUSIONS: These findings suggest there is limited effect of HAART on Th2 to Th1 switching.

Naturally Occurring Tolerance Acquisition to Foods in Children Previously Allergic to Egg and Peanut Is Characterized By Antigen Specificity and Associated with Increased Subsets of Regulatory T Cells

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RATIONALE: Food allergy affects approximately 6-8% of children and is increasing in prevalence. Some children naturally outgrow their food allergy without intervention but mechanisms remain poorly understood. We sought to investigate the role of T regulatory cells in the development of naturally acquired tolerance.

METHODS: Fifty-eight children with either egg or peanut allergy, recent acquisition of natural tolerance to egg or peanut, or no food allergy were studied. Peripheral blood mononuclear cells (PBMC) from these groups were stimulated with relevant antigen for 48 hours and flow cytometry was performed to characterize both surface (CD3, CD4, CD25, CD14, CD19, CD127) and intracellular markers (IL-10 and Foxp3) of the regulatory T cells.

RESULTS: Resting PBMC from naturally tolerant patients had significantly increased thymus-derived T regulatory cells (tTregs) (defined as CD3+CD4+CD25+CD127hiFoxp3+ cells) when compared to allergic or control patients [mean 11.44 vs 2.37 vs 2.62%, respectively, p < 0.02]. Upon stimulation with relevant antigen, naturally tolerant patients also had increased IL-10 expressing CD25+CD127hi cells [6.33 vs 1.65 vs 0.7, p < 0.01]; Foxp3+ cells [mean 12.6 vs 5.42 vs 3%, p < 0.01] and CD4+ cells [mean 4.48 vs 1.59 vs 0.87%; p < 0.01]; this increase was not observed in PBMCs from allergic or control patients. This upregulation was only seen with relevant antigen stimulation and not stimulation with unrelated antigen.

CONCLUSIONS: The increased tTregs at baseline and upon stimulation and increased induction of IL-10-producing cells of several types including Th1 cells from naturally tolerant patients suggests an important role for regulatory T cell subsets in the acquisition of natural tolerance.
Allergic Extracts Require TLR4 to Activate and Increase Expression of CD40, CD80 and CD86 on Bone Marrow-Derived Dcs

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RATIONALE: Toll-like receptors (TLRs) are pattern recognition molecules that bind pathogen-associated molecular patterns and initiate innate immune responses. Very little is known about the allergens requiring specific toll receptors to modulate surface expression of CD40, CD80, CD86 and Ox40 in bone marrow-derived dendritic cells (BMDCs). The objective of this study was to examine whether ragweed pollen extract (RWPE) and cat dander extract (CDE) activate BMDCs by increasing expression of CD40, CD80, CD86 and Ox40 in CD11c+ BMDCs, and whether this activation requires TLR4.

METHODS: Endotoxin-free RWPE and CDE were used in all experiments. BMDCs from wild-type (WT) and TLR4 -/- mice were cultured with PBS and RWPE for 24hrs. Surface expression of CD40, CD80, CD86 and Ox40 on these cultured BMDCs were quantified by flow cytometry.

RESULTS: Compared to PBS, culture with RWPE increased expression of CD40 and CD86, but not CD80 or Ox40 in WT BMDCs. By contrast, CDE increase expression of CD40, CD80 and CD86, but not Ox40. The increase in expression of CD40, CD80 and CD86 by these allergenic extracts was completely blocked in TLR4-/- BMDCs.

CONCLUSIONS: RWPE activates CD40 and CD86 in BMDCs, and this increase requires TLR4. CDE activates CD40, CD80, and CD86 in BMDCs, and this increase also requires TLR4. These results suggest that TLR4 is critical for allergic extracts to activate BMDCs and increase expression of these co-stimulatory molecules.

Biological Variability of Dendritic Cells and Regulatory T Cells in Peripheral Blood of Normal Adults

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RATIONALE: Studies evaluating circulating dendritic cells (DCs) and natural and induced regulatory T cells (nTregs, iTregs) are pattern recognition molecules that bind pathogen-associated molecular patterns and initiate innate immune responses. Very little is known about the allergens requiring specific toll receptors to modulate surface expression of CD40, CD80, CD86 and Ox40 in bone marrow-derived dendritic cells (BMDCs). The objective of this study was to examine whether ragweed pollen extract (RWPE) and cat dander extract (CDE) activate BMDCs by increasing expression of CD40, CD80, CD86 and Ox40 in CD11c+ BMDCs, and whether this activation requires TLR4.

METHODS: Endotoxin-free RWPE and CDE were used in all experiments. BMDCs from wild-type (WT) and TLR4 -/- mice were cultured with PBS and RWPE for 24hrs. Surface expression of CD40, CD80, CD86 and Ox40 on these cultured BMDCs were quantified by flow cytometry.

RESULTS: Compared to PBS, culture with RWPE increased expression of CD40 and CD86, but not CD80 or Ox40 in WT BMDCs. By contrast, CDE increase expression of CD40, CD80 and CD86, but not Ox40. The increase in expression of CD40, CD80 and CD86 by these allergenic extracts was completely blocked in TLR4-/- BMDCs.

CONCLUSIONS: RWPE activates CD40 and CD86 in BMDCs, and this increase requires TLR4. CDE activates CD40, CD80, and CD86 in BMDCs, and this increase also requires TLR4. These results suggest that TLR4 is critical for allergic extracts to activate BMDCs and increase expression of these co-stimulatory molecules.

29 Respiratory Syncytial Virus-Induced Host IFN Signaling Differs Between A549 and BEAS-2B Epithelial Cell Lines

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RATIONALE: Intact host innate immunity, including interferons (IFN) and interferon stimulated genes (ISG), is critical for local control of respiratory syncytial virus (RSV). We compared the innate response to RSV by two respiratory epithelial cell lines, BEAS-2B and A549, to define critical innate components that control local epithelial spread of infection in vitro.

METHODS: BEAS-2B and A549 respiratory epithelial cell lines were infected with RSV expressing GFP (rgRSV) at low MOIs to observe the spread of infection and innate responses over time. Expression of IFNs and ISGs were measured using qRT-PCR and ELISA, and STAT1/2 phosphorylation was determined by western blotting. RSV infection and cellular localization of IRF3, STAT1 and STAT2 were observed by confocal microscopy.

RESULTS: rgRSV spread throughout A549 cells, but BEAS-2B cells contained the RSV in foci of 10-15 cells. Both cell lines highly expressed IFN-β, IFN-α1 and -α2, but the A549 cells expressed more of these IFNs and had higher levels of pSTAT1/STAT2. Paradoxically, the A549 cells expressed lower levels of classic antiviral ISG (e.g. ISG15, MX1), but higher levels of NF-kB associated genes (e.g. CCL2, CCL5). Finally, STAT2 was only detected within uninfected cells, and in BEAS-2B cells more STAT2 localized to the nucleus early than in A549 cells.

CONCLUSIONS: A balance between expression of NF-kB genes and ISG may determine local control of RSV infection by respiratory epithelial cells, which may be critically mediated by the kinetics of STAT2 nuclear localization.

30 Immune Regulation and Tryptophan Metabolism in Asthma

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RATIONALE: Indoleamine 2,3-dioxygenase (IDO), the rate-limiting tryptophan metabolizing enzyme, has been implicated in immune tolerance in pregnancy, cancer, and certain infections through inhibition of T cell proliferation and promotion of the regulatory T cell phenotype. Its role in atopy, specifically asthma, has not been well defined. CD33+myeloid cell subsets have been appreciated as regulators of airway hyperresponsiveness. Further investigations are needed to determine if IDO expression and activity in myeloid cells of allergic asthma contributes to the clinical phenotype of asthma.

METHODS: Serum, circulating CD33+myeloid cells, and bronchoalveolar lavage (BAL) fluid were obtained from non-smoking normal subjects as well as mild asthmatic patients who were not being treated with corticosteroids. IDO activity and IDO expression were determined in these samples. Statistical analyses were performed using Wilcoxon Rank Sum Tests and Chi-square analyses.

RESULTS: No significant difference in serum IDO activity (P=0.69) or expression (P=0.11) was detected between patient groups or upper and lower airway BAL fluid (P=0.22 and 0.13 respectively). Interestingly, a significant increase in IDO activity (P=0.0140) was noted in circulating myeloid cells of allergic asthmatics compared to normal controls.

CONCLUSIONS: The significant increase in IDO activity in circulating myeloid cells of allergic asthmatics suggests that IDO may play a role in asthma pathogenesis. Further investigations are needed to determine if defective secretion of IDO contributes to lack of tolerance in asthma.
**AB10 Abstracts**

The Differential Relationship Between Regulatory T-Cells and Age in Children with Food Allergy

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**Rationale:** Regulatory T (Treg) cells are well known to play an important role in the maintenance of self-tolerance, and patients who lack these cells, such as in IPEX syndrome, have an increased incidence of allergic disease. Although studies have highlighted differences in peripheral blood Treg populations of atopic adults, only a few conflicting studies have examined differences in Tregs among children with allergic diseases.

**Methods:** Peripheral blood mononuclear cells were isolated from 47 patients (0-18yrs) and analyzed using flow cytometry for CD3, CD4, CD25, CD127, CCR6, and Foxp3. Populations of Tregs, defined as CD4+CD25+CD127−Foxp3+, were compared in food allergic children and healthy controls using a 2-tailed Student’s t-test and linear regression modeling.

**Results:** Food allergic children <6yrs had significantly lower percentages (p<0.05) of Tregs compared to healthy controls of similar age. This difference was not observed in older children. There was a significant decrease in both Treg cell percentages (p=0.018, R=0.584) and Treg expression of Foxp3 (p=0.012, R=0.613) with age in healthy controls but not in children with food allergy. Finally, food allergic children >6yrs had significantly less Tregs expressing CCR6 (p<0.05), a gut-homing marker, and this cell population significantly increased with age (p=0.013, R=0.626) in healthy controls but not food allergic children.

**Conclusions:** Younger, food allergic children had significantly lower percentages of Tregs compared to healthy controls, and this difference did not persist with older children. This supports the hypothesis that early childhood is likely a critical time in the development of normal immune regulation and that Tregs are important in this process.

Disseminated Atypical Mycobacterial Infection in Three Patients with Complete DiGeorge Anomaly

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**Rationale:** Complete DiGeorge anomaly is a primary immunodeficiency characterized by athymia with fewer than 50 naive T cells/mm3 associated with either a cardiac defect or hypoparathyroidism. Disseminated mycobacterial infection has not been described in these patients. We present three cases of disseminated atypical mycobacterial infection that developed among children with complete DiGeorge anomaly.

**Methods:** Clinical, laboratory, immunologic and radiologic data of patients with complete DiGeorge anomaly referred to Duke University for transplant were reviewed.

**Results:** In patient (P1), the diagnosis of disseminated mycobacterium avium complex (MAC) was made by lung biopsy 4 months after thymus transplantation after a mass was noted on chest CT obtained to evaluate for a source of fevers. P2 was diagnosed with MAC 2.4 months after thymus transplantation from a mycobacterial blood culture obtained for persistent fever. P2 was given steroids for possible immune reconstitution inflammatory syndrome when fevers recurred 18 days after starting anti-mycobacterial therapy. In P3, a chest CT obtained to evaluate fevers revealed lymphadenopathy and mycobacterial culture of a thoracic lymph node biopsy grew Mycobacterium kanssaii. P1 is on enteral medications for MAC at 22 months post-transplantation and has normal T cell numbers. P2 died 5 months after transplantation with finding of disseminated MAC on autopsy. Flow cytometry revealed naïve T cell development; the autopsy showed thymopoiesis. P3 is 3 months post-transplantation, remains on anti-mycobacterial treatment, and has not yet developed naïve T cells.

**Conclusions:** Azithromycin or clarithromycin prophylaxis should be considered for patients with complete DiGeorge anomaly. Aggressive anti-mycobacterial therapy without steroids is recommended.
34 **Good’s Syndrome Presenting As T-Cell Large Granular Lymphocyte Leukemia**

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**RATIONALE:** Good Syndrome is an adult-onset immunodeficiency defined by hypogammaglobulinemia, low number of B cells, and benign thymic tumor. Rarely associated with malignancies. We report a case of Good’s syndrome that presented as T-cell Large granular lymphocytic leukemia (LGL).

**METHODS:** Lymphocyte subset by flow cytometry, response to recall antigens and mitogens by 3H thymidine incorporation. PMN functions (phagocytosis and oxidative burst), fluorescence in situ hybridization (FISH) analysis for any chromosomal abnormality, and T-cell receptor (TCR) clonal rearrangement by polymerase chain reaction (PCR) on bone marrow aspirate were performed.

**RESULTS:** The patient’s clinical course was complicated by anemia requiring multiple blood transfusions, neutropenia requiring granulocyte-colony stimulating factor, opportunistic infections, including cytomegalovirus retinitis and cutaneous fungal infections. Immunological analysis revealed pan hypogammaglobulinemia, a markedly increased CD3+CD8+ T cells, and low proportions of CD3+/CD16+/CD56+ (NK) cells, and CD3+/CD4+ T cells, and absence of CD19+ (B) cells. Specific antibody responses to pneumococcus polysaccharides were lacking. The response to mitogens (PHA, ConA, PWM) and recall antigens (Candida, tetanus toxoid, mumps) were severely impaired; neutrophil functions are normal. Bone marrow biopsy revealed T-cell LGL involving approximately 20% of hypercellular marrow confirmed by flow cytometry. Clonal rearrangements of both TCR-beta and gamma chains were detected by PCR, consistent with the diagnosis of T-cell LGL leukemia. FISH analysis was normal.

**CONCLUSIONS:** To best of our knowledge this is the first case of T-cell LGL associated with Good’s syndrome. Patient was successfully treated with cyclophosphamide and cyclosporine (for LGL) and intravenous immunoglobulin (for antibody deficiency).

35 **Recurrent Human Papillomavirus Infection and Delayed Diagnosis of Idiopathic CD4 Lymphocytopenia**

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**RATIONALE:** Idiopathic CD4 lymphopenia is a rare disorder featuring an isolated CD4 lymphopenia in the absence of precipitating infections, drugs, or any known etiology. Patients have persistently depressed CD4 counts, and commonly present with recurrent infections, malignancy, or autoimmune disease as a result.

**METHODS:** Molecular diagnostic testing, flow cytometry, and chart review were performed.

**RESULTS:** Patient A presented at the age of 43 with a 25 year history of recurrent infections. History included severe, recurrent HPV affecting the extremities and genital tract, as well as recurrent painful oral aphthous ulcers, recurrent subcutaneous scalp cysts, and persistent molluscum contagiosum, all recalcitrant to therapy. Family history was negative. CBC and evaluation of humoral immunity was normal, and HIV1/HIV2 and HTLV were negative. Other evaluations for newly identified immunodeficiencies were normal. Patient B presented at age 23 with a similar 3 year history of recurrent HPV to the extremities and genital tract, all recalcitrant to both topical and surgical therapy. Family history was negative. CBC and evaluation of humoral immunity was normal, and HIV1/HIV2 and HTLV were negative. Lymphocyte subpopulations were obtained for both patients on 2 occasions 6 weeks apart and notable for isolated CD4 lymphopenia.

**CONCLUSIONS:** We present 2 cases of idiopathic CD4 lymphopenia with no identified precipitating etiologies. One case features a 25 year history of recurrent infections that remained undiagnosed with a normal but limited immunological evaluation. These cases highlight that recurrent HPV infections continue to be a hallmark of this immunodeficiency, and should prompt more extensive immunological evaluation.

36 **The Success of Newborn Screening for Severe Combined Immunodeficiency, Our Hospital’s Experience**

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**RATIONALE:** Severe combined immunodeficiency (SCID) is usually fatal due to serious infection in the first two years of life. Early detection is difficult due to the infant’s relative health initially maintained by maternal IgG. Diagnosis and treatment at an early age is less costly and more efficacious. Population based newborn screening (NBS) for SCID was added in Florida in 2012 with quantification of T cell receptor excision circles. We report the clinical outcome and cost analysis of two patients with SCID diagnosed in Florida.

**METHODS:** Retrospective chart review of two patients diagnosed and treated for SCID at All Children’s Hospital was performed. Patient 1 was born just prior to SCID NBS in Florida; patient 2 was born shortly after. Hospital level of care, complications, treatments, and outcomes were collected as were financial charges including inpatient and outpatient visits up to 2 months post-hematopoietic stem cell transplant (HSCT).

**RESULTS:** Patient 1 was 10 months when diagnosed with X-linked SCID after developing recurrent infections and failure to thrive. He spent 55 days in the critical care setting with multiple infections, respiratory failure, and died relatively quickly prior to HSCT. Patient 2 was diagnosed at 6 days of life with NBS and never required critical care. HSCT was performed without complication at 8 months. Total hospital days were 55 and 46 respectively. Combining all healthcare charges, patient 1 accrued $1,540,049 compared to $867,232 for patient 2.

**CONCLUSIONS:** These two patients highlight the improved outcome and cost savings associated with NBS for SCID.
37 Persistent T Cell Lymphopenia: An Algorithm for Follow up Care
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Rationale: There are patients who have persistent T cell lymphopenia following a positive newborn screen (NBS), that do not qualify for a diagnosis of SCID, DiGeorge syndrome, or other identifiable immunodeficiency disorders. The follow up care for these infants has not been standardized in the literature. We propose, and are utilizing, an algorithm in order to standardize the workup, intervention and follow up of these infants.

Methods: A retrospective chart review from time of NBS implementation in Michigan at the Children’s Hospital of Michigan (September 2011 to July 2014).

Results: 91 out of 189 infants with low TREC detected on NBS in Michigan were followed at our center: 2 were diagnosed with SCID; 1 with combined immune deficiency; 9 DiGeorge Syndrome (3 severe partial; 6 with partial); 2 with lymphopenia secondary to thymectomy; 5 families are refusing follow up care; 5 died with complications of other diseases and 2 pending cases. 65 has persistent lymphopenia without a diagnosis: 48 were discharged from care with normal flow cytometry and 16 were discharged from care because of normal T cell proliferation (PHA > 10 times the control value) despite low T cells; one remains in follow-up with low T cell proliferation assay without a diagnosis. Of those 16 patients, 56.5% were discharged by 12 months of age.

Conclusions: We propose a new algorithm to approach and manage infants with persistent T cell lymphopenia identified by NBS which should minimize morbidity and decrease family anxiety.

38 Tubular Interstitial Nephritis, an Unusual Manifestation of T Cell-Associated Severe Chronic Active Epstein-Barr Virus Infection
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Rationale: Severe chronic active Epstein-Barr virus (SCAEBV), a rare manifestation of EBV infection, is characterized by chronic EBV viremia, histologic evidence of organ invasion, and detection in organs of EBV proteins or nucleic acid. Infection can occur in all lymphocytes, but T cell-associated disease has the poorest survival. Most affected patients present with liver and bone marrow dysfunction, but renal involvement is rare.

Methods: We report a case of T-cell associated SCAEBV infection presenting with proteinuria and hematuria.

Results: A 16-year old Hispanic male was referred by the primary care physician to nephrology clinic due to proteinuria. He had a history of hepatitis one year prior. A liver biopsy showed EBV-infected lymphocytes. A serum EBV viral load was 15x10^6 copies/ml, predominantly affecting T lymphocytes. He received intravenous ganciclovir and acyclovir without resolution. Due to persistent proteinuria and a glomerular filtration rate of 30mL/min/1.73m^2, a renal biopsy was performed and showed tubular interstitial nephritis (TIN) with EBV-positive interstitial staining. Isolated cases of renal involvement in SCAEBV have demonstrated varied pathologies, including TIN, immune complex-mediated glomerulonephritis, and mesangial proliferative glomerulonephritis. No pattern of symptoms distinguished those with renal involvement and SCAEBV. Treatments were diverse, ranging from no treatment to hematopoietic stem cell transplant (HSCT). Our patient underwent cytoreduction with bortezomib and ganciclovir prior to a planned reduced-intensity matched unrelated donor HSCT with donor-derived EBV-specific cytotoxic lymphocytes.

Conclusions: SCAEBV can affect multiple organ systems, but renal involvement is rare. A renal biopsy should be considered for patients with chronic EBV viremia and concurrent presence of proteinuria.
Newborn Screening for Severe Combined Immunodeficiency (SCID) Leads to Early Identification of Ataxia-Telangiectasia (AT) Complicated By Neutropenia: A Case Report

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RATIONALE: AT is an autosomal recessive disorder due to defects in the ATM gene leading to impaired DNA repair and progressive neurological deterioration after the first year of life. Neutropenia is rare and unreported in infants with AT. Here we present an infant with AT complicated by neutropenia detected by newborn screening.

METHODS: T-cell receptor (TCR)-Vbeta spectrotyping was performed using RT-PCR. Analysis of radiosensitivity and ATM expression were performed at the UCLA DNA Repair Clinical Laboratory. Sequencing of the ATM gene was performed by City of Hope.

RESULTS: A healthy-appearing young male presented at 11 days of life with a presumptive positive newborn screen for SCID (TCR excision circle count of 6 and beta-actin of 42,800). Workup demonstrated low T-cell and B-cell counts, normal NK cells and decreased CD4/CD45RA/CD62L+ cells without maternal engraftment. IgG, lymphocyte mitogen stimulation and TCR-Vbeta repertoire were normal. IgA and IgM were undetectable. ANC at presentation was 1080, and decreased to 320 three weeks later. Antineutrophil antibodies were negative and a bone marrow biopsy was normal. Neutropenia persisted until 10 months of age and resolved without intervention. At 2 months of age, testing revealed increased radiosensitivity and absent ATM expression. ATM gene sequencing demonstrated a nonsense mutation at codon 1268(c.3802del) and a frameshift alteration (c.4358_4359; p.Ile1453Lysfs*37).

CONCLUSIONS: Although newborn screening has previously identified infants with AT, this is the first report of transient, asymptomatic neutropenia associated with AT found in infancy. Early identification of AT may identify other novel clinical variants and further define the disease process and treatment strategies.

Seventeen Month Old Child Presents with Plastic Bronchitis Associated with T Cell Lymphopenia, a Novel Case

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RATIONALE: Plastic bronchitis is a rare, potentially fatal condition characterized by casts that resemble the bronchial tree mainly associated with congenital heart disease; however, cystic fibrosis, primary ciliary dyskinesia, and asthma have also been associated. Here we present a novel case of plastic bronchitis associated with T cell lymphopenia.

METHODS: T & B cell subsets were performed at Children’s Hospital Los Angeles.

RESULTS: Seventeen month old male presents with one day of worsening cough, fever, and fatigue in the setting of a persistent cough for three months productive of “white phlegm” resembling the bronchial tree. The patient had several recurrent infections and was on an albuterol inhaler but had never received steroids. Workup revealed pneumonia, sputum culture with group A streptococcus (GAS) and rare Acinetobacter urisingii but otherwise negative infectious workup. Patient had normal: echocardiogram, sweat chloride, IgE allergen testing, nitro blue tetrazolium, and vaccine antibody titers. T & B cell subsets were notable for low: CD8 T cells (258 cells/µL), CD4 T cells (657 cells/µL), and Natural killer cells (122 cells/µL). Bronchial cast was removed and pathology showed predominantly lymphocytes and macrophages, no evidence of fungi or malignancy and culture growing GAS. He responded to amoxicillin/clavulinate therapy with plans to further workup possible causes including cellular immune deficiency, asthma, and primary ciliary dyskinesia.

CONCLUSIONS: While the exact mechanism of fibrinous cast formation is unknown, here we present a novel case that adds to the body of evidence that suggests that recurrent infection and inflammation and immune dysregulation play a role.
**AB14 Abstracts**

**SATURDAY**

**43 Hemophagocytic Lymphohistiocytosis (HLH) in Noonan’s Syndrome (NS) Successfully Treated with Anti-IL-1beta Therapy**

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**RATIONALE:** HLH is a syndrome characterized by uncontrolled macrophage activation. NS is an autosomal dominant disorder involving RAS-MAPKinase pathway associated with multiple end-organ defects, short stature, and dysmorphic features. We present the first reported case of HLH in NS who improved with use of anti-IL-1beta therapy.

**METHODS:** Genetic Screening for Familial HLH; Bone-Marrow Biopsy; NK-cell cytotoxicity assay; Familial Mediterranean fever (FMF) genetic test.

**RESULTS:** Five-year-old female with NS (KRAS c40>), gastroparesis with G-tube placement, mitral valve prolapse and chronic anemia presented with recurrent fevers. Multiple admissions yielded negative work-up for infectious etiology. Fevers associated with joint pain and increased abdominal discomfort. She met 5 of 8 criteria for HLH: Ferritin >20,000ng/ml; hepatosplenomegaly; fevers; decreased NK-cell activity, and hemophagocytosis on bone marrow biopsy. Primary HLH genetic screening was negative. FMF evaluation was negative. Other period fever syndrome evaluations were pending. Systemic steroids led to some improved symptoms. She was initiated on IL-1beta antagonist (anakinra), which led to resolution of fevers and reduction in joint symptoms. She transitioned to long-acting anti-IL-1beta monoclonal antibody (canakinumab), which maintained control of symptoms.

**CONCLUSIONS:** While HLH has not been a reported feature of NS, RAS-MAPKinase pathway is a crucial component of cell signaling that controls transcription of inflammatory mediators. This case illustrates importance of considering autoinflammatory conditions in NS patients with recurrent fevers. Steroids and cytoreductive agents are currently standard of care for HLH. Efficacy of IL-1beta blockade in this case highlights importance of considering these agents as alternative or adjunctive treatment for HLH.

**44 Nijmegen Breakage Syndrome Detected By Newborn Screening for T Cell Receptor Excision Circles (TRECs)**

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**RATIONALE:** Severe combined immunodeficiency (SCID) encompasses a group of disorders characterized by reduced or absent T-cell number and function and identified by newborn screening utilizing T-cell receptor excision circles (TRECs). This screening has also identified infants with T lymphopenia who lack mutations in typical SCID genes. We report an infant with low TRECs and non-SCID T lymphopenia, who proved upon whole exome sequencing to have Nijmegen breakage syndrome (NBS).

**METHODS:** Exome sequencing of DNA from the infant and his parents was performed. Genomic analysis revealed deleterious variants in the NBN gene. Confirmatory testing included Sanger sequencing and immunoblotting and radiosensitivity testing of patient lymphocytes.

**RESULTS:** Two novel nonsense mutations in NBN were identified in genomic DNA from the family. Immunoblotting showed absence of nibrin protein. A colony survival assay demonstrated radiosensitivity comparable to patients with ataxia telangiectasia.

**CONCLUSIONS:** Although TREC screening was developed to identify newborns with SCID, it has also identified T lymphopenic disorders that may not otherwise be diagnosed until later in life. Timely identification of an infant with T lymphopenia allowed for prompt pursuit of underlying etiology, making possible a diagnosis of NBS, genetic counseling, and early intervention to minimize complications.

**45 Newborn Screening for Severe Combined Immunodeficiency in Delaware: Results of the First 3 Years**

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**RATIONALE:** Severe Combined Immunodeficiency (SCID) is fatal if not diagnosed and treated within the first few months of life. Prior to development of the T-cell receptor excision circle (TREC) assay, the diagnosis of SCID was frequently missed, largely due to the often normal physical exam of these infants. Since 2010, 23 states, the District of Columbia, and the Navajo Nation have begun statewide newborn screening for SCID, leading to earlier detection and markedly improved clinical outcomes. In September 2011, Delaware initiated its pilot program and in July 2012, Delaware officially began screening newborns for SCID using the TREC assay. Data from Delaware newborn screening for SCID is unique in that Nemours/AI duPont Hospital for Children (AIDHC) is the only referral center in the state.

**METHODS:** Approximately 33,000 infants were screened in Delaware from Sept 2011 - August 2014. All infants with a positive screen were referred to AIDHC for diagnostic evaluation.

**RESULTS:** Twenty four infants with positive screens were referred. Of these, 5 patients were premature (<36 weeks gestation). Further evaluation identified 2 patients with SCID, 3 patients with partial DiGeorge syndrome, 10 patients with unspecified T-cell lymphopenia, and 9 patients with normal T cell counts.

**CONCLUSIONS:** As newborn screening for SCID is still in early stages, the clinical characteristics, laboratory features, and long-term outcomes of patients with abnormal TREC values identified by newborn screening are not yet well described. The SCID newborn screening program in Delaware has successfully identified 2 infants with SCID and 13 with non-SCID lymphopenia.
46 A Case of Leaky SCID with Variable Presentation in Two Siblings Identified By Newborn Screening
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RATIONALE: Newborn screening (NBS) for severe combined immunodeficiency (SCID) permits to diagnose SCID early in life, thus prompting definitive treatment before development of serious infections. NBS detects not only typical forms of SCID, but also “leaky” forms of the disease. This has important implications on development of optimal forms of treatment for SCID and related disorders.

METHODS: TREC’s screening was performed at birth on the index patient and retrospectively on her older brother. Flow cytometry was used to define lymphocyte populations in peripheral blood. Mutation analysis was performed using a commercially available SCID gene chip, and validated by Sanger sequence.

RESULTS: An infant girl of Guatemalan origin was found to have undetectable TREC’s at birth by NBS. Flow cytometry confirmed severe T cell lymphopenia, but largely preserved proliferative response to mitogens. During the patient’s evaluation family history revealed that her 3-year-old brother had a history of recurrent infections and post-vaccination zoster. Though he was growing and developing well, he was found to have moderate lymphopenia and mildly decreased T cell function. Genetic analysis revealed a new, homozygous missense mutations in the JAK3 gene (Leu201Pro) in both siblings. Functional tests revealed impaired STAT5b phosphorylation.

CONCLUSIONS: The implementation of TREC’s newborn screening at birth has allowed to timely detect not only typical but also leaky forms of SCID. While early diagnosis remains a mainstay in providing effective treatment, our case shows how variable clinical presentations are likely to pose management dilemmas and how treatment guidelines are needed to address non-classical clinical scenarios.

47 Profoundly Low Immunoglobulins, Lymphopenia, Thymoma and No Infections
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RATIONALE: Good’s Syndrome, thymoma associated with an immunodeficiency, is a rare cause of combined B and T cell immunodeficiency in adults. We present two patients with laboratory evidence of an immunodeficiency, however neither patient has had a significant history of infectious complications to date.

METHODS: A retrospective chart review was performed on two patients referred from Hematology clinic for an immunodeficiency evaluation following diagnosis and treatment of thymoma.

RESULTS: Our first patient, a 45 year old female with history of Type A thymoma diagnosed in 2012, demonstrated an IgG of 220mg/dL, IgM of <8mg/dL and IgA of 135mg/dL. Similarly our second patient, a 57 year old female with history of Type B2 thymoma diagnosed in 2010, demonstrated an IgG of 135mg/dL, IgM of 12mg/dL and an IgA of 7mg/dL. Both patients demonstrated a poor response to pneumococcal antigens as well as either a CD4+ or CD8+ T cell lymphopenia. Of note, our second patient has had documented and untreated hypogammaglobulinemia for the past twenty years without experiencing medical complications. Both patients, noting a lack of recurrent or opportunistic infections and no antibiotic requirements for multiple years, have deferred immune globulin replacement in spite of such a profound hypogammaglobulinemia.

CONCLUSIONS: Treatment of Good’s Syndrome typically requires resection of the thymoma and immunoglobulin replacement. These two patients have deferred the latter of the treatments and continue without recurrent or opportunistic infections, which is a strong clinical measure of the functional status of their immune systems, a status we plan to evaluate in the future.

48 Novel Mutation in a Patient with MHC Class II Deficiency
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RATIONALE: Major Histocompatibility Complex Class II molecules (MHCII) are required for CD4+ T-cell development, peripheral T-cell tolerance, and the initiation of cellular and humoral immune responses. Congenital immunodeficiency resulting from functional MHCII deficiency has been described from germ line mutations within trans regulatory elements controlling MHCII transcription. Here, we present a child with congenital MHCII deficiency arising from a novel mutation within the CIITA gene.

METHODS: Flow cytometry to investigate class II expression on peripheral monocytes, and Sanger Sequencing of CIITA gene.

RESULTS: The patient initially presented at 6 months with PCP pneumonia. Family history was notable for consanguinity and a sibling who died from pneumonia in infancy. Studies showed CD4+ T cell lymphopenia (abs. 291), undetectable IgA, IgE, IgG and IgM of 283. The patient’s clinical course was characterized by: 1) local and systemic bacterial, viral and fungal infections 2) failure to thrive 3) progressive liver disease 4) neutropenia and 5) autoimmune thyroiditis. Class II was absent on peripheral monocytes. Sequencing of CIITA demonstrated that the patient possessed a homozygous nonsense mutation (Q228X) and the parents were heterozygous carriers. The patient remains on infectious prophylaxis and IVIG indefinitely.

CONCLUSIONS: We report a novel nonsense mutation in the CIITA gene leading to MHC Class II deficiency and resultant immunodeficiency. Knowledge of this novel mutation may promote genotype-phenotype correlations as well as guide treatment in similarly affected patients.
AB16 Abstracts

49 Individual Cytidine Deaminase and Adenosine Deaminase Variations in a Highly Immunologically Homogeneous Group of Healthy Belarusian Adults

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RATIONALE: Cytidine deaminase (CDA) and adenosine deaminase (ADA) both have a role in immune responses and their regulation. Their activities may have high variability as individualized immunologic parameters.

METHODS: Using a homogeneous group of healthy volunteers from the Minsk, Belarus area without any systemic illnesses (n=33, age 18-31 mean 24±2, M:F ratio 4:1) serum CDA and ADA levels were assessed. The method of Guisti and Gallanti with prolonged incubation time. Also assessed were immunoglobulin heavy chain (IHC) gene rearrangement status by the Langerak and van Dongen method and CD8+, CD19+ cell numbers by flow cytometry.

RESULTS: On the background of normal CD8+ and CD19+ cell numbers and normal polyclonal IHC gene rearrangement status, the serum CDA level manifested itself as a highly variable parameter with vibration amplitude ranging from 0.58 IU/l to 4.91 IU/l (mean 1.82±0.36 IU/l) while the serum ADA level ranged from 4.01 to 25.97 IU/l (mean 11.94±1.92 IU/l).

CONCLUSIONS: Despite similar normal CD8+ and CD19+ cell numbers and normal polyclonal IHC gene rearrangement status there was more than a 6-fold variation in CDA and ADA serum levels. These enzymes may possibly be a biomarker of individually variable immune responses in patients with hidden immunodeficiency.

50 Pediatric Thymic Development of T Cells and Tregs

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RATIONALE: Thymic development of T cells and Tregs has not been well studied in human when compared to murine. It is not fully known whether markers seen in peripheral Tregs are expressed during thymic development and the association of Helios, BCL2 and BCL10 in survival.

METHODS: Fresh thymi obtained from cardiac surgery of pediatric patients (n=50) were processed into cell suspension for FACS analysis. Patients with hidden immunodeficiency.

RESULTS: There was a major change in the frequencies of CD4/CD8 double negative (DN), double positive (DP) and single positive (SP) at <2weeks (n=18) vs. >2weeks (n=32) old. Foxp3+: DN* (5 vs. 2.4%), DP* (34 vs. 73%), CD4SP* (45 vs. 16%) and CD8SP* (13 vs 7%). Foxp3+: DN (1.4 vs. 1.4%), DP* (2.6 vs 0.8%), CD4SP (12 vs. 11%) and CD8SP (2.7 vs. 2.7%). >90% of Tregs were Helios+ with the greatest expression at the DP stage and unchanged with age. High expression of BCL2 and BCL10 was seen in Foxp3+DP. Foxp3+CD4SP, Foxp3-CD4SP and CD8SP, while Foxp3-DP had low expression. Tregs expressed CD39, CD134, CD137, CD278, CD279, CD25, CD152 and CD127 at both DP and SP stage. *p<0.001.

CONCLUSIONS: Helios, BCL2 and BCL10 are upregulated at the DP stage for Tregs, suggesting a survival and selection advantage. Early in the DP stage, Tregs expressed phenotypic markers. This study provides more insights into thymic development in human.

51 Newborn Screening for Severe Combined Immunodeficiency (SCID) in Ohio: Using Algorithms to Standardize Follow-up Limits the Number of False Positive Results

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RATIONALE: T cell receptor excision circle (TREC) analysis on newborn screening (NBS) dried blood spot specimens has proven to be a successful method of screening for Severe Combined Immunodeficiency (SCID). We hypothesized that standardized algorithms for follow-up of abnormal values would decrease the number of false positives results.

METHODS: TREC sequence and actin was amplified from NBS specimens using real-time PCR. Amplification values from samples of infants previously diagnosed with SCID and data from over 12,000 healthy newborns were used to establish a reference range suggestive of SCID. Immunologists in collaboration with the Ohio Department of Health created algorithms for follow-up on abnormal screens. Abnormal results were classified as moderate or elevated risk. Only infants in the latter category were recommended for immediate flow cytometry and consultation with an immunologist. NBS was repeated per the protocols for infants with moderate risk.

RESULTS: In the first year, approximately 140,000 infants were screened. There were 46 moderate risk infants and 4 elevated risk infants. Thirty moderate risk infants died prior to follow-up and 31 infants “normalized” on repeat NBS. Two pre-term infants with abnormal repeat screens were eventually referred for flow cytometry and diagnosed with T cell lymphopenia but not SCID. Four elevated risk infants with no confounding factors had complete absence of TREC: 3 have been diagnosed with SCID and 1 remains without a definitive diagnosis.

CONCLUSIONS: Algorithms created in advance were effective in standardizing follow-up protocols and minimizing the number of false positive results and infants referred for flow cytometry and immunology consultation.
52 Site Specific Gene Correction of Defects in CD40 Ligand Using the Crispr/Cas9 Genome Editing Platform
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RATIONALE: Definitive treatment for CD40 Ligand defects are currently only achieved with hematopoietic stem cell transplantation. As HSCT carries significant risks, there is a clear need for improved therapeutic modalities. Previous studies using CD40L/-/- bone marrow corrected by retroviral-vector transfer of CD40L cDNA in mice resulted in abnormal lymphoproliferation. An alternative approach is targeted gene repair using TALENs (Transcription Activator-Like Effector Nucleases) or CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats), which target specific DNA sequences and create double-strand breaks, combined with homologous donor sequences serving as repair templates.

METHODS: Six TALENs targeting a patient-specific splice site mutation in intron 3 were unsuccessful in creating gene disruption. This is in contrast to TALENs targeting the 5’UTR that achieve up to 31% allelic disruption in cell lines. Recent work has shown that TALENs are sensitive to DNA methylation states and chromatin structure. CRISPRs do not have the same barriers and several guides were targeted to initiate the patient mutation. After electroporation of K562 cells with CRISPR plasmids, allelic disruption was determined by surveyor endonuclease assay.

RESULTS: Patient-specific CRISPRs achieved >50% gene disruption in K562 cells. Co-electroporation with a template donor modified to contain a unique restriction enzyme site demonstrated site-specific gene integration by enzyme digest and gel electrophoresis. Electroporation of XHIM primary cells are underway and re-expression of CD40L will be assessed by flow cytometry.

CONCLUSIONS: These results show that site-specific modification of the CD40L gene is achievable and that physiologic expression of the endogenous gene could provide a viable therapy for immune reconstitution in XHIM.

53 Rapidly Generated Viral-Specific T Lymphocytes for Treatment of Viral Infections in Primary Immunodeficiency
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RATIONALE: Adoptive immunotherapy using virus-specific cytotoxic T-lymphocyte (VSTs) products has been successful in restoring antiviral immunity after hematopoietic stem cell transplantation (HSCT). We hypothesize that VST produced via a rapid protocol will improve clinical immunity after hematopoietic stem cell transplantation (HSCT). We hypothesize that VST produced via a rapid protocol will improve clinical immunity after hematopoietic stem cell transplantation (HSCT). We hypothesize that VST produced via a rapid protocol will improve clinical immunity after hematopoietic stem cell transplantation (HSCT). We hypothesize that VST produced via a rapid protocol will improve clinical immunity after hematopoietic stem cell transplantation (HSCT). We hypothesize that VST produced via a rapid protocol will improve clinical immunity after hematopoietic stem cell transplantation (HSCT). We hypothesize that VST produced via a rapid protocol will improve clinical immunity after hematopoietic stem cell transplantation (HSCT).

METHODS: VSTs were cultured from HSCT donors using peptide pulsed antigen presenting cells and cytokines. VSTs were tested for specificity and non-allergy reaction via IFN-γ ELISpot and cytotoxicity assays. Patients were followed for 45 days following infusion for toxicity monitoring and for >6 months for antiviral response.

RESULTS: Four patients with PIDD who underwent HSCT were recruited to date, and two have been infused with VSTs at 5x10⁶ cells/m2/dose. The first patient was treated for CMV viremia which was refractory to ganciclovir and foscarnet following haploidentical HSCT for SCID. After two infusions of VSTs the CMV load reduced from >1,000,000 to <1000 copies/ml. The second patient was treated for CMV viremia after matched sibling HSCT for MHC-II deficiency, and had viral clearance within 1-month post-VST infusion. One patient developed Grade I skin GVHD post VSTs which resolved within 1 week and was associated with the tapering of immune suppression.

CONCLUSIONS: VSTs are safe and effective for control of viral infections in patients with PIDD following HSCT. Studies of the efficacy of VSTs in this setting as well as prior to HSCT for PIDD patients are currently ongoing.

54 Alternaria alternata Fungal Exposure Associated with Increased Fractional Exhaled Nitric Oxide Among Children in New York City
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RATIONALE: Home dampness and fungi have been associated with asthma morbidity; however, most supporting evidence is based on subjective reports or sensitization to fungi and not direct measurements of exposure. Further, allergic responses to inhalant allergens may be augmented by combustion byproduct exposure. We hypothesized that fractional exhaled nitric oxide (FeNO), an airway inflammation biomarker, would be associated with the abundance of some domestic dustborne fungal species and that these relationships would be modified by a marker of combustion exposure, elemental carbon (EC).

METHODS: As part of the NYC Neighborhood Asthma and Allergy study, 7-8 year-old children were recruited from neighborhoods with higher (11-19%) and lower (3-9%) asthma prevalence. FeNO was measured from asthmatic (n=125) and non-asthmatic (n=103) children, and bedroom floor dust was collected and analyzed by quantitative polymerase chain reaction for 36 fungi (Environmental Relative Moldiness Index panel). A child’s neighborhood annual airborne EC was estimated using NYC Department of Health data.

RESULTS: Quantities of nine fungal species differed significantly across neighborhoods with differing asthma prevalence, including A. alternata (P=0.010). A. alternata was positively associated with FeNO (b=0.063, p=0.033) after adjustment for sex, race/ethnicity, dust mite exposure, se-rootopy, environmental tobacco smoke, ambient NO and season. This association was present among the children with higher EC exposure (b=0.098, p=0.004), but not those with lower EC (b= -0.018, p=0.65), F_{interaction}=0.024.

CONCLUSIONS: Among NYC children, A. alternata measured in house dust was associated with FeNO, specifically among children with higher combustion byproduct exposure, suggesting a possible interaction between these two exposures on airway inflammation.
**55 Dustborne Fungal Diversity in Middle-Income Homes in New York City and Determinants for Domestic Exposure**

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**RATIONALE:** Recent advances in quantitative polymerase chain reaction methodologies help overcome limitations associated with traditional methods of fungal exposure assessment and provide a sensitive and specific approach to evaluate and quantiﬁy fungal diversity. We hypothesize that abundance/types of domestic dustborne fungi vary across NYC middle-income housing by neighborhood, home habits and housing type.

**METHODS:** In this study, 7-8 year-old children (n=347) living in higher (11-18%) and lower (3-9%) asthma prevalence neighborhoods (HAPN and LAPN, respectively) were recruited as part of the New York City (NYC) Neighborhood Asthma and Allergy Study, an asthma case-control study. Bedroom floor dust from homes in HAPN (n=139) and LAPN (n=142) were collected and analyzed by qPCR for 36 fungal species (Environmental Relative Moldiness Index panel). Differences in fungal abundances were tested across factors chosen a priori based on their potential to influence mold growth.

**RESULTS:** Due to low abundances of fungi in dust samples (<10 spores/mg; >10% per sample), 20 of 36 fungal species were included in statistical tests. The abundance of *Mucor amphibiorium* and *Cladosporium sphaero-spermum* were both reduced in homes where shoe removal was reported (p<0.01). Only *Penicillium glabrum* was elevated with participant reports of indoor mold (p<0.001). *Aureobasidium pullulans*, *Penicillium glabrum*, *Wallenia sebi* and *Alternaria alternata*varied by housing type (single, multi-family or apartment) and neighborhood asthma prevalence.

**CONCLUSIONS:** Overall, these preliminary results indicate that multiple environmental factors including anthropogenic behavior modification, housing type, and neighborhood are important variables that influence fungal diversity within middle-income homes in New York City.

**56 Fungal Viability Is Essential in Modulating of Adaptive Immune Responses in Mice**

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**RATIONALE:** Conidial viability and germination have been previously shown in *vitro* to result in release of allergens. The role of fungal viability in modulating the murine adaptive immune responses is less characterized. The aim of this study was to determine if repeated exposures to heat inactivated conidia (HIC) resulted in the expansion of an allergic adaptive immune response.

**METHODS:** B6C3F1/N mice were repeatedly dosed with dry viable conidia or HIC derived from *A. fumigatus*. The conidia were aerosolized using an acoustical generation system and delivered to mice in a nose-only exposure chamber (2/wk, 13 wks). Twenty-four and 48 hours following the final dose, flow cytometry analysis, histopathology, and gene expression studies were conducted to characterize adaptive immune responses.

**RESULTS:** Repeated exposures to viable conidia but not HIC resulted in goblet cell metaplasia and mucus production in the bronchioles. CD4 T cells (Th2) expressing cytokines associated with an allergic phenotype (IL5, IL-13, IL-9 and IL-22) were present in significantly higher numbers in the airways of animals dosed with viable conidia. In contrast, Th1 cells expressed IFN-γ in mice dosed with HIC. Exposures to HIC stimulated the expansion of B cells in mediastinal lymph nodes, but were significantly less compared to animals dosed with viable conidia.

**CONCLUSIONS:** Findings demonstrated that repeated subchronic exposures to viable *A. fumigatus* conidia lead to an allergic phenotype. This response was not observed in mice treated with HIC. This study further demonstrates that fungal viability is a critical component associated with fungal exposures that impacts Th1/Th2 differentiation in mice.

**57 Comparison of Outdoor Ground Level Airborne Fungal Spore Concentrations with Those of a Roof Mounted Regional Collector**

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**RATIONALE:** Airborne fungal spore concentrations are related to allergic disease. To compare the airborne spore concentrations generated by a roof mounted regional spore collector with those taken at ground level on the same day in the same region we conducted the following.

**METHODS:** Pairs of spore counts on 100 randomly chosen days from 2003 to 2014 were evaluated. Regional collections were on a 5-story building centrally located in an urban area using a Burkard collector mounted according to NAB specifications. Ground level collections were with a portable spore trap device located 1 meter from ground level at various locations within 20 miles of the central collector. Spores were enumerated by a NAB certified counter. Statistical evaluations were based upon logarithm of the values.

**RESULTS:** The logarithm of the spore estimates for both sets of data was normally distributed. Total spore estimates for the ground level collector ranged from 46,044 to 171 per cubic meter of air. Total spore estimates for the regional collector ranged from 25,590 to 354 per cubic meter of air. Correlation for total spores was strong (r = 0.66). The best correlations for major spores was for cladosporium (r = 0.62) and ascospores (r = 0.60). On 30% of dates ground level collections were >10% higher and on 15% of dates regional collections were >10% higher.

**CONCLUSIONS:** Although ground level airborne spores are generally in higher concentration a centrally located regional collector gives a good estimation of spores present on a given day.
A Fifteen Year Study of Airborne Alternaria Spores in Tulsa, Oklahoma

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RATIONALE: Airborne pollen and fungal spores, especially Alternaria spores, are well-known triggers of allergic rhinitis and asthma. This study tracked concentrations of airborne Alternaria over time to investigate variability in spore levels and correlations with meteorological conditions.

METHODS: Airborne pollen and spores have been monitored at the University of Tulsa using a Burkard sampler on the roof of Oliphant Hall. Following collection, air samples were made into permanent slides for microscope analysis. Fifteen years of Burkard slides were examined using a single longitudinal traverse at 1000x magnification, and counts were converted into concentrations. For statistical analysis, concentrations were log transformed. Meteorological data were obtained from the National Weather Service in Tulsa.

RESULTS: Analysis of slides from 1998 to 2012 showed that Alternaria spores are very common in the air and are present approximately 340 days each year. Spore data also showed variability in cumulative yearly total, with the highest occurring in 2012 with 120,055 spores, and the lowest in 2007 with 39,581 spores. Additionally, peak concentrations typically occurred in August through early September. Variability in peak daily concentration was notable with a range from 982 spores/m3 in 2004 to 5026 spores/m3 in 2012. Regression analyses with meteorological data indicated that spore concentrations were positively and significantly correlated with maximum, minimum, and mean temperature (p<0.05) each year. There were negative correlations with relative humidity and precipitation, but these were not significant every year.

CONCLUSIONS: Airborne Alternaria spores are a significant component of the Tulsa atmosphere. Addition analysis is needed to develop predictive models for exposure.

Comparison of Year Round Outdoor and Indoor Fungal Spore Count in Kansas City

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RATIONALE: Fungus has consistently been shown to exist both outdoors and indoors. Fungal exposure has been shown to increase risk of respiratory disease in atopic patients. To determine if the indoor mold count correlated in relation to outdoor seasonal mold counts in the Kansas City area we conducted the following study.

METHODS: For the years 2008-2011 homes of persons enrolled in the Kansas City Safe and Healthy Homes Study. Homes were evaluated for indoor airborne spore concentrations for at least 2 rooms and for outdoor concentrations for at least one location per home. Spores were collected using a portable spore trap device and enumerated visually by a NAB Certified counter for total spores and for 23 identifiable taxa. Data was stored and analyzed on an excel spreadsheet.

RESULTS: A total of 382 homes were observed over the period. Cladosporium was the most common collected outdoor spore while Aspergillus was the most common indoor spore seen. The highest outdoor spore count was noted between June-August. The lowest count was in December-February. The highest indoor count was noted in September-November and the lowest was noted in December-February. Most fungi had little variation indoors except for Aspergillus/Penicillium which was noted to increase during September-November months. There was no significant correlation between outdoor and indoor peaks of mold counts.

CONCLUSIONS: There was no significant correlation which may be due to minimal variation of indoor spore count in comparison to outdoor variation. Aspergillus/Penicillium was counted more indoors when compared to outdoor counts, which could be secondary to environmental susceptibility.

Characterization of Antigen Presenting Cells in a Murine Subchronic Fungal Exposure Model

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RATIONALE: Fungal exposures are associated with adverse health outcomes, including allergic sensitization and asthma. Very little is known about the role of antigen presenting cells (APCs) in allergic fungal diseases. The aim of the study was to characterize the recruitment of APCs following subchronic fungal exposures.

METHODS: B6C3F1/N mice were repeatedly dosed (2/wk, 13 wks) with dry aerosols of viable or heat inactivated conidia (HIC) derived from Aspergillus fumigatus. The conidia were aerosolized using an acoustical generation system and delivered to mice housed in a nose-only exposure chamber. Twenty-four and 48 hours following the final dose, flow cytometry and gene expression studies were conducted on bronchial lavage, lung suspension and mediastinal lymph nodes to characterize the molecular and cellular changes associated with APCs.

RESULTS: Repeated exposures with viable conidia but not HIC resulted in significantly higher numbers of myeloid cells including neutrophils and eosinophils in the airways of mice. Alveolar macrophages were the primary APC population demonstrating elevated expression of MHC class II and the co-stimulatory molecules CD80 and CD86. Both, CD103+ and CD11b+ dendritic cell subsets did not exhibit changes in expression of co-stimulatory molecules. In addition, repeated exposures to viable and not HIC resulted in the recruitment of Ly-6C+ inflammatory monocytes.

CONCLUSIONS: The data presented here indicate that AMs are an important APC population controlling the allergic phenotype following subchronic fungal exposures. Gaining a better understanding of the role of alveolar macrophages in fungal allergy may provide new understanding of immunological mechanisms for therapeutic intervention.
Seasonal and Daily Distribution of Allergic Epicoccum Spores in Ambient Air in Vinnitsa, Ukraine
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RATIONALE: Epicoccum tends to be found in grassland and agricultural areas and is a cause of exacerbation of allergic diseases in the summer-autumn period.

METHODS: Spore counts were obtained at Vinnits National Pirogov Memorial Medical University (VNMU) in 2009-2011 daily and in 2012-2013 bi-hourly using a Burkarad trap at 25 meters height above ground from March 1 to October 31.

RESULTS: Epicoccum spores were present in the ambient air through the entire observation period. Spore concentrations were less than 20 spores / m3 from March until mid-July but exceeded 40 spores / m3 later with maximal spore concentrations from the third ten-day period of September through October. 2009 was an exception with a spore concentration value of 70 spores/ m3 on July 14. The peak count in 2014 was seen on October 26, 2014 at 883 spores/m3 while the 2010 peak was September 23, the 2011 was October 2 and the 2012 was October 8 ranging from 105-164 spores/m3. Higher concentrations were seen at mid-to-late day each year including 2012 peaking around 3 p.m. and in 2013 from 9 a.m. to 3 p.m and in 2014 from 11 a.m. to 7 p.m. in March until mid-August.

CONCLUSIONS: Epicoccum spores in the ambient air of Vinnitsa, Ukraine are significant in the late summer and autumn with peaks usually recorded from the third ten-day period of September to the third ten-day period of October and daily distribution patterns characterized by mid- and late- daytime daily peaks.

Outdoor Fungal Spore Exposure in the Midwestern United States
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RATIONALE: Outdoor fungal spore exposure is one of the historic causes of allergic symptoms. To examine the seasonal nature of this exposure, we conducted the following:

METHODS: Spores were collected daily, weather permitting, for 17 years using a Hurst spore trap (Burkard) mounted on the top of a 5 story building in a major Midwestern metropolitan area. Spores impacted onto the coated surface were mounted in glycerin jelly containing Calherbas stain. Airborne spore concentration estimates were obtained by enumerating all identifiable fungi for a portion of the slide using a modification of the 12 traverse method. Spore counts were stored in an Access database and statistical manipulations were conducted in Excel spreadsheet.

RESULTS: During the 17-year study period, over 1 million individual spores were evaluated. Spores were collected during all months of the year; however, the vast majority were collected from May through October (87.8%) with less than 1% of total spores collected from December through February. The lowest spore counts were noted in January (0.2%), increasing during the year to peak levels in August (20%). The years with the highest and lowest spore counts were 2007 (10.4% of total) and 2001 (3.2% of total), respectively.

CONCLUSIONS: Airborne spores can be collected in the Midwest United States throughout the year; however, they are more prevalent in the summer months. Spore counts were highest in August and lowest in December and January.
65  TH2 Type Immune Responses in Patients with Chronic Progressive Pulmonary Aspergillosis
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RATIONALE: Aspergillus species is ubiquitous fungus causing noninvasive and invasive aspergillosis in human lungs. The wide spectrum of Aspergillus-related pulmonary aspergillosis ranges from atopic asthma to invasive pulmonary aspergillosis (IPA). Chronic Progressive Pulmonary Aspergillosis (CPPA) is an entity of gradual and progressive destruction of the affected lung developing from aspergilloma in a cavity or pleural dead space after lung surgery. We here report two patients with CPPA without history of any allergic disease showing profiles of Th2 response.

METHODS: Patient 1; 73 year-old woman with COPD received volume reduction surgery in 2005. In 2009 the chest x-ray film revealed thickened pleura at the apical portion of the operated lung. Patient 2; 73 year-old man had received lobectomy of left upper lobe because of tuberculosis in 1960. In 2010 chest x-ray film revealed diffuse bronchiectasis and thickened pleura with aspergilloma at the apical portion of the operated lung. Serum IgE levels, IgE antibodies were measured, and eosinophil numbers were enumerated longitudinally.

RESULTS: In both patients Aspergillus species was cultured from sputa and serum anti-Aspergillus IgG antibodies were positive. Plasma β-D-glucan levels were 10.8 and 15.6 pg/ml, respectively. Serum IgE levels were 1,604 IU/ml in patient 1 and 4,501 in patient 2. Anti-Aspergillus IgE antibodies were 11.8 UA/ml and 83.6 UA/ml, respectively. IgE antibodies against Dermatophagoides farinae were also positive. Periodic hypereosinophilia were observed.

CONCLUSIONS: In CPPA Th2 response is induced without any allergic symptom unlike to ABPA.

66  Predictors of Bedroom Allergen Exposures in U.S. Homes
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RATIONALE: Bedroom allergen exposures contribute to allergic disease morbidity because people spend considerable time in bedrooms, having close contacts with allergen reservoirs. We investigated housing characteristics and sociodemographic factors associated with bedroom allergen burden in a nationally representative sample of the U.S. population.

METHODS: Data for this cross-sectional analysis were obtained from the National Health and Nutrition Examination Survey (NHANES) 2005-2006. Information on participant and housing characteristics was collected by questionnaire and environmental assessments. Concentrations of 8 indoor allergens (Alt a 1, Bla g 1, Can f 1, Fel d 1, Der f 1, Der p 1, Mus m 1, and Rat n 1; N=6832) in vacuumed dust collected from bedroom bed and floor were measured by immunosays. Allergen burden was classified as high when ≥1 allergens were detected, or ≥2 allergens exceeded levels associated with asthma morbidity/allergic sensitization; and low when ≤2 allergens were detected or no allergens exceeded the thresholds. We identified independent predictors of allergen burden using multivariate logistic regression.

RESULTS: Almost all participants (≥99%) had detectable allergens, 74.2% had 3-6 allergens detected. Approximately 70% of participants had at least one allergen exceeding the morbidity/sensitization threshold values, and 9.7% had at least 3 allergens exceeding these levels. Allergen burden was most consistently associated with the presence of pets and pests, age and type of the home, residents’ age, race, and income.

CONCLUSIONS: Exposure to multiple allergens is common in bedrooms. Allergen burden is influenced by the presence of allergen sources (pets, pests), sociodemographic factors, and housing characteristics.
68 IgE to Furry Animal Allergen Components Was Associated with Asthma in a Population-Based Study of Adults
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Rationale: Atopic sensitization increases the risk of asthma. Our aim was to investigate patterns of sensitization to allergens and animal allergen components in relation to asthma among adults in a Swedish population-based sample.

Methods: A questionnaire targeting asthma was sent to 30,000 randomly selected adults (16-75 y) in western Sweden. Of the 18,087 responders, a randomly selected sample of 2000 subjects and in addition all 1536 subjects reporting asthma were invited to clinical examinations. Serum was received from 906 subjects with asthma and from 1000 without reported asthma (controls) and were tested for three IgE panels of inhalant and food allergens. Samples with a level of IgE ≥ 0.35 kUA/L were further analyzed for IgE against each individual allergen and nine allergen components related to furry animals.

Results: The risk of being sensitized to any of the 21 tested allergens was significantly higher in the asthma group. The risk was highest for individuals sensitized to furry animals (risk ratios 4.5-7.9). The level of IgE to animal allergens was also significantly higher in the asthma group. Co-sensitization to more than one cat and/or dog allergen was associated with a greater risk for asthma. Simultaneous sensitization to the cat allergens Fel d 1 and Fel d 4 was associated with asthma.

Conclusions: In this large population-based study subjects with asthma were commonly co-sensitized to more than one animal allergen component, especially to Fel d 1 and Fel d 4. Component-resolved analysis has the potential to increase precision when assessing asthma in adults.

69 IgE Antibodies to Mammalian Allergens Are a Major Risk Factor for Prevalence, Severity, and Persistence of Asthma in Northern Sweden
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Rationale: IgE to mammalian allergens can contribute significantly to asthma risk. Studying the details of the relationship between animal sensitization and asthma is simpler in an environment where mite, fungal, and cockroach allergens make little or no contribution to asthma risk.

Methods: Quantitative assays for IgE to eight allergens were carried out on 963 sera from 19-year-olds in a population-based cohort in northern Sweden, and associations with questionnaire data from ages 7, 12, and 19 on asthma symptoms, diagnosis, and treatment were tested.

Results: Overall, 79 (53%) of the students with a physician diagnosis of asthma were positive to one or more of the mammalian allergens (cat, dog, or horse danders) tested. Of the allergens assessed, only mammalian allergens, birch, and timothy grass pollen showed a significant relationship with asthma diagnosis. Multivariate analysis showed that high titer (>17.5 IU/ml) IgE to any mammalian allergen had the strongest relationship with asthma at age 19 (odds ratio 5.1 [3.0-8.6]). Furthermore, IgE to mammalian allergens gave an odds ratio of 8.5 [4.9-15] for asthma that started before age 12 and was still present at age 19. Sensitization to Fel d 1 and Fel d 4 was strongly associated with asthma and significantly reduced in cat owners.

Conclusions: Sensitization to cat and dog related allergens, and specifically to the components Fel d 1 and Fel d 4, is a major risk factor for the persistence and severity of asthma in an area where these are the only significant perennial allergens.

70 Association of Sensitization to Specific Pet Allergen Components with Asthma Symptoms in School Children
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Rationale: Animal sensitization is a known determinant of asthma in children. The objective was to study the association of asthma with sensitization to pet allergen components in schoolchildren.

Methods: A random sample of 696 children (11-12 y) from a Swedish population-based cohort was analyzed for sensitization (≥0.1 kUA/L) to cat, dog and horse dander extracts using ImmunoCAP. Sensitized children were further analyzed for IgE antibodies to animal allergen components using ImmunoCAP ISAC112. An expanded ISAAC questionnaire was completed by the parents.

Results: Of 259 animal-sensitized children (37%) the majority (75%) were sensitized to more than one species. Among the 11% (n=77) with current asthma 69% were sensitized to at least one animal extract, as compared to one third of children without current asthma (p<0.001). Current asthma and asthma symptoms upon contact with cats were associated with co-sensitization to Fel d 1 and Fel d 4. Already at moderate levels of IgE antibodies to Fel d 4 (1-15 ISU), at which level children were sensitized also to Fel d 1, the prevalence of asthma symptoms upon contact with cats was significantly increased. Dog-sensitized children were commonly sensitized to several dog components, and the greatest risk for asthma was seen in children co-sensitized to Can f 5 and Can f 1 (f 2).

Conclusions: Among Northern Swedish schoolchildren furry animals were the main perennial sensitizers. Asthma symptoms were associated with sensitizations to multiple components within an animal species. In particular, cat Fel d 4 sensitization was strongly related to asthma symptoms.
Recommendation of Nutritional Alternatives for Children Between 1 and 2 Years of Age with Cow’s Milk Allergy

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Rationale: Cow’s milk allergy (CMA) poses a challenge to adequate nutrition in children between 1 and 2 years of age, as many alternatives generally do not provide comparable nutrition to whole cow’s milk. A summary report from an international consensus panel of allergy specialists (the Diagnosis and Rationale for Action against Cow’s Milk Allergy, DRACMA) recommends a hypoallergenic formula in the absence of breast milk until 2 years of age. We hypothesized that caregivers experienced in counseling children with CMA would recommend a variety of alternatives, some being nutritionally inadequate (i.e. enriched alternative milks).

Methods: We asked 120 pediatric generalists, allergists, gastroenterologists, and nutritionists to complete an online survey to learn their recommendations for first-line alternatives for children with CMA and CM/soy allergy and identify barriers to recommendations.

Results: Thirty-seven responses (31%) were analyzed (86% physicians). Only 14% of providers recommended a hypoallergenic formula for children with CMA. An infant soy formula was recommended by 14%. Soy milk was recommended by 46% and almond milk by 19%. For children with CM/soy allergy, 42% of providers recommended a hypoallergenic formula and 41% recommended almond milk. “Cost,” “availability” and “palatability” were all cited as barriers to recommendations but only 19% felt that barriers “often” caused them to change recommendations.

Conclusions: Nutritional recommendations for children with CMA and CM/soy allergy varied in our small population with the majority of recommendations being nutritionally inadequate based on DRACMA guidelines. Common barriers do not appear to affect recommendations in most cases suggesting that education may lead to more appropriate recommendations.

Impact of Clinical Reactions on IgE Concentrations to Egg, Milk and Peanut in the Observational Cohort of the Consortium for Food Allergy Research (COFAR)

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Rationale: PN-OIT is effective therapy in many peanut allergic patients. Adverse reactions have been reported during build-up (BUP) and maintenance phases (MP). PN–specific IgE (PN sIgE) and component resolved diagnostics (CRD) may have utility in predicting these reactions.

Methods: 177 patients 4 years of age and older with PN allergy confirmed by history, skin test and/or PN sIgE levels and CRD were treated at The New England Food Allergy Treatment Center. Patients were desensitized to a maintenance dose (MD) of 3-4 peanuts daily. Daily diaries were completed. Statistical analyses were performed using Spearman’s rho for correlations and Student’s t or Mann-Whitney U tests for between-group comparisons.

Results: MD was achieved in 164 (93%) patients. During BUP, 71% experienced oral/pharyngeal (OP) itching, 68% experienced gastrointestinal (GI) and 8% respiratory symptoms. Systemic reactions were observed during MP in 5% of patients. GI and OP symptoms most commonly observed at the 6 mg dose. Higher PN sIgE levels were significantly correlated with CRD symptoms (p <0.001), and greater total symptoms (p <0.001) during the BUP. Higher levels of Ara h1, Ara h2, and Ara h3 were significantly correlated with greater total amount of symptoms (p = 0.022, p= 0.019, p= 0.002 respectively).

Conclusions: GI and OP symptoms were prevalent and most common at the 6 mg dose of PN. Elevated initial PN sIgE, and Ara h 1.2 and 3 were good clinical predictors for adverse reactions during BUP and MP of PN-OIT. In spite of symptoms, over 90% reached MD.
Quality of Life and Challenges with Peanut Consumption after OIT

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RATIONALE: Oral immunotherapy (OIT) has demonstrated efficacy in desensitizing patients with peanut allergy. However, follow-up data is needed to assess barriers to continued peanut consumption and long-term impact of OIT on quality of life (QoL).

METHODS: 11 children who completed rapid oral desensitization with omalizumab therapy for peanut allergy participated in follow-up assessments at six-month intervals post-OIT. Children and parents completed food allergy-specific measures of QoL and interviews about peanut consumption status. For each subject, data from the most recent follow-up was analyzed.

RESULTS: Children were aged 9 to 17 years, with a range of 6 to 25 months (mean 15.6) since completion of OIT. One child had discontinued peanut consumption due to allergic reactions, and one was taking 500 mg of peanut flour every 1 to 2 weeks due to aversion to the taste of peanut. The remainder were taking the equivalent of 5 to 20 peanuts per day. Nearly all children (91%) reported disliking the taste of peanut, with 55% of families describing periods of daily stress with child peanut consumption, due to aversion to the taste/smell. Allergy-related QoL (allergen avoidance, social/dietary limitations, anxiety about accidental exposure) was significantly improved at follow-up from pre-OIT, based on child report (p<0.01), adolescent report (p<0.05), and parent-proxy report (p<.001), as was parental burden due to food allergy (p<.001).

CONCLUSIONS: Results suggest improvements in allergy-related QoL following OIT. However, regular consumption of peanut is burdensome for many children, with implications for maintenance of desensitization. Larger studies with longer follow-up periods are recommended to evaluate QoL post-OIT.

Basophil Hyporesponsiveness Following Six Months of Peanut Oral Immunotherapy (OIT) is Associated with Suppression of Syk Phosphorylation

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RATIONALE: Peanut OIT causes clinical desensitization in most subjects and is associated with suppression of basophil and mast cell responses, although the mechanisms of effector cell suppression are not understood. We hypothesized that diminished signaling through FceRI is associated with basophil hyporesponsiveness.

METHODS: Subjects underwent 6 months of OIT then were assessed for desensitization and sustained unresponsiveness after OIT was stopped for 1 and 3 weeks, or 2 and 4 weeks. Whole-blood basophil assays were performed 2 months prior to OIT initiation, at several time points while on OIT, and up to one month off OIT. Basophils were defined as CCR3+ lymphocytes, and assessed for CD63, CD203c, and phosphorylated-Syk after 30 minutes of ex vivo stimulation.

RESULTS: Seven subjects on OIT for at least 3 months were included. Four subjects completing OIT were desensitized, but did not exhibit sustained unresponsiveness. While subjects were on OIT, there was a decrease in %CD63+ basophils stimulated with peanut or anti-IgE (p<0.01); decreased CD203c upregulation following stimulation with peanut (p<0.05); and Syk phosphorylation was reduced in response to both doses of peanut tested (p<0.05). Wheat diameters of skin prick tests to peanut decreased during OIT (p<0.05), whereas peanut-IgE and IgG4 increased. After subjects abstained from peanut OIT dosing for 4 weeks, % CD63+ basophils increased (p<0.05), whereas the other parameters did not change significantly.

CONCLUSIONS: A 6 month course of peanut OIT transiently suppresses basophil activation and is linked with decreased phosphorylation of Syk, suggesting that desensitization may be mediated by disruption of IgE-FceRI signaling events.
Double Oral Milk and Egg Immunotherapy
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RATIONALE: Nearly 30% of patients with food allergy have clinical reactivity to several food allergens. Our goal was to evaluate the safety and effectiveness of the double oral immunotherapy (OIT) for egg and cow’s milk protein.

METHODS: Nineteen patients diagnosed with cow’s milk and egg allergy, were enrolled in OIT protocol with one allergen and subsequently with the other one. Total IgE and Specific IgE levels to milk and egg, were obtained before each challenge. Both OIT were performed with weekly increases at our medical consulting room.

RESULTS: Median age at the beginning of the OIT was 5 years (2-9). Fifteen (80%) children were allergic to more than 3 food allergens simultaneously (nuts 47%, fruits 37%, fish 21%, seafood 15%, legumes 10%). Eighteen patients began with milk OIT. In milk OIT protocol: initial total IgE median was 389 (104-2092), Specific milk IgE 9.27 (0.23-132), casein 13.25 (<0.35-156). For egg OIT: initial total IgE median was 608 (125-2110), OVA 4.1 (1.2-56.6), OVM 3.05 (0.47-99.5). The median of the interval between both procedures was 4 years (1-6). Eighteen patients tolerated both milk and egg without problems. The time that has passed from the last OIT is 0.5-8 years. All patients remain free milk and egg diet. One patient is currently in treatment with Omalizumab, introduced during egg OIT, due to repeated adverse reactions.

CONCLUSIONS: In our experience, consecutive OIT is feasible, even between different food groups. Long term follow-up was effective and satisfactory. Successive OIT it is a useful method for patients with multiple food allergy.

Long Term Follow-up of Patients Successfully Completing Oral Immunotherapy for IgE-Mediated Cow’s Milk Allergy
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RATIONALE: Oral immunotherapy (OIT) has emerged as a therapeutic intervention for patients with persistent IgE-mediated cow’s milk allergy (IgE-CMA). The long-term safety and efficacy of milk-OIT in these patients, however, requires additional investigation.

METHODS: Patients (n=192, >4 years) who reached full dosage (7200 mg) in a milk-OIT program, were instructed to consume at least 3600 mg of CMP daily. After ≥6 months of reaching full dosage, a questionnaire regarding current CMP consumption, allergic reactions, and quality of life issues (QOL) was administered. Skin puncture testing and basophil activation tests (BAT) were performed. Patients who discontinued consuming CMP underwent an OFC.

RESULTS: Overall, 191/192 (99.5%) patients answered the questionnaire. Median time from completing OIT was 24.9 months (range, 6-43.6). CMP was consumed on a regular basis by 175/192 (91.6%) patients. Reactions were noted by 97/192 (51.1%) of patients and 33/192 (17.4%) had multiple (>5) reactions since completion of the program. While the majority of reactions were mild, 16/192 patients (8.4%) required injectable epinephrine. Discontinued consumption of CMP was associated with multiple reactions (p=0.037) and with the use of injectable epinephrine (p=0.027). Allergen-induced basophil CD63 expression was significantly higher in patients who had reactions or required epinephrine (p=0.046 and p=0.005, respectively). CMP wheal size decreased significantly in patients who continued compared to those who discontinued CMP. QOL improved in 169/192 (88.9%) patients.

CONCLUSIONS: Most patients, who reach full dosage during milk-OIT, continue to consume dairy products after completion of the program. The BAT may be a potential predictor of reactivity during long-term milk-OIT.

Influence of Wheat on the Outcome of Oral Food Challenge (OFC) to Baked Egg
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RATIONALE: Eating egg protein in baked form may hasten outgrowing of an egg allergy. Baking egg with wheat flour has been shown to decrease in vitro antigenic activity to heat-resistant ovomucoid. To determine the effects of wheat flour on baked egg tolerance in vivo, we studied the outcomes of OFCs to baked egg in egg allergic children.

METHODS: A 2-year retrospective chart review was performed in 104 egg allergic children, ages 0.9 to 16.8 y (median 5.7 y), who were sensitized to egg by skin test and/or specific IgE and who underwent OFCs to baked egg. The effect of wheat flour or a wheat replacer (rice flour) on OFCs to baked egg in a standardized muffin (2.2 g of egg protein) was assessed.

RESULTS: Eighty-nine (85.6%) children were challenged using an egg muffin baked with wheat flour. Fifteen (14.4%) received a muffin containing wheat replacer. Overall, 68 (65.4%) children passed and 36 (34.6%) failed OFCs to baked egg. In the wheat group, only 30.3% (27/89) failed, while 60% (9/15) of the non-wheat group failed. Females comprised only 37% of the cohort. After adjusting for gender, the odds ratio of failing an OFC to baked egg with a muffin containing wheat replacer was 3.56 (95% CI 1.16,11.69; p=0.026) compared to a muffin containing wheat flour.

CONCLUSIONS: Children undergoing OFCs to egg in baked goods made with wheat replacer may be at an increased risk for failing an OFC. Wheat replacers should only be used when clinically indicated, as in wheat allergic children.

All abstracts are strictly embargoed until the date of presentation at the 2015 Annual Meeting.
**80 Evaluation of Plasma Chemokines and Cytokines in the Setting of Egg Oral Immunotherapy (OIT)**

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**RATIONALE:** The goal of this study was to determine if Th1 and Th2 associated chemokines/cytokines previously reported in allergic infants have utility as biomarkers of response to OIT.

**METHODS:** Plasma samples were obtained from subjects (age 5-11) who participated in CoFAR3, an OIT trial for egg allergy. Chemokin/chemotactants of Th1 cells (CXCL9, 10, 11) and Th2 cells (CCL1, 17, 22) as well as Th2-promoting cytokines (TSLP, IL-33) were measured by multiplex assay at baseline and after 22 months of treatment and analyzed in response to clinical outcomes.

**RESULTS:** Plasma was available for 55 subjects (15 placebo, 40 OIT) at baseline and 51 subjects at 22 months (12 placebo, 39 OIT). CCL1, 17, 22 and CXCL9, 10, and 11 were detectable in all samples at baseline (medians of 4.4, 56.5, 1220, 786, 451, and 393 pg/ml, respectively), while TSLP and IL-33 were detectable in a subset of samples. Spearman rank correlation showed significant positive correlation between cytokines and chemokines with overlapping or related function (CCL17 and 22 (rS = 0.427, p = 4.8x10^-6), TSLP and IL-33 (rS = 0.72, p = 6.4x10^-16), CXCL10 and CXCL11 (rS = 0.51, p = 2.7x10^-5)). There were no significant differences between 0 and 22 months in any of the chemokines/cytokines in either placebo or OIT group, and no significant association of any of these measures with clinical outcome of desensitization or tolerance.

**CONCLUSIONS:** This study reports plasma chemokines for egg-allergic subjects in the setting of OIT. These chemokines and cytokines show significant correlation according to functional groupings (Th1, Th2, and Th2-promoting), suggesting common regulation, but are not significantly altered by OIT.

**81 Baked Egg Oral Immunotherapy (OIT) for Baked Egg (BE) Allergic Children**

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**RATIONALE:** Data shows that frequent ingestion of BE may hasten tolerance to egg for egg-allergic individuals. Treatment lacks for more severely egg allergic children reacting to baked and lightly-cooked egg. We hypothesized that BE OIT will effectively desensitize 80% of BE allergic children after one year.

**METHODS:** Subjects reacting to a standard baked egg product containing 3.8g of egg protein per serving or with ovomucoid IgE>50kU/L were included. Dosing started with ingestion of a 125mg BE product, then home up-dosing at 2 and 4 weeks and 3, 6, and 9 months. At 12 months subjects were challenged to 3.8g of BE. Egg white (EW), ovomucoid-, and ovalbumin-specific IgE, EW SPT, and when possible, BE SPT were obtained at 0, 6, and 12 months. Matched data was compared using Wilcoxon analysis.

**RESULTS:** Seven of 12 subjects completed therapy. Five subjects withdrew secondary to non-compliance with BE ingestion. Adverse events were mild. Reaction rate was 1.8%. Six of 7 subjects (85%) passed the 3.8g BE challenge at 12 months. 6-month mean EW SPT wheal diameter significantly decreased (p=0.0149). 12-month mean EW IgE significantly decreased (p=0.0156). Ovomucoid (p=0.1094) and ovalbumin (p=0.2188) IgE did not significantly change.

**CONCLUSIONS:** Baked egg ingestion may be possible for the most severely egg-allergic individuals. In our cohort, after one year of BE OIT, 6 of 7 children were able to safely ingest a BE product, which had previously triggered a reaction. EW SPT wheal diameter and EW IgE significantly decreased, indicating diminished mast cell reactivity to egg.

**82 Web-Based Reporting Increases Reporting Compliance during the Home Treatment Phase of OIT**

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**RATIONALE:** Reactions during the home treatment phase of oral immunotherapy (OIT) are not uncommon. An ongoing accurate reporting of home treatment outcomes is crucial for OIT safety and success. Previous reports demonstrating that only 20% of patients are truly compliant with paper-based diaries necessitated the development of an efficient and reliable reporting system.

**METHODS:** A website-based electronic reporting system (web-RS) was developed and incorporated a thorough questionnaire querying for pertinent data, including the dose(s) consumed, the presence or absence of adverse reactions, the organ systems involved and the treatment administered. All patients enrolled in an OIT program for at least four weeks at a hospital center from 11/2012 to 01/2014, were introduced to web-RS (n=157). Successful reporting through web-RS was defined by consecutive reporting throughout the first home treatment phase (23 day duration). Comparisons were made to an earlier group of OIT-patients from our program (n=100), who reported by email.

**RESULTS:** Successfully reporting was achieved by 142/157 (90.44%) of patients, in contrast to a 75% success rate with email reporting (p=0.0009). The odds ratio for successful reporting by web-RS was 3.2 (1.6-6.3; 95% confidence interval), as compared to reporting by email. Patient reports were constantly available in real-time for medical staff review. No complaints regarding web-RS feasibility were reported. One risk factor for patient failure to utilize web-RS, was a prior experience of successful OIT treatment, without using web-RS (p=0.012).

**CONCLUSIONS:** Web-RS is a powerful tool for improving OIT safety by achieving a high level of patient cooperation in accurately reporting home treatment results.
Humoral and Cellular Immune Responses in Milk-Allergic Children on an Extensively-Heated (baked) Milk-Containing Diet
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Rationale: Many milk-allergic (CMA) children tolerate baked-milk products. We sought to evaluate the trajectory of humoral and cellular responses to cow’s milk (CM) proteins in children placed on baked-milk diets.

Methods: CMA-children were challenged with increasing amounts of more allergenic forms of milk (MAFM) at baseline. Following the challenges, tolerated baked-foods were incorporated into the diet. Children were randomized 1:1 to have challenges repeated at 6- or 12-month-intervals to advance the diet over 36 months. Antibody concentrations were measured with UniCAP®; basophil activation and T regulatory cells were measured by flow cytometry.

Results: 136 children (70% males) were enrolled (median age: 7 yrs; inter-quartile range, 5-9 yrs). Forty-one (30%) reacted to muffin, 31 (23%) to pizza, 11 (8%) to rice pudding, 43 (32%) to unbaked-CM; 10 (7%) tolerated unbaked-CM at baseline. CM protein-IgE levels and basophil reactivity were negatively correlated with tolerance to MAFM at baseline. Children who became tolerant to unheated-milk (n=37) in the study, had a statistically significant decrease in CM-, beta-lactoglobulin-, casein-IgE antibody levels and maximum basophil activation to CM, and an increase in casein-IgG4 at the time of tolerance to unbaked-milk compared to baseline. There were no differences in peripheral blood T-regulatory cell numbers. Overall, there was no difference in the evolution of immunologic parameters between the randomized groups.

Conclusions: As seen with OIT, tolerance to unheated-milk in children who ingested baked-milk products on a regular basis is associated with decreasing CM protein-IgE and basophil reactivity to CM and increasing casein-IgG4.

Epinephrine Following Completion of Oral Immunotherapy for IgE-Cow’s Milk Allergy
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Rationale: Despite successfully completing food oral immunotherapy (OIT), some patients continue to experience adverse reactions, and may even require injectable epinephrine. Whether reactions requiring epinephrine predict subsequent severe reactions is unknown.

Methods: Patients who had reached full dosage of cow’s milk protein (CMP) (7200 mg=240 ml) during milk OIT were contacted at 12-36 months following completion of treatment and again 12 months later. Patients were asked about current CMP consumption, and occurrence of severe allergic reactions requiring injectable epinephrine. Skin prick testing and basophil activation tests were performed.

Results: Overall 200 patients were contacted and all responded to the questionnaire. Injectable Epinephrine was administered to 13 patients (6.5%). Mean starting dose of OIT in these patients was 130 mg and 7 patients were asthmatics. Interestingly, 8 of the patients did not require epinephrine during the course of oral immunotherapy until they had reached full dose. Timing of epinephrine administration ranged between 2-26 months after completion of treatment. The underlying triggers were considered to be exercise (n=5), viral infection (n=5), menstrual period (n=1), excitement (n=1) or a combination of those. In 4 patients no underlying trigger could be identified. 2 patients discontinued OIT. Only 2 patients reported the use of epinephrine during the additional follow-up year.

Conclusions: Severe reactions to CMP after completion of OIT, although infrequent, still occur. Most of these patients choose to continue treatment without subsequent severe reactions. In most reactions a specific trigger could be identified and avoided.

Single Practice Six-Year Experience Treating Food Allergy with Oral Immunotherapy
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Rationale: Interest in FOIT in the practice setting continues to increase. We report a review of 241 FOIT treated patients who reached their target dose and 53 who did not.

Methods: Retrospective record review of all patients initiating FOIT from 6/10/08 to 6/30/14, approved by the North Texas IRB. Patients received increasing FOIT doses with target doses of (mg of protein) cashew 2000, egg 4545, milk 8000, peanut 2000 pecan 2190, wheat 8000.

Results: 82% of patients reached their target dose. 66% of patients who reached the target dose and 62% of those who did not had a history of systemic reaction to the allergenic food before FOIT treatment. Median FOIT asIgE (kU/L) dropped at least 48% immediately after FOIT completion. The median decrease in asIgE (kU/L) from before FOIT to one month after reaching the target dose was 48% whole egg, 67% egg white, 72% milk, and 51% peanut. Patients who discontinued treatment had a higher pre-FOIT asIgE than those who reached target.

Conclusions: While anaphylaxis before starting FOIT does not help predict which patients will reach the target dose. Those with higher pre-FOIT asIgE may be less likely to reach the target dose. An increase in reactions during escalation may be a predictor of patients that are more likely to discontinue FOIT.
Safety of Specific Oral Tolerance Induction (SOTI) with Partially Hydrolyzed Cereals in Correlation to Wheat-Protein IgE

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RATIONALE: A major drawback of specific oral tolerance induction (SOTI) using standard food products is the possibility of severe side effects. The purpose of this study was to correlate safety of SOTI using partially hydrolyzed Cereals (pHC) in children diagnosed for IgE-mediated allergy to wheat with specific IgE titers to various wheat proteins.

METHODS: In a multicenter, pilot open study, 9 children (aged 1 to 12 years), diagnosed with IgE type wheat allergy (positive challenge or unambiguous history with high IgE titers), were recruited. On the SOTI initiation day they received increasing amounts (0.2 – 25 g) of pHC and thereafter the maximum dose tolerated during 6 months. The primary endpoint was immediate adverse reaction to pHC during SOTI. IgE values and other immunological parameters were evaluated.

RESULTS: Four infants showed allergic reactions after the first ingestion of 0.2g (1), 2g (2) or 8g (1) of pHC and SOTI was not continued. A high level of IgE (>100) to wheat and to hydrolyzed wheat seems predictive for a clinical reaction to pHC. Four infants completed the study taking 25g of pHC each day for 5-6 months and all 4 successfully passed the challenge test at the end of the study (final dose 60-66 g of wheat).

CONCLUSIONS: SOTI using pHC represents a promising option for wheat allergic patients. The IgE allergen profile of the patient (to native and hydrolyzed wheat) could help to predict tolerance to pHC and thus be useful to select patients which could benefit from this approach.

Memory B Cells Are Necessary for the Adoptive Transfer of Murine Peanut Allergy

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RATIONALE: We hypothesize that memory B cells are necessary and sufficient for the adoptive transfer of murine peanut allergy (PNA).

METHODS: Peanut-allergic donor C3H/HeJ mice (with similar anti-peanut IgE levels, symptom scores (SS) and temperature drops (dT) following intraperitoneal (IP) challenge with crude peanut extract (CPE)) were treated intravenously with either anti-CD20 or isotype control (IC) antibody for 18 weeks. Naïve, lethally irradiated recipients were given bone marrow (BM) and splenocytes (SPL) from either anti-CD20 or IC-treated donors. In a separate experiment, B cells (negatively selected, >96% pure) from SPL of peanut-allergic or naïve donors were injected into naïve, irradiated recipients with or without the addition of naïve SPL. Sera were obtained from recipients on days 7 and 17 post-reconstitution, and recipients were challenged IP with CPE on days 8 and 18.

RESULTS: Treatment of donor mice with anti-CD20 for 18 weeks significantly reduced B cell numbers in the blood (p<0.0001, >95% reduction) and the spleen (p<0.0001, >98% reduction) but did not affect IgE levels. Recipients (n=15) given BM and SPL from anti-CD20 treated donors were protected from developing anti-peanut IgE (p<0.0001), SS (p<0.0001), and dT (p<0.0001) compared to recipients (n=14) given cells from IC-treated donors. Recipients (n=9) given purified B cells from peanut-allergic donors plus naïve SPL developed significantly elevated anti-peanut IgE (p<0.001), SS (p<0.002), and dT (p<0.003) compared to controls.

CONCLUSIONS: These results demonstrate that memory B cells are required but not sufficient for the adoptive transfer of murine PNA. Help from a yet-to-be-defined cell population(s) found in naïve SPL is required.
Recombinant Probiotic Bacillus Subtilis Spores with Surface Expression of Ara h2 Reduce Peanut-Induced Anaphylaxis in Mice

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Rationale: Food allergy is a serious and sometimes fatal condition. Peanut allergy is the most common cause of fatal food-allergic reactions. Safe therapies for peanut allergy are urgently needed. Bacillus subtilis spores are regarded as a nonpathogenic and have been widely used as a probiotic in humans. Because of their safety and stability, they have recently been used as vehicle for delivery of heterologous antigens to the gastrointestinal tract.

Methods: The mucosal adjuvant cholera toxin B subunit (CTB) fused with the peanut major allergen Ara h2 was co-expressed on the surface coat of Bacillus subtilis spores using molecular cloning strategies. SDS-PAGE and Western Blotting analyses were used to identify CTB-Ara h2 surface expression on spores. Peanut antigen C3H/HeJ mice were orally administered with these recombinant spores expressing CTB-Ara h2 for 6 weeks and then challenged with peanut for 4 weeks post therapy. Sham treated and naive mice were included as controls.

Results: CTB-Ara h2 fusion protein expression on the spores coat was verified by SDS-PAGE and Western blotting. Oral administration of recombinant spores significantly increased peanut specific IgA (P<0.01 vs sham) and decreased peanut specific IgE (P<0.05 vs sham), but did not significantly affect IgG1 or IgG2a (P>0.05 vs sham) 4 weeks post treatment. Recombinant CTB-Ara h2 spores-treated mice showed significantly reduced symptom scores and plasma histamine levels than sham treated mice (P<0.05 for both).

Conclusions: Oral administration of recombinant Bacillus subtilis spores expressing CTB-Ara h2 protected against peanut induced anaphylaxis. Induction of peanut specific IgA was associated with the immunotherapeutic effects.

Evaluating the Potential Allergenicity of Dietary Proteins Using Model Allergenic and Weak/Non-Allergenic Proteins in Germ-Free Mice

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Rationale: Currently no animal model of allergy has proven to be predictive of human responses in ranking purified dietary proteins in prevalence and potency of food allergy in humans. Our germ-free mouse model may more accurately predict atopic human responses than previously reported conventional mouse models. This study compared sensitizing and eliciting responses to the potent peanut allergen, Ara h 2; moderately potent milk allergen, B-lactoglobulin (BLG); and a non-allergenic dietary protein, soy lipoygenase (LOX).

Methods: Germ-free C3H/HeJ mice were sensitized with 60 μg Ara h 2, BLG, or LOX by three weekly intraperitoneal injections (IP) with alum adjuvant, followed by an IP challenge of 500 μg of indicated protein. Thirty minutes post-challenge clinical scores were graded (0=no symptoms to 5=death) and body temperatures recorded. The presence of protein-specific IgE, IgG1, and mast cell protease concentrations in mouse sera was determined using ELISA.

Results: Upon challenge germ-free mice sensitized with Ara h 2 and BLG exhibited significantly more severe clinical scores (average 4) compared to germ-free mice sensitized with LOX (average 1). All three proteins resulted in different hypothermic responses post-challenge for Ara h 2, BLG, and LOX (average -8.8, -7.6, and -2.2 °C respectively). Antigen-specific IgE positivity and mMCP-1 levels correlated with reactions.

Conclusions: Ara h 2, BLG, and LOX results in germ-free mice indicate that this model can differentiate between potent and non-allergens based on temperature drop, clinical scores, and serum biomarkers. Additional proteins with known human exposure and history of safety or allergy are needed to confirm the prediction accuracy.

Acute Anti-IgE Effect of Topical Application of Formulation of Herbal Extracts in a Peanut Allergic Murine Model

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Rationale: Berberine and indigo naturalis extracts suppress IgE production by a human B cell line. We investigated if a topical cream (Herbal-Cream-IIb) that contains berberine and indigo naturalis extracts could reduce IgE production in peanut allergic mice.

Methods: Peanut allergic C3H/HeJ mice received topical Herbal-Cream-IIb or vehicle sham treatment (n=14/group) daily for 1 week in three sets of experiments, at different time points: weeks 8-9, 16-17, and 19-20 following initial sensitization. Sera were obtained one day prior to and one day after treatment, total and peanut specific antibodies were determined by ELISA. IL-4, IL-10 and IFN-g production by cultured mesenteric lymph node (MLN) cells and splenocytes were measured by ELISA. LC-MS/MS was employed to detect percutaneously absorbed berberine in serum.

Results: Herbal-Cream-IIb decreased serum peanut-specific IgE and total IgE levels by 78% and 52% respectively compared to baseline (p<0.001 for both). There were no significant changes in peanut specific and total IgE levels before and after sham treatment. There were no significant changes in peanut specific IgG1, IgG2a and IgA, or total IgG and IgA levels before and after treatment. MLN cells and splenocytes from Herbal-Cream-IIb treated mice did not show significant difference in peanut protein induced IL-4, IL-10 and IFN-g production compared to MLN cells and splenocytes from sham treated mice. Serum berberine concentrations were peaked at 1 hour following topical Herbal-Cream-IIb application.

Conclusions: Topical Herbal-Cream-IIb application reduced serum peanut-specific and total IgE levels in peanut allergic mice. It may be of value as a convenient therapeutic option for PNA.
Clinical Analysis of Immediate Hypersensitivity to Hydrolyzed Wheat Proteins in Soap

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RATIONALE: In Japan, immediate hypersensitivity after wheat ingestion has recently increased among individuals using a particular soap brand (“Cha no Shizuku”) that contains a specific hydrolyzed wheat protein (HWP) called Glupearl 19S. This study aimed to investigate the ability of diagnostic methods to reflect the clinical symptoms of immediate wheat allergy (IWA) after HWP sensitization.

METHODS: We evaluated 122 patients with suspected IWA induced by Glupearl 19S using serological studies and the skin prick test (SPT).

RESULTS: Overall, 53 patients were diagnosed with IWA due to Glupearl 19S (2 men, 51 women; mean age, 41.6 ± 6.4 years; range, 15–68 years); 35 of these developed severe systemic symptoms. All patients exhibited some degree of facial swelling after wheat ingestion. Specific IgE antibodies for wheat flour, gluten and α-5 gliadin were detected in 45%, 62%, and 0.05% patients, respectively. All 53 patients (100%) exhibited positive reactions to Glupearl 19S in the SPT, while 46 showed positive reaction for Glupearl 19S-specific IgE antibodies in ELISA.

CONCLUSIONS: The SPT and ELISA using Glupearl 19S can reflect the clinical symptoms of IWA caused by this HWP contained in a facial soap used in Japan. Repeated contact with HWP in the soap induced sensitization through skin, eyelids and nasal mucosa followed by severe immediate reactions after having wheat proteins. This immediate hypersensitivity to hydrolyzed wheat proteins gives us a warning that we should consider now on about the usefulness and safety of the cosmetic ingredients of food origin, especially hydrolyzed food proteins.

The Frequency of Food Allergens in Pet Foods

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RATIONALE: Avoidance of food allergens remains the standard of care for food allergy. Recent data suggests 62% of American households own at least one pet (http://www.humanesociety.org). In addition, food allergic children may be at risk for reactions upon ingesting pet food containing food allergens or if licked by pets after eating such foods. Current labelling laws do not regulate pet foods with the major 8 food allergens (milk, egg, wheat, soy, peanut, tree nuts, fish, or shellfish). We investigated whether pet foods in the United States were labelled to contain any of the major food allergens.

METHODS: 747 commercially available dog (n=452) and cat (n=295) foods were identified from major brands and each product was examined for ingredients listed to contain one of the major food allergens.

RESULTS: Of surveyed pet foods, 86.9% and 43.9% contained at least one and two of the 8 foods, respectively. The frequency of individual foods in the screened pet foods was in decreasing order: wheat (47.9%), egg (36.9%), fish (28.9%), soy (28.1%), milk (7.9%), shellfish (0.5%), peanut (0.3%), and tree nuts (0%). Among dog foods, the most common food allergens were wheat (50.6%), egg (33.2%), and soy (25.7%), while among cat foods, the most common were fish (45.8%), wheat (43.7%), and egg (42.7%).

CONCLUSIONS: These novel findings demonstrate that a majority of surveyed pet foods contain at least one of the major food allergens. The clinical significance of these findings remain unknown, warranting further investigation—including determining the concentration of the food allergens within the pet foods.

Predicting Oral Food Challenges to Baked Egg

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RATIONALE: Children who are allergic to egg often are able to tolerate extensively heated (baked) egg products. Although specific IgE (sIgE) to ovomucoid has been suggested, laboratory tests to predict which patients will safely pass an oral food challenge (OFC) to baked egg are not well described.

METHODS: A retrospective chart review of 24 OFCs to baked egg and the most recent ovomucoid sIgE were examined to determine cut-off values for sIgE to predict a positive OFC outcome. Basophil activation tests (BAT) were conducted with whole egg, ovalbumin, and ovomucoid antigens of non-egg allergic (n=23) and egg allergic (n=7) patients. Basophil activation was measured by the expression of CD63. Receiver operative curves were created for ovomucoid sIgE values and BAT results.

RESULTS: Ovomucoid sIgE demonstrated poor accuracy in predicting OFC outcomes to baked egg allergy with a sensitivity of 67%, false positive rate of 29% and AUC ROC=.690. Percentage of basophil activation to ovalbumin showed a sensitivity of 83%, false positive rate of 25%, and AUC ROC=.979. In a small sample size, BAT to ovalbumin may differentiate egg allergic children who could tolerate baked egg.

CONCLUSIONS: BAT may be a reliable prediction tool for an oral food challenge to baked egg in egg allergic children. Further studies with a larger sample size are required to confirm these findings.

Factors Resulting in Deferral of Diagnostic Oral Food Challenges

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RATIONALE: Physician-supervised oral food challenges (OFC) are recommended diagnostic tests, but patient/family motivating factors leading to deferral of the test have not been extensively explored.

METHODS: Participants were parents of children with food allergy consecutively attending the Jaffe Food Allergy Institute who had been offered an OFC but had not undertaken one within twenty-four months. Subjects completed a questionnaire listing 27 possible reasons for deferral, marking all factors that applied, with the option to indicate "other", and identify a most important factor. They provided answers for up to 3 foods offered.

RESULTS: A total of 102 surveys were completed (participation rate 92.7%) for 183 OFC invitations encompassing 30 different foods (most often almond). The children were 38% female, mean age 8.4 years, 31.4% had been treated with epinephrine. Among total OFC invitations, categorical responses were: scheduling issues (56.3%), not interested/not important/impractical for diet (36.7%), fear/emotional issues (25.7%), doubted passing (18.6%), tried on own (10.9%), and others (13.1%). Considering the most important factors (selected for 156 OFC offers, 85%) responses were: scheduling issues (47.4%), not interested/not important/impractical for diet (20.5%), fear/emotional issues (14.1%), doubted passing (4.5%), tried on own (5.8%) and others (7.7%). Other reasons included economic factors and fear of making the allergy worse.

CONCLUSIONS: Excluding scheduling issues, OFCs were deferred primarily for reasons of lack of interest in the food and concern for emotional impact or fear of reactions.
96 Description and Outcomes of Oral Food Challenges in a Tertiary Paediatric Allergy Clinic in South Africa
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RATIONALE: Describe oral food challenges (OFC) at a tertiary paediatric allergy clinic in Cape Town, South Africa: results and proportion of subjects passing challenges despite IgE levels > internationally derived 95% positive predictive values (ID95PPVs)1.

METHODS: Retrospective, descriptive study of children with food allergies undergoing OFC from February 2011 to April 2014 (39 months).

RESULTS: OFC’s (n=202) were performed on 142 children (9 months to 14 years). Egg (64), peanut (37), baked egg (29) and cow’s milk (25) were most common. Thirty eight (18.8%) challenges were positive; 9 of 64 egg challenges (14.1%); 13 of 37 peanut challenges (35.1%); 5 of 29 baked egg challenges (17.2%); and 5 of 25 cow’s milk challenges (20%). Reactions varied from mild urticaria (23, 60.5%) to wheeze (3, 7.9%). Co-morbidities were common; atopic dermatitis (105, 73.9%), asthma (53, 37.3%) and allergic rhinitis (65, 45.8%). Co-morbidity correlated with positive OFC outcome (p=0.01). OFC’s were done in 170 mixed race (MR) (84.1%) and 26 Black African (BA) (12.9%) subjects. Co-morbidity was lower in BA subjects; asthma (3/26 vs. 65/170; p=0.01); PAR/AC (7/26 vs. 79/170; p=0.06) and similar for AD (18/26 vs. 131/170; p=0.38). Thirty six percent (17/47) MR and 42.9% (3/7) BA had negative OFC’s with IgE >ID95PPVs to egg. Forty percent (6/15) MR and 80.0% (4/5) BA had negative OFC’s with IgE >ID95PPVs to cow’s milk and 21.7% (5/23) MR had negative OFC outcomes with IgE >ID95PPVs to peanut.

CONCLUSIONS: Negative challenges with IgE >ID95PPVs in BA subjects may reflect lower manifestation of atopy.

97 What Is the Role of Component IgE Analysis By Immunocap and Microarray Compared to Food-Specific IgE in Peanut and Egg Allergy?
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RATIONALE: Ara h 2 by immunocap was an excellent predictor of peanut allergy, however performance of component IgE by microarray in peanut and egg allergy is still unclear. We sought to compare component IgE by microarray and by immunoCAP to whole food-specific IgE in detecting clinical allergy.

METHODS: Children with peanut and egg allergy ages 1-18 years were recruited from Children’s Hospital Allergy Clinic. Allergy was defined as failed oral food challenge (OFC) with immediate objective symptoms or as food specific IgE >0.35 kU/L and historical immediate objective symptoms after ingestion. Non-allergic was defined as passing OFC. Serum tests were performed by Thermofisher Scientific. χ², Wilcoxon rank-sum and correlation was used for analysis.

RESULTS: Twenty-four peanut allergic children, mean age 6.6 years (1.4-16.5), 67% male, 71% white and 26 egg allergic children, mean age 5.2 years (1.0-17.3), 85% male, 73% white were compared to peanut and egg non-allergic children, respectively. Median [IQR] ara h 2 by immunoCAP (0.7 [0.3-3.5] kU/L) and microarray (0.88 [0.2-4.1] ISU) were significantly higher in peanut allergic than non-allergic group (both p<0.02). There was no difference in whole peanut IgE. Correlation between immunoCAP and microarray ara h 2 was 0.91. Median microarray gal d 1 (0.94 [0-3.7] ISU) was significantly higher in egg allergic than non-allergic children (p=0.02). There was no difference in immunoCAP egg white and gal d 1 between groups.

CONCLUSIONS: Microarrayed ara h 2 and gal d 1 discriminated between allergic and non-allergic children while immunoCAP whole peanut and egg-white IgE did not.

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

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

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

CONCLUSIONS: Our results support both the hypothesis that Ara h 8 could be contributing to oral allergy syndrome between birch pollen and peanut.
Comparison of IgE Epitope Mapping By Peptide Microarray and a Novel Luminex-Based Peptide Assay

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RATIONALE: Peptide microarray-based immunoassays (MIA) identify food protein allergenic epitopes. The diversity of epitope-specific IgE binding and affinity have been shown to correlate with different clinical phenotypes of cow’s milk (CM) allergy. Here we compare IgE binding of milk-allergic subjects utilizing MIA and a novel, high throughput Luminex-based peptide assay (LPA).

METHODS: 80 subjects with different degrees of tolerance to milk products were categorized into 4 groups: A, reactive to baked-milk (BM, n=20, median CM-sIgE=38.7), B, reactive to yogurt-cheese/BM-tolerant (n=17, CM-sIgE=10.5), C, reactive to pasteurized milk/tolerant to BM and yogurt-cheese (n=23, CM-sIgE=7.7), and D, outgrown milk allergy (n=20, CM-sIgE=2.9). Serum samples were tested in both assays, and inter-assay variability, sensitivity, and IgE binding to various epitopes of milk proteins were compared.

RESULTS: Correlations between replicate peptides within the same experiment in MIA were good (R>0.91), while LPA replicates were highly correlated (R>0.99). The identified epitopes with significant differences between groups were similar between the two systems, and overall binding diversity detected was higher in Group A in both assays (p=0.002, Mann-Whitney). LPA, however, showed more pronounced epitope-recognition patterns for high-binding allergic subjects. Non-binding subjects in group A were consistent in both assays, but subjects with undetectable binding in the other groups using MIA showed some positive recognition patterns on LPA due to higher sensitivity.

CONCLUSIONS: The agreement between allergenic epitopes identified is excellent. However, LPA has several advantages including lower serum volume requirement, lower inter-assay variability, increased sensitivity and ease of automation, making the assay more practical as a diagnostic tool for clinical practice.
101 Marked Increase in Basophil Activation during Non-Anaphylactic Allergic Reactions to Peanut in Man
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RATIONALE: The precise mechanisms of IgE-mediated allergic reactions to food remain to be elucidated. In particular, it is unclear how orogastric food allergen exposure causes rapid-onset skin symptoms in the absence of systemic symptoms of anaphylaxis. We hypothesized that widespread systemic basophil activation may be a mechanism through which food allergens lead to systemic effects.

METHODS: Six peanut-allergic individuals were recruited for this pilot study and underwent DBPCFC to peanut. Whole blood was collected before and at the termination of DBPCFC. Ex vivo peanut/placebo-induced basophil reactivity was measured by expression of CD63, CD203cbright and CD107a on CRTC2/CD303/CD123+ basophils. Activated basophils were quantified by flow cytometry.

RESULTS: Proportions of CD63+, CD107a+ CRTC2/CD303/CD123+ basophils were increased following peanut challenge but not placebo challenge (p=0.03). A trend to increased proportion of CD203cbright CRTC2/CD303/CD123+ basophils was observed (p=0.09). The magnitude of basophil activation as measured by the expression of CD63 and CD203cbright following in vitro peanut allergen dose-response stimulation (0, 1, 3, 10, 33, 100, 330 and 1000 µg/mL) was greater in basophils following a positive reaction to peanut compared to placebo challenges, implying a priming effect (AUC, p=0.03).

CONCLUSIONS: We have demonstrated evidence for the involvement of basophils during an acute allergic reaction to peanut, which may explain the high frequency of systemic involvement in food allergic reactions. More work is needed to identify the key events which trigger widespread basophil activation in peanut allergy.

102 Identification and Characterization of a New Oil Body Fraction Peanut Allergen
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RATIONALE: We found patients with severe peanut allergic reactions with negative skin prick test (SPT) with commercial extracts.

METHODS: We studied 7 patients with anaphylaxis upon peanut ingestion and negative SPT with commercial peanut extract. We separated peanut lipid and hydrophobic fraction and performed immunoblotting with all patients’ sera. We performed SDS-PAGE and immunoblotting with the two fractions. We identified the protein recognized in the lyophilic fraction by peptide fingerprint method. We also performed to each patient and to 15 patients with anaphylaxis and positive SPT as controls, specific IgE against whole peanut and peanut components Ara h 3, Ara h 8, and Ara h 9 and Basophil Activation Test against Ara h 1, Ara h 2 and Ara h 9.

RESULTS: Just one patient from the SPT negative group had positive specific IgE against peanut. Those patients with negative SPT and no specific IgE against peanut recognized only the lyophilic fraction. However, those with positive SPT and specific IgE recognized both hydrophilic and lipophilic phase. All seven negative SPT patients recognize a protein around 20 kDa in the lipid fraction. We purified and sequenced the protein with greater than 90% homology with Gly1. Immunoblotting with Ara h 3 did not inhibit negative SPT patients’ sera from binding to Gly1.

CONCLUSIONS: We identified a peanut IgE-binding protein Gly1, present in the peanut oil body fraction. Lipid fraction contains important peanut allergens that might be overlooked. In the commercial extracts preparation, oil body fraction is usually discharged removing important allergens.

103 Making Peanut Allergens Indigestible: A Model System for Reducing or Preventing an Allergic Reaction
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RATIONALE: Peanut allergens are not totally resistant to digestion as previously known. Creating peanut allergen conjugates that are more resistant to digestion may prevent absorption of the allergens into the bloodstream and, thereby, an allergic reaction.

METHODS: Peanut allergen conjugates were prepared by covalently attaching a protease inhibitor, p-aminobenzamidine (pABA), to peanut allergens through activation of a mixture of raw peanut extract and pABA with glutaraldehyde. After dialysis, the pABA-peanut allergen conjugates were subjected to tests for digestion and inhibition of protease. In the model test system, trypsin was used as the protease to digest native peanut allergens in the presence and absence of the conjugates. Digestion profiles and released free amino groups were determined by SDS-PAGE and trinitrobenzenesulfonic acid (TNBS). IgE bindings of the conjugates and native allergens were determined in ELISA.

RESULTS: SDS-PAGE showed that the pABA-peanut allergen conjugate was resistant to digestion, whereas native peanut allergens (Ara h 1 and Ara h 2) were completely digested into peptide fragments by trypsin in 20 min. Digestion of native allergens was inhibited when the conjugate was present. TNBS assay showed that the degree of trypsin inhibition was dependent on the conjugate concentration used. IgE antibodies were not inhibited by the conjugate in ELISA.

CONCLUSIONS: Trypsin was inhibited by the pABA-peanut allergen conjugate. The conjugate was not recognized by IgE antibodies in ELISA. The conjugate can serve as a model system for making peanut allergens indigestible and feasible to be excreted without causing an allergic reaction.
ABSTRACTS

104 Co-Sensitization Patterns to Tree Nuts in a Pediatric Population

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RATIONALE: Tree nut allergy is a common, generally lifelong, and potentially severe food allergy that can be psychosocially and nutritionally limiting. Once deemed allergic to a particular tree nut, patients are often instructed to avoid all tree nuts. A less restrictive approach could be proposed if cross-reactivity patterns among select subsets of nuts were determined.

METHODS: A retrospective analysis of serum specific IgE levels (ImmunoCAP, Phadia, Uppsala, Sweden) to almond, Brazil nut, cashew, chestnut, coconut, hazelnut, macadamia, pecan, pistachio, and walnut was performed. Pearson correlation coefficients (rho, ρ) were calculated. Correlation strength was stratified as weak (ρ<0.5), moderate (0.5<ρ<0.8) or strong (ρ>0.8). Correlations with milk and peanut specific IgEs were used as controls.

RESULTS: Results from 5849 panels of food specific IgEs collected between 2000 and 2012 were retrieved from the clinical records of one institution. Of all relationships assayed between tree nuts, a strong correlation was noted only between cashew and pistachio (ρ=0.955, n=1227, p<0.0001), and between pecan and walnut (ρ=0.940, n=1327, p<0.0001). Other tree nut pairs exhibited weak to moderate correlations similar to that between individual tree nuts and milk, and between individual tree nuts and peanuts.

CONCLUSIONS: A strong relationship exists between cashew and pistachio (Anacardiaceae) and between pecan and walnut (Juglandaceae). Our results imply that it is likely unnecessary to avoid ingestion of phylogenetically unrelated nuts over concern of cross-reactivity. Further studies could potentially elucidate clinical cross-reactivity and possibly eliminate the need for pan-avoidance of all nuts in certain patients.

105 Mirabel Project: Description of a French Population of 785 Peanut Allergic or Sensitized Patients

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RATIONALE: MIRABEL project collects data on peanut allergic (PA) or sensitized (PS) patients, their consumer’s behaviour and peanut contamination in food in order to estimate the risk of allergic reaction in the French PA population. We present the results of the medical questionnaires.

METHODS: 785 patients were included (116 PS, 669 PA, <16y: 86%; 16y: 14%). Age at diagnosis, route of exposition, symptoms, allergic comorbidities, r Ara h2 level, eliciting dose (ED) during oral challenges (OC), reactivity quantity triggering reaction in real life, and dietary recommendations were recorded.

RESULTS: Median age at diagnosis was 3/2 years (PA/PS). At diagnosis, reactions were severe in 30%: serious systemic reaction (48%), laryngeal angioedema (28%), shock (13%), acute asthma (11%), notably after inhalation (n=36) in asthmatic patients. Atopic dermatitis, asthma and another food allergy were observed in 66%, 57.5%, 62% respectively. Median r Ara h2 level was higher in PA than PS (12.9 versus 5.6 kU/L), > 0.23 in 94%, < 0.10 in 11 PA. 225 of the 278 OC performed were positive (193 PA, 32 PS) [ED: 0.03 to 2404 mg of peanut proteins]. In 41/48 negative OC, low cumulated dose could not exclude an allergy. Real life ED (353 PA) was: traces (31%) or quantified (median: 125 mg PP). Strict avoidance was not linked to history or comorbidities, but to a low ED (< 100 mg PP).

CONCLUSIONS: We report the high frequency of comorbidities and severe reactions in PA/PS patients. Reaction to inhalation is a risk factor of severe reaction. Criteria guiding diet need to be clarified.

106 Comparison of Human IgE Binding to Protein Extracts from a Genetically Modified Soybean and Five Non-Transgenic Soybean Lines

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RATIONALE: Codex Alimentarius Commission guidelines (2003) for the food safety assessment of genetically modified (GM) crops recommend testing commonly allergenic species like soybean for potential changes in endogenous allergen accumulation. We compared human IgE binding to extracts of the GM soybean, a non-modified parental control soybean, and four non-modified commercial soybean lines from 3 geographically distinct locations.

METHODS: Sera or plasma from eleven soybean-sensitized and seven control subjects were tested for IgE binding to extracts of the six soybean lines. Proteins were separated by 1-D SDS-PAGE under reducing and non-reducing conditions and exposed to diluted serum or plasma samples. Bound IgE was detected by chemiluminescence with horseradish peroxidase conjugated monoclonal anti-IgE. Direct IgE binding ELISAs were also performed with extracts of geographical replicates of each soybean line and individual clinical samples. Relative IgE binding was evaluated for each subject.

RESULTS: IgE binding patterns of the GM soybean were similar to the patterns obtained with commercial lines. Minor differences were identified in IgE binding to immunoblots of the GM and parental soybean for some subjects. No statistically significant differences were found in ELISA results comparing the GM soybean to non-modified soybeans. Importantly, the IgE binding to the GM soybean fell within the range of variation measured in the commercial lines.

CONCLUSIONS: The endogenous allergen content of the GM soybean was within the range of soybean allergens in commercial, non-GM soybean lines. Thus, the risk of food allergy from consumption of the GM soybean is similar to the risk from consumption of commercial soybeans.
Soy Reactivity May Be Better Identified By Component Testing with Gly m 8 Than Traditional Testing Methods

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RATIONALE: Skin prick testing and serum food-specific IgE (sIgE) levels are sensitive tests for identifying soy allergy, but positive test results are common even when soy is tolerated. We sought to perform soy component testing on patients undergoing an oral food challenge (OFC) to soy to determine if this modality would improve predictability.

METHODS: We recruited children in our university-based, outpatient practice referred for OFC. Challenge outcomes were compared with soy sIgE levels and the sIgE levels to the soy components Gly m 4, 5, 6, and 8. IRB approval was obtained for this study.

RESULTS: We performed component testing on 16 patients, ages 4 to 19 years, who underwent an OFC to soy. Six patients passed the OFC (37.5%), while 10 failed (62.5%). The median soy-sIgE in the patients who passed the OFC (16.5 kUA/L) was lower, but not significantly different from those who failed (31.9 kUA/L). The median Gly m 4, 5, and 6 levels were not significantly different between those with a negative OFC (0.16, 8.95, and 15.05 kUA/L, respectively) and the soy reactive patients (0.40, 18.20, and 25.85 kUA/L, respectively). There were significant differences in the median Gly m 8 levels between patients who passed their soy OFC (2.47 kUA/L) and the soy reactive patients (5.03 kUA/L; P = 0.023).

CONCLUSIONS: Soy component testing using the component Gly m 8 may improve the specificity of soy allergy testing. Further testing is planned with a larger number of soy sensitive patients to validate the utility of soy component testing.

Assessment of IgE Binding Profiles of Lentil Allergic Children: Similarity and Potential Cross-Reactivity Between Dal Proteins

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RATIONALE: Lentils and dal proteins, seeds of edible legumes, are a major protein source in Mediterranean and Asian countries and are increasingly being consumed in the westernized world. Tolerance and sensitization varies among allergic individuals. IgE binding profiles, similarity and potential cross-reactivity between dal proteins, lentil, chickpea and peanut were evaluated and linked to clinical reactivity to assess clinical implications and guide recommendations.

METHODS: Green lentil, toor dal, mung dal, urad dal, chana dal, moth dal, masoor dal, chickpea, and peanut extracts were prepared and evaluated by immunoblotting. Sera from lentil allergic children (IgE >0.35 kUA/L) and a negative control were used. Clinical data, IgE levels and binding patterns were compared among patients. Sequence similarity and consensus was compared between lentil (Lenc1), chickpea provicilin and peanut (Arah1); and lentil (Lenc3) and peanut (Arah9).

RESULTS: Banding patterns and IgE binding patterns of lentil allergic patients showed similarity among taxonomically and phenotypically similar dal proteins (Mong Dal (Vigna congoi) and Mooth Dal (Vigna mungo) radiata); Urad Dal and Toor Dal; Chana Dal and Chickpea). Sequence comparison between Lenc1 and Chickpea provicilin revealed Identity 47.3% and Consensus 57.1% with >90% identity at Lenc1 IgE binding epitopes. Lenc1 and Arah1 showed Identity 33.4%, Consensus 42.4%. Lenc3 and Arah9 (LTP) showed Identity 52.5% and Consensus 61.9%.

CONCLUSIONS: Similar binding patterns among selected dal proteins point to high identity at the protein level making evaluation of IgE binding to discriminate clinical reactivity difficult. Clinical cross-reactivity is likely and has to be evaluated in more detail.

Hypoallergenicity of a New Extensively Hydrolyzed 100% Whey-Based Formula Containing Probiotics

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RATIONALE: The American Academy of Pediatrics (AAP) defined a formula as hypoallergenic if it ensures with 95% confidence that 90% of infants/children with confirmed cow’s milk allergy (CMA) will not react under double-blind, placebo-controlled conditions. We sought to determine whether a new 100% whey protein extensively hydrolyzed formula (EHF) containing B. lactis CNCM I-3446, meets AAP hypoallergenicity criteria.

METHODS: Children with CMA were randomized to double-blind placebo-controlled food challenges (DBPCFC) with a new EHF (Test) and a commercial EHF (Control) in crossover fashion. CMA was confirmed within 6 months prior to enrollment by elevated serum cow’s milk (CM)-IgE levels, positive skin prick test to CM extract, or positive CM oral challenge. Allergic reactions were assessed using a comprehensive scoring system. If both DBPCFCs were tolerated, subjects participated in an at-home week-long Test open challenge.

RESULTS: 77 children (3.30 ± 2.98 years old) with recently confirmed CMA were enrolled. Four out of 75 subjects completing the DBPCFC with Control had an allergic reaction compared to 1 of 67 subjects during the DBPCFC with Test (lower bound 95% confidence interval of 0.921 for Test), meeting the AAP hypoallergenicity criteria. Average intake during the Test open challenge was 250ml/day. One subject reported angioedema, atopic dermatitis, rash around the eyes, and red swollen eyes on open challenge Day 6. This subject did not report any symptoms during the Test DBPCFC and did not discontinue formula during the open challenge.

CONCLUSIONS: The new Test EHF meets the AAP criteria for hypoallergenicity and can be recommended for the management of CMA.
A New Luminex-Based Peptide Assay to Identify Different Degrees of Milk Allergic Reactivity

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RATIONALE: A novel Luminex-based peptide assay (LPA) was used to identify IgE binding to allergenic epitopes in milk-allergic Turkish children who tolerated different forms of milk products in oral food challenges. We sought to establish whether LPA results could distinguish patients’ clinical reactivity to different forms of milk, e.g. baked-milk (muffin), yoghurt-cheese, and whole unprocessed milk.

METHODS: Four groups of milk allergic children were identified by oral food challenge to muffin, yoghurt-cheese and whole milk: 1. Reactive to baked-milk (n=20), 2. Reactive to yoghurt-cheese/tolerant to muffin (n=17), 3. Reactive to milk/tolerant to muffin and yoghurt-cheese (n=23), and 4. Outgrown milk allergy (n=32). Milk, casein, and b-lactoglobulin sIgE and sIgG4 were determined by UniCAP IgE and IgG4 binding to milk protein epitopes were assessed by LPA.

RESULTS: Children reactive to baked-milk had the highest milk, casein and b-lactoglobulin sIgE followed by children reactive to yoghurt-cheese. In analyzing children with positive milk protein epitope-specific binding, LPA revealed the most diverse binding patterns of IgE in the baked-milk reactive group: as1-casein (12-peptides), as2-casein (8-peptides), b-casein (8-peptides), b-lactoglobulin (3-peptides) and k-casein (3-peptides). Binding of IgE was observed only in as1-casein and as2-casein regions in children reactive to yoghurt-cheese and there was no IgE binding to any peptides in groups #3 and #4. IgG4 binding patterns were similar in all four groups.

CONCLUSIONS: Using a novel high-through-put LPA, we were able to successfully distinguish the reactivity and diversity of IgE binding to allergenic epitopes in the most severe milk allergy phenotypes. This assay may be useful in distinguishing different degrees of milk allergy.

Variability of Repeat Egg Sige Levels

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

METHODS: This retrospective chart review included all patients at our teaching institution who had egg sIgE drawn on 2 or more occasions and had a diagnosis code of food allergy (693.1), personal history of allergy to egg (V15.03), or anaphylaxis (995.9), between January 1, 2003, and January 1, 2013.

RESULTS: 1077 patients had 2 or more egg sIgE levels performed. 206 (19.1%) patients who were <2 years old (median age 1.26 years) had an initial sIgE ≥ 2 kU/L (95% predictive of clinical reactivity, median sIgE 15.10 kU/L), and 40 (19.4%) of these patients (median initial sIgE 4.23 kU/L) had any subsequent sIgE < 2 kU/L, which all achieved by 5.54 years old. 394 (36.6%) patients who were ≥ 2 years old (median age 4.87 years) had an initial sIgE ≥ 7 kU/L (95% predictive of clinical reactivity, median sIgE 21.7 kU/L). Of these patients, 97 (24.6%) patients had any subsequent sIgE < 5 kU/L, and 13 (3.3%) patients (median initial age 4.57 years, median initial sIgE 11.1 kU/L) had any subsequent sIgE level < 2 kU/L, which all achieved by 10.56 years old.

CONCLUSIONS: Patients who have lower initial values and are younger are more likely to have a subsequent sIgE level <2 kU/L. Patients should have yearly egg sIgE levels until 11 years of age.

Comparison of Unicap and Immulite Serum Specific IgE Assays for the Assessment of Egg Allergies

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RATIONALE: Food specific IgE levels are frequently used in conjunction with skin-prick testing to guide clinical decisions in allergic patients. The objective of this study was to compare Immulite and UniCAP egg specific IgE assays and to determine whether their measurements can be applied equivalently to guide food challenge and tolerance assessment in egg allergic children.

METHODS: Thirty-seven egg allergic patients between 2 and 13 years of age were enrolled at Sainte-Justine’s pediatric hospital from July 2013 to January 2014. Egg white specific IgE levels were measured in identical serum samples with both UniCAP (Pharmacia) and Immulite (Siemens Healthcare) assay systems, and egg white skin prick tests were performed.

RESULTS: Immulite specific IgE levels (mean of 39.9 kU/L, 95% CI: 27.0-52.7) were significantly higher (p=0.0484) when compared to UniCAP specific IgE levels (mean of 22.8 kU/L, 95% CI: 12.2-33.4). Skin-prick tests to egg white were on average 11.1 mm in diameter (n=37, 95% CI: 9.4-13). All patients who tolerated baked eggs (n=10) had Immulite IgE levels ≥ 28.1 kU/L and UniCAP IgE levels ≥ 8.93.

CONCLUSIONS: Because of variability between the two different assays, it is preferable to use a single assay to monitor a patient’s allergic evolution and to assess the development of tolerance. Results from Immulite and UniCAP assays cannot be substituted.

Specific IgE Ordering Patterns at a Pediatric Reference Laboratory

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RATIONALE: Serum food-specific IgE (sIgE) testing can be a useful part of the workup to diagnose IgE-mediated food allergy. Multiple sIgEs are often bundled together into allergen profiles to facilitate ordering. This can result in over-testing, which can lead to an incorrect diagnosis and possible adverse consequences. We hypothesized that non-allergy/immunology (A/I) physicians were more likely to order allergen profiles instead of single sIgE levels.

METHODS: We reviewed sIgE testing performed between January 1, 2013 and December 31, 2013 by ChildLab, a pediatric reference laboratory. The data was analyzed to assess ordering specialties and whether the sIgE tests were ordered as a single food or as part of an allergen profile.

RESULTS: In 2013, 12542 single-food sIgE tests and 3686 allergen profiles were ordered by 26 physician specialties which we divided into primary and subspecialty care groups. Primary care providers ordered 36.6% of single sIgEs, with subspecialties ordering 63.4%. Of 7949 single-food sIgEs ordered by subspecialties, A/I ordered 91.4%, gastroenterology (GI) ordered 5.9%, and the remaining subspecialties accounted for 2.7%. For allergen profiles, 82.8% were ordered by PCPs. Of subspecialties ordering profiles, GI ordered 9% and A/I ordered 3%. The combined remaining subspecialties accounted for 5% of allergen profiles.

CONCLUSIONS: Allergen profiles and single-food sIgE testing are ordered by many different healthcare providers in both primary care and subspecialty practices. A/I physicians do not routinely order sIgE panels containing foods, however these are frequently ordered by PCPs. Our findings indicate that there are educational opportunities regarding indications for appropriate ordering of sIgE testing.
**114 Alpha-Gal IgE Sensitization in the United States; Surveillance Update**

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**RATIONALE:** Galactose-alpha-1,3-galactose (alpha-gal), is a cross-reactive carbohydrate moiety found in red meats such as beef, pork and lamb and is associated with a delayed IgE response. A link to lone star tick bites was established by researchers at the University of Virginia and although the mechanism of sensitization is not clearly elucidated, it remains the primary suspect.

**METHODS:** A retrospective review of national laboratory data was conducted for alpha-gal IgE testing performed in 2012 and 2013, utilizing data with de-identified patient health information only when the source location could be identified. This data was mapped according to 6 geographical regions and compared to the July 2011 CDC published map of lone star tick populations.

**RESULTS:** For the total data set, 37% displayed positive results for alpha-gal IgE. In the subset of samples that could be tied to a source location, the positive rate declined to 33%. The regions with the highest prevalence of positive samples continue to overlap with the location of the lone star tick with a positive rate as high as 46% in TN and AR. In other non-lone star tick regions the positive rates varied from 8% to 17%.

**CONCLUSIONS:** The overall positive rate of samples tested in 2012-2013 decreased compared to 2009-2011, and the total number of tests increased, suggesting there is more screening of patients potentially exposed to the lone star tick. The overall regional prevalence rates continue to support the hypothesis that lone star tick bites trigger the development of the alpha gal allergy.

**115 Differential Skin Test Reactivity to Pollens in Pollen Food Allergy Syndrome Versus Allergic Rhinitis**

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**RATIONALE:** Pollen food allergy syndrome (PFAS) is a form of food allergy in which uncooked foods cause allergic symptoms generally limited to the oral mucosa. It occurs in a subset of patients with pollen allergy, although not all patients have prominent rhinitis symptoms. PFAS is related to antigenic similarity between the pollen and food allergen. The size of skin test reactions in a group of pollen sensitive subjects with PFAS was compared to a group of pollen sensitive subjects without PFAS. Self-reported rhinitis symptoms between the two groups were compared to identify if symptom severity differed.

**METHODS:** Twenty subjects with PFAS and 20 subjects with seasonal allergic rhinitis without PFAS were enrolled in the study (n=40). All subjects underwent standard skin prick testing to a panel of common allergens including select fresh fruits and vegetables. Subjects completed a Mini Rhinoconjunctivitis Quality of Life Questionnaire (mini RQLQ) as part of their clinical evaluation. Subjects with PFAS and without PFAS were compared statistically.

**RESULTS:** Subjects with PFAS had significantly larger skin prick tests specific to pollens (p < 0.0008). Despite the larger-sized skin prick tests, subjects with allergic rhinitis and PFAS reported milder nasal symptoms in relation to pollen skin tests size when compared to allergic rhinitis controls without PFAS.

**CONCLUSIONS:** Our study outlines basic differences between two seemingly similar patient groups with a particularly striking discordance between skin test size and rhinitis symptoms. This discordance should be explored further to increase mechanistic understanding of allergen cross-reactivity in PFAS.
Prevalence of Sensitisation to Food and Aero-Allergens and Challenge Proven Food Allergy Amongst 11-Year-Old Children on the Isle of Wight
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\textbf{Rationale:} Data regarding prevalence of food allergies in older children is lacking. We undertook a whole population cohort study, investigating the rates of food allergy at the age of 11 years.

\textbf{Methods:} A cohort of unselected children (n = 969) born between September 2001-August 2002 on the Isle of Wight (UK) was followed using a standardised questionnaire covering a history of atopy from birth to 11 years. At 11 years, children were assessed for food allergy and sensitization using questionnaires, skin prick tests (SPT), Sps IgE, CRD, and oral food challenges (FC) in those with a positive SPT that had never eaten the food and those who indicated a previous adverse reaction to foods (regardless of SPT).

\textbf{Results:} At eleven years information was available on 828 (85.5%). Seventy seven of 828 (9.3%) children reported a food-related problem. 587 underwent SPT to a predefined panel of allergens and 24.7% (145/587) were found to be atopic with 24.7% (145/587) sensitised to any aeroallergens and 2.73% (16/587) to any food allergen. Frequency of sensitisation to the individual food allergens were: milk (9 on SPT, 2 positive on prick-prick); egg (2); fish (1); peanut (14); sesame (1); lupin (4). Based on a clinician’s diagnosis, open food challenge or history of reaction on accidental exposure and positive SPT, 27/828 (3.3%) children were shown to have a newly diagnosed or ongoing food related problem at the age of 11 years.

\textbf{Conclusions:} Reported food allergy is common in eleven-year-old children but the rate of relevant sensitisation and food allergy is lower.

Food Allergy, Prevalence, Knowledge and Behavioral Trends Among College Students- a 5-Year Comparison
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\textbf{Rationale:} Food allergy among college students remains poorly described and understood. We sought to update current knowledge of this subject and compare trends to our 2008 published data.

\textbf{Methods:} An online survey was distributed by e-mail to University of Michigan students.

\textbf{Results:} Among 1058 students responding, 39.1% (n=414) reported food allergy, 51.4% (n=213) reported symptoms consistent with NIADD anaphylaxis criteria, and 58.2% reported having a reaction while in college. Tree nut (32.7%), milk (32.5%), peanut (25.3%) and shellfish (14.3%) allergy were most commonly reported. Among students not preparing their own food, only 28.5% (n=64/224) reported that their food preparers were aware of their food allergy. Only 34.5% (n=173) reported maintaining any emergency medication, including 74.6% (n=113) maintaining self-injectable epinephrine (SIE). Only 52.6% (71/137) reported always carrying their SIE, and 50.5% (n=209/414) always avoiding their reported food allergen. Past reported anaphylaxis was not associated with increased odds of either behavior. 82.6% (n=342) reported a campus contact was aware of their allergy. Just 28.5% (n=118) and 33.3% (n=138) reported foods in the dining hall were always labeled for allergen content or allergen-free alternatives were available, respectively. Compared to 2008 data, a significantly higher proportion of students reported past anaphylactic reactions (p<0.001), maintaining SIE (p<0.001), always carrying SIE (p<0.001), always avoiding their allergen (p=0.006), and report allergen content in the dining hall is labeled (P<0.001).

\textbf{Conclusions:} Food allergy remains a growing concern among college students. Though student’s awareness, SIE maintenance/carriage and perpetual allergen avoidance has improved, risk-taking behavior remains problematic among food allergic undergraduates.

Egg-Specific IgA and IgA2 Are Associated with Sustained Unresponsiveness to Egg Following Oral Immunotherapy
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\textbf{Rationale:} In a previously reported CoFAR study, 55 subjects with egg allergy underwent randomized, placebo-controlled egg oral immunotherapy (OIT). Active treatment induced desensitization in most and sustained unresponsiveness (SU) in a smaller subset. We hypothesized that component-resolved analysis of IgE, IgG4, IgA, IgA1, and IgA2 may identify potential biomarkers of SU in OIT subjects.

\textbf{Methods:} Longitudinal samples for 51 egg-allergic subjects (37 active, 14 placebo) were available. Egg white- (EWA), ovalbumin- (OVA), and ovomucoid (OVM)-specific levels of IgG, total IgG, IgG4, IgA, IgA1, and IgA2 were quantified by ELISA. IgE and IgG4 to these antigens were quantified using ImmunoCAP\textsuperscript{5}. Clinical responders achieved SU to egg and/or incorporated egg into the diet by 48 months; all others were considered non-responders. Between-group comparisons were made amongst active and placebo, as well as responders and non-responders.

\textbf{Results:} No placebo subjects achieved responder status. Among the 37 active subjects, baseline IgE-OVM was lower in responders (median 7.1 KUL, n=21) than non-responders (16.6 KUL, n=16, p=0.005). In contrast to post-treatment decreases in IgE, IgG4-EW and IgG4-EW increased in subjects receiving egg OIT, but not placebo. Increases in IgA-EW and IgA2-EW were greater in responders; greater log ratios to baseline values were observed in a repeated measures analysis (p=0.047,0.024, respectively). The sum of IgG4-EW and IgA-EW ratios also increased among responders (p<0.001).

\textbf{Conclusions:} IgG4-EW, IgA-EW and IgA2-EW during egg OIT are associated with clinical response. Lower pre-treatment IgE-OVM may be useful in selecting egg-allergic subjects likely to respond to egg OIT. Future studies are needed to evaluate and validate these potential biomarkers.
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**Eosinophilic Esophagitis (EoE) Histologic Changes More Strongly Associate with Treatment Status Than Peak Eosinophil Count (PEC)**

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**RATIONALE:** EoE is an eosinophil-predominant inflammatory disease. PEC (≥15 per high power field), the gold standard for pathologic diagnosis, correlates imperfectly with clinical parameters. We therefore explored associations between other pathologic features and treatment status.

**METHODS:** Features were scored (0 to 3) in EoE biopsies (19 untreated, 90 treated) for grade (severity) and stage (extent). Data were analyzed using Chi-square and Fisher Exact tests, and multivariate regression models were compared.

**RESULTS:** Grade and stage scores were frequently abnormal (>80%) for eosinophil inflammation (EI), basal zone hyperplasia (BZH) and dilated intercellular spaces (DIS) and were associated with treatment status (P<0.01 for EI, DIS). Eosinophil abscesses (EA), surface layering (SL) and lamina propria fibrosis (LPF) occurred moderately (>30%) pretreatment and were reduced post-treatment. PEC predicted treatment status (P<0.001, r²=0.15, AUC 75%). Exceeding PEC were grade scores for DIS (P<0.001, r²=0.20, AUC 76%) and BZH (P<0.001, r²=0.17, AUC 78%) (proximal biopsies), and DIS (P<0.001, r²=0.18, AUC 78%) (distal biopsies). Superior stage scores included DIS (P<0.001, r²=0.17, AUC 80%) and EI (P<0.001, r²=0.17, AUC 80%) (proximal biopsies). Grade model that best predicts treatment status: BZH, EA, SL, surface epithelial alteration (SEA) (P<0.001, r²=0.29, AUC 87%) (proximal biopsies). Stage model that best predicts treatment status: BZH and LPF (proximal and distal biopsies), DIS and SEA (proximal biopsies), EA (distal biopsies) (P<0.001, r²=0.72, AUC 98%).

**CONCLUSIONS:** Pathology features scores, especially stage, are superior to PEC to detect EoE treatment status. Future directions include validation in additional datasets and further scale development to assess treatment efficacy and patient quality of life.

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**Mucosal Biopsy Microarray Analysis Revealed Elevated Thymic Stromal Lymphopoietin (TSLP) in Infantile Eosinophilic Gastroenteritis**

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**RATIONALE:** Eosinophilic Gastroenteritis (EGE) is clinicopathologically characterized by massive infiltration of eosinophils in the gastrointestinal tract. EGE is often difficult to diagnose due to the lack of specific tests other than endoscopy. We reported that serum levels of both IL-33 and thymic stromal lymphopoietin (TSLP) were significantly elevated in patients with EGE, and that both levels correlated positively with disease activity (AAAAL 2014). To examine whether elevated IL-33 and TSLP can be also detected in the lesions of EGE patients, we compared the gene expression profiles of sigmoid colon biopsies between EGE and control groups.

**METHODS:** We enrolled 5 patients with infantile EGE and age-matched controls (n=3) who underwent endoscopy due to clinical symptoms. The gene expression profile of the biopsies was assessed by using microarray technology with Agilent SurePrint G3 Human GE 8x60k. The differentially expressed genes in the biopsy specimens were systematically compared between infantile EGE and control subjects and analyzed using GeneSpring software.

**RESULTS:** In agreement with our earlier finding of elevated TSLP serum levels in EGE patients, TSLP was the most upregulated immune-related gene in the sigmoid colon biopsies compared with the controls. IL-33 mRNA also showed a tendency to be increased in the EGE patients, although not significantly.

**CONCLUSIONS:** Our results suggest that at least a part of serum TSLP in EGE patients may be derived from their lesions. TSLP likely plays a pivotal role in the pathogenesis of EGE and may be a useful biomarker for EGE diagnosis.

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**Phenotypic Characterization of the Eosinophilic Esophagitis (EoE) Population in the Consortium of Food Allergy Research (CoFAR)**

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**RATIONALE:** We sought to describe the clinical, endoscopic, and histopathological features of patients with EoE in a multi-site CoFAR registry.

**METHODS:** Subjects were 6 months-65 years with biopsy-confirmed EoE from 5 U.S. sites. Concurrent eosinophilia at other gastrointestinal sites was allowed. Resistance to proton pump inhibitor (PPI) therapy was recorded but not required.

**RESULTS:** 506 subjects (71% males, 88% Caucasians, median age 10.5 [0.9-56] years) were enrolled. Median age was 3 years at symptom onset and 7 at diagnosis. 26% reported clinical and histological response to PPI therapy. 5.6% had concurrent eosinophilic gastritis, 0.6% enteritis, 2% colitis. Parents and siblings had reported EoE rates of 3.2% and 3.6%, respectively. Reported symptoms included abdominal pain (55%), reflux (53%), dysphagia (50%), nausea/vomiting (48%), and diarrhea (35%). Food impactions requiring endoscopic removal occurred in 5.9% and esophageal stricture in 10.7%. Esophageal rings/strictures were more common in older subjects (rings: 4.6% ≤16-year-olds, 21.9% ≥16-year-olds, p=0.01; strictures: 3% ≤16-year-olds, 17.9% >16-year-olds, p=0.03). Plaques/furrows and esophageal eosinophils were comparable. Co-morbidities included food allergy (70%), allergic rhinitis (63%), eczema (50%), asthma (48%), sinusitis (31%), urticaria (30%), food anaphylaxis (28%), pneumonia (27%), autoimmune disease (5%), immunodeficiency (3%) and celiac disease (2.2%). Among those tested, food sensitization was very common (>80% sensitized to milk, soy, egg, nuts, barley, and/or) and to aeroallergens (51%).

**CONCLUSIONS:** A large time lag in diagnosing EoE exists. Older patients have fibrostenotic features but similar inflammatory features. In addition to atopy, immune/infectious diseases, and diarrhea are reported more than expected in the general population, warranting further investigation.
Adult Eosinophilic Oesophagitis: A UK Based Case Series
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RATIONALE: Adult onset Eosinophilic Oesophagitis (EoE) presents frequently to the allergy clinics in the UK. However, no study to date has attempted to describe these patients. This case series set out to clinically characterise adults patients with EoE seen over the past four years in a tertiary care center in the UK.

METHODS: We screened Allergy/Immunology clinic lists and the e-document systems at Southampton General Hospital (SGH) for patients given a diagnosis of EoE since 2010.

RESULTS: Thirty six patients were identified with a diagnostic label of EoE. They were predominantly male (77.8%; 28/36) with an age range of 19 – 71 years. The majority (61%, 22/36) suffered from other atopic conditions/or showed a positive total IgE level and suspected a wide range of potential foods triggers. Interestingly, patients often considered food that are difficult to swallow such as meat, chicken and starchy food as the cause of their problems. SPT and specific IgE tests were not helpful in identifying offending foods. As expected the majority (58.3%; 21/36) were sensitised to aero-allergens with 14 being sensitised to tree/birch pollen. Dietary interventions varied widely between these patients, ranging from elemental diets, 6 food elimination diets to test directed diets.

CONCLUSIONS: Male sex and atopy were found to be primary characteristics, but age did not influence the diagnosis. A wide range of foods are suspected to trigger EoE pathology in adults, and dietary measure seems to vary greatly. Studies are needed to identify which foods are implicated in adult EoE in the UK.

Shared Genetic Etiology Between Eoe and Other Allergic Diseases
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RATIONALE: Eosinophilic esophagitis (EoE) is an allergic inflammatory disease characterized by eosinophils in the esophagus. Patients with EoE often have other allergic diseases such as asthma (AS), allergic rhinitis (AR), and atopic dermatitis (AD), and less frequently celiac disease or Crohn’s disease.

METHODS: Using the results of our recently published genome-wide association study, we performed a permutation-based enrichment analysis on EoE risk loci to identify individual shared loci and the global enrichment of loci that reached genome-wide significant association in other diseases.

RESULTS: We identified four loci that were shared between EoE and at least one of the assessed phenotypes. For example, there was strong association (p<10⁻⁷, all diseases) with EoE and AS, AR, AD, and Crohn’s disease at 11q13 which encodes LRRC32 and C11orf30. Furthermore, using EoE risk loci (p<10⁻⁴, we identified significant enrichment for other disease risk loci (p<5x10⁻⁸) including AS, AR, AD, and celiac disease. No enrichment was found for Crohn’s disease, rheumatoid arthritis or ankylosing spondylitis loci. Of the genes in loci with shared association between EoE and AS, AR, Celiac, or AD, 45.1% and 6.1% were expressed and dysregulated in the esophageal biopsies of EoE patients, respectively, which are both significantly higher percentages than expected by chance (p<10⁻⁷).

CONCLUSIONS: We identified EoE risk loci that are shared with 5 related diseases, with a major common locus at 11q13. Shared EoE-risk loci are expressed and dysregulated in esophagi of patients with EoE. These results imply shared etiology between EoE and other allergic related diseases as well as celiac disease.

Ruminating over Refractory Eosinophilic Esophagitis
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RATIONALE: Eosinophilic esophagitis (EoE) is an increasingly recognized disease with characteristics that often include dysphagia, food impaction, and gastroesophageal reflux symptoms. While treatment options such as topical corticosteroids, esophageal dilation, and dietary therapy are often successful, patients with refractory symptoms should be considered for alternative diagnoses.

METHODS: Clinic exam, imaging, endoscopy, biopsy, specific IgE testing, manometry-pH-impedance.

RESULTS: A 26 year-old male with seasonal allergic rhinitis was referred to the allergy clinic for EoE evaluation. Despite six weeks of twice daily H2 and PPI therapy, an EGD demonstrated linear furrowing, esophageal concentric rings, and up to 40 eosinophils/hpf in the proximal and distal esophagus. He underwent esophageal dilation followed by twice daily swallowed corticosteroids with minimal improvement. Despite negative allergy food skin prick and sIgE testing, an empiric wheat elimination diet provided some subjective improvement despite persistent daily vomiting of undigested food. Secondary to refractory symptoms, a 6-month trial of omalizumab was initiated. A repeat EGD revealed rare esophageal eosinophils despite no significant clinical improvement. Secondary to continued daily emesis of undigested food, an esophageal motility study with manometry was performed consistent with ruminium. Patient was referred to Behavioral Health for biofeedback therapy with significant improvement noted.

CONCLUSIONS: Common reasons for increased esophageal eosinophilia include GERD and EoE. Many reports have observed continued EoE symptoms despite resolution of eosinophils on EGD biopsies. To our knowledge, this is the first case of EoE that also had clinical and objective evidence of aerophagia and ruminium.
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Strong Association of Eosinophilic Esophagitis and Food-Pollen Syndrome; Evidence Suggestive of Oral Route of Sensitization to Common Food-Pollen Allergens

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RATIONALE: Eosinophilic esophagitis (EoE) is associated with food and Aeroallergen sensitization. Recent studies have shown a high prevalence of sensitization to common food-pollen allergens including profilins and PR-10 in EoE. This is the first study evaluating the prevalence of food-pollen syndrome in a large cohort of EoE patients.

METHODS: A retrospective chart review of EoE patients evaluated at Northwestern Allergy/Immunology clinic between(2006-2013). 123 EoE cases were enrolled, and 210 consecutive patients presenting with symptoms of rhinitis and tested positive to Aeroallergens were used as control subjects. All patients were skin tested with a standard panel of Aeroallergens and were questioned about symptoms of food-pollen syndrome.

RESULTS: EoE patients had a significantly higher prevalence of sensitization to tree (T), grass (G) and ragweed (RW) 94% sensitized to at least one of these allergens compared to 69% of allergic rhinitis (AR) group (p<0.005). However EoE patients less frequently reported rhinitis symptoms in the specific seasons associated with the above allergens 74% vs. 93% (p<0.05). T.G or RW sensitized EoE patients had food-pollen syndrome at a higher rate (51%) as compared to the AR (10.1%) (Odds 8.02, 95% CI (4.17-15.43)).

CONCLUSIONS: The high rate of sensitization to T.G and RW, the high prevalence of food-pollen syndrome with the decreased frequency of AR symptoms as compared to AR controls is suggestive that the esophageal mucosa might be the route of sensitization to these common food-pollen allergens. This is supported by recent studies showing a high rate of sensitization to profilins and PR-10 in EoE patients. An impaired barrier function in EoE patients may play a role in sensitization to common food-pollen or food allergens.

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Seasonal Exacerbation of Esophageal Eosinophilia in Children with Eosinophilic Esophagitis and Allergic Rhinitis

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RATIONALE: Increasing evidence supports a link between eosinophilic esophagitis (EoE) and environmental aeroallergens in some patients, which can manifest as seasonal exacerbation of EoE. Few studies have examined this link in pediatric EoE patients.

METHODS: We conducted a retrospective chart review of all EoE patients seen at our pediatric institution with clinical suspicion of aeroallergen-induced symptoms. Of 1575 total EoE patients, a subset of 158 patients were suspected to have possible aeroallergen-induced symptoms. All available esophageal biopsy data was collected along with any documented information regarding diet and medications at the time of each biopsy. Fifteen cases were excluded for esophageal eosinophilia due to causes other than EoE (gastroesophageal reflux disease, eosinophilic gastroenteritis, inflammatory bowel disease, and Caelic disease), leaving 143 patients for final analysis. Seasonal exacerbation was defined as esophageal eosinophilia that increased with a change in season from previous biopsies without the introduction of a new food or removal of any medications. Patients whose biopsies showed improvement after initiation of an intranasal steroid or allergen immunotherapy were also considered to have seasonal exacerbation.

RESULTS: Twenty patients were confirmed to have seasonal exacerbation using the above criteria. The remaining 123 cases could not be confirmed due to insufficient data or lack of clear seasonal variation of biopsies. Summer and fall accounted for the most seasonal variability, followed closely by spring.

CONCLUSIONS: Children with EoE and allergic rhinitis may have exacerbations in their esophageal eosinophilia during certain seasons depending on the specific aeroallergens to which they are sensitized.

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Prevalence of Eosinophilic Esophagitis in a Population-Based Cohort from Southern California

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RATIONALE: No population-based studies have been conducted to evaluate eosinophilic esophagitis (EoE) using a validated multi-ethnic cohort representative of the community it serves. The objectives of this study were to determine the prevalence of EoE using such a cohort, and to examine sociodemographic differences.

METHODS: We reviewed administrative data from the medical records of 2,230,660 Kaiser Permanente Southern California members enrolled 2008-2013. EoE patients were identified by ICD-9 code 530.13. Sociodemographic differences were tested using chi-square analysis.

RESULTS: EoE was diagnosed in 1,561 of 2,230,660 patients (prevalence 7:10,000). Cases comprised 1,344 adults (86%) and 217 children (14%). Adult prevalence (8:10,000) was higher than pediatric (4:10,000; p<0.001). Male prevalence (11:10,000) was higher than female (4:10,000; p<0.0001). The racial distribution of our adult membership during the study period overall was 31% white, 33% Hispanic, 8% black, 10% Asian; for children: 19% white, 47% Hispanic, 8% black, 7% Asian. Whites made up the majority of pediatric (52%) and adult (76%) cases, however we found a higher prevalence of disease in children from racial minority groups (22% Hispanic, 13% black, 6% Asian) compared to adults (10% Hispanic, 4% black, 3% Asian). EoE was 4 times more common in those from households of the highest quartile for annual income than in persons from the lowest (p<0.0001). The inverse relationship between prevalence and household income is striking, and deserves further investigation.

All abstracts are strictly embargoed until the date of presentation at the 2015 Annual Meeting.
**AB42 Abstracts**

**129 Prospective Analysis of Eosinophilic Esophagitis (EoE) in Pediatric Patients Living in Rural, Southeastern United States**

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RATIONAL: To characterize demographics, clinical characteristics, and response to management among children living in a rural, southern US region.

METHODS: Subjects, 2-18 years, were enrolled in a prospective database from 2012-2014. Data collected included demographics, symptoms, diet history, allergen profiles, and response to therapy.

RESULTS: 136 subjects were enrolled; mean (SD) age at diagnosis was 7.15 (4.67) years. Atopic co-morbidities included asthma (42.6%), food allergy (39%), allergic rhinitis (46.3%), and atopic dermatitis (33.1%). Predominant symptoms at diagnosis included heartburn/regurgitation (51.1%), abdominal pain (47.4%), nausea/vomiting (48.9%), and dysphagia (31.9%). Commonly sensitized foods included cow’s milk (48.4%), egg (46.1%), soy (37%), wheat (45.7%), peanut (45.3%), tree nuts (40.3%), and sesame (38%). Of 110 evaluable subjects, 28 (25.5%) were proton pump inhibitor-responsive (PPI-R) while 82 (75%) were not. PPI-nonresponsive (PPI-NR) subjects showed higher rates of perennial aeroallergen sensitization than PPI-R subjects (67.3% vs. 30%; p = 0.007) with both groups demonstrating seasonal aeroallergen sensitization in ~50%. Of the PPI-NR group, 72% achieved complete (eos < 15/hpf) or partial response (eos = 15-25/hpf) to treatment with 57% of PPI-NR defined as complete responders; 32/54 (59.2%) responded to an allergen-directed elimination diet, 19/54 (35.1%) to swallowed corticosteroids, 7/54 (12.9%) to 6-food elimination, and 1 to elemental diet. Of those who responded to diet, milk was the most commonly eliminated food (84.3%).

CONCLUSIONS: Children living in a rural, southeastern region of the United States have a high degree of sensitization to environmental/perennial allergens; further work is needed to understand their role in EoE pathogenesis. Milk elimination is beneficial as first-line therapy for dietary management.

**130 Celiac Disease and Immune Disorders in Patients with Eosinophilic Esophagitis**

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RATIONAL: Eosinophilic esophagitis (EoE) is a clinicopathological condition characterized by the combination of upper gastrointestinal symptoms in association with histological findings of > 15 eosinophils/high-powered field found in biopsy specimens. EoE is emerging as an increasingly common cause of esophagitis in children and adults. Little is known about the prevalence of autoimmune conditions in EoE patients and their first-degree relatives (FDR).

METHODS: Utilizing the Utah Population Database (UPDB), we compared EoE patients and their FDR against the matching controls to evaluate possible links between EoE and studied comorbid diseases. The UPDB is a dynamic resource located at the University of Utah and consists of computerized data records for nearly seven million individuals. Cox proportional hazard model was used for analysis.

RESULTS: Using Cox Regression analysis, EoE proband and their FDR both showed highest risk of having Celiac Disease. (n = 12,000, OR 10.6, 95% CI (8.1-13.88), p <2e-16; OR 2.66, 95% CI (2.13-3.31), p<2e-16, respectively). In addition, EoE was associated to a lesser degree with having a diagnosis of lymphoma, IgA deficiency, inflammatory bowel disease, lupus, allergic rhinitis, eczema and iron deficiency. It does not appear to be associated with psoriasis, common variable immunodeficiency (CVID), Ehlers-Danlos syndrome, and others.

CONCLUSIONS: We believe this to be the first reported analysis showing a high degree of association between Celiac Disease and EoE. In addition, EoE was associated with other immune disorders and allergies. These novel findings illustrate the importance of screening for and counseling about other relevant conditions in patients with EoE and their family members.
Use of Food Allergy Testing Beyond the Six Common Food Allergies in Eosinophilic Esophagitis

Marissa A. Love, MD, Osama F. Almadhoun, MD, Selina A. Gierer, DO; University of Kansas, Kansas City, KS.

**RATIONALE:** Food allergy testing related to eosinophilic esophagitis (EoE) in the pediatric population relies on percutaneous testing to a small group of foods thought to cause EoE. Using specific IgE testing is not well-studied in this population. Here we present a child with EoE and food allergies, determined by both percutaneous and specific IgE allergy tests. We performed additional testing to identify key characteristics of EoEFA versus EoEsFA. In addition to routine pathology, immunohistochemistry staining for mast cells on biopsy specimens from both subpopulations was performed. Chi square tests, t-tests, and Mann-Whitney-Wilcoxon tests were used to investigate differences within these subpopulations.

**RESULTS:** From 94 EoE patients, 16 (17%) also had food allergy. EoEwFA patients were more likely to have coexistent allergic rhinitis, eczema, or GERD (p=0.02, 0.03, and 0.04, respectively) than EoEsFA. Additionally, they exhibited a higher total IgE at diagnosis, higher specific IgE and positive skin prick tests to several foods, including egg, soy, peanut, and tree nuts compared to children with EoEsFA. Pathologically, while there was no difference in tissue eosinophils between groups, biopsies showed increases in peak mast cells/HPF and percent of activated MC/HFP in patients with EoEwFA.

**CONCLUSIONS:** Our findings demonstrate differences in both diagnostic features and esophageal pathobiology in EoE patients with or without food allergy. Such differences might help explain the spectrum of characteristics observed in EoE.

The Relationship of Eosinophilic Esophagitis and Food Allergy: Evaluating the Spectrum of Eosinophilic Esophagitis

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**RATIONALE:** Studies support roles for both IgE and non-IgE-mediated food reactions in eosinophilic esophagitis (EoE), yet the interplay between food allergy in EoE, but were common in his diet. His skin test was positive for almond, banana, egg, milk, peanut, soy, and walnut. Despite avoiding these foods, emesis after feeds was reported, but current regimens are challenging. Our objective was to measure the relationship between milk specific IgE antibody and clinical outcomes of cow’s milk elimination diet.

**METHODS:** We treated 20 patients with EoE by removing all foods that contain cow’s milk from the diet for 6-8 weeks. Response was defined as having <15 eosinophils/high power field (hpf) on repeat esophageal biopsy. Symptoms were measured using the PedsQLTM EoE Module. We measured specific IgE to milk and component allergens by ImmunoCAP.

**RESULTS:** Fourteen patients completed the study, and nine (64%) had <15 eosinophils/hpf on repeat esophageal biopsy. Two responders had baseline serum IgE levels >0.35 IU/ml as compared with four non-responders (p=0.09). The geometric mean specific IgE to milk was 0.42 IU/ml in responders and 2.09 IU/ml in non-responders (p=0.008). Similarly, levels of IgE to milk components (Bos d 4, Bos d 5, and Bos d 8) were higher in non-responders. Patients who did not respond to cow’s milk elimination also had specific IgE to wheat (3) and egg (2). There was a weak negative correlation between serum IgE level and change in symptom scores (R=-0.16, p=0.6) such that higher IgE titers were associated with less improvement in symptoms.

**CONCLUSIONS:** Higher levels of specific IgE to milk and components were associated with poorer clinical outcomes from treatment using single food elimination of cow’s milk. In cases in which cow’s milk elimination was not effective, serum IgE results suggested that wheat and/or egg might also be a problem.

Serum IgE Levels and Response to Cow’s Milk Elimination Diet in Patients with Eosinophilic Esophagitis

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**RATIONALE:** Dietary treatment of eosinophilic esophagitis (EoE) is recommended, but current regimens are challenging. Our objective was to identify characteristic of EoEwFA compared to EoEsFA.

**METHODS**: A retrospective chart review of pediatric patients with EoE seen through routine pathology, immunohistochemistry staining for mast cells on biopsy specimens from both subpopulations was performed. Chi square tests, t-tests, and Mann-Whitney-Wilcoxon tests were used to investigate differences within these subpopulations. Methods were used to investigate differences within these subpopulations.

**RESULTS**: From 94 EoE patients, 16 (17%) also had food allergy. EoEwFA patients were more likely to have coexistent allergic rhinitis, eczema, or GERD (p=0.02, 0.03, and 0.04, respectively) than EoEsFA. Additionally, they exhibited a higher total IgE at diagnosis, higher specific IgE and positive skin prick tests to several foods, including egg, soy, peanut, and tree nuts compared to children with EoEsFA. Pathologically, while there was no difference in tissue eosinophils between groups, biopsies showed increases in peak mast cells/HPF and percent of activated MC/HFP in patients with EoEwFA.

**CONCLUSIONS**: Our findings demonstrate differences in both diagnostic features and esophageal pathobiology in EoE patients with or without food allergy. Such differences might help explain the spectrum of characteristics observed in EoE.
**135 Impact of Swallowed Topical Steroid Treatment on Growth in Children with Eosinophilic Esophagitis**

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**RATIONALE:** Swallowed topical corticosteroid treatment is an effective first-line pharmacologic therapy in eosinophilic esophagitis (EoE). However, the impression of a potential adverse effect, growth retardation, has prevented some parents from giving corticosteroids to their children. Clinically, such treatment can alleviate EoE’s symptoms such as food aversion and failure to thrive. Therefore, we hypothesize that treating EoE patients with corticosteroids may lead to the improvement in growth.

**METHODS:** The study includes a retrospective cohort of EoE patients in Children’s Healthcare of Atlanta that were treated with corticosteroid, less than 18 years old, and followed up for at least 6 months. We extracted data, including patients’ demographic information, presenting symptoms, and corticosteroid options, from the health care software Epic. Using before and after treatment standard deviation scores (SDS) for height, we evaluated the significance of our hypothesis by right-tailed Wilcoxon signed-rank test.

**RESULTS:** Seventeen of 66 patients met the eligibility criteria. Of the 17 patients, the mean age was 6.9 years old and the mean follow up duration was 15.0 months. The p-value was 0.0448, which indicated a significant improvement of patients’ growth after the corticosteroid treatment.

**CONCLUSIONS:** No data have been published to address the impact of corticosteroids on the growth of children with EoE. Through our retrospective study, we showed that corticosteroid treatment contributes to an improvement in the growth in children with EoE, possibly by alleviating EoE symptoms such as food aversions and failure to thrive.

**136 Eosinophilic Esophagitis (EoE): Individualizing a Long-Term Treatment Plan**

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**RATIONALE:** EoE, a perplexing chronic inflammatory disease, is increasing in incidence. Identifying factors surrounding symptom recurrence and treatment compliance may optimize individual treatment.

**METHODS:** Retrospective review of 57 adult patients with biopsy-proven EoE in the outpatient setting, all contacted by phone asking about symptom recurrence, seasonal association, medication and diet compliance.

**RESULTS:** 64% men, 36% women with median age of 38 years. Most common initial symptoms: food impaction (45%) and dysphagia (25%). Of 57, 16 were available for follow-up. Of this subset, 56% had recurring symptoms, of which 56% noticed increased dysphagia in the spring, 22% in the fall. 81% stopped using fluticasone, and 63% stopped their proton pump inhibitor (PPI) upon symptom resolution. 44% remain on diet therapy (low acid, avoidance). 67% compliant on fluticasone but not diet still had symptoms. 83% compliant on PPI but not diet still had symptoms. 57% compliant on diet alone still had symptoms.

**CONCLUSIONS:** In adults, there may be different subtypes of EoE. Patients treated with fluticasone, PPI, and food avoidance saw improved esophageal function after a few weeks to months, which often led to therapy cessation. Many improved after PPI alone, hinting at a PPI-responsive EoE. Recurring symptoms have a seasonal pattern, mostly spring and fall, further implicating aeroallergens in EoE pathogenesis. Diet therapy (food allergen avoidance) may make more sense than medical therapy alone given EoE is an antigen/immune-mediated disease. Combination (medication and diet) therapy may have increased efficacy, especially during seasonal transitions. Ultimately, treatment choice and length need to be tailored to each individual.

**137 Characteristics Associated with Treatment Choice in Pediatric Eosinophilic Esophagitis**

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**RATIONALE:** To evaluate factors associated with choice of therapy with either dietary elimination or topical steroids for the treatment of eosinophilic esophagitis(EoE) in children.

**METHODS:** Data from 108 children enrolled in a prospective EoE patient database at a tertiary-care pediatric hospital was analyzed. Bivariate analyses and multivariate logistic regression were used. Patients who chose no treatment were excluded from the analyses.

**RESULTS:** 108 patients with a mean age of 7.8 years (1.1–18.7 years) were included. 77.8% were male. The ethnic distribution was 63.9% Caucasian, 14.8% Hispanic and 21.3% other races. Atopic history included 55.9% with food allergies, 40.6% with asthma, 41.5 % with atopic dermatitis, and 57.7% with allergic rhinitis. Personal history of food allergy, asthma, atopic dermatitis or rhinitis; family history of allergic or GI diseases and demographic characteristics other than racial background were not significantly associated with treatment choice by bivariate analyses. Increased parental education level trended towards a significant association with choosing dietary treatment; however, due to incomplete data it was not included in our logistic regression model. When controlling for demographics, personal and family history, and severity of disease burden in a logistic regression model, Hispanic patients disproportionately chose steroids compared to Caucasians (OR 18.29, p<.001). Children with a history of other gastrointestinal diseases were more likely to choose dietary elimination (OR 6.59, p<.05).

**CONCLUSIONS:** In our pediatric cohort, only Hispanic patients were more likely to choose steroid therapy over elimination diet. A personal or family history of atopy was not significantly associated with choice of EoE treatment.
138 Esophageal Eosinophilia Associated with Congenital Esophageal Atresia/Stenosis and Its Responsiveness to Proton Pump Inhibitor

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RATIONALE: Studies including ours have described eosinophilic esophagitis (as indicated in association with congenital esophageal atresia (CEA) and esophageal stenosis (CES). Proton pump inhibitors (PPI) are sometimes administered to patients who undergo CEA/CES repair since postoperative gastroesophageal reflux disease is common. The aim of this study was to determine the prevalence of EE in patients undergoing CEA/CES repair and those with CES, and the effectiveness of PPI against this condition.

METHODS: Esophageal biopsies from patients with CEA and/or CES following surgical repair or CES in our hospital between 2005 and 2013 were retrospectively reviewed with focus on tissue eosinophilia (≥15 per high-powered field [HPF]) and the use of PPI.

RESULTS: A total of 71 esophageal biopsies (53 patients) were performed between 2005 and 2013. EE was observed in eight biopsies (seven patients) in association with CEA/CES (median: 82; range, 20–250 eosinophils/HPF). All patients except for one were treated with PPI after biopsy and showed clinical improvement. Esophageal biopsies were performed before and after PPI treatment in three of seven patients. The tissue eosinophils were intensively reduced (≤10/HPF) in all three patients. Eight patients with CEA/CES following surgical repair underwent esophageal biopsies after 2010 and five of eight showed EE. Two of the remaining three patients without EE were treated with PPI or steroids prior to the biopsy.

CONCLUSIONS: These findings suggest that patients who undergo CEA/CES repair or those with CES are associated with a significant rate of EE and the effectiveness of PPI against this condition.

139 Esophageal Stricture and Eosinophilic Esophagitis in a Nine-Month Old Girl

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RATIONALE: Eosinophilic esophagitis (EoE) is an increasingly common disorder. In children the reported mean age of diagnosis is 6 years. Food refusal and vomiting predominate in younger children while dysphagia and food impaction affect older children.

METHODS: A 9-month old female presented with spitting up and coughing after feeds for 3 months. Upper gastrointestinal fluoroscopy demonstrated esophageal stricture.

RESULTS: The patient underwent esophageal dilation, and biopsy showed 14 eosinophils/high power field (hpf). She was started on proton pump inhibitor, but persistent stricture led to repeat dilations (ages 10 and 11 months). Esophageal biopsy (age 11 months) demonstrated 105 eosinophils/hpf. She was diagnosed with EoE and treated with oral steroids. For maintenance therapy, she began oral viscous budesonide (OVB). Recurrence of mild symptoms led to esophagram that showed persistent stricture. A six-food elimination diet (without wheat removal) was added with symptomatic resolution. Repeat endoscopy/biopsy (age 18 months) showed narrow esophagus that required dilation and eosinophil count of 2/hpf. She was continued on OVB, but soy, peanut, and tree nuts were added back to her diet. She remained symptom free. Repeat endoscopy/biopsy (age 23 months) showed normal caliber esophagus and 8 eosinophils/hpf. Her continued treatment was OVB and dietary elimination of cow’s milk and egg.

CONCLUSIONS: Our patient is unusual because she presented with esophageal stricture at less than one year of age after dietary exposure to breast milk, cow’s milk based formula, and limited baby food. Her clinical course suggests the need for heightened awareness of, and more aggressive treatment for, esophageal strictures in children.
The Impact of Pediatric Eosinophilic Esophagitis on Bone Metabolism

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RATIONALE: Eosinophilic esophagitis (EoE) presents with a massive eosinophilic infiltration of the esophagus triggered by food antigen(s). Usually recommended treatments - food elimination diet, oral steroids – or the pathophysiology of the disease itself may impact bone metabolism, a situation at risk especially in growing children.

METHODS: A convenience retrospective study analysed 10 female and 4 male patients with EoE being treated according to dietary therapy recommendations, during the phase of progressive reintroduction of eliminated foods following the 6-food elimination diet period. The investigation took place in a single center using bone markers and bone tomodensitometry (TDM).

RESULTS: Patients were aged 7.6 (1.4; 13.6) years [median (range)] and had been diagnosed 2.8 (0.5; 7.0) years earlier. Patients' height and body mass index z-score were in the low normal range, -1.4 (-2.3; -3.1) and -1.1 (-1.8; -0.6), respectively. We did not correlate with disease duration. The mean bone density (DEXA) z-score was -0.6 (0.7; -2.5). Using quantitative TDM, the trabecular bone compartment was the most affected. 250H-vitamin D levels were below the normal range in half of the patients. The urinary calcium excretion and the urinary D-pyridinolines indicated abnormally high bone resorption, being above the normal range in most patients.

CONCLUSIONS: Our study shows that children with EoE present a significant bone mineralization defect associated with high levels of turnover bone markers. For now, we recommend a preventive strategy using vitamin D supplementation and adjustments of calcium intake.

Gene Expression Profiles of Mucosal Biopsy Specimens from Children with Eosinophilic Gastritis

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RATIONALE: Eosinophilic gastritis (EG) is clinicopathologically characterized by both marked gastric eosinophilia and clinical symptoms. The pathogenic mechanism of EG remains obscure, whereas that of eosinophilic esophagitis (EoE) has been well-studied. To elucidate whether EG’s pathogenic mechanism is similar to that of EoE, we performed transcriptome analysis of gastric biopsy specimens from EG patients and compared the identified gene signature with the previous microarray data for EoE patients.

METHODS: We enrolled pediatric EG patients (n = 5) and age-matched controls (n = 5) who underwent gastrointestinal endoscopy due to clinical symptoms. EG was diagnosed on the basis of ≥30 eosinophils/HFP, limited to the stomach. The gene expression profiles of the gastric biopsies were assessed using microarray technology. The differentially expressed genes of EG and EoE were compared by systematic analysis using the NextBio search engine.

RESULTS: Of 42,545 transcripts represented on the microarray, 2,282 were differentially expressed between the EG and control samples ≥2-fold change and adjusted p-value of <0.05). As in the case of EoE patients, eotaxin-3 was the most upregulated (>2,000-fold) gene. Of the 2,282 transcripts composing the EG-related gene signature, only 58, including eotaxin-3, were identified as commonly upregulated genes in EoE.

CONCLUSIONS: Our results suggest that eotaxin-3 plays a crucial effector role in the pathogenesis of EG as well as EoE. However, 97.5% of the gene signature we identified for EG was distinct from that previously identified for EoE, suggesting that distinct mechanisms may be involved in the pathogenesis of EG and EoE.
144 Severe Food Protein Induced Enterocolitis Syndrome (FPIES) in the Pediatric Intensive Care Unit (PICU): A Retrospective Chart Review

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RATIONALE: FPIES is a diagnosis that is often missed and, if untreated, can lead to severe complications.

METHODS: Patients from birth to 7 months admitted to the PICU at Mount Sinai Hospital from June 2012 to June 2014 with the diagnosis codes of failure to thrive, metabolic acidosis, hypovolemic shock, dehydration, vomiting, feeding problems in a newborn, and allergy to milk were selected for medical record review.

RESULTS: Out of 100 infants, 10 were identified with likely FPIES; 5 males, 5 females; all presented with vomiting, stool containing blood or mucous, lethargy, pallor, or dehydration with the majority having 5 symptoms. Age of onset ranged from 3 days to 2 months and delay to admission from 2 days to 2 months. All patients had failure to thrive and had presented to a physician or emergency department prior to PICU admission. One patient was exclusively breastfed, 9 were fed cow’s milk formula. Eight patients had anion gap metabolic acidosis; all required fluid resuscitation, 8 underwent a sepsis workup, and one patient underwent a diagnostic laparotomy. Eight patients were switched to a hypoallergenic formula and demonstrated resolution of symptoms. One patient was re-admitted to the PICU 10 days later and was then placed on hypoallergenic formula with symptom resolution. One patient was never trialed on hypoallergenic formula and remains symptomatic at the age 9 months.

CONCLUSIONS: FPIES is a severe disease that if not promptly diagnosed requires admission to the PICU and must be recognized early in order to prevent complications.

145 Food Induced Gastroenterocolitis Syndrome (FPIES): A Case Series of 51 Children

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RATIONALE: FPIES is a non-IgE mediated gastrointestinal food hypersensitivity whose clinical features are severe vomiting, diarrhea and dehydration within a few hours of ingesting food. The foods most frequently implicated in published series are cow’s milk, soy, rice, fish.

METHODS: A retrospective study of 51 patients, diagnosed by history and/or oral challenge test between 2006 and 2014.

RESULTS: 51 patients, 31 boys and 20 girls (mean age at diagnosis: 12.7 months) were included. The offending foods were fish (27 patients), milk (10), egg (7), beef (3), chicken (1), lentil (1), peanut (1) and chickpeas (1). The most frequent symptoms were vomiting (49 patients), lethargy (18), dehydration (12) and diarrhea (10). 20 patients have other atopic diseases, Skin prick tests y/or specific IgE against implicated food were positive in only one patient. In 12 patients, patch tests were conducted and were positive in 2 cases, 37 oral challenge test were performed in 27 patients 12 or more month after diagnosis. We found that no patient outgrow FPIES by fish by 2 years of age, 44% by 3 years, 66% by 6 years and 88% by 8 years of age. By contrast 80% of FPIES by milk recovered by the age of 2 years and 100% at 3 years old. In case of egg, all patients recovered by 4 years old.

CONCLUSIONS: This is one of the largest series published of FPIES. It seems to be an increase in prevalence of this syndrome. The most frequent culprit food is fish with an elder age of achieved tolerance compared to other foods.

146 Safety of Performing Oral Food Challenges to Food Protein-Induced Enterocolitis Syndrome Patients in the Outpatient Clinic

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RATIONALE: Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE-mediated food reaction typically occurring in young infants that resolves over time, and the oral food challenge (OFC) remains the gold standard for evaluating its resolution. We wanted to investigate utilization and safety outcomes of OFCs in FPIES patients seen in the outpatient setting.

METHODS: Chart review was performed of 1460 patients with a visit diagnosis of “food allergy” or “food hypersensitivity” seen in a tertiary care referral allergy clinic between Oct 2009 and Dec 2013. Twenty five patients were identified with FPIES.

RESULTS: The mean age at diagnosis was 8.5 months. 40% of patients were male, 12% had concomitant IgE-mediated food allergy, and 20% had other atopic conditions (most frequently atopic dermatitis). Most common foods implicated were rice (64%), oat (48%), and cow’s milk (28%). 68% of patients had reactions to more than one food. 38 OFCs were performed, with a mean age of 34 months at first OFC. 12 challenges were performed at home and 26 challenges were performed in the office, 22 as food reintroductions after a previous reaction and 16 as initial food introductions. One OFC failed at home and five OFCs failed in the office. Milk was the most common trigger for failed challenges. Most failed challenges were treated with observation and oral hydration but one challenge required transfer to the emergency department and treatment with intravenous fluids and steroids.

CONCLUSIONS: In carefully selected patients, OFC can be performed in the clinic without intravenous access if emergency services are easily accessible.

147 Atypical Food Protein-Induced Enterocolitis Syndrome (FPIES)

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RATIONALE: FPIES is a non-IgE-mediated food hypersensitivity that typically presents in infancy with severe vomiting and lethargy within hours of eating the offending food. It may also present in a chronic form with diarrhea, emesis, and failure to thrive. Milk FPIES often has a prolonged course, especially with a positive milk-specific IgE. We present an infant with chronic milk and soy FPIES without significant emesis and tolerance of milk products at 12 months of age.

METHODS: Case Report.

RESULTS: This Hispanic male was born at full-term without complications and fed cow’s milk infant formula but frequent, watery, nonbloody stools began by 3 weeks of age. The patient was admitted twice for suspected sepsis and failure to thrive by 6 weeks of age, with associated metabolic acidosis and dehydration. Infectious workup was unrevealing and symptoms resolved after the patient was kept NPO and then switched to amino acid-based formula. Serum IgE to milk and soy were elevated. The patient subsequently did well on amino acid-based formula with avoidance of milk and soy products. Other foods were introduced without difficulty. Just prior to 12 months of age mom reported that successful introduction of yogurt at home without symptoms.

CONCLUSIONS: This case of FPIES is unique because of the lack of emesis in the initial presentation. The tolerance of milk products prior to one year of age is also highly unusual, especially in the setting of elevated serum IgE to milk.
RATIONALE: To comprehend the burden of asthma in the U.S., it is important to have accurate estimates of the costs associated with the condition. The current study aims to provide updated nationally representative estimates of the healthcare expenditures and health resource utilization associated with asthma in the U.S.

METHODS: The 2008-2010 Medical Expenditure Panel Survey (MEPS) was used to estimate the impact of asthma on healthcare expenditures and health resource utilization. Multivariable regression analyses of outcomes were conducted controlling for age, sex, race, ethnicity, income, smoking status, insurance status and comorbidity. Medical expenditure analysis used the Heckman selection method with logarithmic transformation of expenditures. Expenditure data was inflated to $US 2011 using the Medical Care component of the Consumer Price Index (CPI). Utilization data was modeled using negative binomial regression.

RESULTS: Of the 102,767 adults in MEPS (2008-2010), 9,782 individuals (10%) had a diagnosis of asthma. Individuals with a diagnosis of asthma incurred an additional $1,095 ($<0.01) in annual healthcare expenditures attributable to asthma compared to those without asthma. Likewise, individuals with asthma experienced 1.28 ($<0.01) times the number of annual office-based visits, 1.43 ($<0.01) times the number of annual ER visits, and 2.0 ($<0.01) times the number of annual prescription drugs compared to those without asthma.

CONCLUSIONS: In recent national data, asthma is associated with significantly greater expenditures and utilization. Asthma continues to represent a significant direct cost burden in the United States.
152 Improving the Assessment of Overweight/Obesity in Asthmatic Pediatric Patients in a Quality Improvement Project
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RATIONALE: Overweight/obesity is a known co-morbid condition with asthma. Overweight asthmatic children have increased risk of developing asthma exacerbations versus those of average weight. Objective: The aim of this quality improvement project was to facilitate the identification of overweight/obesity in asthmatic pediatric patients.
METHODS: Consecutive patients presenting to an academic-based pediatric allergy clinic in Southeastern United States were included. This clinic was not electronic medical record based. Exclusion criteria included inability to obtain weight. Initially, a random chart audit of asthmatic patients for body mass index (BMI, a marker of overweight/obesity) assessment was performed. Providers and nursing staff were educated about BMI, overweight/obesity, and asthma and asked to record these assessments on checklists. Checklists were collected in 4 phases (1-2 weeks/phase) from June - August 2014. Before proceeding to the next phase, modifications were implemented to facilitate the recording of BMI, overweight/obesity, and asthma.
RESULTS: In the audit period, 4% of patients had their BMI assessed. In phase 1, after education of staff and implementation of checklists, BMI assessments increased to 43%. BMI assessments increased to 44% after repeat education in phase 2. BMI assessments increased to 79% after checklist modification in phase 3. By the end of the monitoring period phase 4, BMI assessments increased from 4% to 83% overall.
CONCLUSIONS: Assessment of an asthmatic pediatric patient’s BMI, to identify those with the co-morbid condition of obesity, is paramount to the optimal care of this chronic disease. Incorporating this specific data in a patient’s chart increases the likelihood of addressing this important issue.

153 Cost-Effectiveness of Bronchial Thermoplasty in Patients with Poorly Controlled, Severe, Persistent Asthma
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RATIONALE: Despite many pharmacological and immunological treatments for asthma, some patients remain not well-controlled and continue to experience asthma exacerbations. Existing FDA-approved treatments for asthma do not address excessive airway smooth muscle mass (ASM), an anatomical feature associated with increased asthma severity and morbidity in some patients. We sought to examine the cost-effectiveness of Bronchial Thermoplasty (BT) to treat poorly controlled, severe, persistent asthma patients. This novel technology uses thermal energy to target and reduce ASM, resulting in a durable reduction in asthma exacerbations.
METHODS: We adopted a payer perspective cost-effectiveness analysis framework, which modeled costs associated with healthcare utilization, patient quality-of-life, and adverse events over a 5-year time horizon, and compared BT plus standard care to standard care among poorly controlled, severe, persistent asthma patients—those patients requiring high dose combination therapy to manage their asthma yet still experiencing asthma exacerbation(s) requiring ER visit(s) in the past 12 months. We utilized Markov model methods to estimate the future costs and quality-of-life impact associated with BT. The model was populated using data from published literature and randomized clinical trials that described exacerbation rates, treatment effects of BT, and patient quality-of-life.
RESULTS: We estimated the cost-effectiveness of BT to be $5,495 USD per QALY; further, approximately 22% of sensitivity analyses estimated BT to be both cost-saving and quality-of-life increasing. These results are favorable when compared to other treatments for this population.
CONCLUSIONS: BT is a cost-effective treatment option for patients with poorly controlled, severe, persistent asthma.

154 Improvement in Asthma Control in Asthmatic Children Following Asthma Camp Attendance
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RATIONALE: We hypothesized asthma control and airway inflammation would improve in children who attended a week long asthma camp. This was measured by spirometry, the Asthma Control Test (ACT), the Mini Asthma Quality of Life Questionnaire (Mini AQLQ), and fractional excretion of nitric oxide (FENO).
METHODS: Spirometry, Mini AQLQ, and FENO were performed on the first and sixth day of camp; the ACT was given on the first day of camp only. Our cohort included 40 children diagnosed with asthma who attended asthma camp; IRB approval and informed consent were obtained from all participants. The Mini AQLQ and ACT surveys were also mailed to participants one month after the completion of asthma camp. Significance was determined by paired t-test, correlation between FENO and ACT was determined by Spearman’s rank correlation coefficient.
RESULTS: There was significant improvement in mean FENO 25.7 ppb to 17.6 ppb (p=0.001). There was significant negative correlation between ACT score and FENO obtained the first day of camp (R=-0.45, p=0.005). There was no significant change in FVC (p=0.70) and FEV1 (p=0.82) or in Mini AQLQ scores (p=0.95) after one week. The results of the one month follow-up Mini AQLQ and ACT surveys are still pending.
CONCLUSIONS: While repeat Mini AQLQ and ACT results one month after asthma camp are pending, the improvement in FENO may suggest an improvement in airway inflammation after attending asthma camp. Daily classes and medication administration may explain these findings. Asthma camp may provide opportunities for children to obtain better control of their asthma.
155 Texting Medication Reminders for Better Asthma Control in Children and Teens: An Update
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Rationale: Non-adherence to medication regimens continues to be a persistent problem among patients with asthma. Technology makes communication with our patients between visits easier and more meaningful for all parties. We examine the effects on asthma control of sending medication reminders and allowing patients to communicate with staff via text messaging, one year after implementation.

Methods: A cohort of 29 participants (up to age 18 years) was enrolled over one year. Text reminders were sent twice daily to the parents and/or teenage patients with understanding that patients should receive medication at receipt of reminders. Retrospective chart review was completed to examine frequency of steroid bursts, ER visits, and hospitalizations for asthma occurring in the year prior to starting the study and number occurring in the year since starting.

Results: 29 participants completed 1 year to date. In the 12 months prior to the study, 21/29 patients had two or more steroid bursts, 28/29 patients had at least one urgent visit, and 20/29 had been admitted. One year after starting the study, 15/29 had two or more steroid bursts, 17/29 had at least one urgent visit, and 11/29 had been admitted. Comparing what we had predicted at 6 months vs the actual numbers at one year, this represents continued improvement in each of these measures, despite the results not being as positive as we originally projected after 6 months.

Conclusions: Our results suggest that communicating with our patients via text reminders is effecting positive change on control of their asthma, one year after initiating the study.

156 Effect of Educational Intervention on Adherence Estimator (AE) Scores and Asthma Control in Pediatric Patients
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Rationale: Non-adherence to asthma medications is a major factor contributing to the morbidity and mortality associated with asthma in the pediatric population. We investigated whether a single asthma education session improved medication adherence and asthma control.

Methods: A cross-sectional study was conducted with outpatient pediatric patients with persistent asthma. Children and their parents (n=36) were asked to complete 2 surveys on 2 occasions separated by 2 months. These surveys consisted of the Adherence Estimator (AE) (Merck), a 3 question survey assessing perceived views of medication concern, commitment and cost; and the Asthma Control Test (aACT/aACT). Patients and parents were educated verbally within 1 hour of initial survey. Follow-up surveys were completed by n=20 (55% of initial group). Data was analyzed with the Wilcoxon Signed Rank test.

Results: Pre-education the median total AE score was 4.9 and mean ACT score was 19.2 indicating a medium risk for adherence problems and well controlled asthma. Mean scores for concern, commitment and cost were 2.4, 2.4 and 0.4 respectively, indicating moderate to low risk for adherence problems. After education the mean total AE score was 3.3 (p = 0.315) and mean ACT score was 20.9 (p = 0.038) indicating an overall reduced risk of medication non-adherence and significant improvement in asthma control. Mean scores for concern, commitment and cost were 1.5 (P = 0.49), 1.7 (p = 0.59) and 0.1 (p = 0.25), respectively, indicating low risk for non-adherence in all categories.

Conclusions: Educating pediatric patients about their medications significantly improved asthma control, but not AE adherence scores.

157 Insurance Barriers in the Management of Uncontrolled Asthma in an Inner-City Population
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Rationale: Admissions for acute asthma exacerbations are ubiquitous in inpatient medicine. Identifying causes of these exacerbations allows for targeted preventative measures to reduce hospitalizations. One suspected factor is access to appropriate pharmacotherapy, which can be limited by under-insurance and lack of insurance. This study serves as an exploratory descriptive analysis of the importance insurance barriers play in uncontrolled asthmatics.

Methods: 134 patients aged 1-80 with a known history of asthma who admitted for acute exacerbations to the general medicine or observation units of Detroit Receiving Hospital, Harper University Hospital, and Children’s Hospital of Michigan were surveyed after providing informed consent. Questions about their asthma history, management regimens, frequency of hospitalizations, and type of insurance were included. Data for minors was provided by their parents. Insurance barriers were defined as patients having either no insurance, prohibitive co-pays for medications, or a refusal by their insurance to authorize medications prescribed by their physician.

Results: 41.8% of all patients reported having at least one insurance barrier related to their asthma care. 12.7% were uninsured, 9.7% reported prohibitive co-pays which ranged from $6-$260, and 19.4% reported insurance refusal to authorize a medication prescribed by their physician.

Conclusions: Insurance barriers are found in both inner-city adults and children with uncontrolled asthma and contributes to their hospitalizations by limiting their access to appropriate pharmacotherapies. Further in-depth investigations into these barriers are warranted based on this exploratory analysis, including whether recent changes in national health-care policy reduce the quantity of insurance barriers for this population.

158 Are We Doing Enough to Protect Asthmatic Patients from Pneumococcal Disease?
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Rationale: The Centers of Disease Control recommends pneumococcal vaccination among adult asthmatics between the ages of 19-64. Asthmatics are at increased risk from pulmonary and invasive pneumococcal disease. We aim to assess adherence to guidelines in a large tertiary care setting.

Methods: We performed an IRB-approved retrospective chart review of randomly selected records from September 2010 - April 2014 using ICD-9 code 493.xx. Demographic, clinical and immunization data were collected and analyzed.

Results: We reviewed 129 cases of asthma confirmed by spirometry and/or provocation studies. The mean age was 45 years [range 19-64 years]; 62% were female and 83% were Caucasian. Fifty eight (46%) were never smokers and 18 (14%) were active smokers. Forty seven (36%) were managed by Pulmonologists; 21 (16%) by Allergists and 61 (47%) by a primary care provider (PCP) or other providers. Fifty three (41%) had moderate or severe persistent asthma. Thirty six (28%) had at least one course of prescribed oral steroids and 2 (1.6%) were hospitalized for asthma in the 6 months prior to data collection. We found that only 23 (17.8%) out of 129 subjects had received pneumococcal vaccination. Four (3.8%) subjects were offered vaccination, but declined. Of the 23 vaccinated patients, 14 (61%) were primarily managed by a specialist, and 9 (39%) were managed by their PCP (p = 0.39).

Conclusions: Despite the inherent limitations of this retrospective study, primary prevention with pneumococcal vaccination remains low among asthmatics. Specialists were more likely to use the vaccine, albeit the rates remained low.
Skin Testing Practices Survey
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RATIONALE: Skin tests are reliable, cost effective techniques for the diagnosis of the IgE-mediated diseases. In order to improve how we perform skin testing we surveyed the allergy community in Pennsylvania for optimal practice ideas.
METHODS: This was a physician to physician survey which met IRB exemption. We conducted an on-line “Practice Site Skin Testing Survey” to identify differences in skin testing practices. Our questionnaire consisted of 10 questions and was distributed to 208 physicians members of the Pennsylvania Allergy & Asthma Association. It explored choice of skin testing devices, number of skin testing encounters per day, and projected duration of testing.
RESULTS: Sixty allergists (29%) responded to our survey, 82% of them were from private practices. 47% of practices allocate 30 to 60 minutes and 28% block 1 to 1.5 hours for new skin testing encounters. 100% of responders reported that they do not have a designated day when they perform their skin tests. 20% reported that they have a separate designated room where they perform their testing. 47% reported using a single test device and 52% use multiple skin test devices. 49% of responders perform skin testing in 4 to 7 patients per day and 93% perform it on the initial visit. 71% of physicians perform intradermal testing only for selective negative prick tests.
CONCLUSIONS: A wide variety of differences exists in skin testing practices. Diversity in the practice sites was very apparent. It appears we are practicing as the majority of allergists in the state.

Physician-Patient Communication Concerning Allergen Immunotherapy: Impact on Treatment Acceptance and Compliance
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RATIONALE: A patient’s knowledge of their treatment is a key factor in compliance and effectiveness. We assessed patients’ understanding and acceptance of allergy and allergen immunotherapy (AIT) on the basis of information delivered by their physicians and then after they had viewed a new, predefined information pack on AIT.
METHODS: We performed an international survey of 261 AIT-eligible patients (France: 57; Germany: 51; Spain: 52; USA: 51; Russia: 50) having consulted a specialist physician within the previous year. “Non-starters” (n = 134) had elected not to initiate AIT and “early abandoners” (n = 127) had ceased AIT before completion. Patients completed an on-line questionnaire before and after viewing the predefined information pack.
RESULTS: The mean time since allergy onset was 14.5 years. 79% of the patients reported a moderate to severe impact of allergy on their personal/professional life. Subcutaneous AIT had been prescribed in 60% of cases. 28% of patients did not know which allergy they were being desensitized for. Early abandoners reported a perception of low effectiveness (39%) and complained about expense (39%) and practical constraints (32%). Additionally, 22% of non-starters feared side effects. Patients considered the new information to be clear (92%), convincing (75%) and reassuring (89%), and felt better informed accordingly (80%). 76% would have been more likely to continue or initiate AIT after viewing the information pack (willingness score: 11.3/20 before and 14.2/20 after).
CONCLUSIONS: In AIT, prescriber-patient communication is suboptimal. After viewing a new information pack on allergy and AIT, patients felt better informed and more likely to initiate or complete AIT.
163 Grass Pollen Exposure in the Continental United States: Species Prevalence and Population Patterns

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RATIONALE: Epidemiological studies have shown that pollen from multiple species within the grass family (Poaceae) are major US aeroallergens. A growing body of immunological data document species-specific responses in grass-allergic patients. Most of the population is exposed to multiple grass species, however there has been no recent update on population exposure by species across the continental US.

METHODS: US Census data released in April, 2012 provided data on the population of each county in the continental US. The US Plants Database (USDA) provided data on the county-level prevalence of the grass species studied. Plant prevalence and population data were correlated to examine the potential exposure of people to different allergenic grass species.

RESULTS: Within the US, 84% of the population is potentially exposed to Lolium perenne (perennial rye grass) 75% to Dactylis glomerata (Orchard grass), 69% to Phleum pratense (Timothy grass), and 68% to Poa pratensis (Kentucky bluegrass). Geographic regions showed distinct exposure patterns. As examples, across the southeastern US, Lolium perenne dominates with 81% of the population exposed followed by 43% to Dactylis glomerata. Exposure in the Northwestern US was more heterogeneous with 99% of the population exposed to Poa pratensis, 95% to Lolium perenne and 94% to Dactylis glomerata. The highly populated East Central region was similarly heterogeneous with 99% exposed to Poa pratensis, 98% to Dactylis glomerata, 94% to Phleum pratense, and 87% to Lolium perenne.

CONCLUSIONS: Knowledge of distinct regional patterns of exposure can help clinicians to interpret patient history and diagnostic information and to plan therapeutic interventions.

164 Developing and Pilot Testing an Electronic Medical Record (EMR)-Based Allergen Immunotherapy Template

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RATIONALE: Allergen immunotherapy (AIT) schedules are commonly documented through paper-based records. We recently developed an electronic medical record (EMR)-based AIT template. We describe the results of pilot testing this EMR-based program.

METHODS: We developed an AIT template for our institution’s Centricity EMR system. During pilot testing, we evaluated patients from two groups (EMR- and paper-based immunotherapy schedules). All patients received a survey (items included injection reaction, satisfaction with the injection encounter). We evaluated the actual time (patient entering treatment room to injection administration) for patients in both groups. We compared results between the paper-based and EMR-based groups.

RESULTS: We observed 32 patients receiving AIT at our Allergy clinic. There were 17 patients (6 males, 11 females; median age 44 years) in the paper-based group, and 15 patients (3 males, 12 females, median age 42 years) in the EMR-based group. Injection reactions occurred in 18% of the paper-based group and 33% of the EMR-based group (p=0.11), and all symptoms were minor (local swelling, pruritus). All patients in both groups reported satisfaction with the injection encounter. Mean actual times in the paper-based and EMR groups were 4.86 minutes and 5.89 minutes, respectively (p=0.0001).

CONCLUSIONS: The EMR-based immunotherapy template was linked to visit time compared to the paper-based form, and all patients in both groups were satisfied. Further studies may indicate whether the EMR-based template is clinically safe and time efficient.

165 Allergic Response to IgE and Skin Prick Test Varies By Ethnicity

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RATIONALE: Although most clinical studies record the self-reported ethnicity of participants, the clinical trial cohorts are not always normalized to the diversity-related differences in allergen response. We analysed a general screening dataset to identify clinically-informative, ethnic patterns in allergy response.

METHODS: Specific antigen (IgE class) and skin prick tests (SPT) were used to screen over 1700 subjects for allergen reactivity to ten (IgE) and thirteen (SPT) allergens. Data were sorted by ethnicity into six categories (Asian, Black/African-American, Indian, Latin American, White/European, and Other) comprising similar sex ratios. Analyses were performed to identify similarities between ethnicity datasets for a variety of allergy related markers.

RESULTS: In general, IgE class correlated positively with mean SPT size, but there were consistent differences between certain ethnic groups for both IgE and SPT reactivity. African American participants had more frequent and greater allergic responses to annual allergens, particularly grass, while Indian participants had higher than average reactivity to perennial allergens. Asians had the lowest response to perennial and annual allergens. Of note is the ragweed allergen which reflected the same pattern of similarities and differences between ethnicities in 14 of 15 categories for both IgE and SPT tests, with 5 of 15 categories showing significant differences (p<0.02).

CONCLUSIONS: Understanding how allergic reactivity differs between ethnicities provides additional considerations for planning and normalizing trial demographics. An awareness of the diversity of the allergic response helps to predict normal variance in clinical trials and reinforces the need for balanced study groups.

166 Characterization of Allergen Immunotherapy at “Big 10” University Health Services

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RATIONALE: Characteristics of Allergen immunotherapy (AIT) administration at University Health Services (UHS) are poorly described. We sought to expand our pilot study that was conducted at the University of Michigan Health Service to the other “Big 10” universities.

METHODS: Online surveys were sent by e-mail to healthcare personnel who administer AIT at UHS.

RESULTS: 7 UHS managers and 17 AIT administrators from 9 different UHS, representing greater than 1200 patients, responded. 88% (15) of AIT administrators were RNs and 22% (2) were LPNs. 94% (16/17) responded that AIT was a significant part of their job, with an average 20 or more AIT shots per day. Times allotted for AIT injections varied from 5 min (1 UHS), 10 min (2 UHS), 15 min (3 UHS) to 20 min (1 UHS). 3 of 9 UHS had affiliated allergists, none were part of UHS staff. 9/17 administrators indicated they usually call the prescribing allergist for clarification of labeling and/or buildup schedule. Onsite supervision of AIT was provided at all UHS: 94% by MD, 6% by NP, although the supervisor was physically present in the suite only 58% of the time. 6 out of 9 UHS tracked systemic reactions, with an estimate of 0-1 reactions per month.

CONCLUSIONS: Variation in AIT administration exists among different UHS of the “Big 10” universities, particularly in time spent in clarification of labeling or buildup schedule orders and level of supervision of AIT administrators.
167 Th2 Cytokines Orchestrate the Secretion of MUC5AC and MUC5B in Chronic Rhinosinusitis with Nasal Polyps

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Rationale: Inflammatory pattern mediated by Th2 cytokines plays an important role in pathogenesis of chronic rhinosinusitis with nasal polyps (CRSwNP). We hypothesized that Th2 cytokines would contribute to mucin oversecretion from patients with CRSwNP.

Methods: Immunohistochemical staining for MUC5AC and MUC5B was performed in human nasal polyps from CRSwNP and cystic fibrosis (CF) patients and controls. IL-5, IL-13, IL-4, IFN-γ, IL-17, IgE, ECP, MPO, MUC5AC and MUC5B were determined in the homogenates of nasal polyps and controls by LuminexxMAP or ELISA. Secretion of MUC5AC or MUC5B was respectively measured in the supernatants of IL-5, IL-4 or IL-13 primed primary cultured human nasal polyp epithelial cells (HNPEs) and nasal polyp tissue fragments. Expression of IL-4 receptor α (IL-4Rα) and IL-13 receptor α1 (IL-13Rα1) in CRSwNP and controls was evaluated by immunohistochemistry.

Results: MUC5AC and MUC5B were both strongly expressed in CRSwNP group. Significant correlations could be found between IL-5, IL-13, IL-4, IgE, ECP levels and MUC5AC or MUC5B in CRSwNP group, but no correlation between IL-17 and MUC5AC or MUC5B in CF-NP group. We further observed an increased MUC5AC secretion in IL-4 or IL-13-treated HNPEs but not in IL-5 treated group. Moreover IL-4 can stimulate MUC5B secretion in nasal polyp tissue fragments while IL-5 and IL-13 had no effect on MUC5B secretion. Nasal tissue serial section studies showed that MUC5AC (+) or MUC5B (+) epithelial cells mainly expressed IL-4Rα, but not IL-13Rα1.

Conclusions: Th2 cytokines IL-4 and IL-13 can directly lead to mucin oversecretion by IL-4Rα from patients with CRSwNP.

168 Natural Killer Cells Regulate Eosinophilic Inflammation in Chronic Rhinosinusitis

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Rationale: Eosinophils play a major pathologic role in the pathogenesis of chronic rhinosinusitis (CRS). Recent study suggests that the impaired effector function of peripheral blood natural killer (NK) cells in patients with CRS is associated with blood eosinophilia. NK cells have been reported to regulate eosinophil activation and apoptosis. Prostaglandin D2 (PGD2) is an important contributing factor to CRS by recruiting and activating eosinophils. This study aimed to investigate whether eosinophilic inflammation in CRS is associated with impaired function of NK cells and dysregulated production of prostaglandin (PG).

Methods: NK cells-mediated eosinophil apoptosis was determined by Annexin V assay. The levels of peripheral blood PGs were assessed by EIA assay. Degranulation of NK cell was determined by measuring the response of CD107a against K562 cells.

Results: Eosinophil apoptosis in patients with CRS was significantly decreased when compared with healthy controls, whose eosinophil apoptosis was largely dependent on NK cells. Tissue eosinophils were positively correlated with blood eosinophils in patients with CRS. In a murine model of CRS, NK cell depletion was associated with an exacerbation of eosinophilic inflammation both in blood and nasal tissues. PGD2 and its metabolite but not PGE2 and a panel of cytokines including TGF-β were increased in CRS patients compared with controls. Effector functions of NK cells were potently suppressed by PGD2 rather than PGE2-dependent pathway in NK cells from both controls and patients with CRS.

Conclusions: Eosinophilic inflammation in CRS may be related to impaired NK cell-mediated eosinophil apoptosis by increased level of PGD2.

169 Natural Killer Cell Deficit Aggravates Eosinophilic Chronic Rhinosinusitis in a Murine Model

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Rationale: Chronic rhinosinusitis (CRS) is a multifactorial inflammatory disease of the nasal and paranasal cavities. Defective immune functions may contribute to the chronic inflammatory state in this disorder. Recently, it has been reported that CRS patients show the impaired function of natural killer (NK) cells, especially in recalcitrant CRS associated with asthma and eosinophilia. We investigated the role of NK cells in mucosal and systemic, particularly eosinophilic, inflammation in an allergic CRS (ACRS) mouse model.

Methods: Mice sensitized to ovalbumin (OVA) by intraperitoneal injection received nasal challenges with OVA for 5 weeks. NK cell depletion was achieved by intraperitoneal injections of anti-asialo ganglioside-N-tetraosyl ceramide antibodies 10 days before OVA sensitization and every 5 days until sacrifice. Sinonasal complex samples were evaluated histologically, and IL-4, IL-5, IL-13, IFN-γ, IL-8, and eotaxin were measured in nasal lavage fluid. A differential white blood cell count was also performed.

Results: ACRS mice showed significantly increased eosinophilic infiltration in sinonasal mucosa, elevated levels of IL-4, IL-5, IL-13, IFN-γ, and eotaxin in nasal lavage fluid, and peripheral blood eosinophilia compared with control mice. The depletion of NK cells induced more prominent eosinophilic inflammation, increased secretion of IL-5, and peripheral blood eosinophilia in ACRS mice.

Conclusions: These results presented that the depletion of NK cells aggravates allergen-induced sinonasal eosinophilic inflammation, suggesting that impaired NK cell activity may be an aggravating factor in eosinophilic CRS.

170 Immunomodulatory Property of Vitamin D in Allergic Fungal Rhinosinusitis

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Rationale: Vitamin D is a potent immunomodulator of cathelicidin (LL-37) in innate immunity. Low Vitamin D levels in Allergic Fungal Rhinosinusitis (AFRS), compared to chronic rhinosinusitis without nasal polyps (CRSwNP) and non-sinus disease healthy control (HC), could compromise epithelial immune barrier resulting in high fungal burden. We hypothesized that vitamin D enhanced innate immunity against fungal growth in AFRS is impaired compared to CRSwNP and HC derived sinonasal epithelial cells.

Methods: Human nasal-sinus epithelium cell (HNEC) cultures derived from AFRS, CRSwNP and HC subjects were stimulated with active vitamin D (1,25-dihydroxy-cholecalciferol) and inactive vitamin D (25-hydroxycholecalciferol). After overnight incubation with Aspergillus Niger conidia, hyphae growth was assessed and fungal activity measured with XTT Cell Proliferation Assay. Cathelicidin (LL-37) mRNA expression was compared at 6 hours and 24 hours of vitamin D stimulation.

Results: Hyphae growth was impaired on visualization in CRSwNP and HC samples after active vitamin D and inactive vitamin D stimulation, but remained uninhibited in AFRS. Fungal activity after stimulation with active and inactive vitamin D measured on XTT was highest in AFRS (n=3), compared to CRSwNP (n=5) and HC (n=2). LL-37 mRNA expression was up regulated in CRSwNP and HC on vitamin D stimulation, but not AFRS.

Conclusions: Fungistatic property in response to Vitamin D was seen in CRSwNP and HC but not AFRS, possibly due to impairment of LL-37 expression.
171 The Roles of Type 2 Innate Lymphoid Cells (ILC2) in Chronic Rhinosinusitis (CRS)

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RATIONALE: Chronic rhinosinusitis (CRS) is one of the most frequent chronic diseases, and little is understood about its pathogenesis. Eosinophils are considered to play a major role in its pathology, but we still know little which is causing chronic immune activation and persistent eosinophilic inflammation in CRS. Recently, type 2 innate lymphoid cells (ILC2s, lineage -, CD45 (+), CD127 (+), CD294 (+)) were identified as a candidate, which produce highly levels of Th2 cytokines such as IL-5 and IL-13, which activates eosinophils. We hypothesized that ILC2s are enriched in blood and nasal polyps in patients with eosinophilic CRS (ECRS) and are associated with its pathology.

METHODS: The patients with CRS or pituitary adenoma (normal sinus) who underwent endoscopic sinus surgery (ESS) in Jikei University Hospital were enrolled. We used PBMC and nasal polyps (NPs) from patients with CRS or normal sinus, and analyzed the amount of ILC2 by flow cytometry. We also investigated the distribution of ILC2s in NPs by immunohistochemistry. EDN and cytokines in NPs were measured by ELISA.

RESULTS: EDN and Th2 cytokines are significantly higher in ECRS than non-eosinophilic CRS (NECRS). The counts of ILC2s were significantly higher in ECRS than NECRS. Immunostained ILC2 were showed in nasal polyps of ECRS, but not in NECRS or normal subjects. The distribution of ILC2 in NPs was observed as chain-like. ILC2’s CD25 surface expression in PBMC was significantly higher in ECRS than NECRS.

CONCLUSIONS: ILC2 are considered as candidate of the commander in ECRS, which strongly induce Th2 inflammation.

172 Changes in Sinus Bacterial Culture Following Mupirocin Treatment in Surgically Recalcitrant Chronic Rhinosinusitis

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RATIONALE: Mupirocin sinonasal irrigations are commonly prescribed for chronic rhinosinusitis (CRS) after functional endoscopic sinus surgery (FESS). Data suggests that systemic antibiotics lead to alteration of the innate sinonasal microbial community, contributing to refractory disease; however, a paucity of data exists addressing the effect of topical antibiotics. We sought to evaluate patterns of sinus microbial colonization in CRS patients treated with mupirocin sinus irrigations after FESS.

METHODS: A retrospective chart review was conducted for consecutive post-FESS CRS patients treated with mupirocin sinus irrigations. The patients with aspirate cultures were performed pre- and post mupirocin therapy. Patients with immunodeficiency and those treated with oral antibiotics in the six weeks prior were excluded from the study.

RESULTS: Twenty-two patients were identified, with mean age of 66 years, and average number of endoscopic sinus surgeries 1.9. Endoscopic evidence of infection was present in 81.8% of cases. The most common isolates prior to mupirocin were coagulase-negative staphylococci (31%) and mixed respiratory flora (31%), followed by Staphylococcus aureus (13%), Pseudomonas aeruginosa (9%), Propionibacterium acnes, Streptococcus pneumoniae, and Klebsiella pneumoniae (4%). Following mupirocin therapy (mean duration 5.1 weeks), the isolates were as follows: Corynebacterium (27%), Pseudomonas aeruginosa (18%), Staphylococcus aureus (13%), Achromobacter xylosidans (9%), Stenotrophomonas maltophilia (9%), Eikenella corrodens, Acinetobacter baumannii.

Enterobacter aerogenes, Klebsiella pneumoniae, and Haemophilus influenzae (4%).

CONCLUSIONS: Mupirocin therapy may alter sinus flora, resulting in infection with unusual and resistant pathogens. The impact of topical antibiotic therapy should be taken into account when treating patients with CRS post-FESS.

173 Pediatric Nasal Poly: How Do They Manifest and Respond to Endoscopic Sinus Surgery

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RATIONALE: Chronic rhinosinusitis with polyp in pediatric population is an uncommon pathology and continues to be a challenging problem. In children inflammatory polyp associated with frequent infection is more common than eosinophilic polyp. This study aims to assess the clinical features and surgical outcome of endoscopic sinus surgery (ESS) in pediatric nasal polyp.

METHODS: Thirty patients younger than 18 years who had ESS for nasal polyps from 2008 to 2013 were available for analysis by medical records. We collected demographic and clinical data including age, sex, Lund-Mackay score of CT, surgery procedure, recurrence and comorbidities including asthma, allergy. Postoperative follow-up period ranged between 6 and 24 months.

RESULTS: There were 23 cases of chronic rhinosinusitis with nasal polyps (CRSNP) and 7 cases of antrochoanal polyps (ACP). The mean age of patients were 15 years with an age range of 6 to 18 years with 22 boys and 8 girls. 24 patients (80%) had bilateral disease. Twenty six patients were treated with ESS and four patients with ESS with concomitant adenoidectomy. Four patients (13.3%) showed recurrence after ESS. CRSNP groups (13.0%) and ACP (14.3%) groups had no significant difference in recurrence rate. CRSNP group showed higher CT Lund-Mackay scores than ACP group. Four patients have allergy and two patients have asthma.

CONCLUSIONS: Nasal polyps in children are more common in teenagers, are usually bilateral, and had good surgical outcome by ESS. The results of this study suggest that pediatric ESS is a safe and efficacious therapy for management of chronic rhinosinusitis with polyp in children with low recurrence rate.
174 Omalizumab for the Treatment of Chronic Rhinosinusitis: A Multi-Disciplinary Practice Review
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RATIONALE: Recently, anti-IgE monoclonal antibody has emerged as a potential therapy for CRS. However, to date evidence for its efficacy in this patient population is sparse. The purpose of this study is to evaluate the clinical treatment effect of omalizumab therapy for patients with recalcitrant CRS treated in a multi-disciplinary clinic.

METHODS: The charts of 194 patients on omalizumab were reviewed. 21 patients diagnosed with CRS and having failed surgical and/or medical therapy were identified. Data extraction was performed and targeted demographic details, asthma, environmental allergy and CRS specific disease related data including self-reported major symptom improvement. Nonparametric data was analysed with the Mann-Whitney test and binary data was analysed with Fisher’s exact test.

RESULTS: The mean treatment duration was 17 months. The most common skin test positive environmental allergens were dust mites (100%) and cats (65%). 75% of the cohort had CRS with polyps. Six patients (30%) had AERD. The mean polyp score decreased from 1.8 to 1.0 (p = 0.106). From the time of treatment initiation to the last omalizumab treatment dose, patients reported a mean 59% improvement in their olfaction, a mean 70.4% improvement in facial pain, a mean 78.2% improvement in nasal obstruction and a mean 68.1% improvement in the symptom of rhinorrhea. Patients reported a mean overall improvement in their sinus symptoms of 74.1%.

CONCLUSIONS: Omalizumab therapy provided a substantial improvement in the self-reported major symptom control for patients with recalcitrant CRS and asthma. A well-designed comparative study is needed to further assess its effectiveness in the CRS population.

175 Systematic Review of Omalizumab for the Treatment of Chronic Rhinosinusitis
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RATIONALE: Chronic rhinosinusitis (CRS) is a complex disorder in which the heterogeneity intrinsic to the disease has impeded efforts to outline optimal evaluation and management strategies. We sought to utilize data from a standardized, validated self-reported questionnaire to predict CRS subtypes.

METHODS: We performed a systematic literature search from a standardized, validated self-reported questionnaire to predict CRS subtypes.

RESULTS: Four distinct clusters were identified; patients with A) severe (n=9) or B) moderate symptoms (n=28) across all SNOT-22 questions, C) minimal symptoms (n=32) and D) with predominantly sinonasal-specific symptoms (n=30) (e.g. nasal congestion, anosmia). Patients in Cluster-A and D showed higher prevalence of polyps, history of aspirin sensitivity as compared to cluster-B or C. Compared to cluster-D however, cluster-A had a higher total SNOT-22 and lower CT scan score (Lund-Mackay). No significant differences were observed in the prevalence of allergic sensitization, blood eosinophil counts, and serum IgE levels amongst the four groups.

CONCLUSIONS: A proportion of CRS patients show more sinonasal-specific symptoms as compared to other SNOT-22 symptoms, and these patients may benefit from medical therapy targeting upper airway inflammation. Identifying subgroups of patients that may need adjunctive therapy to address pain, fatigue, and depression can help clinicians improve clinical outcomes for all CRS patients.
177 Sinonasal Outcome Test Questionnaire Does Not Predict Pathological Diagnosis of Chronic Sinus Disease

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RATIONALE: Chronic rhinosinusitis (CRS) presents as eosinophilic and non-eosinophilic processes, each having distinct prognoses and optimal therapies. Presently, only surgical biopsy allows for their differentiation. The Sino-Nasal Outcome Test 22 (SNOT-22), a patient-reported survey of symptoms and health related quality of life, has been validated as a measure of disease severity in CRS. We hypothesized that components of the SNOT-22, as well as objective measurements of disease observed on sinus CT scan could distinguish eosinophilic from non-eosinophilic CS.

METHODS: Patients who presented to the Otolaryngology Clinic at the University of Virginia with CRS filled out SNOT-22 surveys pre-surgery. The histology of the tissue samples obtained during surgery was analyzed via H&E staining to assess eosinophil count. These data were correlated with the SNOT-22 questionnaire to assess if specific questions could serve as predictors of histologic diagnosis. Also evaluated were sinus CT (Lund-Mackay) scores, asthma status, total IgE, and peripheral absolute eosinophil counts.

RESULTS: We found no statistically significant difference in either total SNOT-22 or any of its components in distinguishing eosinophilic from non-eosinophilic CRS. Similarly, neither total IgE, absolute peripheral eosinophil count, asthma status, nor Lund-Mackay score distinguished histological diagnosis. The only two parameters that approached significance were Lund-Mackay score (p = 0.074) and reduced [mental] concentration (p = 0.067).

CONCLUSIONS: No component of the SNOT-22 or other parameters allowed differentiation of eosinophilic from non-eosinophilic sinus disease. As pathological diagnosis is essential to determining optimal post-operative medical management, histological diagnosis at surgery remains essential in the management of CRS.

178 Action Plans for Managing of Chronic Rhinosinusitis Exacerbations: A Patient Interview Study

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RATIONALE: This study investigated the utility from a patient’s perspective of creating a written action plan to provide anticipatory guidance for patients with chronic rhinosinusitis (CRS) exacerbations.

METHODS: Twenty adult patients presenting to a tertiary care multidisciplinary CRS clinic who had a diagnosis of CRS based on duration of symptoms and CT imaging were interviewed by an investigator separate from the clinical team. Each patient answered a series of questions about the potential utility of an action plan for CRS exacerbations and which type of information such a plan should include.

RESULTS: Eleven patients (55%) called a physician’s office when during a CRS exacerbation for advice. Of the 11 who reported calling, 9 called their ENT physician, 1 called ENT in addition to their primary care provider, and 1 called their ENT and Allergist. Of those interviewed, 95% reported that they would use a written action plan if one was provided. When surveying which components would be helpful to include, 85% would like a list of symptoms indicating a flare-up of CRS, 80% a list of long-term maintenance medications, 90% a list of medications to use during a flare-up would be important, and 75% a list of common medication side effects.

CONCLUSIONS: Most CRS patients attending a specialist clinic would use a written action plan and agreed on common components for such a plan.

179 Allergen Sensitization in Thai Children with Ocular Allergy

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RATIONALE: Allergic conjunctivitis represents common ocular condition accompanied by allergic rhinitis. We reviewed type of allergen sensitization, clinical features and outcomes of children who had allergic conjunctivitis (AC), including seasonal of allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC).

METHODS: Children with history of AC were recruited. Clinical history, results of skin prick test and outcome of treatment were recorded.

RESULTS: One hundred and forty-four patients (age 4 -18 years) were studied. SAC was the most common type of allergic conjunctivitis (52.1%), followed by PAC (31.3%), VKC (11.1%), and AKC (5.6%). Male preponderance was found in all groups. Mean age of onset was 6.9 ± 2.7 years. Most patients sensitized to house-dust mites (84.7%), followed by cockroaches (47.9%), pollen (34.7%), and animal dander (29.9%). The severity of AC was not related to number of sensitized allergens. Standard treatment in all groups was topical olopatadine. However, add-on medications were needed in severe types of AC (VKC, AKC). History of topical corticosteroid use was 68.8% and 12.5% in VKC group and AKC group, respectively. All of them can discontinue topical corticosteroid when topical tacrolimus was applied. Complete remission was found 18.8% in VKC group and 50% in AKC group. Median duration of treatment was 19 months in VKC group and 11 months in AKC group.

CONCLUSIONS: Most Thai children with AC sensitized to house-dust mites. AKC is not uncommon in children and had better prognosis than VKC.

180 Allergen Specific IgE Detection Performance of AllergyQ® System in Korean Allergy Patients

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RATIONALE: AllergyQ enzyme immunoassay (EIA), a screening assay for specific immunoglobulin E (sIgE) for multiple allergens. While ImmunoCAP fluorescent EIA (FEIA) has been widely used for sIgE detection. In this study, we determined to evaluate detection performance of AllergyQ system compared to that of ImmunoCAP.

METHODS: We performed several inter-method comparisons using sera from 260 Korean allergy patients, including asthma (26.5%), allergic rhinitis (42.3%), atopic dermatitis (67.7%) and food allergy (18.1%). We compared the sIgE detection performance for seven major inhalant, five food allergens and four microorganism allergens.

RESULTS: 1,799 paired assay results were analyzed. Most allergen sIgE results showed above 0.5 intraassay correlation coefficient except mugwort and alternaria. Inter-assay class associations were reliable in most allergens (gamma = 0.858-0.983, p<0.001). The inter-method concordance was good to moderate for most allergens (kappa = 0.713-0.898, p<0.001).

CONCLUSIONS: AllergyQ EIA system showed a good detection performance compared with ImmunoCAP FEIA system in correlation and agreement in Korean allergy patients. However, in terms of methodological differences in these two assay systems, careful clinical implication is needed for interpretation of AllergyQ EIA results.
AB57

181 Development of ELISA Assays for Measurement of Can f 1 and Can f 3 in Dog Allergen Extracts

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RATIONALE: Dog allergen extract is a non-standardized extract with no potency measures. Understanding dog allergen extract potency will enhance the safety and efficacy of this product. Our goal is to develop monoclonal antibody-based sandwich ELISA assays for the measurement of Can f 1 and Can f 3.

METHODS: Mouse monoclonal antibodies were generated against natural Can f 1 and Can f 3. Monoclonal antibody-based sandwich ELISA assays for the measurement of Can f 1 and Can f 3.

RESULTS: Screening of Can f 1 antibodies revealed consistent strong responses with natural protein and dog extracts during indirect ELISA. Three pairs were selected for Can f 1 measurement: 6G1 as a capture antibody (2.5 mg/mL) and 4C3, 7D12, or 9A9 as biotinylated primary antibody (each at 1.0 mg/mL). Anti Can f 3 antibodies exhibit greater variability and less affinity: 10-20 μg/mL of each capture and primary antibody are required for consistent measurement of Can f 3. Further studies are needed to optimize both the assays.

CONCLUSIONS: sELISA assays have been developed for determining Can f 1 and Can f 3 contents of non-standardized dog allergen extracts.

182 Comparison Between Intradermal Skin Testing and Serum Specific IgE in Detecting Allergic Sensitization in Patients with Negative Skin Prick Tests

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RATIONALE: Studies show that both skin prick testing (SPT) and serum specific immunoglobulin E (sIgE) complement each other to determine environmental sensitization. However, it is common practice to only use SPT and intradermal skin testing (IDST), assuming that IDST will complement SPT sufficiently. The aim of this study was to compare the capability of detecting allergic sensitization by IDST and sIgE in patients with negative SPT.

METHODS: We retrospectively analyzed 272 SPT and 197 IDST/sIgE results for 11 environmental allergens done in 25 patients aged 10-65 years who presented to our clinic between January-June 2014 for evaluation of perennial or seasonal ocular nasal symptoms.

RESULTS: Of the 272 SPT performed, 45 were found negative. IDST and sIgE testing was done for SPT negative allergens. Of the 197 sIgE/IDST test pairs, 155 (78%) were IDST-/sIgE-, 14 (7%) were IDST+/sIgE-, and 21 (11%) were IDST+/sIgE+. In contrast to sIgE testing, IDST was stronger in detecting sensitizations to most pollen (tree, grass, ragweed) and cockroach. None of these sensitizations would have been detected using sIgE testing alone. While sIgE testing was overall weaker in detecting sensitizations than IDST, sIgE testing was low positive (0.35-1kU/L) in a few cases despite negative IDST for mugwort pollen, dust mite, mouse, and molds.

CONCLUSIONS: While overall IDST was stronger than sIgE testing in diagnosing allergic sensitization when SPT results are negative, for specific allergens sIgE testing may be superior or equal to IDST. The correlation with clinical allergic symptoms in these patients has yet to be determined.
184 Validation of an E-Source Data Collection System in the Environmental Exposure Unit (EEU)
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RATIONALE: Symptom Diary cards used to capture Patient Reported Outcomes(PRO) are traditionally collected via paper cards and loaded into a Clinical Data Management System(CDMS) at Environmental Exposure Facilities. Based on clinically significant(CS) symptom scores participants are examined by physicians to ensure their safety. Typically it takes 15-20 minutes to capture paper system diary cards for 100-140 participants using advanced scanning software. This timeframe limits the interval physicians have to review symptoms and examine participants reporting CS symptom scores. To enhance participant safety and tighten data management a validated system to capture Symptom Diary Cards electronically (e-Source) was developed.

METHODS: An e-Source data collection system was developed to capture electronic symptom scores in real time. The development of the system involved the creation of a secure web service and tablet application that mimicked the look and feel of a paper symptom diary card. This system allows for participants who feel more comfortable with paper symptom diary cards to continue using them if needed.

RESULTS: We have developed a validated, 21 CFR Part 11 compliant, electronic source tablet application that collects participant data securely and stores information in real time in the CDMS. The system has been shown to be extremely efficient, allowing for comprehensive reporting and alerts that can be customized to any study protocol.

CONCLUSIONS: A validated tablet-based e-Source Symptom Diary Card collection system is now available that is specifically designed to monitor and capture symptom scores in a manner that ensures more patient safety and tighter data management.

185 Multiple Cumulative Allergen Concentration Delivery for Nasal Allergen Challenge – a Refinement of the Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) Protocol
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RATIONALE: The Allergic Rhinitis Clinical Investigator Collaborative(AR-CIC) studies the pathophysiology of AR and provides proof of concept for novel therapeutics. Repeatability of symptoms and airflow measurements in Nasal Allergen Challenge(NAC) models are key to ensuring any changes before and following treatment are primarily due to treatment effects. We aimed to further optimize our previously published NAC protocol.

METHODS: 7 ragweed-allergic and 8 non-allergic participants were screened, using four-fold increases in allergen concentration, to determine the qualifying allergen concentration(QAC) at which a Total Nasal Symptom Score(TNSS) of 8/12 and a decrease of ≥50% in Peak Nasal Inspiratory Flow(PNIF) were achieved. At the subsequent NAC visit, participants were challenged with the cumulative concentration of all preceding allergen doses to the QAC, followed by the QAC itself 15 minutes later. TNSS and PNIF measurements were recorded at baseline, 15min, 30min, 1h and hourly up to 12 hours post NAC.

RESULTS: During the NAC visit, TNSS and PNIF reduction reached a peak at 15 minutes(p<0.001 vs baseline and at 15 minutes to 2 hours) followed by a gradual decline. TNSS and PNIF levels recorded during screening were met at 15 minutes during the NAC visit, with no statistical difference, an improvement from previous protocol versions. Participants were phenotyped into early, protracted early, and dual phase reactors.

CONCLUSIONS: Target symptom levels achieved at screening were reliably repeated at NAC, a further optimization of the protocol within the AR-CIC. Participants can be phenotyped to better assess the duration of action of a medication.

186 How Stable Are Allergenic Extracts?
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RATIONALE: Expiration dating of FDA USA standardized allergenic extracts is determined by stability studies performed by manufacturers that monitor potency over time. Nonstandardized extracts or diluted mixes do not have an FDA requirement for studies to determine shelf life. Commercially sold extracts expiration is specified by the FDA. Physician prepared mixes expiration rely on historical practices. The purpose of this study was to help verify the expiration dating of nonstandardized extracts. Additionally the potency change following cold room storage of diluted mixes was studied.

METHODS: Protein and allergen content of 1:20w/v glycerin and 1:10w/v aqueous nonstandardized extracts produced over the past 10 years was determined using SDS-PAGE, major allergen ELISAs and IgE binding using atopic sera pools. Mixes were made from glycerinated extracts and serologically diluted 1:5v/v using commercially available diluents; saline-phenol, human serum albumin, and 10% and 50% glycerin. Potency was determined by major allergen ELISAs for cat, birch, Timothy and mite following storing refrigerated up to 9 months.

RESULTS: Extracts generally maintain consistent patterns for the mandated expiration dating of 6 years for glycerin and 3 years for aqueous bulk concentrates. Major allergen and IgE binding potency helped support the gel data. Human albumin provided significant improvement over all other diluents for dilute extracts.

CONCLUSIONS: The characterization and activity methods used in this study provide evidence supporting the expiration dating for many non-standardized allergens. The stability enhancing ability of 50% glycerin and albumin was confirmed. The most diluted mixes including those containing glycerin are not stable unless they contain added albumin protein.
Characterization of Depigmented-Polymerized Pollen Extracts for Allergen Immunotherapy: Presence of Relevant Allergens and Molecular Size Consistency

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RATIONALE: Chemically modified allergens (allergoids) are extensively used for allergen immunotherapy since they have a reduced allergenicity with respect to native extracts while maintaining their immunogenicity. However, modification makes characterization of these molecules complicated, requiring specific techniques. The objective was to characterize different depigmented-polymerized pollen extracts. These allergoids have been previously purified (depigmentation process) in order to eliminate allergologically irrelevant low-molecular weight components.

METHODS: Depigmented-polymerized extracts of birch (Betula alba), olive tree (Olea europaea), grass (Phleum pratense) and pellitory wall (Parietaria judaica) pollens were manufactured from native extracts. The presence of the relevant allergens in the modified molecule was determined by mass spectrometry and the profile of polymerization of the allergoids was determined by high performance-size exclusion chromatography (HPSEC) using a Bio SEC-3 Column (Agilent) in a HPLC system.

RESULTS: Peptide sequencing confirmed the presence of the relevant allergens and their isoforms in the allergoids. Thus, allergens of groups 1, 2, 6 and 7 were detected in B. alba, groups 1, 2, 4, 5, 6, 7, 11, 12 and 13 in P. pratense; groups 1, 3, 6, 8, 9 and 10 in O. europaea; and groups 1, 2 and 4 in P. judaica. The HPLC profiles of the allergoids showed high consistency between batches with a decrease in retention time (increase in molecular weight) after polymerization.

CONCLUSIONS: The depigmented-polymerized allergen extracts of different pollens have been characterized, demonstrating the presence of the relevant allergens and the consistency batch-to-batch in the molecular size of the modified molecule.

Relative Potency in SPT of Solution and Tablet SLIT Allergen Extracts of Timothy Grass Pollen from 2 European Manufacturers Compared to a US Reference Extract

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RATIONALE: To compare the relative potency in skin tests of solution and tablet extracts of Timothy grass pollen (TIM) of 2 European manufacturers (ALK-Abelló, Stallergènes), with an FDA approved extract (REF) of 10,000BAU/mL.

METHODS: This is a prospective, multicenter, triple blinded, randomized study in which the in vivo extract potency was determined, based on the wheal size obtained in TIM allergic patients. The four tested TIM extracts were: Soluprick, Stalloral 300IR, and Grazax and Oralair 300IR dissolved in 1mL 50% glycerin (under GMP standards). The SPTs were carried out in quadruplicate with the concentrate extracts and three serial half-log dilutions, and +/- controls. The study took place at study sites with different climatologic conditions. To determine if there exists a statistically significant difference between the relative potency of the TIM extracts a parallel line bioassay was carried out using the mean surface of the four wheels of the SPTs per extract and per concentration (Wilcoxon, Asympt. Sig. (2-tailed) at 5% level). Based on the wheel sizes of the concentrate extracts in relation to the REF, BAU values were calculated.

RESULTS: Differences in wheel size between concentrate extracts reached statistical significance for all, except Soluprick-REF. The calculated BAU compared to the REF values for both solutions were between 11,300-16,300BAU/mL and the tablets varied between 4200-7300 BAU.

CONCLUSIONS: Based on SPT wheel sizes grass-tablets seem to be more potent than their reported potency of 2800BAU. There is a difference between the allergen concentration as measured in SPT of both tablets.
191 Serum Zinc and Secretory IgA Levels Are Important Factors in Children with Food Allergy

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RATIONALE: Zinc is an essential nutrient and its deficiency causes malnutrition and results in defects in innate and acquired immune responses. Also, zinc is important for highly proliferating cells, especially in the immune system and influences both innate and acquired immune functions. However, the precise roles and molecular mechanisms of zinc’s function in allergic response have not been clarified. On the other hand, the IgA antibody is massively produced in the intestinal Peyer’s patches, and the secretory IgA (sIgA) plays an important role on mucosal immune responses. It is considered that sIgA regulates the cause of allergic reactions. We studied serum zinc levels and sIgA levels in children with food allergies and studied their relationship with allergy symptoms.

METHODS: It is a retrospective study using medical records of infants (from 6 months to 6 years old) who had been admitted to our hospitals. We classified the groups according to the results of physical examinations with or without allergic symptom (eczema, wheezing, food allergy). In addition, we investigated the white blood cell counts (eosinophils and basophils) and the serum levels of specific IgE, total IgA, sIgA, TARC (thymus and activation-regulated chemokine), and zinc.

RESULTS: Children who were low levels in sIgA and serum zinc have past histories of atopic dermatitis, and their serum levels of specific IgE was significantly higher (p=0.013) but their serum IgA level was significantly lower (p=0.038) compared with children who do not have allergic symptoms.

CONCLUSIONS: Secretory IgA levels and zinc levels are also important to the onset of allergic reactions.

192 Up-Regulation of CysLT2 Receptor Expression and Cysteinyi Leukotrienes-Induced Calcium Signaling By Th2 Cytokines in Human Endothelial Cells

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RATIONALE: Recently we have shown that the nasal CysLT2 receptor localized exclusively in blood vessels and the expression level of the CysLT2 receptor in patients with nasal allergy was higher than that in patients without allergic rhinitis (Shirasaki et al. Allergol Int 2013). We hypothesized that Th2 cytokines could regulate CysLT2 receptor expression and function in vascular endothelial cells.

METHODS: Human umbilical vein endothelial cells (HUVECs) were stimulated with interleukin (IL)-4 or IL-13 for 48 hours, and the levels of CysLT2 receptor expression were evaluated by western blot analysis. HUVECs in 96-well plates were loaded with Ca2+ indicator Fluo-4, and its function in vascular endothelial cells. In non-pretreated HUVECs, any significant changes in intracellular Ca2+ levels were not observed by the stimulation with CysLTS. On the other hand, cellular responses to leukotriene C4 and leukotriene D4 were occurred in Th2 cytokine-pretreated HUVECs.

CONCLUSIONS: CysLT2 receptor expression in vascular endothelial cells can be regulated by Th2 cytokines at protein level. And Th2 cytokines may activate the functions of vascular CysLT receptors.

193 Regulation of Glucocorticoid Receptor (GR) Translocation of Airway Smooth Muscle Cells (ASM) By PPARγ Agonist Rosiglitazone and Insulin

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RATIONALE: We previously showed that PPARγ agonists regulate cytokine production by ASM, but their effects on GR in ASM are unknown. They may also act as insulin sensitizers. We hypothesized that PPAR agonist rosiglitazone would stimulate GR function of ASM and insulin would modulate the PPARγ effects on GR function.

METHODS: Primary ASM were transfected with GR-GFP (green fluorescent protein) vector and treated with buffer, Insulin 100 nm, Rosiglitazone 10 uM, or combination Insulin and Rosiglitazone for 2 hours. Some cells were immediately fixed as baseline, and others were treated with dexamethasone (1um) for 30 minutes to induce GR translocation. Integrated fluorescence intensity over nucleus and cytoplasm was calculated in individual cells (30 cells/block). Fluorescence was expressed as the nuclear:cytoplasmic ratio and normalized to N:C ratio of control and analyzed by one way ANOVA.

RESULTS: Compared to control 100±0, rosiglitazone 144±3.5 caused a significant increase in GR translocation (P-value <0.001). Insulin significantly inhibited the effect of rosiglitazone on GR translocation 101±2.5 (P-value <0.001). Dexamethasone 174±5.7 caused significant increase in GR translocation as compared to control. Rosiglitazone blocked the effect of dexamethasone on GR translocation 141±5.8 (P-value <0.001), whereas insulin had no effect on dexamethasone induced GR translocation.

CONCLUSIONS: Glucocorticoid receptor function in ASM cells is enhanced by rosiglitazone, but rosiglitazone blocked steroid signaling. Insulin blocked effect of rosiglitazone on GR function. These data suggest that PPARγ agonists could influence cytokine production in inflammatory states, and could also blunt or block the therapeutic benefit of steroids.
Airway Sensory Neuronal TRPA1 Does Not Mediate OVA Induced Allergic Asthma

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Rationale: Axon reflex theory was proposed to explain the involvement of afferent airway nerves in regulation allergic asthma phenotypes. Thus, afferent airway nerve ablation affects asthma phenotype in the ovalbumin (OVA) allergic asthma model. The regulation of asthma phenotypes by transient receptor potential A1 (TRPA1) in the OVA model has also been reported. Since TRPA1 is mainly expressed on airway neurons and can mediate neurogenic inflammation, it was suggested that neuronal TRPA1 controls asthmatic phenotypes in the OVA model. Activation of airway nerves by allergen could be a critical requirement for contribution of neuronal channels in regulation of asthma phenotype. According to our observations, OVA cannot activate TRPA1 on airway nerves. Therefore, we hypothesize that neuronal TRPA1 does not participate in regulation of asthmatic phenotypes in the OVA model. Therefore the aim of this study is to evaluate whether neuronal TRPA1 regulates eosinophilia, T cell polarization and airway hyperreactivity (AHR) in the OVA model.

Methods: We used conditional knockout approach to specifically ablate TRPA1 in airway neurons of mice. Global TRPA1 knockout mice served as positive control. In the OVA model, asthma phenotypes were evaluated by total and differential immune cell counts from BALF, measuring Th1 and Th2 specific cytokines in BALF and lung, examining lung pathology and function by Flexivent. Data were analyzed using one- or two-way ANOVA.

Results: We observed attenuation of eosinophilia, Th2 responses and AHR in the OVA model after global, but not neuron-specific ablation of TRPA1.

Conclusions: Non-neuronal TRPA1 mediates allergic asthma phenotypes in the OVA model.

IgE and IgA Produced in B Cells with Mast Cells Are Inhibited By Both Anti-CD40 and Anti-OX40L Abs in Mouse Allergic Asthma

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Rationale: Mast cells are major effector cells on the allergic diseases related to IgE. This study aimed to examine whether IgE or IgA was produced through OX40/OX40L interaction in B cells with mast cells, and the produced IgE or IgA was inhibited by anti-CD40 and anti-OX40L Abs.

Methods: C57BL mice were sensitized and challenged by OVA to induce asthma. Bone marrow-derived mast cells (BMMCs) and primary B cells were co-cultured. Mast cell recruitment into airway were stained by May-Grünwald Giemsa, expression of markers or signaling molecules by immunohistochemistry or Western blot, co-localization of B and mast cells by immunofluorescence.

Results: B cells with activated-BMMCs produced IgE and IgA, which re-activate each receptor on mast cells, through CD40/CD40L or OX40/OX40L. Both blocking Abs (anti-CD40 and anti-OX40L Abs) synergistically reduced IgE, IgA and mediator release, and they additively reduced other responses such as number of mast cells, expression of markers, CD40/CD40L, OX40/OX40L, FcεRI or FcαRI, co-localization of BMMCs and B cells or IgE/IgA-producing cells, compared to those in each Ab treatment or to all responses which are increased in BAL cells or lung tissues of OVA-challenged mice and in B cells and mast cells by co-culture.

Conclusions: The data suggest that IgE and IgA, produced by interaction of OX40/OX40L or CD40/CD40L in B cells with FcεRI-mediated mast cells, may re-activate FcεRI or FcαRI on mast cell surface, followed by more mediator release, and that combination treatment by both blocking Abs may contribute to the treatment of allergic asthma through the different signals.

Bordetella Pertussis Whole-Cell Vaccine Inhibits Specific IgE, Inflammation and Airway Remodeling in a Murine Model of Asthma

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Rationale: Bordetella pertussis whole-cell vaccine (Pw) has shown a protective role in experimental models of ovalbumin-induced asthma. We evaluated the effects of DTPw in a murine model of asthma induced by Dermatophagoides pteronyssinus(Derp).

Methods: The protocol lasted 30 days. BALB/c mice were divided into 6 groups, which were sensitized subcutaneously (s.c.) with saline solution or Derp 50mcg, in three injections. Three groups were submitted to saline, with or without vaccines diptheria-tetanus (DT) and DTPw. The other three groups received Derp with or without vaccines DT and DTPw. Subsequently mice underwent intranasal challenge with saline or Derp for 7 days and were sacrificed 24h after the last challenge. We measured serum specific IgE. IgG1 and IgG2a anti-Derp, cellularity in bronchoalveolar lavage (BAL) and airway remodeling.

Results: Animals sensitized with Derp produced specific immuno-globulins, high density of macrophages in BAL and significant airway remodeling. The group that received Derp+DTPw developed lower levels of IgE and higher levels of IgG2a (p<0.05), in comparison to Derp and Derp+DT groups. There was no difference between all 6 groups in eosinophil cell counts in BAL (p>0.05), but vaccines DT and DTPw decreased the number of macrophages, mainly DTPw (p<0.05). The vaccinated groups had lower airway remodeling compared with controls (p<0.05). Moreover, Derp+DTPw group had lower remodeling than Derp+DT group.

Conclusions: In this murine model of HDM-induced asthma, the vaccine DTPw decreased specific IgE, inflammatory cells in BAL and airway remodeling. Moreover, Pw vaccine increased specific IgG2, suggesting immune tolerance.
Experimental Asthma Induced By Tropomyosins from Cockroach and Shrimp: Insights into in Vivo Cross-Reactivity
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RATIONALE: In vivo cross-reactivity among tropomyosins, major pan-allergens among invertebrates, is not established. Our aim was to investigate the effects of purified tropomyosins from cockroach (recombinant Pera7) and shrimp (natural Litv1) on airway inflammation and hyperresponsiveness in a mouse model of asthma.

METHODS: Balb/c mice, 4 to 6 weeks-old, were sensitized twice with 50μg of rPera7 or nLitv1 intraperitoneally with 1 mg alum, and challenged with 50μg of rPera7 or nLitv1 intranasally for three days. A group was sensitized with rPera7 and challenged with nLitv1 under same conditions. Controls received saline on same days. Twenty-four hours after the last challenge, mice were ventilated with FlexiVent® and in vivo bronchial hyperresponsiveness was evaluated with inhaled methacholine (6.25, 12.5, 25 and 50mg/ml). After ventilation, bronchoalveolar lavage fluid (BALF) was collected and cell counts were performed.

RESULTS: Sensitization and challenge of mice with rPera7 or nLitv1 resulted in increased in bronchial hyperresponsiveness, given by increase in resistance and elastance. Total cells in BALF increased in rPera7 and nLitv1 groups, as compared to controls. There was increase in macrophages (5x10^4 vs 1x10^5 and 3x10^5 for rPera7 and nLitv1, p<0.001) and eosinophils (2x10^5 vs 1.4x10^5 and 9.1x10^5, p<0.001). Mice immunized with rPera7 and challenged with nLitv1 showed no changes in bronchial hyperresponsiveness or cells on BALF as compared to controls.

CONCLUSIONS: Experimental asthma induced by tropomyosins from cockroach and shrimp mimicked the main characteristics of human asthma. Despite the high degree of sequence identity and IgE immunologic cross-reactivity, our data suggested that cross-reactivity of these allergens among invertebrates is unlikely.

Induction of Epithelial-Mesenchymal Transition in House Dust Mite, Ragweed, and Alternaria Sensitized and Challenged Mice
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RATIONALE: Airway epithelial cells differentiate into a pool of myofibroblasts which contributes to airway remodeling. Myofibroblasts are able to invade and migrate outside the epithelium, augmenting subepithelial fibrosis and leading to airway remodeling. Differentiation of epithelial cells into myofibroblasts occurs though epithelial-mesenchymal transition (EMT). EMT involves a loss in E-cadherin with an increase in mesenchymal markers such as vimentin and N-cadherin. An atopic individual is most often allergic to multiple allergens. Therefore, we examined the effect of different allergens to induce EMT and airway remodeling in mice.

METHODS: Female Balb/c mice were sensitized (intranasal) and challenged (aerosolized) with house dust mite, ragweed, and Alternaria extracts (HRA). Airway hyper-responsiveness to methacholine was measured by whole body plethysmography. Histological sections were evaluated by H&E, PAS, and trichrome staining. E-cadherin, vimentin, and N-cadherin expression was assessed in tissues by immunofluorescence.

RESULTS: Sensitization and challenged mice with HRA mice had a significant increase in enhanced pause (Penh) compared to PBS mice. Examination of lung sections from HRA mice revealed increased airway inflammation, mucus hypersecretion, collagen content, and airway thickening compared to PBS mice. There was a decrease of E-cadherin expression along with increased vimentin and N-cadherin expression in the sub-epithelial area in the lungs of HRA mice.

CONCLUSIONS: Thus, exposure to multiple clinically relevant allergens induces a robust EMT response and allergic airway inflammation.

Functional Inhibition of PAR2 Prevents Inflammation and Tissue Remodelling in a Long-Term Model of Cockroach-Mediated Allergic Airway Inflammation
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RATIONALE: A number of serine proteinases inhaled as components of aeroallergens or released by inflammatory cells activate protease-activated receptor 2 (PAR2). We have shown that PAR2 is involved in the development of airway inflammation in short-term mouse models of allergic inflammation. We now hypothesize that functional inhibition of PAR2 will inhibit allergen-induced inflammation and tissue remodelling in a long-term mouse model of asthma.

METHODS: Balb/c mice were sensitized to cockroach extract (CE) intranasally (i.n.) and then challenged 3 times with CE to establish allergic airway inflammation. To investigate the role of PAR2 in the development of airway hyperresponsiveness (AHR), airway inflammation and tissue remodelling we administered i.n. a blocking anti-PAR2 monoclonal antibody (SAM-11) or an isotype control antibody before each of the allergen challenges the mice received during the following 10 weeks. Mice were assessed 24h after the last allergen challenge.

RESULTS: Administration of SAM-11, but not the isotype control antibody, significantly decreased CE-induced AHR, accumulation of eosinophils in the airways, the levels of IL-4, IL-5, and eotaxin in the lung tissue and the accumulation of hydroxyproline in the lungs, the latter as a measure of tissue remodelling. SAM-11 had no effect on IgG1, but decreased IgG2a antigen-specific antibody levels in the blood of these mice.

CONCLUSIONS: A PAR2 monoclonal antibody decreases allergen-induced AHR and airway inflammation as well as markers of tissue remodelling in a long-term mouse model of allergic airway inflammation. Therefore, topical PAR2 blockade in the airways may be a viable therapeutic approach in allergic asthma.
The Absence of Purinergic G Protein-Coupled Receptor 6 on Dendritic Cells Amplifies Antigen-Induced Pulmonary Inflammation

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RATIONALE: We previously reported that mice deficient in the type 6 purinergic (P2Y6) receptor develop more severe airway and lung tissue inflammation compared with wild-type (WT) mice after intranasal administration of dust mite extract. We hypothesized that expression of P2Y6 on dendritic cells (DCs) modulates antigen-induced pulmonary inflammation in mice.

METHODS: Mice lacking P2Y6 expression on CD11c+ cells [p2ry6 (fox/fox);CD11c-cre mice] were sensitized intranasally with PBS alone or containing ovalbumin (OVA) and lipopolysaccharide (LPS) on days 0, 1, and 2, and challenged with OVA on days 14, 15, 18, and 19. Bronchoalveolar lavage (BAL) was performed and total and differential cell counts were determined. In addition, bone marrow-derived dendritic cells (BMDCs) were stimulated with LPS overnight and qPCR was performed to evaluate P2R6 transcript.

RESULTS: Mouse BMDCs expressed P2RY6 transcript that was suppressed by stimulation with LPS. After sensitization and challenge, p2ry6 (fox/fox);CD11c-cre mice had significantly increased BAL total cells and a trend toward increased BAL eosinophilia compared with WT mice.

CONCLUSIONS: Our data suggest that the presence of P2Y6 on CD11c+ cells may inhibit antigen-induced lung inflammation, and that suppression of P2Y6 may be part of the mechanism for the effects of LPS in promoting allergen sensitization.

Rhinovirus Modulation of Dendritic Cell Phenotype and Function

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RATIONALE: Rhinoviruses (RV) are the major cause of asthma exacerbations. Evidence supports a synergistic relationship between viruses and allergen, with dendritic cells (DCs) mediating both viral responses and allergic inflammation. We hypothesise that during respiratory viral infection in atopic asthmatics, virus synergises with allergen exposure resulting in increased disease pathology, which is mediated through the modulation of DC populations.

METHODS: Moderate atopic asthmatic patients and healthy controls were experimentally infected with RV-16. Bronchoalveolar lavage (BAL) was taken at baseline, day 3 and day 8 post infection and DC populations isolated using fluorescence activated cell sorting (FACS) with further flow cytometric analysis.

RESULTS: Following RV infection in asthmatic patients, type I myeloid (m)DCs, which promote Th2 inflammation upon ex vivo allergen exposure, showed significantly increased recruitment to the airways at day 8 post infection (p=0.008). This was also accompanied by an increase in plasma-cytoid (p)DCs compared to healthy controls. Conversely, type II mDCs, which play a role in CD8+ T cell cross priming and the generation of subsequent anti-viral responses, were reduced in asthmatic airways at day 3 (p=0.05) compared to healthy controls.

CONCLUSIONS: We have observed an increase in type I mDCs and a decrease in anti-viral type II mDCs following RV infection in asthmatics, which may provide mechanistic understanding as to why asthmatics have more prolonged and severe respiratory viral infections. These findings will aid our understanding of the pathogenesis of asthma and the role of DCs in viral induced exacerbations.

Impact of Sublingual and Oral Immunotherapy for Peanut Allergy on Blood Dendritic Cells

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RATIONALE: Dendritic cells (DCs) are key antigen presenting cells that direct tolerogenic and effector T cell functions. We evaluated how peanut sublingual (SLIT) and oral (OIT) immunotherapy altered DC responses.

METHODS: Blood was obtained from subjects at baseline and at multiple timepoints during a placebo-controlled trial comparing peanut OIT to SLIT. Plasmacytoid (pDC) and myeloid (mDC) were purified and cultured with autologous CD4+ T cells. Allergen-induced cytokine secretion was measured in co-cultures by multiplexing technology, and expression of MHC II and costimulatory molecules on DCs by flow cytometry.

RESULTS: Peanut-induced secretion of Th2 cytokines decreased in pDC- and mDC-T cell co-cultures after 12 months of maintenance dosing with both OIT and SLIT (p<0.05). Levels of CD40, HLA-DR, and CD86 also decreased on DCs, while expression of CD80 increased (p<0.05). Effects were most striking in mDC-T cell co-cultures from subjects receiving OIT. These markers of immunologic suppression often reversed within weeks following withdrawal from IT, in some cases during ongoing maintenance therapy. Peanut-induced release of other effector cytokines, including IFN-g and IL-10, changed similarly to Th2 cytokines. Changes in DC-T cell responses did not correlate with clinical outcomes. Stimulation of co-cultures with dust mite led to cytokine changes that mimicked those seen with peanut.

CONCLUSIONS: OIT and SLIT for peanut allergy induced marked suppression of dendritic cell activation and Th2 cytokine responses during the initial phases of IT in an antigen non-specific manner. While there was substantial inter-individual variation, in many subjects suppression appeared to be transient, even while on maintenance dosing.
**204 Clinically Relevant Allergen Mixture Induces Robust Immune Response By Increasing CD11c+CD11b+MHCIIhiCD103int Lung Dendritic Cells**

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**RATIONALE:** Dendritic cells (DCs) in the airway epithelium and submucosa detect inhaled allergens and present processed antigens to T-lymphocytes to induce allergic immune responses. The phenotype of DCs is important in determining the nature of a response, whether immunogenic (Th2-mediated) or tolerogenic (Th1-mediated). Here, we examined the effect of multiple allergen sensitization on lung DC population and phenotype in a murine model of asthma.

**METHODS:** Female Balb/c mice were sensitized intranasally and challenged by aerosolizing a combination of house dust mite, ragweed and Alternaria or PBS for a total of 5 weeks. Lung tissue from both groups were harvested and sorted using MACS and FACS to determine phenotype of DC population.

**RESULTS:** Sensitization and challenge with house dust mite, ragweed and Alternaria resulted in increased airway hyper-responsive and mucus secretion. This correlated with increased total cell number and eosinophil infiltration in the BALF of antigen sensitized and challenged mice. Analysis of lung DCs in allergen-sensitized and challenged mice revealed greater percentages of CD11c+ and CD11b+ cells which expressed high levels of MHCII. CD103 expression was marginally increased in CD11c+ cells and increased in the CD11b+MHCIIhiDCs.

**CONCLUSIONS:** Multiple allergen exposure increases the population of dendritic cells associated with promoting an immunogenic response.

**205 Bradykinin Generation in Acute Allergic Reactions and Angioedema: Roles of Mast Cell Tryptase and Chymase**

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**RATIONALE:** There have long been suggestions that bradykinin may be a key mediator in allergic disease, but direct evidence for bradykinin generation has been lacking. We have examined the potential of mast cells to stimulate bradykinin production in vitro and in allergic conditions, and we have investigated in particular the ability of the mast cell proteases tryptase and chymase to cleave high molecular weight kininogen (HMWK) and generate bradykinin.

**METHODS:** Kininogen cleavage was assessed by Western blotting and immunoassay following addition of purified proteases to HMWK, in supernatants of experimentally activated cells of the LAD2 human mast cell line, and also in saliva collected from allergic patients. Concentrations of histamine, tryptase, chymase, carboxypeptidase and HMWK were determined by specific ELISA.

**RESULTS:** The generation of bradykinin was observed following addition of both tryptase and chymase to HMWK, and also following addition of these proteases to plasma samples. IgE-dependent activation of mast cells also resulted in the appearance of bradykinin in cell supernatants. Following onset of food-induced anaphylaxis, there were increased salivary levels of bradykinin and concomitant increases in tryptase and chymase concentration. Patients with a history of anaphylaxis, angioedema and urticaria had bradykinin levels in saliva substantially higher than that of healthy control subjects, a difference reflected in the levels of mast cell proteases.

**CONCLUSIONS:** The release of mast cell proteases in allergic disorders may contribute to the generation of bradykinin in these conditions.

**206 Activated Mast Cells Produce Soluble ST2, a Decoy Receptor for IL-33**

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**RATIONALE:** Interleukin (IL)-33 mediates inflammatory responses in allergic and autoimmune diseases. IL-33 acts through its membrane bound receptor, ST2. A soluble spliced variant of ST2 (sST2) that lacks the ST2 cytosolic and transmembrane domains is thought to act as a decoy receptor to neutralize IL-33 activity. sST2 and IL-33 are elevated in many inflammatory diseases and serum levels of sST2 appear to correlate with disease severity. We investigated whether mast cells produce, and are a significant source of sST2.

**METHODS:** Production of sST2 and IL-33 by stimulated mast cells derived from human peripheral blood CD34+ cells and mouse bone marrow was measured by qPCR, ELISA and Western blotting. A mouse model of passive systemic anaphylaxis was used to investigate sST2 production during anaphylaxis.

**RESULTS:** Antigen and IL-33 evoked substantial release of sST2 from mouse and human mast cells and did so synergistically when added together or in combination with stem cell factor, a growth factor that is critical for mast cell differentiation, growth, survival and homing. Rapid release of sST2 into circulation was also apparent during systemic anaphylaxis in mice. Human mast cells failed to generate IL-33 and IL-33 produced by mouse bone marrow-derived mast cells was retained within the cell.

**CONCLUSIONS:** Mast cells are a significant source of sST2, which may serve as a modulator of exogenous IL-33 activity and, in some circumstances, serve as a possible biomarker of allergic inflammation.

**207 Long-Chain n-3 Polyunsaturated Fatty Acids Inhibit FcεRI-mediated Mast Cell Activation**

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**RATIONALE:** Long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs) inhibit allergic inflammation but their direct effect on mast cells, major effector cells in allergy, is poorly understood. We determined the effect and mechanism of LC n-3 PUFAs on FcεRI receptor I (FcεRI)-mediated signal transduction and mast cell activation.

**METHODS:** Bone marrow-derived mast cells (BMMC) were cultivated from C57BL/6 WT and fat-1 transgenic mice which express fatty acid n-3 desaturase and produce endogenous n-3 PUFAs. Exogenous n-3 PUFAs were supplemented to WT BMMC and human mast cells LAD2 cultures. β-hexosaminidase (β-hex), cysteinyl leukotriene (cys-LT), TNF and chemokine (C-C motif) ligand 2 (CCL2) release were evaluated following FcεRI activation. Lipid rafts were isolated by sucrose gradient centrifugation.

**RESULTS:** LC n-3 PUFAs supplementation reduced β-hex release of LAD2 human mast cells, LC n-3 PUFAs-supplemented BMMC and BMMC from fat-1 transgenic mice released less β-hex and cys-LTs, and produced less TNF and CCL2. Total expressions of Lyn and linker for activation of T cells (LAT), and FcεRI-mediated phosphorylation of Lyn, spleen tyrosine kinase (Syk) and LAT were reduced in fat-1 BMMC. LC n-3 PUFAs did not alter expression of surface and whole cell FcεRI. However, LC n-3 PUFAs suppressed FcεRI localization in rafts of inactivated BMMC, and disrupted FcεRI shuttling to rafts of stimulated BMMC.

**CONCLUSIONS:** Our results suggest that LC n-3 PUFAs inhibit mast cell activation and FcεRI signaling by disruption of FcεRI association with lipid rafts, and suppression of Lyn and LAT expression.

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The Mechanisms Involved in IL-2 Production By Regulatory Mast Cells in Chronic Allergic Dermatitis
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RATIONALE: We have previously reported that MCs dampen inflammation in oxazolone-induced chronic allergic dermatitis in mice. This suppression occurs by a previously unrecognized regulatory mechanism in which MC’s secrete IL-2 which, in turn, supports regulatory T cell activity at the site of inflammation. However, since IL-2 is not a typical product of MC’s, we sought to define conditions that support its production in the setting of allergic dermatitis.

METHODS: Isolated bone marrow-derived MCs (BMMCs) were incubated with various stimulators and IL-2 release was assessed by ELISA. Phosphorylation of signaling molecules was studied by Western blot analysis. Allergic dermatitis was induced in mice by 10 repeated exposures of the ear skin to 0.5% oxazolone in acetone over 4 weeks. Immunohistochemistry was done on paraffin embedded skin specimens.

RESULTS: IL-33 is an exclusive inducer of BMMC IL-2 production. Pre-sensitization of cells with IgE further enhanced secretion of IL-2. IL-33 induced phosphorylation of the MAPKs family members ERK, p38 and JNK, and the inhibitors for these MAPKs dampened IL-2 release. The levels of IL-33 in ear homogenates from oxazolone-treated mice was greater than those of naive ears. Staining of ears disclosed in vivo co-localization of IL-33 and MC in ears with dermatitis.

CONCLUSIONS: MC-derived IL-2 was previously shown to stimulate Tregs in allergic dermatitis, and here we show that its production is stimulated by IL-33. In dermatitis, the levels of IL-33 increase and it co-localizes with MC. We suggest that IL-33 is a pivotal player in the induction of regulatory MC’s.

Microbiome Effects on Hematopoietic Eosinophil/Basophil Progenitor Phenotype: Implications for the Pathogenesis of Allergic Inflammation
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RATIONALE: CD34+ hematopoietic eosinophil/basophil (Eo/B) progenitors express TLRs and cytokine receptors (CyR) differentially in both newborns and adults, depending on allergic risk or disease, thus providing evidence for allergy as a systemic disease. We hypothesized that Eo/B progenitor phenotype would be affected by the host’s gut microbiome, examining this in ulcerative colitis (UC), a prototypical systemic inflammatory disease.

METHODS: Flow cytometry assessment of TLR and CyR surface expression on peripheral blood CD34+ progenitors as well as ex vivo examination of effects on these receptors of exposure to supernatants of bacterial cultures representative of the gut microbiome in adults with UC and age-matched healthy controls.

RESULTS: UC subjects showed a distinct pattern of TLR and CyR expression in comparison to healthy controls, with down-regulation of TLR-4 (MFI of 3.03±0.45 vs 1.87±0.33, P value 0.02) and GM-CSFR (1.71±0.56 vs 0.28±0.26, P value 0.01) and up-regulation of the Eo/B progenitor IL-5R (0.04±0.02 vs 0.77±0.24, P value 0.01). This pattern could be reproduced ex vivo upon exposure of CD34+ progenitors to both normal- and UC-derived gut microbiome supernatants.

CONCLUSIONS: Products of the gut microbiome affect CD34+ progenitor expression of TLR and CyR, with potential effects on Eo/B differentiation. These findings suggest that the gut microbiome may modulate allergic inflammation and disease through systemic effects on hematopoietic progenitors in early life and into adulthood.

A New Disease Cluster: Mast Cell Activation Syndrome, Postural Orthostatic Tachycardia Syndrome, and Ehlers-Danlos Syndrome
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RATIONALE: Patients with postural orthostatic tachycardia syndrome (POTS) and hypermobility often describe symptoms suggestive of mast cell activation. Herein, we describe a new, unique phenotype, characterized by the co-segregation of three disorders: POTS, Ehlers-Danlos syndrome (EDS) and mast cell activation syndrome (MCAS).

METHODS: Participants with diagnoses of POTS and EDS were recruited from throughout North America through a patient support group and evaluated by questionnaire and supporting documentation. A formal diagnosis of POTS by a cardiologist included confirmation via tilt-table test. A formal diagnosis of EDS required assessment by a dermatologist, a Beighton score of >9 and a diagnostic skin biopsy. A questionnaire for MCAS was based on diagnostic criteria and validated symptoms as reported by Akin, Valent and Metcalfe (2010).

RESULTS: 15 participants completed questionnaires with required documentation. All eligible participants were female. 12 of these people had formal diagnoses of POTS (80%), 9 were diagnosed with both POTS and EDS, 6 of 9 patients with both POTS and EDS had validated symptoms of a mast cell disorder (66%), suggestive of MCAS.

CONCLUSIONS: From these pilot data, it appears that a mast cell disorder may frequently co-segregate with POTS and a collagen disorder such as EDS.
AB66 Abstracts

211 Exploring the Impact of Basophil Surface IgE Density on Histamine Release Curve in Response to Anti-IgE
Alireza Sadegh Nejad, MD, PhD; Donald W. Macglashan, MD, PhD; JHAC, Baltimore, MD.
RATIONALE: In a recent study we observed a change in the pattern of basophil histamine release (BHR) in response to a bivalent anti-IgE, HP6061, after treatment with omalizumab. This was an unexpected observation because previous theoretical and experimental studies have shown that density of cell surface does not determine the position of the optimum response to bivalent stimuli that aggregate IgE. In this study we investigate whether IgE density modulates the position of the optimum response to HP6061.
METHODS: Mononuclear cells were prepared on Percoll gradients and were stimulated with a 4-log dose response curve of anti-IgE (HP6061). Histamine release was determined and basophil surface IgE density was quantified by flow cytometry using methods previously described.
RESULTS: A total of 11 subjects were examined and to optimize the distinction in surface IgE density, the top and bottom four results were placed into 2 groups resulting in a 6.4 fold difference in IgE density. The optimum concentration for the high and low density groups was 0.1 μg/ml and 1 μg/ml respectively. Although the dose response curve for the low density group was shifted rightward, overall, the curve was broader than high density group.
CONCLUSIONS: In this cross-sectional study, there is evidence that cell surface IgE density unexpectedly modifies the character of the dose response curve to HP6061. Although a more direct titration of IgE density is needed to confirm this conclusion, the current results support a response curve to HP6061. Although a more direct titration of IgE density is needed to confirm this conclusion, the current results support a

212 Epithelial Cell-Dependent Activation of Human Basophils
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RATIONALE: Mouse models of allergic sensitization have stressed the importance of TSLP in the production of IL-4 (and IL-13) from basophils. Translational evidence for this response in humans has been less forthcoming using in vitro models. We therefore hypothesized a requirement for direct interaction with epithelial cells (ECs) and/or with cytokines other than TSLP in activating human basophils.
METHODS: Basophils (>99% purity), cultured in medium alone or with IL-3±anti-IgE, were co-incubated with recombinant human (rh)TSLP, rhIL-33, or rhIL-25. In similar experiments, basophils were directly co-cultured (1-72h) with A549 ECs. Supernatants were concurrently tested for histamine [automated fluorometry], LTC4 [EIA], and cytokines [ELISA]. Anti-receptor antibodies were tested in neutralization experiments.
RESULTS: rhIL-33 (but not rhTSLP or rhIL-25) augmented IL-13 secretion (9-fold) from basophils co-treated with IL-3 (n=4, P=0.03), with minimal enhancement of histamine and IL-4, including those levels co-induced with anti-IgE. When co-cultured with ECs (at 3-20 passages), basophils showed a similar induction (6-fold) of IL-13 above those secreted with IL-3 alone (n=9, P=0.0004). Enhanced IL-4 and LTC4, was also evident, particularly when co-cultured with ECs having undergone a greater number of passages. Importantly, these basophil responses were not duplicated using EC supernatants, indicating a requirement for cell-to-cell interaction. Antibodies to TSLPR and ST2 were likewise ineffective in suppressing IL-4/IL-13 induction –a finding supported by failure to detect TSLP and IL-33 in A549 supernatants.
CONCLUSIONS: ECs have the capacity to activate human basophils for IL-4, IL-13 and LTC4 production and seemingly do so through yet unknown cell-to-cell interactions not necessarily involving TSLP or IL-33.

213 Correlation Between Serum IgE Concentration and Anti-IgE Mediated Histamine Release from Peripheral Blood Basophils in Allergic Rhinitis and Asthma Patients
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RATIONALE: Allergen-induced histamine release from peripheral blood has been used in support of allergy diagnosis. The expression of high affinity IgE receptors on basophils and mast cells is proportional to serum total IgE (IgE) concentration. The aim of this study was to determine the association between tIgE concentration and anti-IgE mediated histamine release from peripheral blood basophils.
METHODS: Whole blood basophil histamine release employing automated glass-fiber based microtiter plates coated with serial dilutions of rabbit anti-human IgE was performed on 80 allergic rhinitis/asthma patients. The tests were performed with and without pre-treatment with interleukin 3 (IL-3). Histamine release was evaluated in ng/ml (HRng) or as a percentage of total histamine content (HR%). Serum levels of total IgE (IgE) and allergen specific IgE (alge) were evaluated in serum using Phadia ImmunoCAP.
RESULTS: The mean concentration of IgE was 275 kIU/L (95% CI 148 to 401 kIU/L. The mean HRng was 52.6 ng/ml (95% CI 45.2 to 59.9 ng/ml) and mean HR% was 22.8% (95% CI 19.8 to 25.8%) respectively. No correlation was demonstrated between log tIgE and HRng (r - 0.152; p = 0.271). Significant correlation was demonstrated between log tIgE and HR% (r = 0.379; p = 0.0056). Brief pretreatment of the whole blood basophils with IL-3 resulted in a stronger correlation between log tIgE and HR% (r = 0.457; p = 0.007).
CONCLUSIONS: There is a correlation between percentage of histamine release from basophils and total IgE which is enhanced by exposing the basophils to IL-3.
214 Substance P (subP) and Minocycline Suppress Induction of Human Ragweed Specific Memory IgE Responses By Different Mechanisms

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RATIONALE: We reported that CD4+ and CD8+CD60+ T cells and 6 cytokines, including IL-4, were required for ragweed specific (RS) memory IgE responses by PBMC of ragweed sensitized humans, and that minocycline strongly suppressed T cell expression of phosphorylated p38 MAP kinase (p38MAPK) and IL-4, while preventing induction of RS memory IgE responses. Others previously reported that CD4+ T cells and IL-4 were required for human IgE responses and that p38MAPK is associated with IL-4. We hypothesize that subP, previously shown to suppress murine memory IgE responses, will prevent induction of human RS memory IgE responses.

METHODS: PBMC were obtained from ragweed sensitized humans (n=4-6) and CD4+IL-4+ and CD8+CD60+IL-4+ T cell numbers determined by incubation with PMA/ionophore. The effect of subP ± PMA/ionophore on these cells, on CD4+ p38MAPK+ T cells, and on RS memory IgE responses were determined (flow cytometry, ELISA).

RESULTS: CD4+IL-4+ T cells were 5% of CD4+ T cells; CD8+CD60+IL-4+ T cells were >99% of CD8+CD60+ T cells. SubP did not suppress CD4+IL-4+ or CD8+CD60+IL-4+ T cells. Nevertheless, subP suppressed T cell p38MAPK (30-70%) and induction of RS memory IgE responses. As reported, minocycline strongly suppressed IL-4, T cell p38MAPK and RS memory IgE responses.

CONCLUSIONS: SubP and minocycline appear to suppress RS memory IgE responses by different mechanisms. SubP did not suppress IL-4 but minocycline did. Both suppressed IgE responses. It is possible that subP suppresses events occurring downstream of IL-4. Further, it is possible that subP suppresses other cytokines required for memory IgE responses.

215 Microbial Regulation of IgE Production in Early Life

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RATIONALE: Changes in gastrointestinal microbiota have been suggested to drive the increasing prevalence of food allergy. Our aim was to determine the impact of mouse and human intestinal microbiota on modulation of allergic parameters including IgE.

METHODS: C57BL/6 mice were treated with antibiotics (ABX) for 4 weeks. Germ-free (GF) mice were colonized with fecal microbiota from a healthy pediatric donor. Total serum IgE, IgA, and IgG were analyzed by ELISA, blood eosinophils and basophils by flow cytometry, and cytokine expression in Peyer’s patches (PP) by qPCR.

RESULTS: ABX treatment immediately after weaning induced a significant increase in total serum IgE (45.6±10.5 ng/ml (ABX) vs 12.2±2.8 ng/ml (control), and surface IgE on basophils (4377±1371 MFI (ABX) vs 1139±666 (Control)). The levels of other isotypes were unchanged. Circulating eosinophils were also significantly increased (2.37±0.83% (ABX) vs 1.42±0.25% (control)). IL-4 was significantly upregulated in the PP after ABX-treatment of young mice. Susceptibility of IgE and eosinophils to modulation by ABX was rapidly lost, and was not observed in 5 week old mice. GF-mice had progressively increasing levels of IgE with age (187±75 ng/ml at 8 weeks and 623±131 ng/ml at 12 weeks). Colonization with human microbiota at 4 or 8 weeks of age significantly suppressed IgE as measured 4 weeks later (77.75±38.60 and 205.5±148.3 ng/ml).

CONCLUSIONS: In mice there is an early-life window of antibiotic exposure that leads to elevated IgE and other allergic parameters. Transfer of human microbiota to germ-free mice suppresses IgE and offers a unique model system to study regulatory activity of human microbiota.

216 Human Rhinovirus C Specific IgE Is Detectable in High Risk Children

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RATIONALE: Viral respiratory infections and allergic sensitization play important roles in asthma inception and exacerbations. Children who are atopic have increased susceptibility to human rhinovirus (RV) infections. One potential mechanism involves impairment of antiviral responses by high affinity IgE receptor (FcεRI) cross-linking. If RV-specific IgE is produced in atopic individuals, it could function like an allergen, which may have important implications in the development of asthma and severity of exacerbations.

METHODS: Peripheral blood samples were obtained from 134 nine-year-old children enrolled in the Childhood Origins of Asthma (COAST) cohort. Biotinylated RV-C15 was bound to streptavidin on the Phadia ImmunoCap. The presence of RV-C15 specific IgE was detected using standard ImmunoCap methods. Levels ≥ 0.10 kUA/l were considered positive. Relationships among RV-C specific IgE, asthma, and other atopic phenotypes were assessed.

RESULTS: 7.5% (n=10) had detectable RV-C specific IgE, with a range of 0.13 – 1.11 kUA/l. Children with RV-C specific IgE had higher total serum IgE [median: 841 vs. 58 kUA/l in the positive and negative groups, respectively (p<0.0002)], and were more likely to be sensitized to aeroallergens (p=0.04). There were non-significant trends toward higher peripheral blood eosinophils, rhinitis, and asthma in children with RV-C specific IgE.

CONCLUSIONS: RV-C specific IgE was detected in 7.5% of high risk children, and was associated with elevated total IgE and aeroallergen sensitization. It is unclear whether the IgE produced is specific to RV-C or common to all RV species. The biological activity of RV-C specific IgE is unknown and warrants further investigation.
**217 Production of Naturally Occurring Human Allergen Specific IgE Monoclonal Antibodies (mAbs)**

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RATIONALE: Although food and environmental allergies are increasingly common in the developed world, our understanding of the properties and biology of the molecule mediating allergic disease, IgE, is incomplete. A better understanding of this molecule and its functional properties is needed and could lead to more targeted therapies.

METHODS: Human memory B cells were cultured from the peripheral blood of allergic patients, transformed with Epstein-Barr Virus and then expanded in culture. Cultures were screened for IgE production via ELISA using murine anti-human IgE antibodies. The B cells from positively screened cultures were then fused with a myeloma cell line by electrical cytofusion to form human hybridomas, which secrete human allergen specific IgE mAb.

RESULTS: Human memory B cells expressing IgE antibody were consistently isolated from peripheral blood, expanded in culture, then fused to make human hybridomas, which successfully produced large amounts of fully human IgE mAb. Panels of naturally occurring human IgE mAbs are being generated. To our knowledge these represent the first ever naturally occurring human IgE secreting human hybridomas.

CONCLUSIONS: We have made human monoclonal IgE antibodies. Natural human derived monoclonal IgE will assist in investigation of the structural determinates of many clinically important antibody-antigen binding interactions. By improving our understanding of the IgE-antigen interaction, we hope to provide insights needed for the design of better allergen specific immunotherapies.

**218 2-Methyl-1, 3, 6-Trihydroxy-9, 10-Anthraquinone Isolated from Rubia Cordifolia L Inhibits IgE Production**

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RATIONALE: Medicinal herbs provide relief to a large percentage of the world population suffering from inflammatory diseases and a major resource for new drug development. The medicinal herb Rubia cordifolia L. is been widely used to treat inflammation. It also showed direct inhibition of IgE production in vitro and in vivo in our previous study. The aim of this study was to identify the bioactive compound in this herb that inhibits IgE production.

METHODS: Liquid-liquid extraction, silica gel, and Sephadex LH20 column chromatographic methods were used for isolation and purification of compounds. NMR and liquid chromatography–mass spectrometry (LC-MS) techniques were used to identify the compound in Rubia cordifolia L. Rubia cordifolia L compound effects on suppression of IgE production by human B cells (U266 human myeloma cells) and peripheral blood mononuclear cells (PBMCs) from allergic patients was assessed.

RESULTS: The compound that was isolated and purified (purity >95%) from Rubia cordifolia was identified as L. 2-methyl-1, 3, 6-trihydroxy-9, 10-anthraquinone (MT-anthraquinone). This compound showed dose-dependently inhibition of U266 cells IgE production with an IC50 of 3.2μg/mL (11.8μM) without any sign of cytotoxicity. MT-anthraquinone (10μg/mL) also non-toxically abolished IL-4 and anti-CD40 stimulated IgE production by PBMCs from food allergic patients.

CONCLUSIONS: MT-anthraquinone inhibits IgE production in human B cell line and food allergic patient PBMCs. It may have potential for treatment of IgE associated inflammatory diseases, which requires further investigation.

**219 Essential Role of B-Cell-Intrinsic MyD88-Signaling in IgE Responses in Lungs**

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RATIONALE: Allergen-specific IgE is linked to asthma pathogenesis, but the underlying mechanisms of IgE production in response to allergen exposure are poorly understood. This study investigated the role of B-cell-intrinsic myeloid differentiation factor 88 (MyD88) in IgE and IgG1 production evoked by ragweed pollen instillation into lungs.

METHODS: Mice received ragweed pollen every 4 days, for a total of four times, and serum immunoglobulin levels were examined 24 h after final ragweed administration. B-cell-specific MyD88-deficient mice were generated by mixed bone marrow transfer system, in which lethally irradiated Rag2-deficient mice were administered mixed bone marrow cells comprising 80% B-cell-deficient mMT and 20% MyD88-deficient bone marrow.

RESULTS: MyD88-deficient mice showed defective IgE/IgG1 production and germinal center responses to lung instillation of ragweed pollen. However, MyD88 was dispensable for dendritic cell activation and TH2 cell development. B-cell-specific deletion of MyD88 replicated the defective antibody production observed in MyD88-deficient mice. Although ragweed pollen contains Toll-like receptor (TLR) ligands, TLR2/4-deficient mice developed normal allergic responses to ragweed pollen. However, anti-IL-1RI antibody-treated mice and IL-18-deficient mice showed decreased IgE/IgG1 production with normal TH2 development.

CONCLUSIONS: Our data demonstrate that pollen instillation into lungs induces IL-1α/β and IL-18 production, which activates B-cell-intrinsic MyD88 signaling to promote germinal center responses and IgE/IgG1 production.

**220 Role of Patient Education in the Management and Control of Asthma in the Adult Population**

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RATIONALE: Despite advances in asthma medications and devices, studies showing durable improvements in asthma outcomes are lacking. To address this discrepancy, various asthma education programs have been developed. Data regarding the effect of these programs on asthma control and medication adherence are needed. Our study aims to measure the effectiveness of an asthma education session (AES) using established conventional asthma control measures and recently developed asthma medication adherence measures.

METHODS: In this randomized, prospective study, patients (n=11) with Asthma Control Test (ACT) scores <19 were randomized to an education intervention or a control group after consent. Intervention group patients (n=7) received one 60 minute motivational interviewing AES by an allergist and were given an asthma action plan; patients in the control group (n=4) did not receive either. Primary outcome measure was ACT score at 6 month follow-up. Secondary outcome measures included the Asthma Medication Ratio (AMR), Rescue Index (RI), and forced expiratory volume in 1 second (FEV1) by spirometry.

RESULTS: At 6 months, ACT score improved in 50% of the control group and 43% of the intervention group. FEV1 scores improved on average by 7% in the control group vs 12% in the intervention group. No differences were noted in AMR or RI between the two groups.

CONCLUSIONS: Although our education intervention did not demonstrate improvement in all asthma parameters measured, an improvement in FEV1 was observed. Future studies with larger sample size, longer follow-up periods, and stratification based on asthma severity are warranted. The role of education in improving asthma medication adherence measures remains to be determined.
221 Medication Actuations Calculated from Remaining Doses in Discarded Metered Dose Inhalers of Asthmatic Children
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Rationale: Currently available metered dose inhalers (MDIs) do not track the remaining number of doses. Therefore, we assume that asthmatic children use their controller MDIs regularly and we estimate the time that MDIs should be discarded is the discard point labeled on the canister and box to prevent the use of empty MDIs.
Methods: Fluticasone propionate is the controller MDIs used in this study. Children with asthma symptoms had a regular schedule to replace controller MDIs according to the discard point labeled on the canister. We asked asthmatic children attending our clinic from May 2013 to April 2014 to collect their discarded controller MDIs. The remaining medication in each discarded MDI was calculated from the canister weight. We collected demographic data and percentage of actuated doses to evaluate whether our intervention has an influence on the patient’s adherence.
Results: One hundred discarded MDIs were collected from 52 asthmatic children. Nearly all of the remaining medication (n=48, 92.3%) was controlled disease. More than 80% of the fluticasone propionate in the MDIs was used until nearly empty (>80% of labeled dose). There was no correlation between patient characteristics and the percentage of actuated doses.
Conclusions: Most of the discarded MDIs of controlled asthmatic children had less than 20% of the remaining medication of the labeled doses. The canister weight is a useful and reliable method to track a child’s medication supply. This procedure should be implemented into the health care system to prevent the discard of unused medication.

222 Analysis of Open Oral Food Challenges Performed in a Pediatric Allergy Clinic
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Rationale: While the incidence of food allergies is increasing, inaccurate diagnosis remains a concern. An oral challenge is the gold standard for confirmation of presence, or resolution, of a food allergy. There are well published food-specific IgE predictive values that can help guide when to perform an oral challenge. We wanted to compare total and food-specific IgE of patients undergoing oral food challenges in our pediatric allergy clinic to the predictive norms.
Methods: We conducted a retrospective chart review of all pediatric patients, aged 1-18 years of age, who underwent oral challenges in our Allergy Clinic from 2012-2014. Data on total and food-specific IgE, challenge results, and requirement of epinephrine for systemic reactions was analyzed.
Results: A total of 110 challenges were performed. The median total IgE was 52kU/L (range 8.4-12,240). The median values for the 8 major allergens were: milk 0.92kU/L (range <0.10-7.81), peanut 0.61kU/L (<0.10-17.8), tree nuts 1.53kU/L (0.34-10.9), egg 0.71kU/L (<0.10-4.78), soy 0.58kU/L (<0.10-54), wheat 10.7kU/L (7.27-93.2), and fish, including shellfish 0.97kU/L (<0.10-1.79).
Conclusions: We report a high rate of success at 78.1% in oral challenges performed at our site. Even though the median value of the food-specific IgE was low, our failure rate was only 7 patients required treatment with epinephrine. While predictive food-specific IgE values help guide the decision to perform oral challenges, they are not entirely successful in predicting the potential for reactions, including systemic ones requiring use of epinephrine.

223 Resident Knowledge Regarding Use and Interpretation of Diagnostic Testing for Food Allergies
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Rationale: Pediatric residents do not always receive formal training on food allergy diagnosis and management. The aim of this study is to evaluate resident knowledge regarding the use and interpretation of diagnostic testing for food allergy compared among the three levels of resident training.
Methods: A questionnaire of five clinical vignettes related to food allergies was created and distributed electronically to the Nationwide Children’s Hospital Pediatric Residency program.
Results: Of 150 total residents, 41 residents responded to the questionnaire (27%). Of the responders, 32% were year 1 residents, 39% were year 2 residents, and 29% were year 3 residents. Total number of correct responses for the entire sample size was 56%. Although there were no differences in total correct responses between the three years, there were significant differences for each individual question. Third year residents were over two times more likely to answer the question exploring the role of food allergy testing in mild atopic dermatitis then first year residents. However, third year residents were less likely to answer the question correctly regarding appropriate testing following an apparent peanut allergic reaction. Other questions were answered incorrectly universally. For example, more than half of all respondents would not advise food avoidance for a child who experienced an anaphylactic reaction but had negative serum IgE testing.
Conclusions: Our small survey did not show improvement in understanding of food allergy diagnosis and management with advanced training and demonstrated a need for improved resident education. In particular, residents appear to prioritize serum IgE testing over patient history.

224 Twitter As a New Medium for Public Health Advocacy: Asthma, Food Allergy and Allergic Rhinitis
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Rationale: Social networking and microblogging enables the rapid distribution of information to the public. The role of Twitter and its present application in asthma, food allergy and allergic rhinitis education was analyzed.
Methods: We used the Twitter search function to gather the top 100 tweets (English language) with words asthma, food allergy and allergic rhinitis with hashtags (#asthma, #foodallergy and #allergicrhinitis) (searched August 26, 2014). Tweets were analyzed based on author and stratified into 2 groups: public versus professional (tweets by physicians, journals and health policy organizations).
Results: Tweets assigned to the professional category were 38% of total for asthma (38/100), 28% for food allergy (28/100) and 18% for allergic rhinitis (18/100). Tweets messaged by allergists constituted 8% of total for asthma (8/100), 8% for food allergy (8/100) and 15% for allergic rhinitis (15/100) of the total, respectively. Links to professional sources were below 40% of top 100 tweets.
Conclusions: Twitter has capacity to be embraced as a new tool for public health education. Professional sources have not yet seized the opportunity that Twitter presents with respect to asthma, food allergy and allergic rhinitis-related communication. Allergists are a small part of the online asthma and allergy-related conversation (8-15% of tweets). Fewer than 40% of tweets were from professional sources, indicating a quality gap on this communication channel and an audience in need of expert input. Health advocates can use Twitter to construct public health campaigns to engage specific Twitter users who participate in asthma, food allergy and allergic rhinitis conversations.
225 The Role of AHPCO Technology in Reducing Allergic Rhinitis Cases As Air Purifier, Surface Sterilizer and Ice Maker Sterilizer

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RATIONALE: Fifteen year’s aeroallergen data that we collected using a Burkard Spore Trap showed a steady increase in aeroallergen concentration in the Texas Panhandle area. Data showed a strong correlation with the allergy and asthma cases that have doubled since 2007. We developed a novel AHPCO or Advanced Hydrated Photocatalytic Oxidation technology to produce filter less air purifiers, surface sterilizer for cell phones and in meat processing facilities and in ice makers to reduce contamination.

METHODS: We analyzed the daily aeroallergen by using the coated Melinex tape from the Burkard Volumetric Spore Trap. Exposed, stained Melinex tape was observed under a BX-40 Olympus microscope. We developed and assessed the AHPCO Technology for potential uses as air purification unit, surface sterilizer and net reduction of bacteria, fungi during food processing. Petri dish culture, meat blocks, fruits were placed in the chamber to assess the capacity of sterilization. Images were captured with FITC, TRITC Filters with a BX40 and SZ-CTV Olympus Microscopes and SEM.

RESULTS: A fluctuation and gradual shift in aeroallergen index with the warmer climate and a shift in flowering seasons were noticed that contributed the increased allergy cases. AHPCO Technology produces negative ions that can be successfully applied in devices to reduce indoor aeroallergen and bacterial contamination in food processing and ice makers.

CONCLUSIONS: Environmental factors contribute to a high concentration of aeroallergens that led to increased allergy cases among the residents of Texas Panhandle. AHPCO Technology can be successfully used in air purifiers, food processing and in ice makers in reducing contamination.

226 Correlation of Development of Allergic Disease to Parental History of Cancer in Chinese Immigrant Populations Residing in Brooklyn

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RATIONALE: The association of allergic disease with cancer is understudied. We investigated the relationship between development of allergic disease/asthma in adult Chinese immigrants to Brooklyn and their parental history of cancer.

METHODS: Retrospective survey was conducted for adult immigrants to Brooklyn from People’s Republic of China (n=164) who developed allergic disease (allergic rhinoconjunctivitis, asthma, eczema, food allergies) after immigration to Brooklyn, and Chinese immigrants to Brooklyn without allergic disease (n=128). All participants completed a survey regarding history of cancer of any kind in their fathers and mothers, personal history of cancer, and development of allergic disease. Total serum IgE in subjects and controls were determined by ELISA. Fisher’s exact test and Wilcoxon rank-sum 2 sided tests were used for statistical analysis.

RESULTS: There was a significant association between development of allergic disease in adult Chinese immigrants and any parental history of cancer (p=0.0052); allergic disease was also associated with development of paternal cancer (p=0.024). Approximately half of paternal cancers in allergic subjects were gastrointestinal solid tumors (11/21); 4 of 21 were lung cancers. Allergic disease did not associate with history of maternal cancers (p=0.26). There was no correlation between personal history of malignancy (7/290) and IgE levels (p=0.598).

CONCLUSIONS: These findings suggest there may be genetic influences on development of allergies and malignancies.

227 Association Between Asthma Prevalence and Environmental Tobacco Smoke (ETS) Exposure in Schoolchildren from the Pittsburgh Region

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RATIONALE: Environmental tobacco smoke (ETS) is a known risk factor for pediatric asthma. Despite access to healthcare and effective treatments, asthma prevalence remains high among Pittsburgh inner-city children. The purpose of this study was to evaluate the association between ETS and asthma prevalence among Pittsburgh schoolchildren.

METHODS: This study was approved by the institutional review board of Allegheny General Hospital and informed consent/assent was obtained from all subjects prior to participation. Fifth graders from six Pittsburgh elementary schools were enrolled. Demographic information was collected through a self-reported survey. Prior diagnosis of asthma and risk of newly diagnosed asthma were assessed using an abbreviated, validated survey. Salivary cotinine levels were assayed by commercially available Enzyme-Linked Immunosorbent Assay (ELISA). Results were compared using a t-test.

RESULTS: A total of 146 subjects were enrolled (49.3% female, 50.7% male, 64.4% Caucasian, 31.5% African American, 78% public insurance). Saliva was available for cotinine analysis in 129 (88%) subjects. Of those, 29 (22%) had confirmed ETS exposure. The overall asthma prevalence among ETS and non-ETS exposed subjects was 62% and 32%, respectively (p<0.05). The prevalence of newly diagnosed asthma among ETS and non-ETS exposed subjects was 21% and 8%, respectively (p<0.05).

CONCLUSIONS: These results demonstrate a significantly higher prevalence of asthma among schoolchildren from the Pittsburgh region with documented ETS exposure as compared to those without ETS exposure. Future efforts to improve asthma outcomes in the Pittsburgh region must incorporate smoking cessation strategies.
CONCLUSIONS: Previous research indicates that diet during infancy influences taste and food preferences. This study aimed to investigate whether consuming a milk free diet during infancy impacts on eating habits in later childhood.

METHODS: Two groups of children were recruited from the Food Allergy and Intolerance Research (FAIR) study on the Isle of Wight, UK. Prospective infant feeding data was available from the FAIR study birth cohort. Group one had been fed a milk free diet for Cows’ Milk Allergy (CMA) in infancy. Group two, a control group, were fed a normal diet during infancy. Children with current food allergies were excluded. Parents and children completed validated eating habits questionnaires. Descriptive statistics and Mann Whitney tests were calculated.

RESULTS: 83 children of mean age 11.6 years were recruited (26 previously milk free and 57 control). Children who were fed a milk free diet and/or a specialized formula for CMA in infancy had significantly higher levels of food avoidant behavior than controls (median scores 52 and 43 respectively, p < 0.01). They also had significantly lower liking scores for chocolate, full fat milk, ice cream and cream (p < 0.05).

CONCLUSIONS: Children who followed a milk exclusion diet for food allergy during infancy have higher levels of avoidant eating behavior and a dislike for the excluded food group approximately 10 years after a normal diet has resumed. Efforts should be made to incorporate previously excluded foods into the diet once a food allergy has been outgrown.

Pilot Study Demonstrates High Prevalence of Asthma in Inner-City Schoolchildren from Pittsburgh Region

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RATIONALE: Pediatric asthma continues to be a public health concern in the inner-city region of Pittsburgh. The purpose of this pilot study was to develop efficient and accurate methods to assess the prevalence of asthma among schoolchildren in Pittsburgh.

METHODS: This study was approved by the Allegheny Singer Research Institute–West Penn Allegheny Health System Institutional Review Board. Informed consent/ascent was obtained from all subjects prior to participation. Fifth grade students were recruited from six elementary schools across Pittsburgh. Parents were surveyed using an abbreviated, validated survey by Galant et al. to assess the risk of asthma, and where applicable, level of disease control. Demographic and clinical characteristic differentials in percentage of children ‘at risk’ for asthma, previously diagnosed with asthma by a physician, and with asthma not well controlled were evaluated for significance using Chi-square test statistic.

RESULTS: 146 subjects were enrolled (64.4% Caucasian, 31.5% African American (AA), 49.3% female, 50.7% male, 78% public insurance). Results demonstrate that 34.3% of subjects were at risk of asthma, 24.7% had a previous diagnosis and 9.6% were newly identified. Additionally, 44.4% of those previously diagnosed with asthma had poorly controlled disease. Finally, asthma prevalence was higher in AA and those with public insurance (p<.05).

CONCLUSIONS: Results indicate a high prevalence of asthma and poor disease control among schoolchildren. AA race and public insurance were identified as risk factors. Future studies need to expand upon this sample size, explore the impact of environmental factors on health outcomes, and ultimately improve asthma outcomes in at-risk populations.

Trends in the Workforce of Certified Asthma Educators (AE-Cs) in New York State (NYS) & Relationship to State Funding Support

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RATIONALE: Asthma presents a major healthcare burden in NYS. The NYS Department of Health (NYSDOH) commissioned a taskforce to develop strategies to increase the number of AE-Cs, follow workforce trends and promote integration into practice. State contract funding supported preparation and examination costs.

METHODS: National Asthma Educator Certification Board online registry of current AE-Cs in NYS as of 3/14/14 was compared against 2010 data. Workforce changes were examined against extrinsic factors. Demographic distribution by primary training was compared against 2010 data. AE-C/asthma population ratios were calculated based on estimates from the US census and Behavioral Risk Factor Surveillance System reports.

RESULTS: A sharp rise AE-Cs in NYS from 106 in January 2010 to 294 in March 2014 coincided with contract amendment funding by NYSDOH. Most AE-Cs were trained in Nursing or Respiratory Care. The greatest increase was seen in the percentage of successful 1000-hour candidates (12.2% in NYS). The AE-C/asthma population ratios in NYS went from 1:10,115 to 1:4016 assuming an asthma prevalence rate of 8.2% in 2010 and 9.0% in 2014.

CONCLUSIONS: The 2009 legislation providing coverage of Asthma Self Management Training (ASMT) services by a licensed Medicaid healthcare provider with AE-C, as well as the use of NYSDOH funding has impacted on the number of certified asthma educators in NYS. The professional composition of the workforce is evolving with significant increase in 1000-hour qualified certificants. Despite improved AE-C/asthma population ratios in NYS, additional numbers are needed to address the needs of underserved communities.
Preliminary Results of the Teen Food Allergy Education Survey
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RATIONALE: Food allergic teens are at increased risk of fatal anaphylactic reactions. Using teen input, the CAAEC wanted to develop an education program to help teens gain appropriate knowledge and skills to handle food allergies safely.
METHODS: Based on teen focus group interviews and literature review, an online survey was created. The link was distributed to food allergy professionals and lay organizations in Canada.
RESULTS: 89 teens completed the survey; 55 lived in Manitoba and Ontario (65%), 16 (24%) lived elsewhere in Canada, 8 lived outside of Canada (11%). Peanut allergy was common amongst the teens (73%) and 8 (9%) did not have an epinephrine auto-injector. 59 (74%) of the teens preferred a small group setting (5-15 people) and the majority of teens were interested in topics such as, “Social situations” (73%), “Traveling” (64%) with food allergies. The majority of teens thought the group should meet once/month (47%), weekday evening (58%) and that a young adult with a food allergy expert should lead the group (44%). Additionally, teens preferred getting new information through email (51%), talking to someone (53%), YouTube (53%) and through websites (62%); accessing online information with Smartphones (86%) and laptops (72%). 70% of the teens had looked at online food allergy resources.
CONCLUSIONS: Many of the teens preferred small group settings to talk about food allergies and were experienced with technology to search for information online. The results of this data will help in the development of a food allergy education program for teens based on their learning preferences.

Interaction of Leptin Genetic Variants and DNA Methylation Influences Lung Function and Asthma at 18 Years of Age
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RATIONALE: The leptin gene (LEP) encodes a 16 kDa pleiotropic adipokine that is considered to play a regulatory role in inflammation and allergy. Human and experimental animal studies have linked leptin to asthma. We therefore investigated whether genetic polymorphisms of LEP in concert with DNA methylation within LEP can explain the risk of lung function and of asthma.
METHODS: Blood samples were collected from a random sample of boys and girls at age 18 from the Isle of Wight birth cohort, UK. Four LEP SNPs were genotyped. DNA methylation of CpG (cytosine-phosphate-guanine) sites was assessed using the Illumina Infinium HumanMethylation450 bead chip. Linear regression models were used to test the interplay of SNPs and CpG sites on lung function. SNP and CpG interactions that survived false discovery rate (FDR) correction were further examined for association with asthma at age 18 using log-linear models.
RESULTS: The interaction of CpG cg00666422 with SNP rs11763517 (AG vs. GG) was significantly associated with lung function. For subjects with rs11763517 genotype AG, FEV1/FVC ratio (p = 0.007), and FEF25-75% (p = 0.003) decreased with increasing methylation levels of cg00666422. Relative to subjects with rs11763517 genotype AA or GG, subjects with genotype AG also had increased risk of asthma at age 18 as methylation levels of cg00666422 increased.
CONCLUSIONS: The penetrance of leptin genetic variants is modified by DNA methylation. Future studies are required to assess the pathways that further explain the development of asthma mediated by the leptin gene.
**234** IFNγ and Foxp3 Methylation, Expression in Buccal Mucosa in Inner-City Children with Allergic Asthma

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**RATIONALE:** DNA methylation of IFN and Foxp3 has been associated with environmental exposures and asthma-related outcomes. However, it is not known whether epigenetic regulation and expression of these genes in the buccal mucosa can be used as a biomarker for allergic disease in children.

**METHODS:** Buccal mucosa cells from mouse sensitized asthmatic children (ages 5-17) who were recruited to participate in the Mouse Allergen and Asthma Intervention Trial (MAAIT) were collected at baseline and 6 months after intervention or control. DNA and total RNA were extracted. Pyrosequencing of CpG sites of IFNy (promoter) and Foxp3 (promoter, upstream enhancer) and real-time PCR was conducted.

**RESULTS:** At baseline, in this mixed cell population, negative correlations between expression and DNA methylation at each gene were not found. Instead, IFN and Foxp3 buccal gene expression positively correlated (N = 113, r = 0.59, p<0.0001). Unexpectedly, IFN promoter methylation (CpG-54) positively correlated with Foxp3 gene expression (N=113, r = 0.20, p= 0.03). Methylation at CpG sites in the Foxp3 promoter (CpG-207) negatively correlated with methylation at Foxp3 enhancer site (eg. CpG 4575; N = 125, r = -0.64, p <0.0001). The same trends repeated 6 months later.

**CONCLUSIONS:** Several patterns of regulatory gene methylation and expression in buccal cells were exhibited in this cohort of mouse-sensitized, allergic asthmatic children. Future work examining internal validity and reliability of these markers, associations with allergen levels, and associations with allergy and asthma-related outcomes following intervention may yield information on potentially novel biomarkers for pediatric allergic asthma.

**235** Sophora Flavescens Alkaloid-Rich Fraction Induction of IL-10 Production and Prevention of Dexamethasone Suppression of Asthma Patient PBMC IL-10 Production Is Associated with Altered DNA Methylation at foxp3 Gene Promoter

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**RATIONALE:** Allergic asthma is associated with increased Th2 and impaired Th1/Treg responses. Corticosteroids suppress inflammation, but also cause unwanted generalized immunosuppression. ASHMTM, a 3-herb formula, had a beneficial immunomodulatory effect in asthma patients. This study focused on IL-5 and IL-10 as signature Th2 and Treg cytokines to characterize ASHMTM immunomodulatory components.

**METHODS:** Peripheral blood mononuclear cells (PBMCs) were isolated from physician diagnosed asthma patients (n=21). The effect of ASHMTM and individual herb constituents on anti-CD3/28 Dynabeads-stimulated production of IL-5 and IL-10 by PBMCs was determined.

### RESULTS

- **Sophora flavescens** (SF), an herbal constituent with significant immunomodulatory effects, was fractionated to 4 fractions (F) and their effects on PBMC IL-10 and IL-5 production in the presence or absence of dexamethasone were determined. Pyrosequencing was employed to determine DNA methylation levels at the foxp3 gene promoter.

**RESULTS:** ASHMTM dose dependently reduced IL-5 and increased IL-10 secretion (p<0.05-0.001). SF-F2 (contained alkaloid compounds) was most effective in increasing IL-10 but had no effect on IL-5, whereas SF-F4 (contained flavonoid compounds) was most effective in suppressing IL-5 but did not affect IL-10 production. Dexamethasone-treated PBMCs produced significantly less IL-5 as well as IL-10 (p<0.05). Co-culture with dexamethasone and SF-F2 significantly prevented dexamethasone suppression of IL-10, but not IL-5 production. Furthermore co-culture with SF-F2 and dexamethasone significantly reduced DNA methylation levels at the foxp3 gene promoter.

**CONCLUSIONS:** The SF alkaloid-rich fraction was responsible for ASHMTM induction of IL-10 production by PBMCs, and also prevented dexamethasone suppression of IL-10 production in association with acquired epigenetic modification of the foxp3 gene promoter.

### Abstracts AB73

**236 Early-Onset Asthma Is Associated with a Specific Polymorphisms of TLR-4 (Asp299Gly) in Ukrainian Adults**

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**RATIONALE:** Toll-like receptor 4 (TLR4) is the principal receptor for bacterial endotoxin and an important intracellular signal pathway of the innate immune response in asthma. The TLR-4 gene is located on chromosome 8, which is believed to be related to the occurrence of asthma. There may be an association of the TLR-4 (Asp299Gly) SNP and early-onset asthma patients.

**METHODS:** 262 early-onset (<40 years old) and 69 late-onset persistent asthma patients were assessed. The control group included 285 non-atopic volunteers. Single nucleotide polymorphism of TLR-4 (Asp299Gly) was detected by PCR.

**RESULTS:** In early-onset asthma patients the AA genotype was detected in 203 patients, AG in 56 and GG in 3; in late-onset asthma AA in 58, AG in 10, GG in 1; and in controls group AA in 242, AG in 40, and GG in 3. The allele frequencies were 88% (n=524) for the A allele and 12% (n=62) for the G allele in the controls. The risk of asthma for the G allele (OR = 1.529, C.I. = [1.023-2.284], χ²= 4.34, p=0.037) but was not increased in late-onset asthma (OR = 1.085, C.I. = [0.558-2.108], χ² = 0.06, p= 0.810).

**CONCLUSIONS:** The TLR-4 (Asp299Gly) polymorphism is associated with adult early-onset asthma in Ukraine.
237 DNA Methylation Modifies the Effect of Genotype on Atopy Risk
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RATIONALE: Genome-wide association studies (GWASs) have detected single nucleotide polymorphisms (SNPs) that influence allergic disease risk. However, environmentally induced DNA methylation may modify the effect of genotype on disease risk. More accurate assessment of allergic disease risk may be possible by taking local DNA methylation into account.

METHODS: Genome-wide DNA methylation and ten atopy risk SNPs detected in a recent meta-GWAS were profiled in a subset (n = 367) of the Isle of Wight (UK) birth cohort. Cytosine-phosphate-guanine (CpG) sites within genes and intergenic gaps surrounding each atopy SNP were selected. The effects of SNP genotype, DNA methylation at local CpGs and SNP×DNA methylation interactions on risk of atopy at age 18 were examined using logistic regression models.

RESULTS: Effects on atopy risk were directionally consistent with previous data for 8/9 SNPs, although only one SNP reached statistical significance. Thirty local CpGs across eight loci were significantly associated with atopy (p < 0.05). Parsimonious models detected two significant SNP×DNA methylation interaction effects on atopy, at SLC25A46/TSLP and LPP. Having ≥ 1 G allele at SNP rs10056340 (SLC25A46/TSLP) is protective against atopy at lower cg04022379 methylation levels, but increases atopy risk at higher methylation levels, relative to genotype TT. Genotype GG at rs9865818 (LPP) was associated with increased risk of atopy at low methylation levels, relative to genotype AA.

CONCLUSIONS: Two significant SNP×DNA methylation interaction effects on atopy were detected. This suggests DNA methylation, as a surrogate for environmental exposure, can be used to improve the accuracy of genetic risk profiling in allergic disease.

238 Oral Tolerance and Unresponsiveness to Allergen Challenge after Immunotherapy Are Not Associated with a Change in B10 Cell Number in Mice
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RATIONALE: IL-10 secreting B cells (B10 cells) have been described to have an immunosuppressive role in several inflammatory diseases including ulcerative colitis and rheumatoid arthritis. We hypothesized that B10 cells would be increased in orally tolerant mice compared to naive and peanut-sensitized mice. Similarly, we hypothesized that peanut immunotherapy would increase B10 cells in mice compared to placebo.

METHODS: To induce oral tolerance, BALB/c females were gavaged with crude peanut extract (CPE) or sham tolerized with PBS for three consecutive days followed by sensitization with either PBS, or CPE and cholera toxin once weekly for three weeks. For the immunotherapy model, C3H/HeN females were sensitized with CPE and cholera toxin for 4 weeks followed by intraperitoneal immunotherapy with peanut or placebo for 4 weeks. Mice were bled and challenged with intraperitoneal CPE. Serology and challenge results were compared between naive, tolerant, and sensitized groups as well as immunotherapy and placebo groups. Spleen and peritoneal cells were isolated and stimulated in culture with PMA, ionomycin, and LPS. B10 cells were quantified by flow cytometry as the percentage of CD19+ cells that were IL-10+.

RESULTS: No significant differences were observed in %B10 splenocytes between naive (n = 9; mean±SD: 4.55±0.93), sensitized (n = 9; 5.17±0.95), or tolerant (n = 8; 4.85±0.46) mice. Likewise, %B10 splenocytes did not differ following IT (n = 4; 5.12±1.27) compared to placebo (n = 4; 5.73±1.22).

CONCLUSIONS: Percentages of total splenic B10 cells do not appear to be important in oral tolerance or immunotherapy in these mouse models; however, antigen-specificity and functionality may reveal a role for B10 cells in tolerance.

239 Allergic Disease-Related Phenotypic Differences Emerges in Type 2 Immune Responses
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RATIONALE: Major factors limiting the use of allergic disease-causing T cells as a therapeutic target and clinically useful biomarker are the lack of accepted methods that allows their identification and discrimination from non-pathogenic TH2 cell types.

METHODS: Ex vivo MHC-class II tetramer staining was used to detect, characterize and sort allergen-specific CD4+ T cells. Transcriptome and surface marker immuno-phenotyping of allergen-specific CD4+ T cells from allergic and non-allergic subjects revealed a “pathogenic footprint” that could be analyzed by flow cytometry. These T cell biomarkers were then assessed to determine the extent to which the allergic T cell signature can be used as surrogate biomarker to evaluate the efficacy of ASIT.

RESULTS: We have identified a distinct subset of TH2 cells that is confined to atopic individuals. This allergic T cell signature is characterized by the unique expression of five markers that exhibit functional attributes distinctive of conventional TH2 cells. As such, we have denoted cells with this stable a distinct allergic disease-related phenotype the TH2A cell subset. Transcriptome analysis reaveled distinct pathway in the initiation of pathogenic responses to allergen compared to conventional TH2 cells. Elimination of TH2A cells was indicative of clinical responses induced by immunotherapy.

CONCLUSIONS: These results provide new therapeutic avenues for response-monitoring and treatment of allergic diseases using TH2A cells.
240 Variations in the Heat Shock Protein 90 Gene Are Associated with Asthma in Populations of African Ancestry
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RATIONALE: Autophagy is a core cellular process contributing to cellular homeostasis which may be critical to the pathogenesis of asthma. It has been reported genetic markers in the autophagy-related gene 5 (ATG5) are associated with asthma, and autophagy activity is increased in epithelial cells of asthmatic airways, suggesting modulation of autophagy could have the potential to become a new therapeutic approach for asthma treatment. We identified rare and common variants in autophagy pathway genes associated with asthma in populations of African ancestry.

METHODS: We performed whole-genome sequencing on 168 asthmatic cases and 160 non-atopic controls as part of the ‘Consortium on Asthma among African-ancestry Populations in the Americas’ (CAAPA). We examined 84 genes in the autophagy pathway for association with asthma. Single-variant tests for common variants (minor allele frequency (MAF) ≥5%) were performed using logistic regression adjusting for first two principal components, and low frequency variants (those with MAF<5%) were tested using Fisher’s exact test.

RESULTS: We identified a total of 77,634 single nucleotide variants (SNVs) encompassing 5,214 kb of sequence including 15,573 common and 62,061 (79.9%) rare variants. Five SNVs (rs190565313, rs184564544, rs145402512, rs145846796, rs189323755) in the gene HSP90AA1 encoding heat shock protein 90, a protein involved in chaperone-mediated autophagy activity, were associated with protection for asthma (P<10-4). An additional 5 SNVs (including one novel SNV at Chr.14: 102543837) located downstream of HSP90AA1 also provided evidence for association with P < 10-4.

CONCLUSIONS: Our findings suggest variants in HSP90AA1 may be associated with asthma in populations of African ancestry.

241 Negative Regulation of Eosinophil Production By TLR2
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RATIONALE: Although eosinopenia is a diagnostic and prognostic clinical marker in sepsis, the mechanism of sepsis-associated eosinopenia is unknown. The pattern recognition receptor Toll-like receptor 2 (TLR2) participates in the pathogenesis of sepsis, but its role in eosinophil production is unclear. We hypothesize that TLR2 activation on hematopoietic progenitors inhibits eosinophil production.

METHODS: Progenitor-enriched low-density bone marrow (LDBM) cultures were stimulated with IL-5 for 4 days to generate eosinophil precursors (preEos) and then treated with the TLR2 agonist, Pam2CSK, for 24 or 48 hours. PreEos apoptosis and proliferation were determined by flow cytometry via Annexin V staining and nucleoside analog incorporation, respectively.

RESULTS: Pam2CSK-treated LDBM cultures evidenced a dose-dependent decrease in eosinophil yield with a peak reduction of 57.5 ± 8.6 (mean ± SEM) percent compared to controls (P<0.0001, n = 3 independent experiments). The TLR2 ligand’s inhibitory activity was mediated by a combination of decreased proliferation (P<0.0001, n = 3 independent experiments) and increased apoptosis (P<0.001) of preEos. Notably, TLR2 stimulation of mature eosinophils did not yield increased apoptosis, suggesting that the inhibitory effect is specific to the immature preEos. Mechanistic studies revealed that inhibiting eosinophil production was dependent upon myeloid differentiation primary response 88 (MyD88), but not TLR4 or Toll/IL-1 receptor domain-containing adaptor inducing interferon beta (TRIF), expression by the hematopoietic cells.

CONCLUSIONS: Our data suggests that TLR2 and MyD88 activation in hematopoietic cells contribute to eosinopenia associated with bacterial infections.

242 Peak TMA Specific IgG Responses May Predict the Likelihood of TMA Exposed Workers Developing TMA Specific IgE Responses
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RATIONALE: Workplace exposure to Trimellitic Anhydride (TMA) can elicit specific IgG and IgE antibody responses leading to occupational asthma (OA). An immuno-surveillance program to identify and remove workers with TMA-specific serum IgE, who are at risk for OA, has been previously developed. This purpose of this study was to determine whether the kinetics of specific antibody responses can differentiate workers who only develop TMA specific IgF from those that progress to specific IgE.

METHODS: 140 TMA exposed worker’s sera were previously analyzed for TMA-specific IgG and IgE responses as part of an ongoing immuno-surveillance program. Serum antibody levels were plotted against duration of exposure and statistically compared between active (specific-IgG only) and removed (specific-IgG and IgE) workers.

RESULTS: Of the 132 actively TMA exposed workers, 18 developed specific IgG within 190 days after initial exposure and reached a peak concentration after 363 days. Analysis of 18 workers relocated to a non-TMA exposure area after development of specific IgE revealed that each worker produced specific IgG within 182 days prior to producing TMA-specific IgE which was detectable on average after 334 days. The initial TMA specific IgG response was significantly greater in magnitude in removed workers compared to active workers (p<0.001).

CONCLUSIONS: The magnitude of the TMA-specific IgG response is greater for workers who subsequently develop specific IgE responses. Further work is required to determine if the initial peak specific IgG response in TMA exposed workers predicts the likelihood of later developing TMA-specific IgE over time.
**New Studies on Dust from Middle East Deployment Areas**

**Mark B. Lyles, MA, MS, DMD, PhD**; US Naval War College, Newport, RI.

**RATIONALE:** In the Middle East, dust and sand storms are a persistent problem delivering significant amounts of mineralized particulates via inhalation into the mouth, nasal pharynx, and lungs. Recent reports suggest that this exposure can cause Constrictive Bronchiolitis in veterans.

**METHODS:** Chemical composition, mineral content, or microbial flora of Kuwaiti and Iraqi dust was determined. Multiple site samples were collected and chemical and physical characterization including particle size distribution as well as identification of biologic flora to include bacteria, fungi and viruses was conducted.

**RESULTS:** The mineralized dust is composed of calcium carbonate over a matrix of metallic silicate nanocrystals containing a variety of trace and heavy metals constituting ~3% of the PM10 particles by weight, of which ~1% each of bioaccessible aluminum and reactive iron. Microbial analysis reveals a significant biodiversity of bacteria and known pathogens. Of the microbes identified, several have hemolytic properties and most have significant antibiotic resistance. Viral analysis indicates a tremendous biodiversity of bacteria and known pathogens. Of the microbes identified, several have hemolytic properties and most have significant antibiotic resistance. Viral analysis indicates a tremendous biodiversity of bacteria and known pathogens.

**CONCLUSIONS:** Taken together, these data suggest that at the level of dust exposure commonly found in the Middle East (i.e., Iraq, Kuwait, and Afghanistan), in addition to the microbial and metal content, mineralized dust constitutes a significant health risk, both acute and chronic, to deployed troops and native inhabitants.

**The Cardiac Protein Alpha-T-Catenin Contributes to the Pathogenesis of Occupational Asthma**

**Stephen S. Folmsbee**, Cara J. Gottardi, PhD; Northwestern University, Chicago, IL.

**RATIONALE:** 10-25% of adult asthma is occupational-induced, but its pathogenesis is not well described. Recently, a genome-wide association study identified single nucleotide polymorphisms in the cardiac adhesion protein α-T-catenin (α-T-cat) that correlated with the incidence and severity of toluene diisocyanate (TDI) occupational asthma. However, the mechanism of α-T-cat dysfunction in asthma has not been found.

**METHODS:** α-T-cat knockout (KO) mice, which have an established model, TDI-exposed α-T-cat KO mice show increased airway hyperresponsiveness to methacholine by plethysmography and forced oscillation. Cell culture and animal studies indicate a high level of toxicity. Lung biopsies show alveolar sacs filled with mineralized particles.

**RESULTS:** The mineralized dust is composed of calcium carbonate over a matrix of metallic silicate nanocrystals containing a variety of trace and heavy metals constituting ~3% of the PM10 particles by weight, of which ~1% each of bioaccessible aluminum and reactive iron. Microbial analysis reveals a significant biodiversity of bacteria and known pathogens. Of the microbes identified, several have hemolytic properties and most have significant antibiotic resistance. Viral analysis indicates a tremendous biodiversity of bacteria and known pathogens.

**CONCLUSIONS:** Taken together, these data suggest that at the level of dust exposure commonly found in the Middle East (i.e., Iraq, Kuwait, and Afghanistan), in addition to the microbial and metal content, mineralized dust constitutes a significant health risk, both acute and chronic, to deployed troops and native inhabitants.

**The Potential of a Low-Cost Particle Counter to Quantify Airborne Particulate Matter in a Laboratory Animal Facility**

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**RATIONALE:** To determine the potential of a low-cost particle counter (Dylos DC 1700) to quantify airborne fine particulate matter concentrations in a laboratory animal facility.

**METHODS:** We collected airborne fine particulate concentration data using two photometric devices, (Dylos DC 1700 and Sidepak AM510 Personal Aerosol Monitor), from fourteen locations within a laboratory animal facility covering a range of typical tasks including animal handling, cage cleaning, laundry and office work.

**RESULTS:** We compared over 12,000 1-minute concentration measurements collected from the laboratory animal facility over a total of 28 days. Approximately half of the randomly selected paired-measurements (n=6041) were used to generate a calibration equation converting the Dylos particle number (0.5-2.5 μm per cu ft) to a mass concentration of PM_{2.5} as measured by the Sidepak, producing an R² value of 0.44. This equation was applied to the remaining 6042 paired measurements (validation dataset) with the mean difference (limits of agreement) between the Dylos and Sidepak measurements being -0.55 (-26.30-25.20) mg/m³ with 0.5% of values outside the limits of agreement. The Dylos appears to underestimate PM_{2.5} particularly at the higher end of exposure range.

**CONCLUSIONS:** Pilot data suggest that the low-cost Dylos DC 1700 may be a useful machine to provide feedback on airborne fine particle concentrations in laboratory animal facilities. The real-time data from the Dylos may be particularly helpful in training laboratory personnel to adhere to safe working practice, with a view to ensuring low aerolallergen exposure when working in laboratory animal facilities.
247 **Functional Analysis of Calpain-14 in Eosinophilic Esophagitis**

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**RATIONALE:** We recently identified a genome-wide association susceptibility locus for eosinophilic esophagitis (EoE) at 2q23 wherein CAPN14 (p = 2.5 X 10−10) is located. CAPN14 encodes for a member of the calpain protease family. We have shown that CAPN14 was specifically expressed in the esophagus, dynamically regulated as a function of disease activity and genetic haplotype and after exposure of epithelial cells to IL-13. Herein, we aimed to determine the function of calpain-14.

**METHODS:** We overexpressed calpain-14 in an esophageal progenitor cell line (EPC2) grown in an air-liquid interface (ALI) culture and analyzed barrier effects by transepithelial electrical resistance (TEER), fluorescein isothiocyanate (FITC)-dextran flux, and hematoxylin and eosin stain. We also identified potential calpain-14 targets via western blot and immunofluorescence (IF) of the ALI culture.

**RESULTS:** Calpain-14 overexpression induced a 2-3 fold (p = 0.0034) decrease in TEER and a 2-3 fold (P < 0.0001) increase in FITC-dextran flux, consistent with calpain-14 induced impaired barrier function (IBF). Histological analysis revealed that calpain-14 overexpression induced acantholysis, intraepithelial clefting, and epidermolysis. Western blot following calpain-14 overexpression revealed degradation of the desmosome protein, desmoglein-1 (DSG-1), as a low molecular weight band was increased 30-fold (p = 0.0009) compared to control. IF for DSG-1 demonstrated reduced expression and less defined desmosome-like structures following calpain-14 overexpression versus control.

**CONCLUSIONS:** The genetic pathoetiology of EoE is mediated by genetic variation of CAPN14 and associated with an IL-13–inducible esophageal response involving calpain-14 induced IBF mediated by DSG-1 cleavage. The study was supported by the Consortium of Food Allergy Researchers (CoFAR) by US NIH grant U19 AI066738 from NIAID and NIDDK.

248 **TRAIL Signalling Is Pro-Inflammatory in Eosinophilic Esophagitis**

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**RATIONALE:** Eosinophilic esophagitis (EoE) is an inflammatory disorder of the esophagus defined by eosinophil infiltration and tissue remodelling which presents with symptoms of esophageal dysfunction. We have shown previously that tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) promotes allergy and virus-induced exacerbation of allergic airways disease by upregulation of the E3 ubiquitin-ligase midline-1 (MID-1), which binds to and deactivates the catalytic subunit of protein phosphatase 2 A (PP2Ac) resulting in increased NF-κB activation. Here we examine the significance of this proinflammatory pathway in EoE.

**METHODS:** We identified the expression of TRAIL and MID1 in human esophageal biopsy samples through post hoc analysis of microarray data. We then employed an Aspergillus fumigatus (Asp F) induced EoE mouse model to investigate the role of TRAIL in experimental EoE.

**RESULTS:** Analysis of gene array data demonstrated upregulation of TRAIL and MID-1 mRNA in a cohort of children with EoE as compared to controls. TRAIL-deficient (-/-) mice had markedly reduced eosinophils and mast cells in the esophagus and were protected from features of remodelling including thickening of the muscularis externa, enlarged esophageal circumference and increased collagen deposition. Esophageal MID-1 expression and NF-κB activation were reduced in the TRAIL-/- Asp F treated mice, while PP2Ac levels were increased compared to wildtype controls. This was associated with reduced expression of eosinophil CCL11, CCL24, IL-5, IL-13, TSLP and TGF-β mRNA in TRAIL-/- mice.

**CONCLUSIONS:** TRAIL is upregulated in EoE and regulates hallmark features of experimental EoE including elevated eosinophilic expression of CCL11 and TH2 cytokines, cellular inflammation, fibrosis and smooth muscle hypertrophy.

249 **Active Eosinophilic Esophagitis Is Characterized By Epithelial Barrier Defects and Eosinophil Extracellular Trap Formation**

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**RATIONALE:** Eosinophilic esophagitis (EoE) typically exhibits esophageal dysfunction owing to an eosinophil-predominant inflammation. Activated eosinophils generate eosinophil extracellular traps (EETs) able to kill bacteria. There is evidence of an impaired barrier function in EoE that might allow pathogens, including microbes and their products, to invade the esophagus. We aimed to investigate the presence and distribution of EETs in esophageal tissues from EoE patients and their association with possible epithelial barrier defects.

**METHODS:** Anonymized tissue samples from 18 patients with active EoE were analyzed. The presence of DNA nets associated with eosinophil granule proteins forming EETs and the expression of filaggrin, the protease inhibitor lympho-epithelial Kazal-type-related inhibitor (LEKTI), antimicrobial peptides, and cytokines were evaluated by confocal microscopy following immune fluorescence staining techniques.

**RESULTS:** EET formation occurred frequently and was detected in all EoE samples correlating with the numbers of infiltrating eosinophils. While the expression of both filaggrin and LEKTI was reduced, epithelial antimicrobial peptides (human beta-defensins-2, -3, cathelicidin LL-37, psoriasin) and cytokines (TSLP, IL-25, IL-32, IL-33) were elevated in EoE as compared to normal esophageal tissues. There was a significant correlation between EET formation and TSLP expression (p = 0.002) as well as psoriasin expression (p = 0.016). On the other hand, a significant negative correlation was found between EET formation and LEKTI expression (p = 0.016).

**CONCLUSIONS:** Active EoE exhibits the presence of EETs. Epithelial cytokines and antimicrobial peptides are present together with indications of epithelial barrier defects which may have contributed to the activation of eosinophils. The formation of EETs could serve as a firewall against the invasion of pathogens.
Salivary Microrna As a Biomarker for Monitoring Response to Treatment in Eosinophilic Esophagitis

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RATIONALE: Eosinophilic esophagitis (EoE) is a clinicopathological condition where endoscopic biopsies of the esophagus are required for diagnosis and monitoring the response to treatment. No non-invasive method of monitoring currently exists. MicroRNA (miRNA), regulates expression of many cytokines important in allergic disease and have been found in saliva. We hypothesize that salivary miRNAs are differentially expressed in EoE and can be used as biomarkers to monitor the response to treatment.

METHODS: After IRB approval, fifteen adult patients with EoE (symptoms consistent with EoE, endoscopic biopsy with ≥15 eosinophils per high powered field, and previous proton pump inhibitor therapy) were randomly enrolled to provide a saliva sample before and after 2 months of swallowed fluticasone therapy. Differences of miRNA expression were compared to healthy controls (n=17) using Wilcoxin rank sum testing. Expression changes before and after treatment were analyzed by paired T-test. A significance cutoff of <0.05 was used for all analyses.

RESULTS: All but two patients had complete resolution of their symptoms on swallowed fluticasone. MiRNA expression profiles comparing EoE patients and healthy controls demonstrated > 2-fold up-regulation of miR-570-3p, -3613-5p, -4668-5p, and -30a-5p in EoE (p<0.01). After treatment, expression of miR-658 increased while expression of miR-3613-5p and -4668-5p decreased (p= 0.0027, 0.0314, and 0.0488 respectively). These results suggest miRNA expression differences can be seen at time of diagnosis and can change with treatment of EoE.

CONCLUSIONS: Salivary miRNA represents a promising new tool for non-invasive, biomarker monitoring of EoE. These differentially expressed miRNAs may have pathogenic roles in EoE.

Transcriptome Analysis of PPI-Responsive Esophageal Eosinophilis Reveals the Presence of an Eosinophilic Esophagitis Transcriptome Reversible By PPI Mono-Therapy and the Identification of PPI-Response Predictor Genes

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RATIONALE: Proton pump inhibitor (PPI) -responsive esophageal eosinophilia (PPI-REE) is a newly recognized enigmatic condition that comprises 50-50% of patients with PPI-treated esophageal eosinophilia. PPI-REE and Eosinophilic Esophagitis (EoE) present with similar clinical, histological and endoscopic features, posing a diagnostic and treatment dilemma according to consensus guideline. While EoE is accepted to be an immune-mediated allergic disorder, the molecular etiology driving PPI-REE remains unclear.

METHODS: A recently published EoE diagnostic panel (EDP) composed of 94 genes characteristic of EoE was utilized to acquire the EoE signature in achieved esophageal biopsy samples obtained from 4 institutions. The molecular signature of PPI-REE, before and after PPI mono therapy, was compared to NL and EoE references by bioinformatics analyses. RESULTS: The EDP achieved 100% accuracy in distinguish NL and EoE controls collected from 4 institutions. PPI-REE shared a similar allergic inflammatory molecular signature of EoE, including the genes for eosinophil cytokines (CCL26), barrier molecules (DSG1), tissue remodeling (POSTN), and mast cells (CPA1and TPSB2). After mono-PPI therapy, the PPI-REE transcriptome and mastocytosis almost completely normalized. We also identified a set of candidate genes to differentiate EoE from PPI-REE before treatment, in which KNCJ2 (Kir2.1) represents the most prominent lead with a 3-fold difference between EoE and PPI-REE. Immunohistochemistry staining validated this finding at the protein level and semi-quantification revealed a similar trend of dysregulation in the esophageal epithelium.

CONCLUSIONS: We have determined that PPI-REE is molecularly homologous to EoE and that PPI therapy has the capacity to remarkably reverse the cardinal pathways associated with allergic inflammation.
253 Asthma Needs Assessment on the Navajo Indian Reservation
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RATIONALE: Native American children carry high prevalence of asthma. Care of asthmatics is known to pose different challenges among various cultures, races and ethnicities. We sought to identify gaps in order to improve asthma care for Navajo children on our nation’s largest reservation.

METHODS: One hundred randomly selected charts of 6–17 year-old children with asthma were reviewed for: frequency of exacerbation, severity assessment, spirometry, controller medication, hospitalization and emergency department presentation. Asthma service needs were assessed through surveys with providers, pharmacists, and respiratory therapists; and “town-hall” meetings with providers.

RESULTS: The 12-month review of 100 charts identified 143 clinic visits for asthma/wheeze, 19 urgent care visits, 15 emergency department visits, 5 hospitalizations and 70 prescriptions for systemic steroids. Spirometry was included in 8%, severity assessment in 63%, asthma control test in 28%, asthma action plan in 27%, and controller medication was prescribed where appropriate in 89% of visits. Among 26 providers surveyed, over 90% identified patient education and coordination of care as targets for improvement; 60% identified office tools/spirometry as a means to improve care. Sixty percent of providers indicated interest in obtaining asthma education.

CONCLUSIONS: Primary needs of Navajo asthmatic children include access to care coordination, asthma education, and spirometry. Providers were highly likely to prescribe a controller medication where appropriate, but spirometry, asthma control tests, and asthma action plans were under utilized. These results will inform phase two of our project to improve care of asthmatic Navajo children.

254 Electronic Asthma Self-Management Program Can Improve Asthma Control and Quality of Life in Young, African Americans
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RATIONALE: Strategies to overcome barriers to optimal asthma care and improve compliance in young, African American adults are needed. The feasibility and effectiveness of an electronic asthma self-regulation program in improving asthma control and quality of life among young, African Americans are unknown.

METHODS: Participants between 18 and 30 years of age with uncontrolled persistent asthma, and self-identified as African American were included in the study. The 6-week intervention utilized an online digital platform, Breathe Michigan program, configured based on social cognitive theory using principles of self-regulation, specifically designed within the cultural context of this population. Educational videos, surveys, peak-flow logs, symptom reporting, barrier identification, and support messages were utilized. Outcomes were assessed using participant feedback, and asthma control and quality of life were measured using the Asthma Control Test (ACT) and the Asthma Quality of Life Questionnaire (AQLQ).

RESULTS: 44 subjects were enrolled, and 36 subjects completed the program (81.8%). The average age of participants was 24.7 years. Seasonal and weather changes and multiple life responsibilities were listed as the most common barriers to optimal asthma care (68.2% and 40.9% respectively). All participants found the program to be helpful during the 1-month post-intervention telephone follow-up. Mean ACT score at enrollment was 16.1, and at 1-month post-completion was 19.3 (p <0.0001). Mean AQLQ score at enrollment was 4.0, and at 1-month post-completion was 5.1 (p <0.0001).

CONCLUSIONS: The electronic asthma self-management program, Breathe Michigan, is a feasible intervention to help improve asthma control and quality of life in young, African Americans.

255 Characteristics of Symptomatic Children Undiagnosed with Asthma and Known Asthmatics in Inner-City Schools
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RATIONALE: Inner-city studies have identified that many children with asthma symptoms are undiagnosed asthmatics through confirmatory evaluations. We evaluated differences in health symptoms and reported exposures in inner-city school-age children diagnosed with asthma and those with highly predictive symptoms of asthma but were undiagnosed.

METHODS: Validated school-based screening survey data was analyzed. Subjects with asthma symptoms without a provider-diagnosis of asthma were defined by affirmative responses that have been suggestive of asthma in previous studies of inner-city asthmatic children.

RESULTS: 7032 children were screened. 269 (3.8%) reported asthma symptoms without a provider-diagnosis of asthma. 1442 (21.7%) reported a doctor’s diagnosis of asthma. Asthmatics had significantly higher rates of exertional symptoms (76% vs. 66%, p <0.001), missed school (50% vs. 25%, p<0.001), hospital care for asthma (52% vs. 17%, p<0.001) compared to undiagnosed asthmatics. These associations did not significantly vary by race (all interaction effects p>0.60).

Compared to undiagnosed asthmatics, asthmatics had a higher proportion of: asthma family history, 56% vs 36% (p<0.001); food allergies, 20% vs 13% (p=0.01); having a pet at home, 30% vs 23% (p=0.02). Undiagnosed asthmatics had a higher proportion of possible eczema, 56% vs 48%, (p=0.02); sore throat 79% vs 65%, (p=0.0); headache 77% vs 66%, (p=0.002). There was no difference between groups in allergic rhinitis or sieving pests in the home.

CONCLUSIONS: School-age children with asthma symptoms without a provider-diagnosis differ from children with asthma in regards to health history and symptoms (allergic/non-specific) and reported exposures. These differences may highlight a different asthma phenotype suggesting further evaluations may be important.
256 Characteristics of Inner City Children with Life-Threatening Asthma

Mary E. Bollinger, DO1, Arlene Butz, ScD, CRNP2, Cassie Lewis-Land, MS3, Francesca DiPaula, BS2, Shawna Mudd, DNP, CRNP3; 1Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD; 2Johns Hopkins University School of Medicine, Baltimore, MD; 3Johns Hopkins University School of Nursing, Baltimore, MD. RATIONALE: Prior intensive care unit (ICU) admission is a risk factor for fatal asthma. The purpose of this study was to examine characteristics of underserved children with a history of ICU admissions for asthma.

METHODS: Baseline survey data, salivary cotinine and allergen specific IgE serology were obtained from underserved children with uncontrolled asthma enrolled in a randomized clinical trial of a multifaceted Emergency Department/ home-based asthma intervention. Baseline characteristics of children with and without a prior history of ICU admission were compared using chi-square and ANOVA.

RESULTS: Subjects included 100 primarily African American (95%), Medicaid insured (97%) children (Mean age=6.25 yrs; 56% male) with uncontrolled asthma. Atoxy was high with 80.2% sensitized to >1 environmental allergen. Most (54.1%) had detectable cotinine levels. Almost one-third (31%) had prior ICU admissions. ICU subjects were more likely to be sensitized to dust mite (p=0.009) and ragweed (p=0.002) and trended toward higher cockroach sensitization (p=0.09) than non-ICU subjects. Allergen sensitization between the two groups did not differ for mouse, cat, dog, alternaria, aspergillus, grass or tree. Cotinine levels were similar. Albuterol overuse was prevalent (>70%); appropriate controller medication use was poor (32%). Although ICU subjects were more likely to have seen their PCP for asthma in prior 3 months, only 31% had seen an asthma specialist in the previous 2 years.

CONCLUSIONS: Children at risk for fatal asthma should undergo asthma specialty evaluation to optimize controller medication use, assess environmental sensitivities/exposures and implement individualized trigger avoidance procedures to improve morbidity and mortality in this high risk population.

257 Regulation of Tissue Plasminogen Activator Expression in Human Epithelial Cells

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RATIONALE: Recent data from our laboratory demonstrated fibrin deposition in nasal polyp tissue in chronic rhinosinusitis (CRS) associated with a significant decrease of tissue plasminogen activator (t-PA) expressed on eosinophilic epithelial cell (EoE) patients. Bronchoalveolar lavage (BAL) was obtained from allergic asthma patients following segmental allergen challenge (SAC).

METHODS: NEC cultures from control and CRS showed no differences in expression of various stimulants especially Th2 cytokines associated with CRS.

RESULTS: NEC cultures from control and CRS showed no differences in expression of various stimulants especially Th2 cytokines associated with CRS.

CONCLUSIONS: In vivo, suggesting that in vivo barrier dysfunction in CRS is epithelial cell–extrinsic. The correlation of OSM with markers of epithelial leak in both CRS and allergic asthma suggests that OSM may mediate epithelial dysfunction in mucosal disease.
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**Staphylococcus Aureus Induces a Th2 Response Via TSLP and IL-33 Release in Human Airway Mucosa**

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**RATIONALE:** Chronic rhinosinusitis with nasal polyps (CRSwNP) is mainly characterized by a Th2-skewed inflammation. We hypothesized that *S. aureus* induces Th2 response in CRSwNP via epithelial derived cytokine production.

**METHODS:** The levels of epithelial derived cytokines TSLP and IL-33 and consecutively Th2 cytokines were assessed in human mucosal *S. aureus* infection model. The localization of IL-33 and cell death in human CRSwNP tissue after *S. aureus* infection was evaluated by immunofluorescence staining. Human bronchial epithelial cell line BEAS-2B with *S. aureus* infection was examined for the epithelial cell-derived cytokine production pathway.

**RESULTS:** The levels of TSLP and IL-5 significantly increased in supernatants of CRSwNP tissue after *S. aureus* infection. Even though an increased protein level of TSLP was found in control tissue after *S. aureus* infection, no change of IL-5 cytokine was observed. IL-33 was released into the extracellular space between epithelial cells, where TUNEL-positive cells were localized after *S. aureus* stimulation in CRSwNP tissue. At the same time, IL-33 cytokine was over-expressed in CRSwNP tissue homogenates after *S. aureus* infection. Increased levels of IL-33 and TSLP were induced by *S. aureus* in a dose-dependent manner in BEAS-2B cells with an increase phosphorylation process of p50, p65 and p38.

**CONCLUSIONS:** We here demonstrate for the first time that *S. aureus* can directly induce epithelial cell-derived cytokine in human nasal tissue, and propagates Th2 response only in CRSwNP tissue, not in controls. The NF-κB and MAPK pathway might be involved in the production of TSLP and IL-33 after *S. aureus* infection.

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**Omeprazole Has Anti-Inflammatory Effects on Type 2 Cytokine-Stimulated Human Airway Epithelial Cells**

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**RATIONALE:** Chronic rhinosinusitis with nasal polyps (CRSwNP) is a disease frequently characterized by mucosal eosinophilia likely influenced by elevated levels of type 2 cytokines, notably IL-13, and CCR3 ligands of the eotaxin family. Recent reports demonstrate proton pump inhibitor omeprazole has suppressive effects on eotaxin-3 responses to type 2 cytokines in esophageal squamous cells. We aimed to evaluate IL-13-mediated expression of eotaxin-3 by human bronchial epithelial cell line (BEAS-2B) and primary human sinonasal epithelial cells (SNEC) and the inhibitory effects of omeprazole on IL-13-induced eotaxin-3 responses in airway epithelial cells.

**METHODS:** BEAS-2B and SNEC from inferior turbinate (IT) scrapings from control and CRSwNP patients and polyp scrapings were cultured with IL-13 (1-100ng/ml) with or without omeprazole (0.1-50mM) for 48 hours. Messenger RNA expression was measured by real-time PCR and protein secretion by ELISA at baseline and at 48hr.

**RESULTS:** Eotaxin-3 expression was dose-dependent and most significantly induced following IL-13 (25ng/ml) stimulation compared to media-only controls in both BEAS-2B (196.1±22.8 vs 16.8±19.5 pg/ml respectively at 5ng/ml IL-13; p<0.0001, n=3) and SNEC (3.2±1.9 vs 0.1±0.1 ng/ml respectively; p<0.0001, n=15). However, SNEC derived from CRSwNP and control patients showed similar IL-13 responses. Omeprazole, especially in the acid-activated form significantly (≥5μM), dose-dependently suppressed IL-13-mediated eotaxin-3 mRNA expression and protein secretion from BEAS-2B cells and SNEC (p<0.05).

**CONCLUSIONS:** IL-13 potently stimulates eotaxin-3 production in airway epithelial cells and is strongly inhibited by omeprazole at concentrations achievable in vivo using conventional oral dosing. The mechanism by which omeprazole modulates epithelial-derived chemokine production is under active investigation.
Ozone induced mRNA for IL-33 and TSLP (activators of ILC2) in both mice and human airway epithelial cells. Pulmonary ILC2 addback by intratracheal transfer restored ozone-induced airway inflammation and AHR. Our data suggested epithelial activation of ILC2 may play a critical role in mediating air-pollution-induced exacerbation of respiratory diseases.
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**SATURDAY**

**265** A Bell-Shaped Dose-Dependent Induction of Allergen-Specific Tetramer+ CD4 T Cells and Activated Lung ILC2s Following Epicutaneous Allergen Sensitization in HLA-DR4 Transgenic Mice

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**RATIONALE:** Disrupted epithelial barriers are a potent risk factor for allergic sensitization in mice and humans. Allergy to cat dander is one of the most common forms of allergy and is strongly associated with the development of asthma; therefore we determined whether it was possible to sensitize humanized mice to cat dander via disrupted skin.

**METHODS:** 1.5-150 mg cat dander extract (CDE) was placed on the tape-disrupted skin of adult female HLA-DR4 transgenic mice (Taconic). Mice were given identical doses of CDE intranasally, and airway inflammation, physiology, and leukocyte phenotypes were assessed. Antigen-specific CD4 T cells were identified using Fel d 1 tetramers.

**RESULTS:** Despite identical levels of induced damage to the epithelium, mice receiving low (1.5 mg), or high (150 mg) dose CDE did not display significant AHR or airway eosinophilia, and had a paucity of tetramer+ CD4 T cells. In contrast, mice receiving an intermediate dose (15 mg) CDE displayed significant AHR/erosinophilia in the BAL and lung, and had markedly higher numbers of tetramer+ CD4 T cells. While all the tetramer+ T cells displayed a Th2 phenotype, the high dose group contained more IL-10 producing tetramer+ T cells. The 15 mg group also contained higher numbers of activated lung ILC2s in the lung.

**CONCLUSIONS:** Induction of AHR, eosinophilia, allergen-specific Th2 cells and activated ILC2s is a dose-dependent phenomenon following epicutaneous allergen sensitization, with high dose allergen leading to inhibition of Th2 outcomes. These findings may be relevant to the observation that greater exposure of children to cats is protective against the development of cat allergy.

**266** Rapamycin Preferentially Inhibits IL5+ Th2 Cell Proliferation through the mTORC1/S6 Kinase Pathway

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**RATIONALE:** Rapamycin inhibits T cell proliferation by blocking the mechanistic target of rapamycin (mTOR) kinase. We examined rapamycin inhibition of Th2 cell proliferation for mechanistic and therapeutic insights.

**METHODS:** Proliferation and mTOR pathway phosphorylation were examined by flow cytometry, using dye dilution and phospho-specific staining, respectively.

**RESULTS:** Rapamycin inhibited antigen specific proliferation of Th2 cells more than Th1 cells (p < 0.001). Moreover, IL-5+ Th2 cell responses demonstrated the greatest inhibition. At a low rapamycin concentration (1 nM), IL-5+ Th2, IL-5- Th2, and Th1 proliferation were inhibited 97%, 90%, and 51%, respectively (p = 0.009). Rapamycin did not induce Th2 cell apoptosis or affect Th1/Th2 differentiation. The mTOR complex 1 (mTORC1) downstream effector S6 ribosomal protein (S6RP) was more highly phosphorylated in IL-5+ Th2 cells than in any other CD4+ T cell population. Conversely, rapamycin inhibition of S6RP phosphorylation was greatest in Th2 cells. In contrast, the phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and mTOR was unaffected by rapamycin. siRNA knockdown of the mTORC1 components S6 kinase 1 (S6K1) and S6RP reiterated the preferential inhibition of IL-5+ Th2 proliferation (P = 0.008).

**CONCLUSIONS:** Rapamycin preferentially inhibits IL5+ Th2 cell proliferation through the mTORC1/S6K1 pathway. The mTORC1 pathway is highly activated in IL-5+ Th2 cells, making this Th2 subpopulation particularly sensitive to mTORC1 inhibition. The exquisite sensitivity of IL-5+ Th2 cells to rapamycin inhibition suggests that rapamycin and the mTORC1/S6K1 pathway should be considered as a potential therapeutic target for Th2 inflammatory diseases, such as asthma and eosinophilic gastrointestinal diseases.