**AB174 Abstracts**

**562 Relationships Between Impaired FEF25-75 and Feno in Children with Asthma**

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**RATIONALE:** Airflow obstruction and airway inflammation are main characteristics of asthma. Forced expiratory flow between 25% and 75% of vital capacity (FEF25-75) has been used as an indicator of small airway disease and proposed as more sensitive indicator of persistent airflow obstruction than forced expiratory volume in 1 second (FEV1). Impaired FEF25-75 (<65% of predicted value) should be considered abnormal bronchodilator response. The measure of the fractional concentration of exhaled nitric oxide (FeNO) may be considered a surrogate marker for airflow inflammation. The aim of this study was to examine relationships between FEF25-75 and FeNO levels in children with asthma.

**METHODS:** A total of 118 children with asthma were recruited by Allergy Clinic of Korea University Anam Hospital. They underwent spirometry and measurement of blood eosinophils, serum IgE and eosinophil cationic protein (ECP) concentrations. Exhaled NO was measured using a chemiluminescence analyzer (NIOX MINO analyzer, Aerocrine, Sweden) during single breath exhalation. We also examined sensitization to 13 common allergens using skin prick testing in each subject.

**RESULTS:** The mean (±SD) FEV1, forced vital capacity (FVC) and FEF25-75 were 88.3±15.3%pred, 95.5±14.5%pred and 85.2±27.0%pred, respectively. The mean (±SD) FeNO concentrations in impaired FEF25-75 children (FEF25-75<65%pred, (ln)3.62±0.42 ppb, n=28) was significantly higher than that of normal FEF25-75 children (FEF25-75>65%pred, (ln)3.26±0.59 ppb, n=90) (p=0.008). An inverse correlation was observed between FEF25-75 and FeNO in children with impaired FEF25-75. (r= -0.405, p=0.032).

**CONCLUSIONS:** Impaired FEF25-75 values were associated with high FeNO levels in children with asthma. FEF25-75 might be considered an indirect marker of airflow inflammation.

**563 Comparison of Two Handheld Fractional Exhaled Nitric Oxide Measurements in the Assessment of Asthma Patients**

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**RATIONALE:** There has been increasing interest on the use of fractional exhaled nitric oxide (FENO) in detecting airflow inflammation and guide for anti-inflammatory treatment. The aim of this study is to compare two different FENO devices in the assessment of suspected asthma patients.

**METHODS:** A total of 67 patients who had been prescribed methacholine bronchoprovocation test (MBPT) in a tertiary hospital from November 2013 to February 2014. FENO measurements performed using by both handheld devices (NIOX-MINO® and NObreath®) before MBPT in a same day. We compared paired value of FENO between two devices and evaluate the association between FENO value and clinical characteristics.

**RESULTS:** The value of NIOX-MINO® mostly showed higher results than the NObreath® (mean difference 9.0 ± 19.6, p=0.001). We observed good correlation between the results from two devices (p<0.0001). FENO value from both devices revealed significant positive correlation with sputum eosinophil (%) and serum total IgE, whereas negative correlation with PC20. In the prediction of positive MBPT, the NIOX-MINO® showed higher specificity but, lower sensitivity than the NObreath® (NIOX-MINO®: sensitivity 50%, specificity 96.9% in criterion > 41 ppb; NObreath®: sensitivity 78.9%, specificity 56.2% in criterion > 9 ppb).

**CONCLUSIONS:** FENO values obtained by NIOX-MINO® were higher compared to those of NObreath® with a good correlation. In contrast to the NIOX-MINO®, the value from NObreath® showed higher sensitivity but, lower specificity for prediction of positive MBPT.

**564 Exhaled Nitric Oxide, Lung Function, and Asthma Control in Children and Adolescents**

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**RATIONALE:** Exhaled nitric oxide (FeNO) and scores of asthma control are tools recently introduced in the clinical evaluation of asthma patients, but their relationships with other traditional markers are still not completely understood in children. Our objective was to evaluate relationships among FeNO, lung function and the level of asthma control in children and adolescents.

**METHODS:** FeNO, spirometry, bronchodilator response (BDR) and asthma control (ACT/C-ACT) were measured in 147 children and adolescents with asthma.

**RESULTS:** Median of age was 12 years (6 to 18 years) and 92 were males. Uncontrolled asthma (ACT/C-ACT ≤ 19) was observed in 45 (31%) patients. FeNO values ranged from 1ppb to 196ppb (median of 30ppb). Significant correlation was found between FeNO and FEV1 (r = -0.18), FeNO and BDR (r = 0.15), ACT and FEV1 (r = 0.16), ACT and FEF25-75 (r = 0.25). BDR was significantly correlated with FEV1 (r = -0.46), FEV1/FVC (r = -0.44) and FEF25-75 (r = -0.33). Patients with uncontrolled asthma had higher BDR than controlled ones (median: 8% vs 5%; p=0.01). Those with a positive BDR (FEV1 ≥12%) had higher FeNO than those without positive BDR (45.3ppb vs 24.4ppb; p=0.01). Among patients without regular use of controlled medication, FeNO correlated significantly with FEVI/FVC (r = -0.34), FEF25-75 (r = -0.34) and ACT (r = -0.25).

**CONCLUSIONS:** Correlations among FeNO, lung function and asthma control scores are weak in asthmatics, probably because they represent different domains of the disease. Among patients without regular use of control medication, FeNO have a stronger correlation with lung function parameters.
The Predictors for Asthma Control By Stepwise Treatment in Elderly Asthma Patients

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RATIONALE: The geriatric population is increasing and asthma severity increases with age.

METHODS: We determined the predictors for asthma control and exacerbation in elderly Korean patients with asthma. In addition, we investigated the factors that affect asthma-specific quality of life (A-QOL). This is a prospective, multi-center real-life study for 6 months with stepwise pharmacologic treatment. A total of 296 asthmatic patients aged ≥ 60 were recruited from five University Centers in Korea. Improved-asthma-control group was defined if patients maintained well controlled status or improved control status for 6 months; the remaining patients were defined as not-improved-asthma-control group.

RESULTS: Smaller number of medications for co-morbidities and higher physical functioning (PF) scale were significant predictors for the improved asthma control group (OR = 0.863, P = 0.004; OR = 1.028, P = 0.018, respectively). Asthma control test (ACT) score ≤19 at baseline was a significant predictor of asthma exacerbation at 6 months (OR = 3.938, P = 0.048). Asthma duration (F = 5.656, P = 0.018), ACT (F = 12.237, P = 0.001) at baseline and the presence of asthma exacerbation (F = 5.565, P = 0.019) during the 6 months of treatment were significant determinants for the changes of A-QOL.

CONCLUSIONS: The number of medications for co-morbidities and performance status determined by PF scale as well as ACT are important parameters for assessing asthma control in elderly asthma patients.

Factors Associated with Asthma Control in Children: Findings from a National Web-Based Survey

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RATIONALE: Although achieving and maintaining control of asthma is considered to be the goal of asthma treatment, determinants of asthma control are not fully understood. Our aim was to assess factors associated with asthma control among pediatric patients in the general population.

METHODS: In June 2012, a web-based survey was conducted to identify Japanese children aged 6 to 11 years who currently have asthma and evaluate control of their disease using the Childhood Asthma Control Test (C-ACT). Associations were evaluated among uncontrolled asthma (C-ACT score <20) between environmental factors, demographics and comorbid allergic diseases.

RESULTS: Among the 3,066 children with current asthma, 447 (14.6%) had uncontrolled asthma. Multivariable analysis identified factors such as low birth weight, obesity, and pet ownership before birth to be associated with uncontrolled disease [adjusted OR [95%CI]: 1.65[1.25-2.18], 1.44 [1.05-1.99], and 1.68[1.24-2.29], respectively]. Comorbid allergic diseases, especially rhinitis were a significant risk of uncontrolled asthma [adjusted OR = 3.88[95%CI= 2.50-6.00] for severe rhinitis]. The severity
568 Poor Sleep Quality As a Risk Factor for Poorly-Controlled Asthma in Children
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569 Development of a Clinical Lab Assay for Assessment of Eosinophil Peroxidase in Sputum
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571 Role of FEF25-75 and Bronchodilator Response in Childhood Asthma Control and Morbidity Among Inner-City Children with Asthma

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RATIONALE: FEV1 or FEV1/FVC are used to assess asthma control and recent exacerbation requiring oral corticosteroids also predicts future exacerbations. We sought to determine if FEF25-75 or bronchodilator reversibility (BDR) are more sensitive indicators of uncontrolled asthma.

METHODS: 605 urban children age 6-17 yrs (n=605) with persistent asthma underwent spirometry. Associations between FEV1% predicted, FEV1/FVC, FEF25-75, and BDR (a post-bronchodilator increase in FEV1 >12%) and asthma control were analyzed.

RESULTS: Subjects were predominantly black (77.9%) and low income (60.2%). Half of the children had FEF25-75 <80% predicted. 78.1% had FEV1 >80% predicted, 58.7% had FEV1/FVC ≥ 80. Adjusting for sex, age, race, income, education level, insurance, and BMI, subjects with a low FEF25-75 had higher odds of uncontrolled asthma (ORadj 2.1 95%CI 1.2-3.1, P<0.001) and recent oral corticosteroid use past 3 months (ORadj 2.2, 95%CI 1.2-4.0, P=0.010), compared with those with normal FEF25-75. Additionally, positive BDR was associated with uncontrolled asthma (ORadj=1.8, 95%CI 1.1-2.8, P=0.014) and recent oral corticosteroid use (ORadj =1.6, 95%CI 1.0-2.5, P=0.047). Adjusting for the factors above, FEV1 and FEV1/FVC, subjects with low FEF25-75 had higher odds of oral corticosteroid use in past 3 months (ORadj FEV1% predicted =1.9 95%CI 1.2-3.1, P=0.011) ORadj FEV1/FVC= 1.8(95%CI=1.1-3.0, P=0.030)) and oral corticosteroid use in the past 2 weeks (ORadj FEV1% predicted =3.0(95% CI =1.2-7.3) P=0.014 ORadj FEV1/FVC= 2.6(95% CI = 1.2-5.9)(P=0.019). Positive BDR did not remain significant.

CONCLUSIONS: In children with persistent asthma, a low FEF25-75 may add additional information for asthma control, independent of FEV1 % predicted and FEV1/FVC.

572 Lung Function in an Asthmatic Cohort in Puerto Rico

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RATIONALE: There is a high prevalence of morbidity from asthma and lower lung function among Puerto Ricans compared to other ethnic groups in the United States. Obesity has been identified as a risk factor for asthma among Puerto Ricans. We hypothesized that female gender, older age and obese asthmatics will have a greater decreased in lung function compared to male, younger and non-obese asthmatics.

METHODS: We compared demographic variables with lung function parameters obtained by spirometry among 194 asthmatics evaluated at a tertiary care academic center in the Medical Sciences Campus at the University of Puerto Rico.

RESULTS: Subjects had a mean age of 49.3 years. There were 154 females and 40 males. The average BMI was 31. The average FEV1 was 74% predicted and airway reversibility was 13.7%. No difference in airway reversibility was identified by age or gender (p=0.116 and p=0.53). However, females had significantly higher BMI compared to males (32.7±8.7, compared to 27.7±5.7; p=0.013).

CONCLUSIONS: The relationship between gender, obesity and airway reversibility deserve further evaluation. Emphasis on weight control in asthma management, particularly among females may be useful.

573 A Single Breath Method to Assess the Relative Contribution of Central and Peripheral Airways to Overall Exhaled Breath Temperature

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RATIONALE: Multiple exhalations can be used to discriminate between central (Caw) and peripheral airways (Paw) and assess the contribution of each to disease states. As multiple breathing maneuvers are time consuming and induced subjective discomfort, this study assesses a method of using a single deep breath maneuver.

METHODS: The equipment employed included a fast reacting inflatable balloon valve system operated by computer which steers the expired airflow through channels with sensitive temperature sensors. During initial deep inhalation, the inspired volume is measured and the sequence of valve openings is adjusted to yield volumes of air characteristic of Caw or Paw during expiration. Absolute differences of and ratios between EBT of Caw and Paw were calculated and compared in 6 partly controlled asthmatics and 6 healthy controls.

RESULTS: The absolute differences between EBT of Paw and Caw ranged from 1.82°C and 3.45°C, with a median of 2.19°C in the healthy controls and from 3.22°C and 4.56°C, with a median of 4.03°C in the asthmatics (P=0.019). The Paw/Caw ratios showed a trend of higher EBT in Paw of asthmatics, but the differences did not reach statistical significance due to the small number of subjects. The reproducibility of the measurements was high and the subjects did not experience any subjective discomfort.

CONCLUSIONS: Measuring differences between central and peripheral EBT by an upgraded method of short duration may allow more insight into the nature of the inflammatory processes in obstructive respiratory diseases.
574 Age-Dependent Cut Points for Airway Hyperresponsiveness to Distinguish Asthma from Healthy Children in Methacholine Challenge Test

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RATIONALE: Cut-off points of bronchial provocation tests to distinguish asthma in children have not been fully investigated. We evaluated age-dependent cut-off values of bronchial provocation test to determine asthma in children.

METHODS: A total of 3,045 subjects aged from 6 to 19 years old were undertaken a methacholine challenge test. All subjects were classified into 3 groups. The group 1 included patients with clinical asthma diagnosed by pediatric allergist. The group 2 included 280 subjects with a history of recent wheeze, physician diagnosed asthma, or recent asthma treatment by a Korean version of ISAAC questionnaire from population-based researches. The group 3 included healthy subjects (n=2,398) except group 2 from population studies. Receiver operating characteristics curves to determine the cut-off values for clinical asthma were analyzed between group 1 and 3, and also analyzed between group 2 and 3.

RESULTS: From ROC analysis between group 1 and 3, cut-off values for determining asthma were increased with age. Cut-off of PC20 were 5.95 mg/ml at age 6, 9.13 mg/ml at age 7, 11.77 mg/ml at age 8, 12.62 mg/ml at age 9, 14.94 mg/ml at age 10, 16.61 mg/ml at age 11, and 24.60 mg/ml at age more than 15 years. All cut-off points were statistically significant. In ROC analysis between group 2 and 3, a significant cut-off value was only found in subjects aged more than 15 years.

CONCLUSIONS: The reference values of PC20 for determining asthma distinguished from healthy children were increased with age. Age-dependent interpretation is needed in evaluating AHR in children with asthma.

575 Comparison of Clinical Characteristics Between Positive and Negative Response to Mannitol Provocation Test in Asthmatics

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RATIONALE: Airway hyperresponsiveness (AHR) is a consistent and defining feature of asthma. Mannitol increases osmolarity at the airway surface, and results from the release of mediators from inflammatory cells. The efficacy and safety of mannitol was demonstrated in non-asthmatic and asthmatic adults and children.

METHODS: We conducted a retrospective analysis of 250 asthmatics who performed mannitol provocation test at Dong-A University hospital from 2010 to 2013. We compared the clinical characteristics between those who showed positive and negative response to mannitol provocation test.

RESULTS: Ninety five patients (38%) were negative, 152 (60.8%) were positive response to mannitol, and 3 (1.2%) failed to complete the test. The proportion of atopy, newly diagnosed asthma, and inhaled corticosteroid use was significantly higher in patients with positive response than those with negative response to mannitol. The mannitol provocation test was repeated in 13 patients in which 6 converted to negative results. There was no significant difference in baseline lung function, the frequency of asthma exacerbation, asthma severity, eosinophil count of serum and sputum between those with persistent AHR and with negative conversion of AHR.

CONCLUSIONS: In this study, 60.8% of the asthmatics showed positive response to mannitol provocation test. Atopy, newly diagnosed asthma, and inhaled corticosteroid use were related to AHR to mannitol.

576 Standardized Airway Resistances Are Practical Parameters for Asthmatic Children Who Cannot Perform Spirometry

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RATIONALE: The forced oscillation technique (FOT) is expected to be a useful tool to evaluate lung function of children who cannot perform spirometry. Since values obtained by FOT seem to be affected by various growth-related factors, it is difficult to assess changes in the lung function of asthmatic children by the absolute values during growth periods. Therefore, we standardized FOT values by using a regression formula withdrawn from healthy controls’ data, and thereby analyzed usefulness of the standardized FOT values for follow-up of lung function of asthmatic children.

METHODS: 15 asthmatic children at 4 to 12 years old of age were recruited to the study. After taking the guardian’s informed consent, airway resistance (R5, R5-R20), and reactance (X5) were measured by Mostgraph 01 at the recruitment and 5-15 months later. Spirometry was performed in children over 7 years old. All data were standardized by using the regression formula withdrawn from normal controls.

RESULTS: 12 well-controlled asthmatics showed a decline in standardized R5 (%R5), R5-R20, and reactance (X5) were measured by Mostgraph 01 at the recruitment and 5-15 months later. Spirometry was performed in children over 7 years old. All data were standardized by using the regression formula withdrawn from normal controls.

CONCLUSIONS: Standardized FOT data can be used as practical parameters for long-term follow-up of asthmatic children.
577 Esophagastroduodenal Mucosal Behavior after Bronchial Challenge with House Dust Mites in Allergic Asthmatic Patients

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RATIONAL: Several trials show that respiratory allergy could induce a Th2-mediated inflammation of the digestive tract and that this would be the expression of a systemic response. The aim of this study was to evaluate the occurrence of an inflammatory infiltrate (eosinophils and lymphocytes) in esophagastroduodenal mucosa in allergic asthmatics after bronchial challenge (BC) with house dust mite.

METHODS: This study included eight allergic asthmatic patients. They were submitted to skin prick test to aeroallergens to confirm atopy. Subsequently, patients underwent two upper endoscopies with biopsies of the esophagus, stomach and duodenum, one before and one after the specific BC (with house dust mite). Patients remained in hospital for 24 hours after the bronchial challenge, and the second upper endoscopy was performed 24 hours after the BC.

RESULTS: Mean age was 41.8 years old and 62.5% were female. All patients were sensitized to mites. Seven patients were submitted to a specific BC with Dermatophagoides pteronyssinus and one patient with Blomia tropicalis. In seven patients there were no difference between first and second upper endoscopies in relation to the macroscopic findings, as well as in relation to histological analysis. One patient presented increase in the lymphocytes number after the specific BC (10 to 40 intraepithelial lymphocytes/100 epithelial cells).

CONCLUSIONS: These patients, submitted to specific BC with a perennial aeroallergen, did not trigger an eosinophilic infiltrate in esophagastroduodenal mucosa. However, a lymphocytic infiltrate was observed in duodenum after dust mite inhalation, suggesting a systemic response to a perennial aeroallergen.

578 Effect of Dithiothreitol on Sputum Interleukin-13 Protein Measurement

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RATIONAL: Cytokine protein measurement in human sputum is a commonly used method to identify markers of inflammation. Dithiothreitol (DTT) is a strong reducing agent, used during sputum processing for its mucolytic and homogenizing effect through reduction of disulfide bonds. The potential effect of DTT on cytokine structure raises concerns about the accuracy of using ELISA-based methods to measure cytokines in sputum processed with DTT. Due to the previously published variable impact of DTT treatment on some cytokine measurements in sputum samples, we sought to assess the effect of DTT on the measurement of sputum IL-13 protein.

METHODS: We used a commercially available ELISA kit (Human IL-13 kit, Diaclone, France) to measure IL-13 in replicate aliquots of two induced sputum samples from human volunteers and a sputum pool each spiked with IL-13 in serial dilution. All samples and standards were measured in portions treated with 2.5% DTT, 2.5% DTT and 20 minute shaking treatment or non-DTT diluted. The ELISA was run according to the manufacturer’s instructions.

RESULTS: The presence of DTT in standards decreased the measurement of IL-13 to 54-67%. The IL-13 concentration in standards with shaking was reduced to 25-38%. Although sputum sample recovery of spiked IL-13 was reduced to 33-37%, there was no significant difference between the diluent- and DTT-treated samples.

CONCLUSIONS: DTT decreased ELISA-based measurement of IL-13 standards but did not significantly impact protein measurement in sputum samples. The poor recovery of cytokine from sputum may reflect the presence of proteases or other interfering substances.

579 The Flavonoid 7,4’- Dihydroxyflavone Inhibits Human Airway Epithelial Cells MUC5AC MuUnits Production Via Regulation of NF-κB, STAT6 and HDAC2

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RATIONAL: Mucus overproduction is a significant component of the pathophysiology of obstructive lung diseases, for which there are few medications available. Previous studies showed that glycyrrhizin, a triterpenoid in Glycyrrhiza uralensis (G. uralensis) inhibits mucin 5AC (MUC5AC) mRNA and protein expression. Other potential mucus production inhibitory compounds in G. uralensis have not been fully investigated.

METHODS: Using a various of column chromatographic methods, flavonoid 7,4’-dihydroxyflavone (7,4’-DHF) was isolated from G. uralensis. MUC5AC mucus production and gene expression were stimulated with phorbol 12-myristate 13-acetate (PMA) in human airway epithelial cells NCI-H292. The cells were pretreated with 7,4’-DHF for 30 minutes prior to PMA exposure. Supernatants were collected 24 hours later and MUC5AC protein levels and gene expression were measured using ELISA and RT-qPCR, respectively. Phosphorylated-(p)-IκBα, p-STAT6, p-ERK1/2 and HDAC2 expression changes by 7,4’-DHF treatment were measured by Western blot assay.

RESULTS: 7,4’-DHF 28 times more effectively suppressed MUC5AC protein production than glycyrrhizin (IC50 value of 1.4 μM vs 38 μM, respectively) in a non cytotoxic manner. 7,4’-DHF (10 μM) significantly decreased PMA-stimulated MUC5AC mRNA expression (p<0.01). 7,4’-DHF (10 μM) inhibited PMA stimulated NF-κB and STAT6 activation (p<0.05-0.01) and enhanced HDAC2 expression (p<0.05). 7,4’-DHF did not affect ERK1/2 activation.

CONCLUSIONS: G. uralensis flavonoid 7,4’-DHF suppressed PHA stimulated epithelial cell mucus protein, MUC5AC production. This effect was more potent than glycyrrhizin. 7,4’-DHF also suppressed MUC5AC gene expression, which is at least partially linked to suppression of NF-κB and STAT 6 activation and upregulation of HDAC 2. 7,4’-DHF may have a potential for relieving symptoms in mucus hypersecretion related obstructive lung diseases.
**580** The Effect of Age on Airway Inflammation in Younger and Older Patients with Asthma

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**RATIONALE:** Mounting data suggest that older asthma patients experience greater disease morbidity and mortality, possibly reflecting distinct underlying pathophysiology. There is limited information on the effects of aging on inflammation in asthma.

**METHODS:** To define age-related patterns of airway inflammation, we recruited younger (20-40 years, n = 17) and older (>60 years, n = 28) inner-city subjects with asthma. Asthma history, co-morbidities, asthma healthcare utilization, environmental conditions, atopy (detectable IgE to >1 aeroallergen), current asthma medications, degree of airway obstruction reversibility, asthma-related quality of life (mini-AQLQ), and asthma control (ACT) were collected. After a 2-week run-in period to measure adherence to inhaled corticosteroids, subjects underwent sputum induction. Sputum Treg cells were measured by flow cytometry, gaiting on double stained CD3+CD4+ cells and identifying Foxp3+ and CD127low cells, using isotype controls.

**RESULTS:** Older subjects with asthma had increased numbers of hospitalizations, urgent care visits and systemic corticosteroid bursts in the past year, and a decreased asthma related quality of life (mini-AQLQ 3.9 (aged) versus 5.2 (young, p < 0.05)). There was no difference in atopy between age groups (p = 0.34). Sputum analyses showed that older subjects with asthma had a significantly higher percentage of neutrophils (p = 0.03) and a trend for increased eosinophils (p = 0.08) compared with younger subjects. Additionally, older subjects with asthma had increased sputum Treg cells compared to younger subjects.

**CONCLUSIONS:** Asthma in the elderly may be associated with differences in airway inflammation, including an over-representation of Tregs. Additional studies are needed to determine whether these patterns of airway inflammation contribute to a greater morbidity in older patients with asthma.

**581** Systemic IL-17 Signaling Relates to Gender, Disease Severity and Use of Oral Steroids in Children with Asthma

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**RATIONALE:** Signalling via the archetype Th17 cytokine IL-17 is involved in human pulmonary host defense and has also been implicated in adult patients with asthma. Here, we characterized the level of systemic IL-17-signalling in relation to gender, disease severity and use of oral steroids in children with asthma.

**METHODS:** Serum concentrations of IL-17 protein were measured using ELISA in samples from a Swedish study of children with severe (n = 57) or controlled (n = 39) asthma and healthy controls (n = 27). The children with asthma performed spirometry, metacholine provocation and exhaled nitric oxide. Analyses of IL-17 in serum and Asthma Control Test (ACT) were also done for the clinical characterization.

**RESULTS:** Serum concentrations of IL-17 were detectable (>0.55 pg/ml) in 90 out of totally 123 samples. As expected, healthy controls tended to have lower concentrations of IL-17 than asthmatics. The concentrations of IL-17 were higher in children with severe compared to controlled asthma (p < 0.05). Among patients with asthma, those with high IL-17 concentrations (above 75th percentile, n = 30) had lower ACT scores than those with undetectable IL-17 (p < 0.05). Patients with high IL-17 concentrations were more frequently treated with oral steroids during 12 months of observation than those with undetectable IL-17 (p < 0.05). Finally, IL-17 concentrations were higher in female (n = 40) compared to male (n = 56) patients with asthma (p < 0.005).

**CONCLUSIONS:** The level of systemic IL-17-signalling relates to gender, disease severity and use of oral steroids in children with asthma. Further study is required to validate and clarify the mechanisms behind and the clinical utility of these observations.

**582** Mouse Sensitization and Exposure Are Associated with Prescribed Treatment Step and Asthma Severity Among Low Income, Minority Children

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**RATIONALE:** Mouse sensitization and exposure are associated with uncontrolled asthma in urban children, but whether they are associated with measures of asthma severity, an intrinsic characteristic of the disease, is unclear.

**METHODS:** 733 children (5-17y) with uncontrolled asthma underwent evaluation of mouse sensitization, defined as mouse-specific IgE ≥0.35kU/L or skin prick test ≥3mm. Sensitized children had mouse allergen measured in bedroom dust. Relationships between mouse sensitization, mouse allergen levels, and asthma severity measures (controller medication requirement (treatment step) and Composite Asthma Severity Index (CASI)) were examined using regression models adjusted for age, sex, atopy (number of positive skin tests, excluding mouse), study site, race, and insurance.

**RESULTS:** The study population was predominantly minority (70.3% black, 20.3% Hispanic), low income (60.7% income <$30,000), and mouse sensitized (60.0%). Mean(SD) treatment step was 3.2(1.7), equivalent to low-dose inhaled corticosteroids (ICS)+long-acting beta-agonist or medium-dose ICS. Mean(SD) CASI score was 6.6(3.6), reflecting moderate persistent asthma. Mouse sensitization was associated with higher treatment step (3.4 vs. 2.8 among sensitized vs. non-sensitized, p < 0.001), independent of potential confounders (β [95% CI]: 0.41 [0.11-0.72], p = 0.01). Mouse sensitization was associated independently with CASI score (β [95% CI]: 0.88 [0.21-1.55], p = 0.01). Among mouse-sensitized participants, higher log2 (bedroom floor mouse allergen levels) were independently associated with treatment step (β [95% CI]: 0.10 (0.04-0.16), p = 0.001).

**CONCLUSIONS:** Mouse sensitization and mouse allergen levels are associated with increased treatment step and asthma severity. Further studies are needed to determine if reducing mouse allergen exposure among mouse-sensitized asthmatics can reduce controller medication needs and disease severity.
583 Immunodeficiency Associated with FOXP1 (3p13) Deletion
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RATIONALE: The translation factor Forkhead box protein 1 (FOXP1) is implicated in the development of many organ systems. While murine models have demonstrated that FOXP1 is an essential regulator of macrophage, B and T cell development, reports describing the immunologic phenotype in human subjects with 3p13 deletion are exceedingly sparse.

METHODS: Genetic testing was performed by microarray (Signature Genomics).

RESULTS: A 9 year old Caucasian male with 3p14.1-3p12.3 deletion, syndromic features and limited speech underwent thorough immunologic laboratory evaluation due to frequent leukocytosis and recurrent pneumonia resulting in a pneumatocele. His pneumococcal antibody titers revealed a weak response to the conjugate vaccine, and poor memory indicated by loss of protection following polysaccharide vaccine and low memory B cells. Lymphocyte proliferation studies showed normal response to mitogens, with reduced T cell proliferation to recall antigens.

CONCLUSIONS: This subject demonstrates FOXP1 (3p13) deletion with recurrent pneumonia and abnormalities of the B and T cell compartments. Considering the role of this transcription factor in immunity demonstrated by murine models, FOXP1 deficiency could be classified as a primary immunodeficiency disease, especially with the support of additional case reports.

584 A Novel Case of Idiopathic CD4 Lymphopenia Presenting with Disseminated Coccidioidomycosis
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RATIONALE: Idiopathic CD4 lymphopenia (ICL) is a rare clinical syndrome characterized by CD4+ T cell count of less than 300 cells/mm³, or less than 20% of total T cells, without evidence of HIV infection or other immunodeficiency. Patients with cellular immunodeficiency are at higher risk for invasive fungal infection. We present the first reported case of ICL associated with disseminated coccidioidomycosis.

METHODS: A 44 year old woman with a ten year history of leukopenia, recalcitrant human papilloma virus (HPV), pharyngeal abscess, and disseminated coccidioidomycosis, presented for evaluation. There was no history of recurrent sinopulmonary, gastrointestinal or cutaneous infection, autoimmune disorder or malignancy.

RESULTS: White blood cell count was 2800 with an absolute lymphocyte count of 400. Absolute CD4+ and CD8+ T cell counts were decreased at 23 and 46, respectively. HIV viral load and antibody levels were undetectable. NK cell count was normal but function was depressed. Normal studies included quantitative immunoglobulins, pneumococcal titers, complement function, neutrophil oxidative burst, HTLV I/II Ab, and bone marrow biopsy. No monoclonal populations were detected. Lymphocyte antigen and mitogen panel showed absent lymphocyte responses to Candida. CSAF analysis detected coccidioidomycosis antibodies.

CONCLUSIONS: Our patient had persistently decreased CD4+ T lymphocytes in the absence of other etiologies consistent with a diagnosis of ICL. She had low CD8+ T-lymphocytes, not routinely observed, conferring higher mortality rates. Lifelong prophylactic fluconazole, trimethoprim-sulfamethoxazole, and azithromycin were prescribed. Therapies with IL-2 or HSCT have been proposed. The pathogenesis and management of ICL are not well defined and require further research.

585 Resolution of Treatment-Resistant Recurrent Aphthous Stomatitis with Colchicine in a Patient with Muckle-Wells Syndrome: A Case Report
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RATIONALE: Recurrent aphthous stomatitis (RAS) is a common disorder that has a significant impact on a patients’ quality of life. It is unclear what percent of patients diagnosed with cryopyrin-associated periodic syndromes (including Muckle-Wells Syndrome) are affected by RAS or the preferred treatment approach in this population. We present a case of treatment-resistant RAS in a patient with Muckle-Wells Syndrome responsive to colchicine.

METHODS: Case report.

RESULTS: A patient with Muckle-Wells Syndrome initially presented for management of severe attacks (urticarial, lacy erythematous rash, arthritis, arthralgias, myalgias, conjunctivitis) and ongoing RAS resulting in interference of daily life. Patient had improvement in symptoms upon starting canakinumab treatment; however, despite appropriate treatment for Muckle-Wells Syndrome, patient continued to have ongoing RAS. Patient was trialed on amlexanox 5% paste and magic mouth (viscous lidocaine, diphenhydramine, Maalox 1:1:1) with some subjective improvement in RAS. Given improvement on amlexanox, a trial course of montelukast was attempted that resulted in mild improvement in RAS, but without resolution of symptoms. Due to ongoing symptoms, a course of oral steroids was attempted but was ineffective. Patient was then started on colchicine 0.6mg once daily. Four months after initiation of colchicine, patient had a dramatic improvement in RAS (decrease frequency of lesions, improved physical examination, and improved quality of life).

CONCLUSIONS: We present a patient with Muckle-Wells Syndrome managed on canakinumab with treatment-resistant RAS that was responsive to colchicine. We propose that colchicine should be considered as medical management in patients with Muckle-Wells Syndrome with unremitting RAS.
We believe these are the first reported cases of autoinflammatory syndrome induced by adjuvants (ASIA). Systemic symptoms, which met criteria for Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA), were positive. Preoperative: low CD3, CD4, CD8 subsets while IgG normalized. Significant clinical improvement only occurred after wide resection of silicone and autoimmune disease. Public awareness of silicone-associated autoinflammatory and autoimmune diseases may be a part of larger spectrum, involving elements of innate and adaptive immunopathy.

Method: A case of FCAS confirmed by genetic studies, with asymmetrical inflammatory polyarthritis fulfilling the American College of Rheumatology (ACR) criteria for RA. Clinical features included recurrent febrile episodes of episodic erythematous, papular, painful and burning rash, prominent on extremities and buttocks 3–4 hours after exposure to cold. Rash was always associated with low-grade fever, chills and profuse sweating. Morning stiffness lasting approximately 1 hour, and joint pains and swelling involving bilateral metacarpophalangeal, proximal interphalangeal and wrist joints were reported. Labs included normal cell count, C3, C4, immunoglobulin levels, negative anti-nuclear antibody and monoclonal proteins. Rheumatoid factor and Anti-cyclic citrullinated peptide was strongly positive. ESR/CRP was elevated. He was diagnosed with RA based on history and ACR criteria. Patient was heterozygous for L355P mutation in NLRP3.

Conclusion: To our knowledge, this is the first reported case of concurrent FCAS and RA. Increased activation of NLRP3 by mutation has been observed in other rheumatologic diseases. This further supports that autoinflammatory and autoimmune diseases may be a part of larger spectrum, involving elements of innate and adaptive immunopathy.

Method: A retrospective chart review of patients diagnosed with Evans Syndrome by Pediatric Hematology/Oncology at a tertiary hospital between 1/1/00–1/31/12 was performed. The medical charts were reviewed to determine the type, frequency of infections, quantitative immunoglobulins (IgA, IgG, and IgM), lymphocyte subsets, as well as specific antibody titers. Standard methods were used to estimate proportions, and their associated 95% exact Binomial confidence intervals.

Conclusion: Of the 11 patients diagnosed with Evans Syndrome, 9(82%) were males; mean age at diagnosis was 12years (SD=4). Of the 11 patients, serum immunoglobulins were obtained in 10 prior to treatment. Of these, there were 2 subjects(20%; 95% Exact CI: 2.5% to 56%) who were hypo-gammaglobulinemic for age and were subsequently diagnosed with Common Variable Immune Deficiency (CVID). Of the ten patients in which immunoglobulin levels were obtained, five (50%; 95% Exact CI: 19% to 81%) had abnormal levels of at least one immunoglobulin; four had low IgA, four had low IgM; six had a history of pneumonia(55%); three had been hospitalized for an infection(27%).

Conclusion: In this series, we observed a relatively higher prevalence of CVID/hypo-gammaglobulinemia, and immunoglobulin dysregulation in patients with Evans Syndrome. Obtaining screening immunoglobulins, a thorough history of infections and ongoing immunological surveillance is suggested when caring for patients with Evans Syndrome.
590 Prompt Diagnosis of Autosomal Dominant Hyper IgE Syndrome Leads to Reduced Infection and Improved Clinical Phenotype

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RATIONALE: Dominant negative mutations in STAT3 lead to elevated serum IgE, eczema, and recurrent skin and lung infections, known as autosomal dominant Hyper IgE Syndrome (AD-HIES). We aimed to characterize the phenotype of AD-HIES diagnosed in infancy and the impact of early diagnosis.

METHODS: We retrospectively reviewed 16 subjects referred for AD-HIES before age 2 years. Demographic, clinical and laboratory data concerning diagnosis and treatment were collected and compared between children with and without a family history of AD-HIES.

RESULTS: Sixteen patients were diagnosed with AD-HIES before age 2 years, with 9 (56%) having a known family history. The median diagnosis age was 5.8 months and comparable between groups. Antibacterial prophylaxis was started in 94% and antifungal prophylaxis in 32% of the 16 patients. Nine of the 16 (56%) had documented skin abscesses; 5 probands and 4 with family history. All 7 probands had at least one pneumonia with 43% first presenting with Pneumocystis jiroveci. Six (67%) with family history had pneumonia. Newborn rash and eczema were common; severity was worse in probands. At current ages 1.3-30 years, 3 have pneumatoceles and 1 has bronchiectasis. Four of the 9 (current ages 1.3-6.6 years) with a family history were diagnosed genetically as newborns before symptoms developed and had antiseptic baths and antibacterial prophylaxis initiated in the first months of life. None reported skin abscesses or bacterial pneumonia, and eczema was mild.

CONCLUSIONS: Prompt diagnosis of AD-HIES leads to earlier initiation of antibacterial prophylaxis and antiseptics, which are effective at reducing infection and improve clinical phenotype.

591 Improvement of Recurrent Infections after IVIG Supplementation in a Patient with Leukocyte Adhesion Deficiency Deficiency III with a Novel Missense Mutation in FERMT3

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RATIONALE: Leukocyte adhesion defect type III (LAD-III) is a rare primary immunodeficiency disorder. The cause is from mutation in FERMT3 leading to functional defects of integrins. Its main clinical features are from phagocytic dysfunctions. Although integrins present on many cell players including cytokines and growth factors. It has been observed that suppressed immune function results in delayed unorganized tissue repair. Reports of the use HBOT are unusual in wound infections complicating primary immunodeficiency. We found HBOT to be beneficial in promoting soft tissue healing in these two patients. Additionally, this adjunctive therapy was safe, with no adverse effects. Because of the delayed healing and the complications that accompany soft tissue infections in the immune compromised host, adjunctive treatments such as HBOT should be considered.

METHODS: Molecular characterization was performed by exome sequencing. Western blotting of FERMT3 protein was analyzed. Flow cytometric analyses of T and B cell subset and neutrophil were measured. RESULTS: The patient presented with recurrent life-threatening infections, osteoporosis and impaired wound healing starting at 2 months old. Mutation analysis confirmed homozygous novel missense mutation in FERMT3. Besides persistent leukocytosis and impaired platelet aggregation which are the classic phenotypes, she had low immunoglobulin levels. Flow cytometry revealed low percentages of class switching memory B cells(CD27+IgD-), marginal zone-like B cells(CD27+IgD+IgM+),CD27- memory B cells (CD27-IgD-), and plasmablast(CD24-CD38hi). IVIG replacement leads to a significant reduction in episodes of infection and eventually no infection for the 3 years of follow up.

CONCLUSIONS: Patient with LAD-III may have associated humeral immune defect. Our study emphasized the importance of adaptive immune defect contributing to recurrent infections in the patient. Adaptive immune function especially serum immunoglobulins should be evaluated in patients with LAD-III. IVIG can be considered as an alternative treatment or as an adjunctive therapy while awaiting bone marrow transplantation.

592 Adjunctive Hyperbaric Oxygen Therapy (HBOT) in Patients with Primary Immunodeficiency

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RATIONALE: Wound healing is a carefully orchestrated process involving many cell players including cytokines and growth factors. It has been observed that suppressed immune function results in delayed unorganized tissue repair. Benefits of adjunctive HBOT include enhancement of cellular defenses, toxicity to anaerobes and increased leukocyte cidal activity. Here we describe 2 patients with primary immunodeficiency who received adjunctive HBOT in conjunction to wound care to accelerate the wound repair process.

METHODS: Effects of HBOT on wound healing in two female patients aged 9 and 17 years with primary immunodeficiency and necrotizing fasciitis were reviewed.

RESULTS: These case presentations highlight the role of HBOT in the immune compromised host. HBOT was effective in promoting wound healing which is impaired in these patients. Furthermore HBOT was safe; neither of the patients had any adverse effects related to the HBOT.

CONCLUSIONS: Reports of the use HBOT are unusual in wound infections complicating primary immunodeficiency. We found HBOT to be beneficial in promoting soft tissue healing in these two patients. Additionally, this adjunctive therapy was safe, with no adverse effects. Because of the delayed healing and the complications that accompany soft tissue infections in the immune compromised host, adjunctive treatments such as HBOT should be considered.
593  Chronic Granulomatous Disease in China: New Study and a Systematic Review
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RATIONALE: CGD is a rare inherited primary immunodeficiency which is caused by the defect in one of the subunits of NADPH oxidase complex. Here, we aim to find out clinical, laboratory and genetic characteristic of CGD in Chinese population, thus further improving the level of diagnosis and treatment for CGD.

METHODS: We collected and analyzed 38 Chinese CGD patients who were diagnosed in hospitals affiliated to Shanghai Jiao Tong University School of Medicine from 2005 to 2014. Moreover, we reviewed and analyzed all the paper regarding the Chinese CGD study published on English and Chinese journals, summarized and provided further insight in the clinical course of CGD in China.

RESULTS: Through reviewing all of CGD studies in china, 181 Chinese patients in this study or reported previously are included and analyzed. The ratio of male to female patients is 12:9.1. The mean age of onset in CGD patients was 5.24 months, while the mean age at diagnosis was 2.53 years. The most susceptible tissues and organs are lung (86.33%), digestive tract (53.85%) and skin (60.82%). Notably, Bacille Calmette-Guerin infections occurred in 50.97% of the patients. Of all the patients, 145 have been diagnosed by gene test, in which 129 patients with CYBB mutations (88.3%), 8 patients with CYBA mutations (5.5%), 6 patients with NCF1 mutations (4.2%) and 2 patients with NCF2 mutations (2%). In our study, there are no relationship between genotype and phenotype.

CONCLUSIONS: BCG infection is a serious problem in these immunodeficient patients. Mutation analysis is an important tool for CGD diagnose.

594  Gastric Adenocarcinoma in the Setting of X-Linked Agammaglobulinemia (XLA) and HIV
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RATIONALE: X-linked agammaglobulinemia is a primary immunodeficiency resulting in the absence of B-cells and increased susceptibility to infections. There are sixteen published reports of XLA linked to malignancy. Here we present an additional patient with XLA and HIV who was diagnosed with gastric adenocarcinoma.

METHODS: Quantitative immunoglobulins, T/B cell phenotyping, CDH1 gene testing, esophagogastroduodenoscopy (EGD), and biopsy of the antrum of the stomach.

RESULTS: A 24-year-old male with XLA, diagnosed at the age of 2, was maintained on IVIG replacement. He contracted HIV at the age of 23 and was treated with HAART, which kept his CD4 count >1000 and viral load undetectable. He developed recurrent Campylobacter bacteremia and leg cellulitis, which was treated with IV antibiotics. He developed low IgG troughs, despite increasing IVIG dose and frequency. He then developed iron deficiency anemia so EGD was performed, which showed gastric inflammation with erosions and ulceration. Biopsy showed poorly differentiated gastric adenocarcinoma, her2/neu negative, positive for Helicobacter Pylori. Genetic testing for mutations in the CDH1 gene, which are associated with hereditary diffuse gastric cancer, was negative. He underwent subtotal gastrectomy, lymphadenectomy, Roux-en-Y gastrojejunostomy, and had negative lymph nodes and margins making his final diagnosis stage 1A gastric cancer. He was placed under surveillance with no evidence of recurrence of the disease.

CONCLUSIONS: There are multiple case reports linking early onset GI malignancy to XLA with inflammation possibly due to chronic infection as a predisposing factor. Further studies are needed to validate the cost effectiveness of early screening for GI malignancy in patients with XLA.

595  Immunomodulators Use Unmasking Immunodeficiency in 3 Patients with Low IgA: Misdiagnosis or Complication?
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RATIONALE: IgA deficiency is the most common congenital immunodeficiency in the world, usually asymptomatic, it can be associated with functional antibody deficiency and autoimmune diseases.

METHODS: We present 3 cases with low IgA, who develop sepsis and abscesses after immunomodulators prescribed for auto-immune diseases, or post-transplantation.

RESULTS: The 1st case is a 16 year old female presenting with steroid dependent neutropenia, and recurrent upper respiratory infections. Two months after receiving the 2nd dose of rituximab, she presents perianal abscess and sepsis. The 2nd case is a 21 year old girl with multiple granulomas and repetitive sino-pulmonary infections since age 15. She is treated with Methotrexate and glucocorticoids for a diagnosis of Wegener’s disease, develops sepsis and invasive aspergillosis 3 months later. The 3rd case is a 21 year old renal transplant, on mycophenolate mofetil and tacrolimus, developing 2 years after severe skin abscesses. All 3 cases had low IgA and normal IgG levels at baseline. Post-immunomodulators and at onset of infections, the IgG level was very low. Evaluation of specific IgG to encapsulated organisms, diphtheria and tetanos showed no response. All cases were started on intravenous immunoglobulins 600mg/kg/month with partial improvement of infections.

CONCLUSIONS: With the recent proposition of the term “persistent immunodeficiency after treatment with immunomodulatory drug” (PTITD), it would be necessary to evaluate the immune system in patients with isolated low IgA before starting immunomodulators. The detection of rare immunodeficiency syndromes before using these drugs or their persistent suppression of the immune system would avoid severe outcomes in those patients.
596 A Young Male with Systemic Lupus Erythematosus Presenting with Seizures Secondary to Posterior Reversible Encephalopathy Syndrome (PRES)

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RATIONALE: Posterior reversible encephalopathy syndrome (PRES) is an under recognized condition in systemic lupus erythematosus (SLE) that can mimic neuropsychiatric lupus. Identification of distinct clinical and radiographic patterns is important, as one would need to escalate than decrease immunosuppressant therapy in neuropsychiatric lupus. The majority of PRES cases secondary to SLE reported in the literature involve female patients. We report a young male patient presenting with PRES secondary to uncontrolled hypertension due to lupus nephritis.

METHODS: MRI brain demonstrated findings consistent with PRES.

RESULTS: Patient is a 20-year-old male with history of SLE (lupus colitis, lupus nephritis), HTN, and end-stage renal disease (creatinine 2.9) who presented to the emergency department (ED) with new onset tonic-clonic seizures. His blood pressure in the ED was 197/114 for which he was started on a Cardene drip and admitted to ICU for further management. Subsequent MRI brain revealed multiple foci of increased FLAIR and T2 weighted signal intensity in the subcortical and periventricular matter bilaterally, and bilateral cerebellum; edema in the parietal and occipital regions consistent with PRES.

CONCLUSIONS: Systemic lupus erythematosus should be considered in the differential diagnosis of young patients who present with PRES. Clinicians should have a low threshold for MRI especially when neurological symptoms occur in young patients with SLE; even more so in those with active lupus, lupus nephritis, renal failure, and/or poorly controlled hypertension. We feel that consideration of this diagnosis in young adults with lupus, including males should be recognized, as prompt recognition is crucial to deliver appropriate management.

597 Chronic Breast Abscess in a Previously Healthy Adolescent Female Due to X-Linked Chronic Granulomatous Disease with Extreme Lyonization

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RATIONALE: X-linked chronic granulomatous disease (X-CGD) is a primary immunodeficiency caused by mutations in the CYBB gene. Patients with X-CGD are almost always male infants who present with abscess-forming infections involving catalase-positive bacteria and fungi. We present the case of a previously healthy adolescent female who suffered a prolonged breast infection that led to her diagnosis and eventually to appropriate therapy.

METHODS: A 15yo previously healthy AA female presented to clinic with intermittent abscesses on trunk and extremities, worsening over the past two years. On presentation to clinic, an abscess measuring 2.5cm x 0.5 cm x 2.5 cm on her left breast had been draining and healing intermittently for 4 months while being treated with oral antibiotics. Debridement was performed with cultures that grew MSSA and the patient was placed on appropriate therapy. However, her abscess persisted until subsequent surgical debridement revealed the presence of Serratia marcescens which responded to appropriate therapy.

RESULTS: Screening immunoglobulin and complement tests were normal. A DHR flow cytometry test revealed twin peaks typical for X-CGD with only 10% of neutrophils demonstrating a normal oxidative burst. Genetic testing revealed a heterozygous mutation in CYBB gene (c.1359DelG) consistent with X-linked CGD carrier with extreme lyonization.

CONCLUSIONS: Most CGD patients are males who present in early childhood with a persistent infection due to a catalase-positive organism, but CGD should be considered in relatively healthy older females with persistent abscesses/cellulitis since X-CGD carriers with extreme lyonization may have a milder phenotype than females with autosomal recessive CGD.

598 Interleukin-21 Receptor Defect: A Report of Two Brothers

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RATIONALE: Many rare types of immunodeficiency are not clearly understood. Only two cases of inherited IL-21 receptor defect were documented previously. We describe the first cases from South America.

METHODS: The patients are male siblings of consanguineous parents.

RESULTS: During their years of repeated admissions, diagnostic studies were inconclusive. Cystic Fibrosis testing was negative. Pancreatic fecal elastase studies and ciliary biopsy were normal. Immunologic evaluation showed low IgG, elevated IgE, poor antibody response, and normal complement.

CONCLUSIONS: IL-21 receptor (IL-21r) is a type I cytokine receptor for IL-21 found on the surfaces of T, B, and NK cells. IL-21r, a T-cell derived cytokine, binds to IL-21r, and is a modulator between innate and acquired immunity. IL-21 synergizes with IL-7 and IL-15 to expand and activate CD 8 T cells. IL-21 also works with IL-4 to activate B-cells and induce class switching. It has a role in the differentiation of B cells into memory cells and plasma cells. IL-21 plays a significant role in immune cells. These cases describe a rare immunodeficiency, which should be considered in patients with recurrent infections, like cryptosporidium, bronchiectasis, and liver disease.

599 Hemophagocytic Lymphohistiocytosis in Adults: Spectrum of Severity, Therapy and Outcome

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RATIONALE: While the molecular etiology of primary HLH (pHLH) is well characterized, secondary HLH (sHLH) is less defined. sHLH is a heterogeneous entity whose severity and presentation vary depending on the underlying disease. Thus, the HLH 2004 therapeutic protocol (developed for pHLH) may not be the best approach for all sHLH patients. We report 13 sHLH patients with a spectrum of severity, outcome, and a modified therapeutic approach.

METHODS: Adult patients seen in the Immunology Division between 2010 and 2014 who fulfilled the 2004 HLH criteria were included.

RESULTS: HLH was identified in patients with underlying autoimmune disease (n=9) and/or malignancy (n=3), or with no known underlying disease (n=2). Six patients had concomitant viral infections; three were on azathioprine. Therapy was tailored as per clinical picture and lab data including ferritin level trend rather than absolute number. Four patients responded to treatment of underlying disease only. Nine patients received a modified HLH therapy with Anakinra (Interleukin-1 receptor antagonist), immune-modulatory dose of IVIG, cyclosporine, and parenteral steroids. All patients with auto-immune disease (n=6) had resolution of HLH and clinical improvement. Three patients with underlying malignancy (lymphoma and CLL) did not survive, but two elected palliative care prior to completion of therapy.

CONCLUSIONS: Early recognition of HLH and prompt treatment of underlying disease might be sufficient to halt its progression without additional therapy. sHLH is different than pHLH and hence management strategies should be individualized according to underlying cause and severity. A modified HLH protocol was associated with 100% survival in patients with auto-immune disease.
**600 Variable Presentations of Gain of Function STAT1 Mutations within a Single Institution with Features Beyond Chronic Mucocutaneous Candidiasis**

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**RATIONALE:** Gain of function (GOF) mutations in signal transducer and activator of transcription 1 (STAT1) are a major cause of chronic mucocutaneous candidiasis (CMC). Susceptibility to viral, bacterial, fungal, and mycobacterial infections can also occur. In addition to infection susceptibility, patients with GOF-STAT1 mutations suffer from many other manifestations.

**METHODS:** Retrospective chart reviews were performed on 5 patients with GOF-STAT1 mutations to identify specific infectious susceptibility and other immunologic manifestations.

**RESULTS:** All 5 patients identified have mutations in the coiled-coiled or DNA binding domains of STAT1, all consistent with gain of function. Four patients have CMC. 1 has recurrent herpes simplex virus stomatitis, 1 had *Mycobacterium fortuitum* mediastinal lymphadenitis, and 2 have recurrent sinopulmonary infections. One patient presented at 5 months of age with immunodeficiency-polyendocrinopathy-enteropathy-X-linked (IPEX) like manifestations including severe refractory diarrhea, diabetes, and failure to thrive. Successful matched unrelated bone marrow transplant was performed at 3 years of age. In addition to CMC, one patient had a giant hand granuloma caused by *Trichophyton tonsurans*. Other non-infectious manifestations include generalized seizures, autoimmune hemolytic anemia, recurrent thrombosis, and squamous cell carcinoma.

**CONCLUSIONS:** In addition to CMC, these patients demonstrate broad infectious susceptibility and many other manifestations of GOF-STAT1 mutations including IPEX-like disease, vascular disease, autoimmunity, and malignancy.

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**601 A Young Adult Male with Chronic Mucocutaneous Candidiasis (CMC) with Signal Transduction and Activator of Transcription 1 (STAT1) Mutation and Progressive Multifocal Leukoencephalopathy (PML)**

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**RATIONALE:** Living into the second decade, with presumed autosomal dominant transmission, and concurrent polyomavirus (JC virus) diagnosis is rarely reported in CMC STAT1 mutations including IPEX-like disease, vascular disease, autoimmunity, and malignancy.

**METHODS:** Sequencing of STAT1 was performed on a pure T cell lineage at *SickKids* revealing a heterozygous STAT1 DNA binding domain mutation at Thr385Met.

**RESULTS:** A young man presented with a history of failure to thrive, hypothyroidism, recurrent: stomatitis, shingles, and pneumonias, bronchiectasis on computed tomography (CT), and recent hospitalization for empyema and cold immune-mediated hemolytic anemia requiring Rituximab. Previous labs revealed a low immunoglobulin A (IgA) of 28ng/dL, abnormal pneumococcal protection with adequate vaccine response, normal lymphocyte subset testing (CD markers), normal lymphocyte mitogen stimulation, and absent response to candida with lymphocyte antigen stimulation testing. Family history revealed a mother with CMC, hypogammaglobulinemia, and early death due to end organ failures with associated recurrent pseudomonas pneumonia. STAT1 analysis revealed a heterozygous DNA binding domain mutation at Thr385Met. After diagnosis, he developed PML presumably due to his predisposition risk with STAT1 mutation, in combination with the Rituximab therapy he received. His JC virus caused progressive neuromuscular deficits. Interferon Alpha therapy was initiated at the National Institutes of Health.

**CONCLUSIONS:** We believe that this is the first probable autosomal dominant transmission of STAT1 DNA binding domain Thr385Met mutation. This patient was the oldest patient living with this mutation. Also unique to this case, is the patient’s associated polyomavirus activation. The patient’s treatment plan may be important for therapeutic strategy for future PML cases.

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**602 STAT3 Signaling Hypersensitivity in a Child with Unicentric Castleman’s Disease**

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**RATIONALE:** 10yo boy with enlarged right axillary lymph nodes (LN) was diagnosed with Unicentric Castleman’s Disease (UCD) after resection revealed hyaline vascular type. UCD is a rare, isolated lymphoproliferative disorder with poorly understood pathogenesis that has been linked to excessive release of IL6. In this study we phenotype the patient’s Tregs, Th1, Th2, Th17 and STAT3 response to cytokines.

**METHODS:** PBMCs (patient and three controls) and cells from patient’s resected LN (collagenase-digested) were analyzed by flow cytometry for intracellular Foxp3, cytokines, and p-STAT3. BD PhosFlow method was used to detect p-STAT3 after 30min stimulation: 10ng/mL IL6, IL9, IL21 or IFNa2.

**RESULTS:** Lymphocyte subsets and serum cytokines performed by ARUP Laboratories were remarkable for elevated soluble CD25 (1136pg/mL, ref ≪1033) and IL13 (13pg/mL, ref ≪6). Patient’s CD4+PBMC: 5.8% Foxp3+Tregs, 4.6% IFNg+, 0.5% IL17A+, 31.2% TNFa+, 2.5% IL2+, 0.8% IL13+ and 1% IL4+. LN: 0.4% CD16+NK, 0.2% CD16+NKT, 72% CD19+, 16% CD4+ and 5% CD8+. CD4+LN: 21% Tregs, 10.7% IFNg+, 0.8% IL17A+, 8.4% TNFa+, 1.4% IL2+, 0.1% IL13+ and 0.4% IL4+. PBMC: %p-STAT3 MFI change from controls with IL6, IL9, IL21, IFNa2, respectively: monocytes (51, 0, 0, 22%), CD4+ (30, 0, 24, 29%), and CD56+NK (0, 16, 83, 40%). Expression of IL6 receptor was similar amongst patient and controls.

**CONCLUSIONS:** The level of STAT3 hypersensitivity might be the underlying etiologic cause of the inflammatory response and lymphoproliferation within the lymph nodes. Genetic analysis is needed to investigate the possibility of a gain-of-function in STAT3.
603 The Major Allergens of Birch Pollen and Cow Milk, Bet v 1 and Bos d 5, Are Structurally Related to Human Lipocalin 2, Enabling Them to Manipulate T-Helper Cells Depending on Their Load with Siderophore-Bound Iron

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RATIONALE: Human lipocalin 2, LCN2, is highly expressed in encounter sites of allergens, as lung and gut. It has immuno-regulatory properties when carrying iron via siderophores (holo) or not (apo). We investigated whether major allergens of birch pollen and milk, Bet v 1 and Bos d 5, might structurally and biologically interfere with LCN2.

METHODS: Structural comparison to LCN2 was performed using FATCATflex, CE algorithm and TM-Align methods. Ligand binding was analyzed with AutoDock Vina. Iron-binding was determined by Prussian blue staining. Activated human PBMCs were stimulated for 18h with apo- or holo-allergens, or controls. Subsequently, cells and supernatants were analyzed with flow cytometry and for their cytokine-content.

RESULTS: Both tested allergens shared great structural homology to LCN2. Thus, besides Bos d 5, we could also classify Bet v 1 as a lipocalin-like protein. Both allergens were capable of binding iron via siderophores. When incubated with PBMCs, only the apo-forms of the allergens, but not the holo-forms, were able to promote CD+ cells and the secretion of IL13.

CONCLUSIONS: We conclude that Bet v 1 and Bos d 5 not only structurally mimic human LCN2, but also functionally by their ability to bind iron via siderophores. The apo-forms promote Th2 cells, whereas the holo-forms appear to be immunosuppressive. These results provide for the first time a functional understanding on the principle of allergenicity of major allergens from entirely independent sources, like birch and milk.

604 Analysis of GST Allergen Cross-Reactivity in a North American Population for Molecular Diagnosis

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RATIONALE: IgE reactivity to glutathione S-transferases (GST) has been reported in tropical and subtropical environments, where it is not clear if cross-reactivity or co-sensitization occurs. In the US, Bla g 5 is the most important GST allergen, and lack of co-exposure to GSTs from certain species allows a better assessment of cross-reactivity versus co-sensitization.

METHODS: The crystal structures of Bla g 5, Der p 8, Blo t 8, and Ascaris suum GST (GSTA) were determined and surface residues compared. Sera from North American cockroach and mite allergic patients were tested for IgE reactivity to these GSTs. A panel of six murine anti-Bla g 5 mAb was compared for cross-reactivity with the other three GSTs using antibody binding assays.

RESULTS: The allergen structures revealed few contiguous regions with similar exposed residues that would make cross-reactivity unlikely. Bla g 5 sensitive sera did not react with Der p 8, and vice versa. None of the sera reacted with Blo t 8 or GSTA. Only Der p 8 inhibited IgE binding to Der p 8. The anti-Bla g 5 mAb failed to interact strongly with the other three GSTs.

CONCLUSIONS: The lack of IgE cross-reactivity to Bla g 5, Der p 8, Blo t 8 and GSTA in allergic patients from temperate climates, is in agreement with the low shared amino acid surface identity. Previous results from tropical environments may be due to co-sensitization. This highlights the need for species-specific GSTs for accurate molecular diagnostics.

605 Structural and Stability Studies of Profilins Amb a 8 and Art v 4

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RATIONALE: Profilins form one of the largest family of allergens. Members of this family have highly conserved sequences and are responsible for many cross-reactions between different sources of pollen and food allergens. The goal of this analysis is to map IgE epitopes on allergenic profilins originating from pollens, and provide a detailed explanation at the molecular level of cross-reactivity of this allergens.

METHODS: X-ray diffraction analysis was used to investigate the molecular structures of Amb a 8 and Art v 4. Thermal shift assays were used to analyze the stability of both weed allergens and their homolog Bet v 2. Gel filtration, dynamic light scattering and electrophoresis were used to determine the oligomeric state of these allergens.

RESULTS: Structural data revealed that the both Amb a 8 and Art v 4 share the same overall fold and are structurally similar to Bet v 2. Our studies revealed that pollen profilins may form oligomeric assemblies.

CONCLUSIONS: Determination of 3D models of Amb a 8 and Art v 4 allowed for their comparison with the already known structure of Bet v 2. This comparison confirmed a high degree of structure and sequence conservation among analyzed pollen profilins. In addition, we have found that the profilins we analyzed in solution may be present in different oligomeric states. However, detailed molecular structure of the oligomeric assemblies is yet to be determined. Sequence and structure conservation, as well as the propensity of profilins to oligomerize seem to be crucial for triggering IgE-dependent inflammatory response.
606 Expression, Purification, and Characterization of Recombinant Coptotermes Formosanus Tropomyosin

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RATIONALE: Tropomyosin is a pan-allergen from arthropods including shellfish and cockroaches that has a high degree of sequence similarity. Our goal was to express, purify, and characterize recombinant tropomyosin from the Formosan subterranean termite Coptotermes formosanus.

METHODS: A full length recombinant C. formosanus tropomyosin was codon optimized, cloned, and expressed in Escherichia coli. The recombinant protein was purified by LPLC using a 6x-histidine affinity tag and a nickel affinity column. Purified recombinant protein was analyzed by circular dichroism, mass-spectrometry, immunoblot with IgG and IgE antibodies, and was subjected to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) digestion.

RESULTS: Codon optimization allowed high levels of the recombinant tropomyosin to be expressed and purified. The recombinant protein was recognized by commercial antibodies to tropomyosin and serum IgE. A fragment of the protein migrating at approximately 13 kDa was resistant to both SGF and SIF digestion in vitro.

CONCLUSIONS: We have expressed and purified milligram quantities of recombinant termite tropomyosin from the Formosan subterranean termite Coptotermes formosanus. C. formosanus tropomyosin is 98-99% identical to cockroach tropomyosin and 83-84% identical to shellfish tropomyosin, and continued analysis of the protein will determine if it is a clinically significant allergen.

607 Quantitative Binding Assay for Measuring Specific IgG Antibodies to Alpha-Gal Using the Neoglycoprotein Gal-α-1,3-Gal-β-1,4-GlcNAc-Human Serum Albumin

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RATIONALE: Tick bites are known to induce IgE production to alpha-gal. Elevated alpha-gal IgG1 compared to natural alpha-gal IgG2 production has been reported in alpha-gal IgE+ subjects. We here report further investigation of the relationship between alpha-gal IgG and both reactions to red meat and exposure to ticks.

METHODS: IgG from serum was absorbed onto recombinant Protein G-Sepharose and incubated with radiolabeled allergen. The radioactivity of bound allergen was measured using a gamma counter. A control curve was generated in parallel to assign unitage. Additional testing of serum immunoglobulins was performed via ImmunoCAP and nephelometry.

RESULTS: Alpha-gal IgG was measured in a Northern Sweden cohort and in subjects presenting to allergy clinics in Virginia with delayed reactions to red meat. Alpha-gal IgG was significantly higher in alpha-gal IgE+ subjects versus alpha-gal IgE- subjects, and longitudinal serology in several alpha-gal IgE+ subjects demonstrates parallel alpha-gal IgE and IgG response trends. Among the alpha-gal IgE+ subjects, alpha-gal IgG was higher in those with alpha-gal IgE-total IgE ratios >25%, but was not related to reported severity to red meat. Compared to the alpha-gal IgE- subjects in Virginia, alpha-gal IgG was lower in the group from Northern Sweden, where alpha-gal IgE-mediated hypersensitivity is absent and ticks are rare.

CONCLUSIONS: Alpha-gal IgG is strongly related to alpha-gal IgE and is significantly lower in prevalence and titer in subjects without tick exposure. The absence of a relationship between alpha-gal IgG and severity of reactions to red meat suggests that the alpha-gal syndrome may not be a suitable candidate for conventional immunotherapy.

608 Cloning, Expression and Purification of Recombinant per a 5 from Periplaneta americana

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RATIONALE: Glutathione-S-transferases (GSTs) are known to possess allergenic potential. Per a 5 from Periplaneta americana, a GST was cloned, expressed, purified and characterized using immuno-biochemical techniques.

METHODS: RNA was isolated using fresh cockroach powder and cDNA was prepared. Per a 5 was PCR amplified with gene specific primers and cloned in pET 22b+ expression vector. The recombinant Per a 5 (rPer a5) was expressed and affinity purified using Ni-NTA resin. Immunoblot and ELISA with rPer a5 were performed using cockroach positive patient sera. ELISA inhibition assay was also done to check the potency of rPer a 5 as an allergen.

RESULTS: PCR amplified gene has 651bp, similar to accession No: AAX33729. Homology of Per a 5 protein sequence exhibited 60-84% similarity to insect GSTs and 31% to human delta class GSTS. Secondary structure prediction of Per a 5 protein showed 9a helix and β sheet structure. rPer a 5 protein expressed in E.coli cells yielded 0.8mg/L of culture. Purified protein on SDS PAGE showed 25 kDa and 50 kDa (dimer) when expressed at 37°C & single band was observed at 25 kDa when expressed at 32°C. The rPer a 5 was recognized by cockroach hypersensitive patients sera in immunoblot. rPer a 5 showed 40% IgE binding with individual patients' sera in ELISA. rPer a 5 inhibited the IgE binding to surface coated cockroach extract in ELISA inhibition.

CONCLUSIONS: rPer a 5 gene was sub-cloned, expressed and purified to homogeneity. The protein showed allergenic potential and is a minor allergen from P. americana.

609 Cross-Reactivity Among Pho d 2 and Profilins from Different Pollen Allergenic Sources

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RATIONALE: Profilins are considered as an important panallergen being the IgE cross-reactivity among these proteins associated with multiple pollen sensitization. Sequence homology among pollen profilins is very high, ranging 67.4-89.5%. The objective was to evaluate the cross-reactivity among profilin from palm tree pollen (Phoenix dactylifera) and other pollen allergenic sources where profilin has been described.

METHODS: Palm tree profilin (Pho d 2) was purified by affinity chromatography in a poly-L-proline column, sequenced by LC/MS-MS and used to produce rabbit polyclonal antibodies. Identification of profilin in eleven pollen extracts (Ambrosia artemisiifolia, Artemisia vulgaris, Betula alba, Chenopodium album, Cynodon dactylon, Helianthus annuus, Mercurialis perennis, Olea europea, Parietaria judaica, Phleum pratense and Plantago lanceolata) was carried out by IgG ELISA and immunoblot using the polyclonal antibodies. Cross-reactivity studies were performed by IgG immunoblot inhibition assays, using the purified profilin for inhibition.

RESULTS: Highly purified profilin (>95%) from palm tree pollen (Pho d 2) with a molecular weight of 14 kDa was obtained. Identity and purity of profilin was confirmed by LC/MS-MS. Presence of profilin was confirmed in all the extracts, except Chenopodium, by direct ELISA (O.D. >0.1). Identification of profilin by IgG immunoblot, and cross-reactivity with Pho d 2 by immunoblot-inhibition, was observed in Ambrosia, Betula, Cynodon, Helianthus, Mercurialis, Plantago and Phleum extracts.

CONCLUSIONS: Cross-reactivity among Pho d 2 and other profilins was demonstrated in Ambrosia, Betula, Cynodon, Helianthus, Mercurialis, Plantago and Phleum extracts by immunoblot inhibition studies. Purified Pho d 2 could be used for diagnosis and treatment of pollen profilin sensitization.
610 Immunoglobulin E to Allergen Components of House Dust Mite in Children with Allergic Disease

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RATIONALE: House-dust mites (HDM) are important sources of indoor allergens. Thirteen components have been identified from Dermatophagoides pteronyssinus(der p). Our aim was to define the prevalence of IgE to components of Der p in Korea and investigate the clinical features of them in children with allergic disease.

METHODS: We performed a prospective evaluation of 80 HDM sensitized patients with history of allergic rhinitis (AR), atopic dermatitis (AD), asthma and urticaria (UC). Patients underwent ImmunoCAP for total IgE, Der p, Der f, Der p 1, Der p 2, and Der p 10.

RESULTS: Seventy nine patients had detectable serum IgE to Der p, 80 patients were sensitized to Der f, 66 patients were sensitized to Der p 1, 63 patients to Der p 2, and 7 patients were sensitized to Der p 10. Der p 1 specific IgE was significantly lower in the UC group compared with the AD and AR group. Total IgE was significantly higher in the Der p 10 sensitized group. Der p 10 serum IgE level was highly correlated with crab and shrimp specific IgE. There was a significant positive correlation between total IgE and specific IgE to Der p and its components and Der f.

CONCLUSIONS: Sensitization to HDM and its components in Korea is similar to previous studies from temperate climate. The determination of Der p 1, Der p 2, and Der p 10 specific IgE helps in obtaining additional information in regards to allergic disease.

611 Utility of Recombinant Allergens in the Diagnosis of Patients with Rhinococonjunctivitis and / or Asthma Sensitive to Pollens Cupressus, Platanus, Olea and Phleum

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RATIONALE: The study of recombinant allergens improves the diagnostic accuracy of allergic diseases. The aim was to compare the parameters of sensitization to Cupressus, Platanus, Olea and Phleum.

METHODS: A retrospective study where skin prick tests (SPT) and specific IgE (sIgE) to native (n) and recombinant of Cupressus, Platanus, Olea and Phleum were performed to 10 patients with symptoms of rhinoconjunctivitis and asthma from December to July.

RESULTS: SPT were positive for: Cupressus (9/10), Platanus (5/10), Olea (9/10), and Phleum (9/10). sIgEs were positive for Cupressus (n: 100%), Platanus (n: 69.4%/Pla a1:10%), Olea (n: 90%/rOle e1: 90%) and Phleum (n: 90%/rPhl 1 Phl: 80%; rPhl 5: 70%; rPhl p7: 20%; rPhl p12: 30%). No statistical differences were found among qualitative results of SPT and sIgE to Cupressus, Platanus, Olea and Phleum (p> 0.05). The correlation coefficients were: Cupressus [n/rCup a1, r: 0.87 (P<0.01)]; Platanus [n/rPla a1, r: 0.76 (P<0.05)]; Olea [n/r Ole e1, r: 0.95 (P<0.01)]; Phleum [n/rPhl p1, r: 0.91 (P<0.01)]; Phleum [n/rPhl p7, r: 0.97 (P<0.01)]. No statistical differences were found between Phleum[n/rPhl p7] and (n/rPhl p12).

CONCLUSIONS: The results of SPT, sIgE native and recombinant major in patients with RC and/or Asthma, are effective in the diagnosis of sensitization to four pollens, preferably with the recombinant major of Cupressus, Olea y Phleum.

612 Factors Contributing to Poor Asthma Control in Children

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RATIONALE: Asthma is one of the most common chronic diseases in children. Control over the disease is the target, so we should seek to identify factors that negatively affect asthma control.

METHODS: Included subjects were asthmatics aged 5-14 years, scheduled to visit the asthma clinic during the period from April 2013 to June 2014, at King Abdulaziz Medical City, Jeddah, Saudi Arabia. Data were collected from 147 patients through chart review, and telephone interviews. Classification of asthma control was according to theGINA guidelines 2014. Children in each classified group were compared regarding BMIs, presence of allergic rhinitis, sinusitis, GERD, tobacco smoke exposure, medication compliance, technique using medications, and their geographical distribution.

RESULTS: Out of 209 eligible patients, 185 were recruited (38 did not respond to the telephone calls, 147 patients’ data were finally analyzed); 24 children met the exclusion criteria. 27.9% poorly controlled, 27.9% partially controlled and 15% poorly controlled. Assessing the impact of combined risk factors using chi-square test, subjects group with 3 or more risk factors present had higher prevalence of poor asthma control (p=0.02).

CONCLUSIONS: Controlling asthma is challenging because of different contributing variables. The current study provides a highlight on asthma control and associated factors in western Saudi Arabia; however, a multicenter prospective study is required for further assessment of this important health problem.

613 Aerobiological Study in Lima (PERU)

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RATIONALE: Knowledge about seasonal and annual fluctuations in airborne pollen and fungal spores in any geographical area is essential for effective diagnosis and treatment of allergy diseases. Our objective was identify and register the most important aeroallergens in the atmosphere of Lima urban city.

METHODS: The pollen and fungal spores counts were made according to standardized technique with Burkard spore trap for 7 days and the analysis procedures recommended by the Spanish Aerobiology Network. The trap was installed on the roof of a building, which is 20m high, in the west-south of the Lima urban area. The sampling period was performed from February 2012 to March 2013.

RESULTS: The 3 most important fungal spores during all the periods of sampling, in order of abundance, were: Cladosporium herbarum (75.15%), Nigrospora sp (22.31%), Alternaria alternata (2.57%), with higher frequency in autumn and summer.

The greatest pollen counts were recorded in winter and summer. We found 10 leading taxa: Poaceae (22.6%), Oleaceae (20.9%), Compositae (Artemisia spp) (19.38%), Urticaceae (16.45%), Betulaceae (Casuarina) (9.03%), Myrtaceae (Eucalyptus) (7.21%), Betulaceae (Alnus) (2.19%), Chenopodiaceae-Amaranthaceae (1.88%), Asteraceae (Ambrosia) (0.15%), Polygonaceae (Rumex spp) (0.10%).

CONCLUSIONS: We report the first aerobiological study in Lima city performed with Burkard spore trap for 7-days technique. The west-south population of Lima urban city is exposed to several aeroallergens with predominance of fungal spores. The results of this study should be compared with data from the forthcoming years, to identify seasonal and annual fluctuations, and extend the traps to other locations.
Entopy: Where Art Thou Entopy?

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RATIONALE: The concept of local respiratory allergy ("entopy") has long been recognized. Entopic individuals have strongly suggestive histories of allergic rhinitis (AR), lack systemic evidence for allergy as measured by skin testing (SKT) or RAST, yet who demonstrate specific sensitivity to nasal allergen provocation tests (NAPT). We report the results of NAPT that significantly differ from those previously reported.

METHODS: Thirty CAAC patients (11M/19F, age 9-71 yrs [mean39]) with a clinical history strongly suggestive of seasonal and/or perennial AR but negative SKT were chosen for NAPT. Prick SKT were performed with standard concentrations. In addition, intradermal SKT (1:1000 w/v) were performed to selected allergens if prick tests were negative. NAPT were administered in concentrations established in previous studies, including mixtures of trees, weeds, timothy grass, or cat. Symptoms scores were the primary outcome measure. Six control subjects with known AR and positive SKT also received NAPT.

RESULTS: Thirty patients received a total of 52 NAPT; 18 (35%) were positive. 11/50 (22%) patients had at least one positive NAPT; 7/18 (39%) tree pollen, 4/15 (27%) timothy grass, 4/11 (36%) weed pollens, 3/8 (37%) cat. All control subjects had positive challenges. Four patients with positive NAPT have been treated with allergen immunotherapy. One patient developed a Grade II systemic reaction.

CONCLUSIONS: Positive NAPT were found less frequently than previously reported. Few of the earlier reported studies performed intradermal skin testing prior to NAPT. We hypothesize that prior NAPT studies identified many patients with systemic allergy that would have been recognized had intradermal SKT been done.

Allergic Skin Prick Test Results in Thai Children with Respiratory Allergy in 2011-2013

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RATIONALE: Aeroallergen sensitization is the major cause of atopic asthma and allergic rhinitis (AR). The change in global environment may affect aeroallergen pattern. The objective of this study was to determine the distribution of aeroallergen sensitization in Thai children with asthma and/or AR in 2011-2013.

METHODS: The skin prick test (SPT) results in Thai children (age 0-18 years old) with asthma and/or AR in 2011-2013 were evaluated. Demographic data, asthma and AR severity, SPT result were recorded.

RESULTS: There were 1,314 children with asthma and/or AR; 62.1% male, enrolled into the study. The mean age was 7.4±3.7 years. Seventy seven (5.9%) patients were diagnosed as having asthma alone and 819 (62.3%) patients were AR alone. The most common severity of both asthma and AR was mild persistent (76.5% in asthma and 66.0% in AR). Fifty eight percent of the children with asthma and/or AR were sensitized to aeroallergen. The most common aeroallergen was Dermatophagoides pteronyssinus (48.8%), followed by Dermatophagoides farinase (45.4%), American cockroach (23.5%), German cockroach (16.1%) and Bermuda (10.9%). The distribution of major aeroallergen (House dust mite and Cockroach) sensitization was not different among the children with asthma alone, AR alone and asthma with AR. The distribution was also not significantly different among the year 2011, 2012 and 2013.

CONCLUSIONS: House dust mite and American cockroach are the most common aeroallergen sensitization in children with asthma and/or AR. The distribution of aeroallergen sensitization was not different among the children with asthma, AR and asthma with AR in 2011, 2012 and 2013.

Exposure and Sensitization to Dust Mites in Peruvian Cities

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RATIONALE: Knowledge of the prevalence of different species of dust mites according geographical areas is important to support the diagnostic and specific treatment of allergic diseases that dust mites trigger.

METHODS: Dust samples were collected from mattresses in peruvian cities, using an adapted vacuum cleaner. After collection samples were frozen for 48 hours. Mites were extracted from 10 mg of each sample, under stereo microscope, by suspension method with saline solution. Mites were identified by optical microscope. We performed skin prick test (SPT) with standardized mites extracts (ALK-Abelló and LETI Laboratories;Spain) to subjects who lived in the homes where dust samples were taken.

RESULTS: Presence of dust mites was confirmed in almost 90% of samples collected. The predominant species was Dermatophagoides pteronyssinus in cities of peruvian coast, followed by Euroglyphus maynei and Blomia tropicalis. In the jungle, we identified 2 not previously cited species as Malagophagus intermedius and Tarsonemus sp, being this last the predominant specie. Regarding SPT, most of the participants were positive to D. pteronyssinus, followed by D. farinase, B. tropicalis, E. maynei and T. putrescentiae, with different percentages by geographic areas.

CONCLUSIONS: To our knowledge this is the first study on mite count in dust and pattern of sensitization performed in Perú. The acarologic fauna and sensitization profile show different percentages depending on geographic areas. D. pteronyssinus is the dominant species in the coast, and Tarsonemus sp. in the jungle, though other species can be considered important. Further studies about mite species and sensitization profile (SPT, specific IgE) will elucidate the clinical importance of these findings.

Differences in Both Total and Specific IgE Against Mites, Cockroach and Ascaris Lumbricoïdes in Columbian Asthmatic Children in a Poor Communities within the Colombian Caribbean Coast

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RATIONALE: Asthma and Ascariosis are important health problems in underdeveloped countries. The environmental conditions also promote the co-exposure to mite allergens and AL. A high levels of Ig E has been demonstrated, but its effects on the inception, evolution, and immune modulation is still unknown. We hypothesize that perennial immunological boosting from AL immunomodulate the IgE response in poor asthmatics children living in the Colombian Caribbean area.

METHODS: This was a case control study. 280 asthma cases and 280 controls. All of them recruited from schoolchildren aged 5–11. Total Ig E and and specific IgE to D f, cockroach and AL were measured using commercially available ImmunoCAP assay. Statistical analysis was carried out using SPSS 20. Measures of central tendency were used. U test of Mann-Whitney test was used (Kolmogorov-Smirnov = 0.000). Qualitative variables were compared using chi2 test.

RESULTS: The average age of the cases was 6.81 years (SD = 3.63). The frequency of males in the cases was 50.4%. When it was compared the different types of specific IgE to allergens, statistically significant differences in anti AL was found with higher values in controls. For other specific IgE no differences were identified. In comparing the proportions of subjects positive for IgE anti AL between cases and controls were not statistically significant (Chi2 = 1.618, p = 0.203).

CONCLUSIONS: taking together these results we could say that ascariosis modulates in a peculiar way the immune response to allergens among children living in this poor communities.
**618** Skin Reactivity to Inhalant Allergens in Allergic Children and Adolescents from a Specialized Outpatient Clinic – Value of the Skin Index

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**RATIONALE:** Skin prick test (SPT) is an important tool in the evaluation of allergic sensitization and can be influenced by several factors. Skin index (ratio of allergen-induced wheal diameter and corresponding histamine diameter; SI) have been used to improve the diagnostic power of SPT, especially in young children. The main objective of this study is to evaluate the SI in identifying allergic sensitization in patients followed at a specialized pediatric clinic.

**METHODS:** SPT of 1662 patients performed, between 2009 and 2014, to inhalant allergens, negative control (saline) and positive control (histamine, 1mg/mL) (IPI-ASAC® Brazil) were analyzed. Mean wheal diameters of allergens, histamine and negative control were recorded, according to different ages. All patients had a histamine positive SPT (wheal diameter equal or higher than 3mm).

**RESULTS:** Skin reactivity to histamine increased significantly with increasing age. *D. pteronyssinus* and *B. tropicalis* were the predominant inhalant allergens identified. SI cut-off points for the diagnosis of allergic sensitization were 0.6 for dust mites. However, when applying SI alone as the single diagnostic criteria for sensitization, we observe a reduction in the prevalence of sensitized patients.

**CONCLUSIONS:** SI should be used in addition to SPT for the diagnosis of allergic sensitization in those patients who, despite of having low skin reactivity, had SI above 0.6 for *D. pteronyssinus* or *B. tropicalis*.

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**619** Allergic Sensitization and Home Allergy Triggers in Preschool Population in Hermosillo, Sonora, Mexico

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**RATIONALE:** Allergic diseases are a cause of hospitalizations for chronic diseases in children in the Western World. Children with allergic disease are affected by pathophysiological, environmental, individual and family factors which should be determined to prevent allergic crises. The objective of this study is determining home allergy triggers in a preschool population.

**METHODS:** Approved by the Ethics Committee. A total of one hundred and eighty six children (ages 3-6 years) serum samples were analyzed by Phadia ImmunoCAP®. Positive samples were later determined for specific IgE antibodies in a panel of ten common allergens in northwest Mexico by Phadia ImmunoCAP® 100. Allergy triggers were collected using Healthy Home questionnaire done by UMass-Lowell.

**RESULTS:** Eighteen children were sensitized. Most children studied were sensitive to pollens, specifically *Artemis vulgaris, Fraxinus americana* and *Phleum pratense*. In some cases the correlation between the presence of allergen sensitization and observed at home and having a pet as the dog and cat, so the amount of dust in the home mainly because the streets are not pave. We also found sensitized children without clinical symptoms of allergy or undiagnosed.

**CONCLUSIONS:** This study underlines the importance of early detection of allergic sensitization in asymptomatic children or children with symptoms but no diagnosis and leads to take steps to avoid the allergic march. In addition to inform parents about the potential risks in home that can trigger symptoms.
Efficacy Correlates with Plasma Levels in Opus-1, a Proof-of-Concept Study of Oral Kallikrein Inhibitor BCX4161 As a Prophylaxis Against Attacks of Hereditary Angioedema (HAE)

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RATIONALE: BCX4161 is an oral kallikrein inhibitor in development as a prophylactic agent in HAE. BCX4161 significantly reduced the mean HAE attack rate versus placebo in the Opus-1 study. We evaluated the relationships between clinical efficacy, plasma BCX4161 concentrations, pharmacodynamic markers of activity and safety in Opus-1 subjects.

METHODS: Opus-1 was a double-blind, placebo-controlled, randomized 2-period crossover study conducted in 24 HAE subjects who received 28 days of treatment with BCX4161 400 mg TID and placebo. Subjects recorded HAE attack incidence daily. Pharmacokinetic and pharmacodynamic samples were drawn at trough (C_{trough}) and a PK profile (0.5, 1, 2 and 3 hours post-dose) was assessed on Day 14. PT and aPTT samples were drawn throughout the study.

RESULTS: Plasma kallikrein inhibitory levels were highly correlated with C_{trough} (r = 0.73, p < 0.002). C_{trough} was significantly correlated with the percent difference in HAE attack rate (Pearson correlation coefficient -0.42, p = 0.04), with an IC_{50} of approximately 34 ng/mL. The BCX4161 E_{90} for kallikrein inhibition was 51 ng/mL. Four of 6 subjects (67%) with C_{trough} greater than 51 ng/mL had an attack reduction on BCX4161 of at least 0.5 attacks/week compared to 5 of 18 subjects (33%) with concentrations below 51 ng/mL. C_{max} and AUC_{0-24h} did not correlate with efficacy. There were no effects of BCX4161 on aPTT or PT.

CONCLUSIONS: Despite a small sample size of 24 subjects, there were relationships suggestive of a pharmacokinetic/pharmacodynamic/efficacy association, and adequate, maintained BCX4161 exposure correlated with degree of benefit. BCX4161 did not affect coagulation parameters.

Pharmacokinetics of Subcutaneous C1 Esterase Inhibitor Concentrate for the Prevention of Angioedema Attacks in Patients with Hereditary Angioedema

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RATIONALE: To evaluate the pharmacokinetics of two doses of subcutaneous, plasma-derived C1 esterase inhibitor human (C1-INH) with the dispersing agent, recombinant human hyaluronidase (rHuPH20) in patients with hereditary angioedema (HAE).

METHODS: In this randomized, double-blind, dose-ranging, crossover study, patients ≥12 years of age with a confirmed diagnosis of HAE were randomly assigned to receive subcutaneous injections of 1000 U C1-INH with 24,000 U rHuPH20 (treatment A) or 2000 U C1-INH with 48,000 U rHuPH20 (treatment B) every 3 or 4 days for 8 weeks and then crossed over to the other treatment for another 8-week period. Pharmacokinetic assessments included plasma C1-INH antigen and functional C1-INH activity. C4 was used to assess pharmacodynamics.

RESULTS: The study was terminated early as a precaution related to non-neutralizing antibodies to rHuPH20. Forty-seven patients had been randomized and treated. Evaluable samples for pharmacokinetic analyses were available for 26 and 29 patients who received treatments A and B, respectively; 18 of these received both treatments. Observed mean C_{max} of functional C1-INH activity at steady state (week 8) for treatment B was 1.28-fold higher than for treatment A (0.408 and 0.319 U/mL, respectively), while the mean baseline-adjusted C_{max} of functional C1-INH was 2.0-fold higher (0.240 and 0.122 U/mL, respectively). Similar results were observed for C4.

CONCLUSIONS: Patients receiving either 1000 U or 2000 U of C1-INH with rHuPH20 can achieve pharmacologically active functional C1 INH. Patients receiving 2000 U of C1-INH had approximately double exposure for baseline adjusted functional activity compared with those receiving 1000 U.

Clinical Usage of a C1 Esterase Inhibitor Concentrate for Hereditary Angioedema: Final Results from a Large International Registry

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RATIONALE: The plasma-derived, pasteurized, nanofiltered C1-Inhibitor concentrate (pnC1-INH; Berinert®/CSL Behring) has been used for the management of hereditary angioedema (HAE) for over 35 years in Europe and since 2009 in the US. An international patient registry was created to gather more administration and safety data for Berinert.

METHODS: The registry was conducted at 34 US and 7 European sites. Both prospective and retrospective data were collected from 2009 to 2014 on indication, dosing, HAE attacks, treatment location (home or medical facility), and adverse events (AEs).

RESULTS: Data were collected on 14,819 Berinert infusions in 318 patients (65.1% female; mean age 38.8 y, including 18 patients aged 3 to 12 y (275 infusions). Most infusions (11,720; 79.1%) were administered as on demand treatment of attacks. Approximately 95% of infusions were self-administered in a home setting. Failure to self-infuse was reported rarely (13 events in 6 patients; <0.1% of all infusions). The mean US dose of pnC1-INH per infusion (18.2 IU/kg) was higher than that in Europe (10.1 IU/kg). At least one laryngeal attack was experienced by 85/318 (26.7%) registry subjects. A total of 252 AEs were reported for an overall rate of 0.02 events per infusion; nine AEs were considered related to pnC1-INH. The rate of AEs did not correlate with the pnC1-INH doses. One of three reported TEsIs, deep vein thrombosis, was considered possibly related; however, it had a plausible alternative causality, a central IV port.

CONCLUSIONS: These registry findings indicate widespread safe use of pnC1-INH in HAE, mostly as home treatment.
**624 Efficiency and Safety of Long Term Prophylaxis with Berinert during Hereditary Angioedema Pregnancies: Data from the French Register Cobra**

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**RATIONALE:** Almost 40% of hereditary angioedema women worsen during pregnancies. This disease is life-threatening for the mother but also for the baby. Patient’s quality of life impaired dramatically. On demand treatment with C1Inh concentrate is often efficient but sometimes long term prophylaxis (LTP) is needed. Danazol is not recommended during pregnancies. We described our experience of LTP with Berinert (C1Inh concentrate, Behring CSL).

**METHODS:** We have collected data from the French national register COBRA. From 2004, every patients infused with Berinert was recorded in this cohort.

**RESULTS:** We obtained data from 4 pregnancies. Three women who have hereditary angioedema type I, have had 4 pregnancies at the median age of 28 years. Before pregnancies, patients presented very few attacks. Since the very beginning of their pregnancies, the disease worsened with 1-2 attacks per week: abdominal, ENT or combined. 1000-1500 units of Berinert per week were not efficient to limit acute attacks. With 1500 units of Berinert, 2 times a week, patients have experienced no more attacks until deliver. Each delivery has been prepared with short term prophylaxis with Berinert. Mothers and babies remain safe without any attack. No side effects were reported.

**CONCLUSIONS:** LTP with Berinert during pregnancies is very effective and safe. Patients needed at least 2500-3000 units per week.

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**625 Efficacy and Safety of a C1 Esterase Inhibitor Concentrate for Long-Term Prophylaxis in Hereditary Angioedema: Findings from a Large International Registry**

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**RATIONALE:** The plasma-derived, pasteurized, nanofiltered C1-Inhibitor concentrate (pnC1-INH; Berinert®/CSL Behring) is approved as on demand treatment for hereditary angioedema (HAE) attacks in the US and as on demand and short-term prophylaxis in Europe. This is to report data gathered through an international patient registry regarding the use of pnC1-INH for long-term prophylaxis (LTP).

**METHODS:** The registry (34 US and 7 European sites) collected prospective and retrospective data between 2009 and 2014 on patients using Berinert for any reason, including LTP. Data were gathered on indication, dosing, attacks, treatment location (home or medical facility), and adverse events (AEs).

**RESULTS:** The registry included 48 patients (81.3% female; mean [SD] age 45.4 [16.2] y) who reported Berinert LTP usage, reflecting 4111 Berinert LTP infusions and a total of 474.1 months of LTP administration. The median LTP dose per infusion was 1000 IU (range, 500 to 3000 IU). The breakthrough attack rate per month was 0.53 and the majority of breakthrough attacks were abdominal (0.19 per month). The mean (SD) time between the most recent LTP Berinert administration and a breakthrough attack onset was 73.7 (32.3) hours. A total of 82 AEs were reported in 16 patients using LTP (0.02 events per infusion; 0.17 events per month); only 3 AEs were considered related to pnC1-INH (non-cardiac chest pain; post-infusion headache; deep vein thrombosis in a patient with an IV port).

**CONCLUSIONS:** In this international registry, individualized dosing of pnC1-INH for LTP in HAE patients was safe and efficacious with a low breakthrough attack rate of <1 attack/month.
**627 The Icatibant Outcome Survey: More Than 1500 Icatibant-Treated Attacks in Patients with Type I or II Hereditary Angioedema**

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**RATIONALE:** Icatibant is a bradykinin B2 receptor antagonist used to treat attacks of hereditary angioedema (HAE) due to C1-inhibitor deficiency in adults. The Icatibant Outcome Survey (IOS; NCT01034969) is an international observational study monitoring the safety and effectiveness of icatibant in a real-world setting. Here we report data from the first 1716 icatibant-treated attacks in patients with HAE type I or II.

**METHODS:** IOS is conducted at 47 centers in 11 countries. Patient characteristics and icatibant treatment outcomes were recorded at clinic visits. Descriptive retrospective analyses were performed on data collected from July 2009–July 2014.

**RESULTS:** Icatibant was used to treat 1716 angioedema attacks in 353 patients with type I or II HAE. Mean age at enrollment was 41.1 years (range 16.5–79.0), and 60.2% of patients were female. Proportions of very mild/mild, moderate, and severe/very severe attacks were 8.4%, 29.8%, and 61.8%, respectively (N=1545 attacks). Of attacks with anatomical location data (N=1682), 56.8% affected the abdomen, 40.4% affected the skin, and 6.8% affected the larynx. Most icatibant injections were self-administered (N=1289/1585 attacks; 81.3%). Median time to icatibant administration was 1.0 hour (N=794 attacks). Median time to symptom resolution was 4.9 hours (N=860 attacks). Median attack duration was 7.0 hours (N=692 attacks). Icatibant was well tolerated, with no unexpected safety outcomes.

**CONCLUSIONS:** IOS has accumulated a large database of patients with HAE, providing insight into the characteristics of this rare disease. In addition, treatment outcomes of icatibant in the real world were consistent with those from the Phase III studies.

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**628 History of Misdiagnosis in Patients with Hereditary Angioedema Participating in the Icatibant Outcome Survey**

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**RATIONALE:** Hereditary angioedema (HAE) causes swelling in the skin and airways and pain in the abdomen due to mucosal swelling. HAE is frequently unrecognized and misdiagnosed, leading to delays in diagnosis, inadequate treatment, and potentially unnecessary procedures. We evaluated the history of misdiagnosis in patients who are participating in an international registry, the Icatibant Outcome Survey (IOS; NCT01034969).

**METHODS:** IOS is an observational study in which the safety and effectiveness of icatibant has been evaluated since 2009. As part of IOS, patients record any misdiagnoses received before being diagnosed with HAE.

**RESULTS:** In April 2014, 318 of 512 patients with HAE type I/II enrolled in IOS had provided misdiagnosis data. Of these, 151/318 (47.5%) had received ≥1 prior misdiagnosis. The most common misdiagnoses were allergic angioedema (n=86/151, 57.0%), appendicitis (n=46/151, 30.5%) and other forms of non-allergic angioedema (n=35/151, 23.2%). A wide variety of other misdiagnoses were reported, with approximately 20% being gastrointestinal disorders (excluding appendicitis). There were no substantial differences in misdiagnoses between males (46.6%) or females (48.1%), or for HAE type I (46.9%) vs HAE type 2 (53.8%). Having family members with HAE reduced the likelihood of misdiagnosis vs those without (45.2% [n=114/252] vs 62.0% [n=31/50]; p=0.030). Patients with a prior misdiagnosis had longer median delay to HAE diagnosis (13.3 years) than patients without (2.3 years; p<0.001).

**CONCLUSIONS:** From this large database, almost 50% of patients with HAE type I/II have previously been misdiagnosed, most commonly with allergic angioedema or appendicitis.

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**629 Development and Characterization of an Anti-FXIIa Monoclonal Antibody for the Treatment of Hereditary Angioedema**

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**RATIONALE:** Hereditary angioedema (HAE) is characterized by recurrent and potentially life-threatening bradykinin-mediated edema. There is an unmet medical need for a safe and long-lasting prophylactic treatment for HAE. We have generated a potent and specific FXIIa blocking antibody and demonstrated that targeting FXII represents a promising prophylactic therapy for HAE.

**METHODS:** A human phage display antibody library was used to generate a fully human recombinant anti-FXIIa antibody. Enzyme assays were performed to determine its specific and potent inhibition of FXIIa. The in vivo efficacy was investigated in various murine edema models.

**RESULTS:** The anti-FXIIa mAb 3F7 is a potent and highly specific inhibitor of the proteolytic activity of FXIIa. 3F7 binds to rabbit, mouse and human activated FXII. Administration of 3F7 abrogated skin edema induced by contact activation triggered by mast-cell released heparin in mice. 3F7 was also shown to abolid bradykinin-mediated increase of vascular permeability induced by the angiotensin-converting enzyme (ACE) inhibitor captopril in C1-inhibitor deficient mice. Comparison of 3F7 with current HAE therapeutics in these murine edema models revealed that 3F7 has potent and prolonged efficacy. CSL312, a variant of 3F7 with improved affinity and potency, effectively inhibited dextran sulphate-triggered FXII contact activation and bradykinin formation in plasma of healthy donors and HAE patients.

**CONCLUSIONS:** The preclinical data provide strong evidence that CSL312, a potent and specific inhibitor of FXIIa, represents an effective prophylactic treatment option for HAE.
Bars to the Self-Administration of Medication in the Treatment of Hereditary Angioedema (HAE)

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Rationale: Early therapy of Hereditary Angioedema (HAE) reduces morbidity, improves outcomes, reduces absenteeism, and mortality. This can be accomplished best with self-therapy. Previously we have examined barriers to self-therapy from the perspective of the nurse and physician, but data is lacking on what patients perceive as major barriers to self-administered therapy for HAE. Our aim was to identify those barriers.

Methods: After IRB approval, we performed a phone survey of HAE patients from a database of patients who were recently seen in clinic. Patients seen for research only were excluded. Patients of all ages were included. The survey focused on anxiety, depression, stress, concerns of route of therapy, the ability to inject themselves, and what they perceive as barriers to providing self-care.

Results: Not all patients could be contacted. We contacted 92 patients and 59 agreed to participate. With 69% of those patients currently undergoing self-administered treatment, the results show an increased reduction in depression and anxiety, a higher satisfaction rating with treatment, and greater compliance with treatment. In those not yet on self-administered therapy, the majority wanted to be, despite being satisfied with the care received in the Emergency Department. They also felt care at home would be optimal. The main concern of both groups was not being able to treat themselves when they need it.

Conclusions: From our data it is obvious that most patients are willing to self-treat. This suggests that physicians should encourage self-treatment of HAE to improve outcomes and quality of life of patients with HAE.

Assessment of Adherence to Hereditary Angioedema Guidelines in Pediatric Population

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Rationale: Hereditary angioedema (HAE) is a life threatening and disabling disease caused by a deficiency of C1 esterase inhibitor which may occur at any age, but attacks usually begin during school-age years or adolescence. Evidence based recommendations have been established that describe key components in therapeutic management in specific regards to screening labs and follow up recommendations. The aim of this retrospective study is to determine whether pediatric HAE patients receive quality care consistent with the international consensus expert recommendations.

Methods: Retrospective electronic chart review was performed on 20 pediatric patients with confirmed diagnoses of HAE between 2000 and 2013. This quality assurance study met IRB exception.

Results: Of the 20 patients, only 65% were given an acute action plan. Medication use for acute attacks included 58% who received C1 esterase inhibitor (C1-INH), 5% received ecallantide, and 32% received icatibant. For prophylaxis of attacks, only 15% patients received C1-INH. Hepatitis-B, Hepatitis-C, and HIV screening was performed in only 45% patients. Only 55% patients had annual follow up visits with allergist.

Conclusions: Routine annual follow up, medication action plans, and screening labs prior to blood product administration are lacking in the management of pediatric HAE patients. Quality assurance programs as well adherence to recommended guidelines are important to provide quality care for pediatric HAE patients.

Hereditary Angioedema with Normal C1 Inhibitor: An Italian Survey

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Rationale: Hereditary Angioedema (HAE) is characterized by sudden swellings of the subcutaneous or submucosal tissues. Most patients have C1-inhibitor (C1-INH) deficiency. HAE with normal C1-INH (nC1-INH-HAE) has been described associated to mutations in factor XII (FXII-HAE) or with unknown genetic defect (U-HAE). This study reports the clinical and genetic features of Italian patients with nC1-INH-HAE.

Methods: Subjects were from the Italian network of HAE collecting 1000 entries. Fourteen males and 14 females, from 14 independent families and with normal functional levels of C1-INH, were studied. Twenty-five had history of angioedema. All subjects were investigated for mutations in the whole F12 coding region and SERPING1.

Results: No subject had mutations in SERPING1. In F12 we identified mutation p.Thr309Lys in 3 women with angioedema and 3 (2 females and 1 male) asymptomatic relatives of one of them. In the remaining 22 patients the genetic analysis of F12 gene was unsuccessful and no mutation was found. Sequencing analysis revealed the presence of different SNPs that were previously described and do not affect protein activity or function.

Conclusions: Our data identify 3 subjects with FXII-HAE, 3 asymptomatic carriers of F12 mutation and 22 subjects with U-HAE. They indicate that FXII-HAE is less frequent in Italy than in other European countries. This uneven distribution probably results from the fact that p.Thr309Lys, the most common mutation related to FXII-HAE, derives from a common founder. In U-HAE subjects the screening of entire F12 excludes this gene as related to HAE; the underlying cause of this disease remains to be defined.
Hereditary Angioedema Deaths: A Review from the Romanian Registry

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RATIONALE: Hereditary angioedema (HAE) is a life-threatening rare disorder that may be lethal in over 30% of cases. We describe the occurrence of fatalities, caused by upper respiratory tract obstruction attacks, in our HAE patient database.

METHODS: The Romanian HAE Registry includes up to date 86 patients with both types of C1 inhibitor deficiency, belonging to 41 affected families. Deaths linked to HAE of their relatives were recorded throughout a template similar to the former European HAE registry. We also describe 4 cases deceased in the past 4 years.

RESULTS: We have identified 24 relatives of our 86 cases who died in the last year. All abstracts are strictly embargoed until the date of presentation at the 2015 Annual Meeting.

CONCLUSIONS: Lack of the awareness of both physicians and patients about the risks and unavailability of specific treatment was responsible for deaths of our HAE cases. Comprehensive intensive educative national programs and reimbursement of drugs recently registered are essential to reduce mortality in lower income countries.
636 Successful Management of Hereditary Angioedema (HAE) and Thrombophilia during Pregnancy: A Case Study
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RATIONALE: The rate of HAE attacks often increases during the second and third trimesters of pregnancy. Therapeutic options during pregnancy are limited, and patients require vigilant care and close monitoring. Hospital delivery is highly recommended, and in the case of caesarean section prophylaxis with C1-inhibitor (C1-INH) is advised. Here we present the case of a 33-year-old woman with type I HAE who suffered seven spontaneous abortions between November 2000 and October 2011.
METHODS: Thrombophilia screening and C1-INH self-administration training was performed at the Haemophilia Centre Rhine Main.
RESULTS: The patient presented in March 2013, experiencing ~2 HAE attacks per month. A diagnosis of thrombophilia was initially excluded; however, additional screening identified a heterozygous MTHFR C677T mutation, which can be associated with spontaneous abortion. The patient was therefore treated with daily subcutaneous low-molecular-weight heparin, which was adjusted during her subsequent pregnancy. The frequency of HAE attacks increased during the second and third trimesters, which were treated with individualised C1-INH replacement therapy. The patient self-administered pasteurised nanofiltered C1-INH concentrate for each attack early during the prodrome phase. All attacks were treated successfully and safely; the combination of this with the thrombophilia treatment allowed the patient to undergo caesarean section and give birth to a healthy girl at week 39.
CONCLUSIONS: This unusual case involved a patient with both HAE and undiagnosed thrombophilia, who suffered from a long history of spontaneous abortions. Correct diagnosis and treatment allowed childbirth, highlighting the importance of multi-disciplinary cooperation in HAE treatment and the need for an individualised management plan during pregnancy.

637 Genetic Analysis As a Practical Tool to Diagnose Hereditary Angioedema with Normal C1 Inhibitor: A Case Report
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RATIONALE: Hereditary angioedema (HAE) with normal C1-INH poses a diagnostic challenge in clinical practice. We report a patient presenting recurrent episodes of severe abdominal pain and ascites as her major symptoms.
METHODS: 35 years-old female with recurrent abdominal pain attacks since age of 16. In the past 5 years, abdominal attacks became severe, associated with vomiting and diarrhea. Computed tomography revealed ascites and bowel wall edema. She presented episodes of lip and face edema, lasting for 48-72 hours, despite IV corticosteroids and anti-histamines. Symptoms worsened with start of estrogen/progestogen contraceptive. Patient had asthma in childhood, and currently has mild rhinitis; in use of Pantoprazole and Panaverium Bromide. Genetic analysis comprised sequencing of the 8 exons of the C1-INH coding gene SERPING1, and of exons 9-10 of the gene encoding coagulation Factor XII (F12).
RESULTS: During attacks, serum amylase and lipase were normal, with elevated white cell counts (13.970/mm3, 91% neutrophils) and C-reactive protein, without eosinophilia. Endoscopy revealed esophagitis involving distal esophagus and mild pan-gastritis, colonoscopy was normal. In our Allergy Clinic she was switched to progestogen-only pill, with remission of symptoms. C1-INH and C4 were normal, C1-INH activity was 30%.
CONCLUSIONS: Identification of an F12mutation is the only way to diagnose HAE with normal C1-INH. Our results provided support to prescribing HAE specific treatment for acute attacks to our patient, in addition to progestogen-only oral contraceptive.

638 Clinical and Genetic Investigation in a Family Segregating Different Types of Angioedema, Including a Case of Hereditary Angioedema Type-III
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RATIONALE: Hereditary angioedema with normal C1-INH (HAE Type-III) manifests with recurrent angioedema but normal C1-INH concentration and activity. One subgroup of HAE Type-III has mutations in the gene encoding factor XII (HAE-FXII), mostly involving exon 9. In another subgroup no mutations are found in this gene (HAE-unknown). We present a case of HAE Type-III with a family history of vibratory and undifferentiated angioedema, so far unreported clustering of angioedema.
METHODS: Genomic DNA of the patient and two affected sons was extracted from peripheral blood and exon 9, intron 9 and exon 10 of F12 was sequenced using Sanger sequencing.
RESULTS: A 41 year old woman with severe episodes of swelling had normal C4 and C1-INH level and function. Angioedema resolved after a large ovarian cyst was removed. One son developed vibratory angioedema, a rare form of physical urticaria with no known genetic cause. Her second son and two other relatives in Italy have angioedema of yet undiagnosed type. Direct sequencing for exon 9, intron 9 and exon 10 of the Factor XII gene showed no protein changing mutations. A different gene may be the underlying reason for this disease; exome sequencing employing the Illumina platform is used to search a new protein coding changes to explain the phenotype of this family.
CONCLUSIONS: This is the first case of a familial cluster of different types of angioedema, proposing an interesting question of genetic variations predisposing to angioedema and a new potential causal gene for angioedema in this family.

639 Hereditary Angioedema Type III in Young Male Siblings
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RATIONALE: Hereditary angioedema type III is familial, non-histaminergic angioedema with normal C1-INH. Initially described in women, it has since been found in men. Mean age at onset is 26.8 years. We present the case of two brothers < 12 years with recurrent angioedema consistent with hereditary angioedema type III.
METHODS: A 7 y.o. AAM presented with recurrent episodes of angioedema in the extremities. No family history of recurrent angioedema was identified, although mother reported limited knowledge of paternal family history. His 5 y.o. brother subsequently began having recurring episodes of extremity angioedema. Episodes were typically precipitated by trauma and were associated with functional limitation and missed days of school.
RESULTS: Imaging during an episode of angioedema in each brother was consistent with soft tissue swelling. Muscle enzymes, CMP, tryptase, C4, and C1 inhibitor level and function were repeatedly normal. Neither brother responded to oral antihistamines or oral steroids. Episodes were not associated with systemic symptoms and self-resolved within 1-2 weeks. At the onset of next attack, treatment with plasma derived C1-INH or bradykinin receptor antagonist will be tried.
CONCLUSIONS: Recurrent angioedema not responsive to antihistamines or steroids in these brothers is likely secondary to hereditary angioedema type III. Treatment with kalilkinin inhibitors, bradykinin receptor antagonists and plasma derived C1-INH has been successful in patients with HAE type III. Awareness of onset of HAE type III in children is important in facilitating early diagnosis and treatment, and decreasing morbidity.
640 A Type III Hereditary Angioedema 45y-Old Female Patient Presents with a Rare Complication of Acute Atraumatic Compartment Syndrome in Arms and Legs: A Case Report
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RATIONAL: HAE is a rare life-threatening condition characterized by recurrent angioedema affecting the face, upper airways, GI, and limbs due to bradykinin dysregulation. If not diagnosed and managed appropriately, severe complications may ensue. Three types of HAE have been described: Type I and II due to C1-esterase-inhibitor (C1-INH) deficiency, and a third type with normal C1-INH level and function and an unclear pathophysiology. Idiopathic bradykinin-mediated angioedema also occurs but confirmatory diagnostic tests are lacking. We present a rare serious complication in a patient with recurrent severe extremity angioedema and resulting acute-atrumatic compartment syndrome (ACS) due to apparent bradykinin-mediated angioedema.

METHODS: Case description with clinical assessment and diagnostic tests: C1-INH quantitative/qualitative tests, C4 serum levels, CT scans of extremities.

RESULTS: An adopted 45 y-old female presented with severe angioedema episodes since puberty. Her symptoms were typical of HAE including serious gastrointestinal angioedema that led to numerous abdominal surgeries. Distinctively, she developed ACS in all four limbs at different times, requiring fasciotomy because of severe nerve and vascular compromise. Lab assessment excluded C1-INH deficiency but HAE with normal C1-INH could not be ruled-out due to lack of available family history. Clinical evaluation demonstrated complete unresponsiveness to histamine-targeted therapies. The patient was treated with ecallantide for acute angioedema and eventually placed on treatment with prophylactic C1-INH. Both medications were clinically effective with dramatic reduction in angioedema episodes and severity and presented no further complications from ACS.

CONCLUSIONS: This case highlights the need for prompt clinical and diagnostic evaluation of angioedema and specific treatment to prevent serious complications.

641 Hereditary Angioedema with Normal C1-INH with Versus without a Specific Mutation in the F12 Gene
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RATIONAL: Hereditary angioedema (HAE) with normal C1 inhibitor (HAE(C1)) may or may not be linked with a mutation in the coagulation factor XII gene. To assess the clinical, genetic, and laboratory differences between HAE(C1) with a F12 mutation (HAE-FXII) and HAE(C1) without a F12 mutation (HAE-unknown).

METHODS: Sixty-nine patients with HAE-FXII coming from 23 families and 196 patients with HAE-unknown coming from 65 families were studied.

RESULTS: Pedigree analysis revealed an autosomal dominant inheritance with incomplete penetrance for both conditions. The penetrance of HAE-FXII was 83.8% in women and 4.3% in men. The male to female ration was 1:68 in HAE-FXII and 1:6.2 in HAE-unknown. The mean age of onset of clinical symptoms was 20.3 years in HAE-FXII and 29.6 years in HAE-unknown. Death by asphyxiation occurred in 2/69 (2.9%) patients with HAE-FXII and 5/196 (2.6%) patients with HAE-unknown. In 48/58 (82.8%) women with HAE-FXII and 52/144 (36.1%) women with HAE-unknown who took oral contraceptives or received hormonal replacement therapy or were pregnant there was an impact of those high-estrogen periods on their disease. Slightly decreased C1-INH activity and C4 were observed in more patients with HAE-FXII than with HAE-unknown. In both patient groups abnormalities were seen in FXI and FXII activity, plasminogen activator inhibitor and in the activated partial thromboplastin time in some patients. No abnormalities were found in the tests for C1-INH, a2-macroglobulin, antithrombin III, and angioteins-converting enzyme in both groups.

CONCLUSIONS: HAE-FXII and HAE-unknown differ in several respects including clinical symptoms whereas the laboratory findings were mainly similar.
643 Profile of Seasonal Differences in Angioedema Presenting to an Inner City Hospital

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RATIONALE: We have previously reported there is a significant increase in angioedema, but not urticaria, in the summer months. In order to further understand this increase, we have investigated the difference in the clinical profiles of patients who are diagnosed in summer months vs. those treated in the winter months.

METHODS: A retrospective EMR chart review of the cases of patients treated for angioedema from 2007 to 2012 in either summer (June, July, August) or winter (December, January, February). Data gathered included probable triggers (medication, food, or unknown), body location (face, lips, mouth/throat, and/or extremities/others), duration of symptoms before seeking medical care, and AM or PM presentation at the hospital. Statistical methods included Chi-square test, Fisher’s Exact test, and Mann-Whitney test.

RESULTS: A total of 96 cases of angioedema were diagnosed in summer, vs. 65 in winter (p=0.015). There was no significant difference in associated triggers between the two seasons (p=0.186). There was a significant increase in facial edema occurring with winter presentation (24/65 vs. summer 19/65 (p=0.016). There was no difference in AM vs. PM presentation (AM summer 49/88 (59%) vs AM winter 34/63 (41%), p=0.87) or duration of symptoms (mean ± SD: summer: 2.0 ± 2.2 hours vs. winter 1.6 ± 1.6 hours) before presentation (0.09).

CONCLUSIONS: Seasonal influence on angioedema includes increase in frequency in summer months and increased facial angioedema in winter months.

644 Epidemiology of Angioedema without Wheels in an Allergy and Immunology Clinic

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RATIONALE: We describe the epidemiology, clinical course, family history and response to treatment of patients consulting with angioedema without wheals (AWW) to an Allergy and Immunology Clinic.

METHODS: We reviewed the case records of all patients consulting our office with a preliminary diagnosis of AWW from January 1997 to April 2013. We recorded sex, age, at onset of symptoms, family history of angioedema, number of visits to the office, type of angioedema presumed and response to treatment. We classified angioedema according to its pathophysiology. We also described those patients with angioedema mimics.

RESULTS: From a total of 17.823 new patients, 303 had a presumptive diagnosis of AWW, with an estimated frequency of 1.70%. Forty percent were men and 60% were female. Average age at first visit was 40.6y0 but was earlier in hereditary angioedema than in other causes of AWW. Average number of visits was 2.39. Fifty seven patients referred a family history. We attributed idiopathic angioedema to 55.7% of patients, 24.3% were drug related, 15.7% were due to C1 inhibitor deficiency, 2.14% were drug related + idiopathic angioedema, 1.43% were type III and 0.71% had exercise induced angioedema. Ninety six percent of 53 evaluable idiopathic angioedema patients referred a benefit with anti-histamine therapy. Twenty three (7.59%) had a mimic of angioedema.

CONCLUSIONS: AWW was a rare consultation case. Most of our patients had anti H1 responsive idiopathic angioedema and none had allergic angioedema. Women prevailed over men. Family history and average age of symptoms onset was different among different types of angioedema.

645 Angioedema without Urticaria, at the Emergency Department Marta Suero, MD, Maria Elisa Caralli, MD, Sarah Micozzi, MD, Dasha Roa Medellin, MD, Mercedes Saenz de Santa Maria, MD, 1 Hospital General Universitario Gregorio Maraño, Allergy Department, Madrid, Spain, 2 Hospital General Universitario Gregorio Maraño. Allergy Department., Madrid, Spain.

RATIONALE: To determine the incidence and characteristics of Angioedema without urticaria (AE) at the Emergency room (ER) of a third level Hospital.

METHODS: Observational study between January-december 2013. Patients older than 14 years old, with AE, were included.

RESULTS: A total of 149.194 patients were attended. Incidence: 0.18%. Mean age: 47.8 years. Females: 66.77%. Facial location in 83.69%, 21.74% had pharyngolaryngeal involvement, one needed intubation. The 84.78% responded to antihistamines and corticoids, 6.75% did not respond (mean age 43.9 and 53.45, p<0.05), no gender differences. The response in 7.43 % was uncertain. At the ER drug allergy was suspected in 17%, food in 15%. The average stay in the ER was of 251 and 455 minutes, for the anhistamine responders and non-responders respectively. P<0.005; 148 were finally studied at the Allergy Unit. After reevaluation, 11.48% were diagnosed with anaphylaxis and withdrawn from the study. Not having a suspected cause at the ER was a motive to seek for the allergy workout (OR: 1.72, p<0.05). After the study, 67.17% were histaminergic (10.34% drugs, 8.04% food, 13.79% Anisakis simplex, 39.08% idiopathic), and 32.82% non-histaminergic (4.65% hereditary AE, 90.69 % ACEi related AE,4.65% idiopathic). Only 12.36% of drug or food allergy suspicion was confirmed.

CONCLUSIONS: The incidence of AE without urticaria is low. It is more often histaminergic, in females, with frequent facial and uncommonly severe faring-laryngeal involvement. Not having a suspected cause at the ER is a motivation to seek for allergy study. The etiology impression at the ER is rarely confirmed.

646 A Novel Form of Recurrent Angioedema

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RATIONALE: Angioedema occurring without co-existent urticaria may be due to hereditary angioedema (HAE), acquired C1 esterase inhibitor deficiency (AAE), or drug-induced angioedema (DIA). We describe a cohort of patients with recurrent idiopathic angioedema, distinct from the known causes listed above.

METHODS: Patients were accrued prospectively from an academic allergy practice. After institutional research ethics board approval, patients’ charts were reviewed and demographic, clinical and laboratory data extracted.

RESULTS: 29 patients were recruited between 2007 - 2014. The mean age at presentation was 52.34 years (range 19–84 years) and 48% were male. The mean number of episodes per year was 21.4 (range 2–96) and mean duration of episodes was 24.48 hours (range 3–96). About half of episodes (46%) began overnight. Areas of involvement were upper airway (24%), lips (23%), tongue (14%), eyelids (11%), feet (9%) and hands (8%). None of the patients had low C3, C4, or CH50; none had positive ANA; C1 esterase inhibitor level and C1q were normal in all patients tested. 13% of the patients had low C3, C4, or CH50; none had positive ANA; C1 esterase inhibitor level and C1q were normal in all patients tested.

CONCLUSIONS: Herein, we report a novel form of angioedema without urticaria, mediated by histamine and leukotrienes. Clinical, demographic and therapeutic characteristics differentiate this from other recognized causes of angioedema.
Anaphylaxis to Mint in a 5 Year Old Boy: A Case Report
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RATIONALE: Mint is derived from the aromatic plant *Mentha spicata*; together with oregano, rosemary, basil and thyme, it is a member of the *Lamiaceae* family. Allergic contact dermatitis to mint is well described. We report the first case of delayed IgE-mediated anaphylaxis to mint in a pediatric patient.

METHODS: A 5 year-old boy with a history of asthma presented with multiple adverse food reactions. His first reaction was at age 4; several hours after swallowing a few pieces of mint gum he awoke with 5 episodes of emesis and an erythematous rash to arms and legs. Several hours after chewing mint gum he vomited multiple times. Several hours after eating a chocolate mint he vomited. In contrast to these delayed reactions, the child had immediate reactions of repeated vomiting 30-45 minutes after using mint toothpaste. Subsequent reactions to mint involved repeated emesis, erythematous rash to arms and legs, and diarrhea.

RESULTS: Skin prick testing (SPT) was performed to fresh leaves of mint, oregano, rosemary, basil, and thyme; peppermint gum; aeroallergens; milk; egg; wheat; soy; and peanut. Five controls had SPT to fresh mint leaf for comparison. The patient had a positive SPT to fresh mint leaves and to standardized dust mite mix. No control subjects reacted to fresh mint. Serum specific IgE to mint, sage, basil, oregano and thyme were undetectable. The patient was advised to strictly avoid mint; an allergy action plan and epinephrine autoinjector were provided.

CONCLUSIONS: We report the first case of delayed IgE-mediated anaphylaxis to mint in a pediatric patient.

Deficiencies in STAT3 Signaling Confers Resistance to Histamine/PAF Induced Vascular Permeability in Autosomal Dominant-Hyper IgE Syndrome (AD-HIES)
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RATIONALE: To determine whether a reduction in STAT3 signaling due to small molecule inhibition or in vivo loss of function, promote an exaggerated allergic inflammatory response and increase susceptibility to allergic phenotypes. We hypothesized that the IL-4RaY500F mutation would enhance the susceptibility of mice to food-induced anaphylaxis.

METHODS: We examined signs of anaphylaxis (hyperthermia, hematocrit concentration), evidence of mast cell activation (intestinal mast cell and mast cell protease-1 (mcpt-1) levels) in passive systemic and oral-antigen-induced anaphylaxis and histamine-induced hypothermia models in WT and IL-4RaY500F mice. In vitro studies were used to determine the interaction of histamine and phosphatidylinositol 3-kinase (PI3K) activity on endothelial barrier function in a human vascular endothelial cell line.

RESULTS: In the present study, we show that ablation of IL-4Ra–induced PI3K signaling by germline point mutation within the IL-4Ra motif (IL4RaY500F mice) did not alter susceptibility to IgE-mediated, food-induced anaphylaxis. Moreover, diarrhea occurrence, antigen-specific IgE and intestinal mastocytosis were comparable between WT and IL-4RaY500F mice. However, mice unable to stimulate IL-4Ra–mediated PI3K signaling had accelerated disease progression. Moreover, the IL-4RaY500F mice demonstrated a more rapid decrease in body temperature than the WT mice. Notably, the accelerated anaphylactic response was associated with more rapid histamine-induced hypovolemia. Mechanistic in vitro and in vivo analyses revealed that endothelial PI3K signaling negatively regulates histamine-induced response.

CONCLUSIONS: These results define an unanticipated role for IL-4Ra-mediated PI3K signaling in negative regulation of IgE-mediated anaphylactic reactions.

Loss of IL-4Ra-Mediated PI3K Signaling Accelerates the Onset and Progression of IgE/Mast Cell-Mediated Reactions
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RATIONALE: Clinical and experimental evidence indicates that interleukin 4 (IL-4) receptor (IL-4R) chain motif polymorphisms that negatively regulate the IL-4/IL-13 signal are sufficient to induce a gain-of-function, promote an exaggerated allergic inflammatory response and increase susceptibility to allergic phenotypes. We hypothesized that the IL-4RaY500F mutation would enhance the susceptibility of mice to food-induced anaphylaxis.

METHODS: We examined signs of anaphylaxis (hyperthermia, hematocrit concentration), evidence of mast cell activation (intestinal mast cell and mast cell protease-1 (mcpt-1) levels) in passive systemic and oral-antigen-induced anaphylaxis and histamine-induced hypothermia models in WT and IL-4RaY500F mice. In vitro studies were used to determine the interaction of histamine and phosphatidylinositol 3-kinase (PI3K) activity on endothelial barrier function in a human vascular endothelial cell line.

RESULTS: In the present study, we show that ablation of IL-4Ra–induced PI3K signaling by germline point mutation within the IL-4Ra motif (IL4RaY500F mice) did not alter susceptibility to IgE-mediated, food-induced anaphylaxis. Moreover, diarrhea occurrence, antigen-specific IgE and intestinal mastocytosis were comparable between WT and IL-4RaY500F mice. However, mice unable to stimulate IL-4Ra–mediated PI3K signaling had accelerated disease progression. Moreover, the IL-4RaY500F mice demonstrated a more rapid decrease in body temperature than the WT mice. Notably, the accelerated anaphylactic response was associated with more rapid histamine-induced hypovolemia. Mechanistic in vitro and in vivo analyses revealed that endothelial PI3K signaling negatively regulates histamine-induced response.

CONCLUSIONS: These results define an unanticipated role for IL-4Ra-mediated PI3K signaling in negative regulation of IgE-mediated anaphylactic reactions.
650 The Role of Basophils and Pro-Allergic Cytokines, TSLP and IL-33, in Cutaneously-Sensitized Food Allergy
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RATIONALE: We frequently encounter patients presenting with immediate allergic reactions after first consumption of a food in children. Recently, the concept of cutaneously-sensitization with a food antigen has been established. However, the mechanisms underlying the process have not been fully elucidated. The purpose of this study is to clarify the mechanism of cutaneously-sensitized food allergy, focusing on basophils and pro-allergic cytokines, TSLP and IL-33.

METHODS: We established cutaneously-sensitized food allergy mouse model. Briefly, mice were epicutaneously sensitized with ovalbumin (OVA) at SDS-treated shaved skin followed by oral challenge with OVA. In some experiments, TSLP receptor-deficient mice and IL-33 deficient mice were used. We measured OVA-specific IgE antibody titer for evaluation of sensitization, and the change of rectal temperature as an indicator of systemic anaphylaxis.

RESULTS: Epicutaneously-sensitized mice produced OVA-specific IgE and developed IgE-mediated anaphylaxis after oral challenge. Basophil-depleted or TSLP-receptor-deficient mice did not produce OVA-specific IgE and were protected from oral challenge-induced anaphylaxis. IL-33 deficient mice produce normal levels of OVA-specific IgE, however, IL-33 deficient mice and mice treated with recombinant soluble IL-33 receptor were protected from anaphylaxis. Thus, basophils and TSLP have pivotal roles in Th2 development in the skin during the sensitization phase of food allergy. In contrast, while IL-33 is dispensable for promoting cutaneous antigen sensitization, the cytokine is essential for inducing IgE-dependent anaphylaxis in the gut.

CONCLUSIONS: Basophils/TSLP-pathways can be targeted to manage food allergy development prophylactically in pre-sensitized high-risk individuals, such as eczematous infants. In addition, the IL-33 could be a target for post-sensitized individuals to prevent anaphylaxis.

651 C-Care: Impact of Labeling in Food Induced Anaphylaxis in Children Treated at the Montreal Children’s Hospital
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RATIONALE: To assess the role of food labels in precipitating food-induced anaphylaxis.

METHODS: The C-CARE (Cross-Canada Anaphylaxis REgistry) study documents triggers and management of anaphylaxis. As part of this registry, data was collected on epinephrine auto-injector (EAI) prescriptions in three EDs: Montreal Children’s Hospital (MCH), Sacré-Cœur Hospital (HSC) and Sainte-Justine Hospital (HSJ), from February 2013 to April 2014. Multivariate logistic regression analysis was performed to determine the association between type of EAI prescribed and demographic and clinical characteristics of cases, using Stata, version 12.0 (StataCorp, College Station, TX).

RESULTS: Among 445 cases, 321 were prescribed epinephrine EAI since they did not have one. Of these, 48 (15%) were prescribed Allerject™ as were patients presenting with anaphylaxis to emergency departments (ED) in Montreal, QC, Canada.

CONCLUSIONS: C-Care™ auto-injector was approved by Health Canada for sale in Canada in February 2013. Objective was to determine factors associated with the prescription of C-Care™ versus EpiPen™ in patients presenting with anaphylaxis to emergency departments (ED) in Montreal, QC, Canada.
Anaphylaxis Cases Presenting to the Emergency Center over a Two Year Period in Montreal, Canada

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RATIONALE: To assess the percentage of anaphylaxis cases among all emergency room visits at Sacré-Cœur Hospital in Montreal, Quebec, Canada, and characterize triggers and management.

METHODS: As part of the Cross-Canada Anaphylaxis REGistry (C-CARE), cases of anaphylaxis presenting at Sacré-Cœur Hospital ED were identified prospectively and retrospectively between May 2012 and May 2014. Data on demographics, reaction characteristics, and management was collected for cases meeting the definition of anaphylaxis (Sampson et al. 2006). Logistic regressions were conducted to determine an association with epinephrine use.

RESULTS: Over two years, the percentage of anaphylaxis cases among all ED visits (121, 979) remained constant (0.14% (95% CI 0.1%, 0.17%)). Median age was 35.3 years (IQR 21.8, 35.3) and 86.9% (80.6, 91.4) were adults (≥18 years). The majority were females (63.7% (55.9, 70.9)). Food was the most common trigger (52.7% (44.8%, 60.5%)), followed by drugs and venom (17.0% (11.7%, 23.8%) and 7.9% (4.4%, 13.4%) respectively). Among all reactions, 12.0% (7.6%, 18.1%) were severe and 76.6% (69.4%, 82.7%) were moderate. Among all cases, epinephrine was administered in 66.9% (59.1%, 73.9%) and antihistamines in 95.2% (90.5, 97.8) (difference 28.4 (19.9, 36.8)). Epinephrine was administered in 75% (50.6%, 90.4%) of severe cases, compared to 68.5% (59.6%, 73.6%) of moderate cases. Female patients were more likely to be managed without epinephrine (odds ratio; 2.1 (1.0, 4.2)).

CONCLUSIONS: Anaphylaxis in general, particularly food-induced anaphylaxis, is a substantial problem. There is a relative underuse of epinephrine, especially in females. Educational programs prompting the use of epinephrine in all cases of anaphylaxis are required.

Tryptase Levels in Children Presenting with Anaphylaxis to the Montreal Children’s Hospital -2014 Update

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RATIONALE: To determine if children with anaphylaxis have tryptase levels ≥11.4 ng/ml (the published threshold for anaphylaxis); to examine predictors of elevated tryptase levels; to compare tryptase levels during and post-anaphylaxis.

METHODS: Children presenting with anaphylaxis to the Montreal Children’s Hospital between April 2011 and September 2014 were recruited. Symptoms, triggers, and management of the anaphylactic reactions were documented. Total tryptase levels were measured 30-120 minutes following onset of symptoms. Multivariate linear and logistic regressions were used to evaluate the association between tryptase levels and age, gender, reaction trigger, reaction severity, and history of atopy. Levels during reaction and approximately 14 months after the reaction were compared using the paired t test for a subgroup of consenting patients.

RESULTS: 143 children had serum tryptase levels measured. Twenty-four children (16.8% (95% CI 11.3%, 24.1%)) had elevated levels (≥11.4 ng/ml). Only severe reactions were associated with levels of ≥11.4 ng/ml [OR 9.6 (95% CI 2.5, 37.3)]. Of the 41 patients with post reaction tryptase levels, the mean tryptase level was 8.4 ng/ml at time of reaction, and 3.2 ng/ml post reaction [difference of 5.2 (95% CI 3.0, 7.4)]. 29 had levels < 11.4 ng/ml during reaction, which were substantially higher than post reaction levels [difference of 1.8 (0.8, 2.8)].

CONCLUSIONS: Our results do not support the use of total tryptase as a diagnostic marker in children at the time of anaphylaxis as it exceeds published thresholds in only a minority of cases, however, it may be useful in establishing the diagnosis when compared to post reaction levels.

Variability in the Recognition and Management of Food Induced Anaphylaxis in Pediatric Emergency Departments and Urgent Care Centers

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RATIONALE: Common practices for managing food allergic reactions in pediatric emergency departments (EDs) and urgent care centers (UCCs) have not been established.

METHODS: We performed a retrospective chart review of a tertiary care pediatric ED and affiliated UCCs for visits that were likely related to an IgE-mediated food allergy between May 1, 2011 and May 31, 2013.

RESULTS: Of 5,582 charts reviewed, 587 were considered a likely food allergic reaction. Almost three-fourths (431) met the definition for anaphylaxis, although only one-third were coded as anaphylaxis. Treatment included steroids (78%), anti-histamines (75%) and epinephrine (35%). Approximately half of the patients were observed for more than two hours, 31% were observed for more than six hours, 20% were admitted, 23% were advised to see an allergist and 77% were given an epinephrine prescription. Children with cutaneous symptoms (OR 2.7, p < 0.0001) and peanut reactions (OR 1.6, p = 0.017) were more likely to be diagnosed with anaphylaxis, whereas children with gastrointestinal symptoms (OR 0.41, p < 0.0001), oral-pharyngeal symptoms (OR 0.33, p < 0.0001), asthma history (OR 0.44 p < 0.0001) or food allergy history (OR 0.34, p < 0.0001) were less likely. There were significant differences in epinephrine treatment (OR 8, p < 0.0001), observation (OR 15.9, p < 0.0001), admission (OR 10.3, p < 0.0001) and epinephrine prescriptions (OR 5.5, p < 0.0001) between those diagnosed with and without anaphylaxis.

CONCLUSIONS: There is variability in the recognition of food-induced anaphylaxis in pediatric emergency settings, with significant differences in management based on diagnosis. Presenting symptoms and associated clinical conditions may influence diagnosis.
How Good Is the Management of Anaphylaxis in the Emergency Room (ER)? a UK Center Experience

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RATIONALE: The National Institute for Clinical Excellence (NICE) provides clinical guidelines to ensure consistent, high quality, evidence based care for patients. In 2011, NICE published guidance for the emergency management of anaphylaxis. We looked at the overall management from presentation through to discharge, and in particular the practice of prescribing adrenaline (epinephrine) autoinjector to patients upon discharge.

METHODS: We performed a single center retrospective audit of anaphylaxis management at the ER at the Royal London Hospital (RLH) from 01/08/2013 to 31/10/13.

RESULTS: 241 patients presented with the diagnosis of an allergic reaction. 19 (8%) fitted the ICD10 criteria for anaphylaxis. Of these, 53% were female. Mean adult age 32 years (range 16-52). 2/19 (10%) were children under 16. Appropriate emergency treatment with adrenaline autoinjector was administered in 100% of the cases. However, only 55% of patients were offered adrenaline autoinjectors on discharge. Referral to the allergy clinic was made in 72% of the cases.

CONCLUSIONS: The timely recognition of patients presenting with anaphylaxis to the ER and the immediate management was excellent. The aftercare, however, was not fully in keeping with the NICE guidance. Only half of the patients were prescribed an adrenaline autoinjector as an interim measure until seen by an allergy specialist. We recommend an electronic tick-box proforma to ensure the key points of emergency and aftercare management is covered such as adrenaline autoinjector prescription and referral to a specialist clinic. A flow chart of the guidelines has been created by the department for easier reference.

Anaphylaxis in a Rural Emergency Department: The Dartmouth-Hitchcock Experience

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RATIONALE: Few studies have examined the presentation of anaphylaxis in rural settings. In this study, we characterize the population of patients diagnosed with anaphylaxis in a rural emergency department.

METHODS: We conducted a retrospective review of patients diagnosed with anaphylaxis in the emergency department of Dartmouth-Hitchcock Medical Center between April 2011 and May 2014.

RESULTS: We identified a total of 61 patients, 18 of whom were under 18 years of age. There were 33 females and 28 males. The median age was 26 years (range 1 to 76). Triggers in children included foods excluding tree nuts and peanuts (33%), medications (22%), tree nuts or peanuts (11%), and insect stings (11%). Triggers in adults included insect stings (44%), tree nuts or peanuts (14%), and other foods (14%). A disproportionate number of adult visits (58%) occurred during the summer (p < 0.01), and the majority of these were due to insect stings. Among adults, insect stings were more common in men compared to women (68% versus 25%, p = 0.01). Tryptase levels were measured in 3% of cases. Epinephrine was administered in 82% of cases. A prescription for an epinephrine autoinjector was provided to 93% of patients upon discharge. Only 12% of the 25 patients without a history of anaphylaxis were referred to an allergy specialist.

CONCLUSIONS: In this rural setting the plurality of emergency visits for anaphylaxis occurred in the summer, and insect stings were the most common trigger, particularly in men. Emergency providers frequently prescribed epinephrine, but few allergy referrals were placed.

Management of Children with Anaphylaxis in an Urban Emergency Department

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RATIONALE: Recent studies suggest minority children are more likely to report having a food allergy, but less likely to be diagnosed. Appropriate recognition and management of anaphylaxis, including food-induced anaphylaxis, is therefore critical, especially among minority children.

METHODS: Our study population includes patients evaluated in the Children’s National Emergency Department from January 2013 to June 2013 with the ICD-9 diagnosis codes of “Anaphylaxis”, and “Anaphylaxis to [specific food].” A retrospective chart review documenting demographic, clinical reaction, and clinical management variables was performed. Patients were also classified into a “Met anaphylaxis criteria” group based on clinical presentation meeting the FAAN/NIAID definition of anaphylaxis. Relationships between epinephrine use, allergy clinic referral, observation time, discharge planning, and each of the demographic, clinical reaction, and management variables were analyzed.

RESULTS: Of 103 patients coded as having anaphylaxis, only 64 met diagnostic criteria for anaphylaxis. More patients received antihistamines and steroids than epinephrine in the ED. Patients who met anaphylaxis criteria were more likely to receive epinephrine in the ED, tended to be observed in the ED longer, and were more likely to receive an epinephrine prescription and an Anaphylaxis Action Plan upon ED discharge.

CONCLUSIONS: Education of ED providers regarding anaphylaxis diagnostic criteria and epinephrine as first-line treatment for anaphylaxis is warranted, as less than half of patients were treated with epinephrine, and other adjunctive medications (antihistamines, steroids) were administered more frequently than epinephrine. More research is needed to continue to elucidate barriers to education and patient management between Emergency Departments and allergists.
**659** An Evaluation of the Treatment of Anaphylaxis in a Pediatric Emergency Room Setting

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**RATIONALE:** Anaphylaxis is a potentially fatal systemic reaction causing significant morbidity and mortality. Epinephrine is the standard treatment; however, epinephrine may be underused in anaphylactic patients in the Pediatric Emergency Department (PED). We assessed if anaphylaxis is appropriately recognized, diagnosed and treated in the PED.

**METHODS:** Retrospective chart review of patients aged 0-20 with diagnosis codes of Anaphylaxis (995.0X, V13.81), Allergy Unspecified (995.3), Drug Allergy (995.2X, V14.X), Food Allergy (995.6X, 995.7X, V15.0X), or Urticaria (708.X) seen in the pediatric emergency department from January 2012 – April 2013. Patients who did not meet the NIH 2006 definition of anaphylaxis were excluded. Cases meeting criteria were reviewed for triggers, presenting symptoms, treatment and clinical course.

**RESULTS:** Of the 668 charts examined, a total of 155 patients met criteria for anaphylaxis based on the NIH 2006 definition. Only 54.8% of patients who met criteria for anaphylaxis received epinephrine. 18 (11.6%) received a formal discharge diagnosis of anaphylaxis and 137 (88.4%) received another discharge diagnosis. For patients formally diagnosed with anaphylaxis, 83.3% received epinephrine, versus only 51.1% who were given a non-anaphylaxis discharge diagnosis (p<.001). 35 of 44 patients (79.5%) reporting a known trigger for anaphylaxis received epinephrine versus 50 of 111 (45.0%) of patients without a known trigger (p<.001).

**CONCLUSIONS:** A knowledge gap exists in recognizing symptoms constituting an anaphylaxis case. When correctly identified and diagnosed, anaphylactic patients were more likely to receive epinephrine than when diagnosed with Allergy or Urticaria. Provider education on the clinical presentation of anaphylaxis may improve treatment.

**660** Inpatient Management and Discharge Planning for Children Admitted for Food-Induced Anaphylaxis

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**RATIONALE:** There is a lack of literature detailing the management of food-induced anaphylaxis from admission to a pediatric inpatient unit through discharge.

**METHODS:** We performed a retrospective review of all patients admitted to a large pediatric tertiary care center for food-induced anaphylaxis from Jan. 1, 2013-Dec. 31, 2013.

**RESULTS:** Of the 91 total patients (mean age=6.1 yrs), the most common inciting agents were unspecified foods(30%, N=27), peanut(26%, N=24), tree nut(14%, N=13), dairy(14%, N=13), seafood(7%, N=6), and mixed nuts(5%, N=5). 59%(N=54) reported a known history of food allergy(FA). 98%(N=89) received epinephrine before admission with a mean of 1.15 doses. 7%(N=6) required epinephrine following admission, with 67%(4/6) due to peanut ingestion and two of those requiring an epinephrine drip in the ICU. The average length of admission was 0.92 days. The Allergy service was consulted for 4% of admissions with the primary diagnosis of anaphylaxis. Self-injectable epinephrine teaching was documented prior to discharge for 79%(N=72) and a prescription was provided for 90%(N=82). Allergy follow-up was recommended for 69%(N=63) with a referral placed to our Allergy clinic for 63%(40/63). For those with a reaction to an unspecified food, allergy follow-up was recommended for 78%(21/27).

**CONCLUSIONS:** The majority of patients admitted for food-induced anaphylaxis received self-injectable epinephrine teaching and a prescription at discharge, but almost 1/3 had no plan for allergy follow-up. Inpatient Allergy consultation is underutilized for this patient population as seen by the 4% consult rate. Increased Allergy involvement could have positive consequences on identification of the inciting agent, proper education, appropriate food avoidance, and follow-up.
Pre-Hospital Use of Epinephrine for Treatment of Anaphylaxis in Children and Adolescents
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RATIONALE: Epinephrine is the most effective and first line treatment for anaphylaxis but is underutilized by patients and medical personnel.

METHODS: We performed a retrospective review of the electronic medical record for all patients ages 0-25 with ICD-9 codes 995.0, 995.6, who presented to the Emergency Department (ED)/Urgent Care (UC) at a tertiary care pediatric academic referral center during the time period 2009-2013.

RESULTS: Four-hundred-and-eight patients (mean age = 7.25 years, 62% male) were identified. Thirty-six percent (N=148) received epinephrine prior to arrival at ED/UC, 65% (N=264) had a prior history of anaphylaxis, and 47% (N=191) had a prior epinephrine auto-injector prescription. Peanuts (N=149, 37%) and tree nuts (N=85, 21%) were the most common causes identified. Symptoms included: skin/mucosal (N=378, 93%), respiratory (N=305, 75%), and gastrointestinal (N=147, 36%). Of all patients, 42% (N=175) were discharged to home, but only 13% (N=55) had a follow-up appointment scheduled with an Allergist/Immunologist. Hospitalization occurred for 33% (N=134), with 2.7% (N=11) requiring intensive care.

Patients who received epinephrine prior to arrival at ED/UC differed in the following characteristics: history of prior anaphylaxis (N=264, 65%; OR = 4.267, p = 0.001), reaction at school (N=49, 12%; OR = 2.636, p = 0.002), disposition to home (N=175, 42%; OR = 1.876, p = 0.007), and age 13-17 years (N=91, 22%; OR = 1.802, p = 0.019). Patients who did not receive epinephrine prior to arrival at ED/UC differed in the following characteristics: required transfer to another medical facility (N=88, 22%; OR = 7.70, p < 0.0001), reaction at home (N=120, 29%; OR = 1.758, p = 0.0178), epinephrine auto-injector available at time of reaction (N=52, 13%; OR = 2.327, p = 0.0206), and involvement of two or more organ systems (N=335, 82%; OR = 1.986, p = 0.0106).

CONCLUSIONS: Despite being established as the most effective and first line treatment of anaphylaxis, the majority of children and adolescents did not receive epinephrine prior to arrival at the ED/UC. Increased education for patients and emergency responders is paramount to improving treatment for this life-threatening condition.

Ketotifen: A Role in the Treatment of Idiopathic Anaphylaxis
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RATIONALE: Idiopathic anaphylaxis is a life-threatening disease characterized by acute and recurrent episodes of urticaria, angioedema, airway compromise, gastrointestinal symptoms and shock. It is diagnosed when a patient has signs and symptoms of anaphylaxis without any apparent triggers. The treatment consists of antihistamines and oral steroids. However, long term prednisone therapy has a variety of known debilitating side effects. Ketotifen is an oral anti-allergic drug which also inhibits the release of mast cell and basophil mediators. It has been shown to be effective for treating chronic urticaria and idiopathic anaphylaxis.

METHODS: We describe 6 patients with corticosteroid dependent idiopathic anaphylaxis who were successfully treated with ketotifen. Detailed history, physical examination and extensive laboratory evaluation failed to indicate any specific trigger of their anaphylaxis.

RESULTS: The duration of symptoms prior to treatment ranges from one month to 13 years. All patients had angioedema, urticaria or both. Some patients had various concomitant medical conditions but none of them was shown to be casually related with their anaphylactic episodes. Some of them were on long term prednisone treatment. All patients were placed on 2-4 mg ketotifen orally twice daily. Among the 6 patients, one had an episode of recurrence requiring increased dose of ketotifen. Five of the 6 patients were able to come off prednisone without recurrence of symptoms.

CONCLUSIONS: Our clinical experience suggests that ketotifen is effective in inducing remission of corticosteroid dependent idiopathic anaphylaxis. More studies are needed to determine the role of Ketotifen in the treatment of idiopathic anaphylaxis.

Delayed Urticarial and Anaphylactic Reactions to Red Meat: Age of Onset, Severity, and Immunology Among 353 Cases and 140 Controls
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RATIONALE: Patients with IgE to galactose-alpha-1,3-galactose report delayed reactions, which vary from itching or gastrointestinal distress to frank anaphylaxis.

METHODS: Patients who presented to allergy clinics in Virginia with histories compatible with delayed reactions to red meat (n = 353) or with recurrent urticarial or anaphylactic reactions of other types (n = 140), many of which appeared to be idiopathic, completed a questionnaire. Sera were assayed for IgE antibodies, total IgE, and alpha-gal specific IgG.

RESULTS: IgE was measured to alpha-gal and mammalian allergens, to six inhalant allergens, to five foods, and to two venoms. Results for IgE and IgG to alpha-gal were analyzed in relation to symptoms and related to evidence of preexisting atopy. The presence of IgE antibodies to inhalant allergens was not correlated with sensitization to alpha-gal. Severity of reactions (urticaria, n = 87 or anaphylaxis, n = 249) was not associated with the titer of IgE antibodies to alpha-gal. In addition, neither the ratio of alpha-gal IgE to total IgE nor IgG antibodies to alpha-gal were correlated to reaction severity. Of those with anaphylaxis, 45% reported their first food reaction after age 40, and in 85% of cases the reactions started 2 hours or more after eating meat. The severity of reactions was not related to age of onset or delay before reactions.

CONCLUSIONS: Patients with delayed anaphylaxis to red meat present a novel disease with late onset, delayed expression, no immediate symptoms of food allergy, and a very high incidence of previous exposure to ticks. Atopy was not a predictor of IgE responses or food reactions.
Rapid Onset Anaphylaxis to Red Meat in Three Siblings from Uganda

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Rationale: IgE to galactose-1,3-galactose (“alpha-gal”) is associated with delayed onset anaphylaxis and has been measured in sub-Saharan Africa, but without reports of anaphylaxis. We present three siblings with rapid onset anaphylaxis to red meat while in a Uganda refugee camp.

Methods: Commercial extracts, raw and cooked meats were used for SPT. Allergen specific IgE (sIgE) was measured to foods, alpha-gal, cat, Fel d 1, Fel d 2 and parasites. Alpha-gal was then absorbed with beef thyroglobulin conjugated to sepharose beads.

Results: The siblings developed anaphylaxis within an hour of consuming goat, beef or pork. SPT for all siblings was positive to commercial beef, pork; raw beef, goat; cooked beef, and cat.Sibling 3 SPT was also positive to cooked goat. sIgE for all siblings was positive to alpha-gal (5.62; 8.38; 6.70 KU/L), beef (4.82; 6.72; 7.00 KU/L), pork (4.60; 5.86; 6.46 KU/L), cow’s milk (3.02; 4.38; 5.26 KU/L), cat (1.62; 2.58; 3.92 KU/L) and echinococcus (2.02; 3.26; 5.04 KU/L). Pork albumin, Fel d 1, Fel d 2, ascars, and anisakis sIgE were negative. Goat sIgE was not available. After depletion of alpha-gal from the sera, beef, pork, cow’s milk, cat and echinococcus sIgE were negative. The siblings consume chicken, fish and cow’s milk. There is no clear history of tick bites.

Conclusions: To the best of our knowledge, these are the first reported cases of red meat anaphylaxis from Africa. The early onset of their symptoms may indicate another spectrum of red meat allergy with IgE to alpha-gal in a specific population.

Recurrence Rates of Anaphylaxis in Children

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Rationale: Determine recurrence rate of anaphylaxis in children.

Methods: As part of the Cross-Canada Anaphylaxis Registry (C-CARE), parents of children identified prospectively at the Montreal Children’s Hospital Emergency Department and Sacré-Cœur Hospital with anaphylaxis were contacted annually after presentation and queried on subsequent allergic reactions. Cox regression analysis was conducted to determine factors associated with recurrence.

Results: Among 266 children presenting with anaphylaxis, 96 completed follow-up questionnaires (36.1%). Respondents were younger (median age 3.6 vs. 6.5 years) and more likely to have had severe anaphylaxis at baseline (10.4% vs. 2.9%) than non-respondents. Respondents reported 42 episodes of anaphylaxis in 25 patients, with an annual incidence of recurrent anaphylaxis of 28.8%. Those with recurrent anaphylaxis had a median age of 4.2 years and most were males (57.1%). Children with recurrent anaphylaxis were less likely to have peanut as a trigger for anaphylaxis (hazard ratio 0.29, 95% CI 0.11,0.82). Among recurrent reactions, food was the principal trigger (90.5%) and most reactions were moderate in severity (73.8%). Injectable epinephrine was used outside of a healthcare facility (HCF) in 52.4% of recurrent reactions and 90% of patients were brought to a HCF. Among patients brought to a HCF, 75.0% received epinephrine during the reaction.

Conclusions: We report an annual incidence rate of 28.8%, higher than previously reported. Patients with anaphylaxis triggered by peanut have lower recurrence risk potentially due to higher vigilance or ease in avoiding products containing peanut. Limited sample size and low response may have affected these estimates.
668 Food Dependent Exercise-Induced Anaphylaxis - a Component Resolved Approach to Evaluate Suspected Cross-Reactive LTP Sensitization
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RATIONALE: In non-specific food dependent exercise induced anaphylaxis (NS-FDEIAn), a high prevalence of LTP sensitization is seen in European series. We aimed to assess IgE cross-reactivities using (in-vitro) depletion with the suspected native allergens and an allergen microarray.

METHODS: Three cases of FDEIAn were analysed: (1) a 25-year-old man with generalized urticaria and angioedema during a soccer match, preceded by peach-based soft drink ingestion; (2) a 19-year-old polysensitized woman with an anaphylactic shock while playing soccer, ingested walnuts in the previous 90 minutes; (3) a 57-year-old man with bakers-asthma with four episodes of anaphylaxis during exercise after ingesting wheat-containing food. All individuals performed diagnostic work-up by skin prick tests (SPTs) and specific IgE (sIgE). Immunodepletion consisted in serum pre-incubation with the suspected native allergen in a solid-phase (ImmunoCAP®) and samples being re-tested with ImmunoCAP®-ISAC.

RESULTS: Case 1 showed LTP sensitization in SPTs and ISAC (Prup-3, Cor-a-8, Jug-r-3, Ara-h-9); after peach pre-incubation there was 100% depletion of sIgE to all LTPs. In case 2, SPTs and sIgE were positive to Cor-a-8, Jug-r-3, Ara-h-9); after peach pre-incubation there was 100% depletion of sIgE to all LTPs. In case 3, SPTs and sIgE were positive to Rosaceae, non Rosaceae fruits and nuts; ISAC detected LTP sensitization (Prup-3, Cor-a-8, Jug-r-3, Ara-h-9); walnut depleted sIgE ranging from 60% (Ara-h-9) to 100% (Cor-a-8). In case 3, SPTs and sIgE were positive to wheat (ω-5-gliadin was negative); ISAC showed LTP sensitization (Prup-3, Jug-r-3, Ara-h-9, Tri-a-14); wheat immunodepletion occurred only to Tri-a-14.

CONCLUSIONS: The in-vitro immunodepletion assay allowed to assess potential cross reactivity between LTP containing allergens. This easy assay may guide the diagnostic study and eviction recommendations in LTP-sensitized patients with NS-FDEIAn.

CONCLUSIONS: Anaphylaxis due to cholinergic triggers is uncommon and under-reported with only several case reports in the literature. Reactions are multisystem with cutaneous, upper and lower airway and cardiovascular involvement in most patients. Manifestations may be life-threatening and reactions may be severe. Prompt identification will obviate unnecessary investigations and effective education will help to avoid triggers of anaphylaxis.

670 Food-Induced Anaphylaxis in Thailand: A 10 Years Data from a Tertiary Care Center in Bangkok
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RATIONALE: There is limited data on food-induced anaphylaxis, a severe life-threatening reaction, in Thailand.

METHODS: Based on the ICD-10 report, we retrospectively studied the demographic data, clinical features, management and outcome of patients with food-induced anaphylaxis treated in a tertiary care center in Bangkok, Thailand, during January 2003 to December 2012.

RESULTS: Sixty-two patients, with median age of 32 years, were enrolled, and 33 (53.2%) were male. Family history of food allergy was 5/25 (8%) and of atopy was 8/25 (32%). Most patients had no underlying disease, but if they had, atopy was the most common. Nine from 37 (24%) had prior food-induced anaphylaxis and only 6 of them (66%) used adrenaline kit. Seafood was the most common culprit, responsible for 32/62 (52%), followed by fried insects 5/62 (8%) and fruit 3/52 (5%; banana, avocado and rambutan). Cutaneous manifestation was the most common system of presentation (56/62 or 90.3%). Reaction occurred within 2 hours in 78% (46/59), mostly less than 15 minutes in 20/59 (34%), of patients. Less than 70% (40/60) of patients received adrenaline for emergency treatment and less than 40% (22/58) received adrenaline kit after hospital discharge. Recurrent rate from 19 followed-up cases was 8 (42%), of whom 75% (6/8) received adrenaline kit, and however only 50% (3/6) used it. One patient (1.7%) died due to ventilator-associated pneumonia from prolonged intubation because of prolong hypoxemia.

CONCLUSIONS: Seafood is the most common cause. Further improvement of anaphylaxis awareness and management among healthcare providers are warranted.
671 **Cyclical Anaphylaxis: A Review of the Literature and a Novel Approach to Treatment**

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**RATIONALE:** Cyclical anaphylaxis is a rare peri-menstrual reaction. Standard treatment is either medical or surgical ovulatory suppression. We present a literature review of cyclical anaphylaxis and the first case treated by IgE-inhibition with omalizumab, with a favourable response.

**METHODS:** A review of published literature was performed to identify cases of anaphylaxis associated with menstruation. Demographics, clinical features, diagnostic investigations, terminology, management, and response to treatment in all reported cases were reviewed.

**RESULTS:** We have identified 22 published cases of cyclical anaphylaxis. None of these cases were treated with omalizumab. Our patient is a 34 year old female who presented with a 3-year history of generalized pruritic skin eruptions, peri-orbital and lip swelling, throat tingling and diarrhea in the 2-4 days preceding menstruation. She has a history of asthma, allergic rhinoconjunctivitis and allergy to peanut and tree nuts. Investigations for systemic mastocytosis, mast cell activation syndrome, pheochromocytoma and carcinoid syndrome were negative. Her total serum IgE was 1990 IU/mL. Intradermal testing with progesterone was negative. There was no response to cetirizine 20 mg daily. The patient refused induction of menopause or surgical oophorectomy. Therapy with omalizumab has continued for eleven months. She had one reaction shortly after commencing treatment, then no further reactions. Asthma control has significantly improved.

**CONCLUSIONS:** We present the first case of cyclical anaphylaxis with a response to omalizumab. As an alternative to surgically- or medically-induced menopause among women of child-bearing years, omalizumab is a treatment option without adverse life-changing consequences for this rare but potentially life threatening condition.

672 **Causes of Anaphylaxis in the Pediatric Population**

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**RATIONALE:** The etiology of anaphylaxis is mostly recovered from the medical impression at the emergency departments (ED). In our area, in adults, we found only a 55% concordance with the diagnosis given after the allergologic study. We aimed to study the etiology of anaphylaxis in children.

**METHODS:** An observational descriptive study of all the anaphylaxis cases attended at the pediatric ED of a third-level hospital in Madrid was carried out. Electronic clinical records from March 2012 to March 2013 were searched through keywords related to allergy and considered anaphylaxis if WAO criteria were fulfilled. Patients were sent to the Allergy Unit.

**RESULTS:** Eighty anaphylaxis cases (60 patients) were retrieved at the ED. 87.5% were studied at the Allergy Service. Mean age 4.8±4.2, 52.9% boys, 78.6% previous history of atopy. Food allergy was identified in 93.1% of the cases: milk (47.8%), egg (25.4%), nuts (14.9%), fruits (7.5%) and fish (4.5%). Only 62.7% had been suspected of food allergy at the ED, while only 52.2% had been recommended to avoid the correct food group. Idiopathetic anaphylaxis was finally assigned to 2.8% of the cases, a 91.9% reduction from the ED impression (34.7%). Overall, there was a concordance of 47.6% between the ED impression and the definitive diagnosis (Cohen’s kappa 0.493).

**CONCLUSIONS:** Children very often get the allergologic study done after an anaphylactic episode. The concordance between the cause determined at the ED and the confirmed cause after the allergologic study is low. Food allergy is a more frequent cause of anaphylaxis than previously suspected.

673 **Survey on Experiences and Knowledge Status about Anaphylaxis By Academic Members in Korea**

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**RATIONALE:** Anaphylaxis occurs at anytime and at anywhere. Immediate epinephrine injection and avoidance of causative agent are important in management and prevention. In this study, we analyzed experiences and knowledge status about anaphylaxis among academic members.

**METHODS:** We performed the survey for physicians who attended the annual allergy congress. The questionnaire consisted of number of annually experience, test of serum tryptase, use of epinephrine, knowledge status of how to inject epinephrine, effort to find culprit agent and the most frequent causing agent.

**RESULTS:** Total 70 physicians answered the survey and most physicians (64, 91.4%) worked at tertiary hospital. 36 physicians (51.4%) were specialist in department of internal medicine and 22 physicians were pediatrician. 59 physicians (84.5%) were experience of management anaphylaxis (mean 21.7 cases per year) and physicians in half (59.6%) replied that the effort to find the cause of anaphylaxis. The frequent experiencing agents were drug (60%) and food (34%). 28 physicians used serum tryptase as diagnostic marker. Only a half of physicians (55.2%) prescribed epinephrine in anaphylaxis. 47% physician used route other than muscle to inject epinephrine. Well-known physicians how to use epinephrine were just 55%. 88% of physicians provided information and educated emergency action plan for anaphylaxis.

**CONCLUSIONS:** Many physicians did not know about anaphylaxis management and epinephrine use. It is needed to pay more efforts to this serious issue. We believe that constant education of anaphylaxis management will lead to improved standards of anaphylaxis patient care.
Epinephrine Autoinjector Use One Year after Training: A Randomised Controlled Comparison of Two Different Devices

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RATIONALE: Previous cross-sectional studies found patients have poor understanding of how to use epinephrine autoinjectors (EAI). We tested whether prescribing different EAI devices affected patients’ ability to administer epinephrine 1 year after training.

METHODS: We randomly allocated 158 mothers of food-allergic children prescribed an EAI for the first time, to Anapen or EpiPen with appropriate training. Treatment was allocated by a third party using a computer-generated randomisation sequence. The primary outcome, ability to effectively administer epinephrine in a recorded simulated anaphylaxis scenario, was assessed by a paediatric allergist independent of the trial and host institution. ISRCTN12504076.

RESULTS: We evaluated ability to administer epinephrine at 1 year in 110/158 (70%) randomised participants. Overall success rates were low, and similar between the 2 groups - 28/51 (55%) using Anapen, and 35/59 (59%) using EpiPen (P=0.64). The primary reason for failure differed between groups (P<0.001). Failure to remove all safety caps was most common for Anapen (33%); using the wrong end of the device for EpiPen (17%). When actual device-specific epinephrine delivery times were taken into account, success rates were 30/51 (59%) for Anapen and 42/59 (71%) (P=0.17). Digital injection - the only adverse event recorded – occurred in 8/59 (14%) in the Epipen group, and 0/51 (0%) in the Anapen group (P=0.007).

CONCLUSIONS: EAI success rates were similar for Anapen and EpiPen 1 year after training, but digital injection was more common with EpiPen. Optimal EAI design should include a single safety cap, and easy identification of the needle end.

The Race Study: Comparison of Carrying Time, Confidence in Epinephrine Auto-Injector (EAI) Use, and Experience with EAI Training Among Patients with Auvi-Q Versus EpiPen

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RATIONALE: Limited data are available on real-world experience with EAIIs among patients at-risk of anaphylaxis.

METHODS: The Real-World Assessment of Patients’ Carrying Time and Confidence with EAI’s (RACE) was a non-interventional, cross-sectional survey among patients aged ≥7 years who filled ≥1 prescription for Auvi-Q (N=1,000; children: n=597; adults: n=403) or EpiPen (N=1,000, children: n=105; adults n=895) between 2013–2014. Outcomes included: EAI carrying time in the last 7 days (primary); confidence in EAI use and EAI training experience (secondary). Data were examined by multivariate analyses due to significant differences between the two patient groups in their socio-demographic and clinical characteristics.

RESULTS: After adjusting for confounding factors, patients prescribed Auvi-Q were significantly more likely to carry their EAI all the time as compared with patients prescribed EpiPen (overall: adjusted odds ratio [aOR]=1.91; children: aOR=3.26; and adults: aOR=1.78, all p<0.01). Similarly, patients prescribed Auvi-Q carried the EAI significantly longer vs EpiPen (adjusted average number of carrying days, overall: 5.24 vs 4.30; children: 5.24 vs 3.46; and adults: 5.46 vs 4.82, all p<0.01).

If having an anaphylaxis event, adults prescribed Auvi-Q were more likely to be very confident than those prescribed EpiPen in using the EAI themselves (aOR=2.02, p<0.01), and someone else administering them the injection (aOR=2.25, p<0.01). Additionally, patients prescribed Auvi-Q were significantly more likely to find EAI instructions clear, receive EAI training, and find the EAI trainer useful than those prescribed EpiPen (all p<0.05).

CONCLUSIONS: This study suggests significant real-world differences between Auvi-Q and EpiPen in patients’ carrying time, confidence in use, and training experiences.
**AB210 Abstracts**

**677 The Race Study: Predictors of Carrying Epinephrine Auto-Injector Devices (EAs)**

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**Rationale:** Carrying the EAI is critical for patients at-risk of anaphylaxis, yet studies show that a significant proportion of patients do not always carry EAs. Identifying factors associated with carrying EAs could improve treatment adherence.

**Methods:** The Real-World Assessment of Patients’ Carrying Time and Confidence with Epinephrine Auto-Injector Devices (RACE) was a non-interventional, cross-sectional survey of patients aged 27 years who filled 21 prescription for Auvi-Q (N = 1,000; children n = 597; adults n = 403) or EpiPen (N = 1,000; children n = 105; adults n = 895) between 2013–2014. Patients were surveyed on their EAI carrying time over the last 7 days prior to completing the survey. Predictors of ‘carrying the EAI all the time’ and longer ‘EAI carrying time’ were identified by multivariate analyses.

**Results:** Overall, predictors of significantly higher odds of carrying the EAI all the time were: being female (adjusted odds ratio [aOR] = 1.88, p < 0.0001), older age (47–56 years: aOR = 2.54, p = 0.0004; 57–64 years: aOR = 2.88, p = 0.0002), having Auvi-Q as EAI (aOR = 1.91, p < 0.0001), having previously experienced anaphylaxis (one time: aOR = 1.87, p < 0.0001; 2 times: aOR = 2.09, p < 0.0001), having food allergy (aOR = 1.29, p = 0.0319), and having asthma (aOR = 1.40, p = 0.0019). Higher education level (Associate/Bachelor: aOR = 0.63; Masters and higher: aOR = 0.63) and household income ≥ $69,000 (aOR = 0.52) were associated with significantly lower odds (all p < 0.05). These factors were also predictors of longer ‘EAI carrying time’. Similar predictors were observed in children and adult sub-populations.

**Conclusions:** Several factors appear to influence EAI carrying time, including the type of EAI prescribed. By addressing these factors, treatment adherence could potentially improve.

**678 Designing Auto-Injectors for Children: Effect of Form Factor on the Human Factors of Efficient Drug Delivery**

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**Rationale:** Because commercially available epinephrine auto-injectors differ in form factor, and are important for children the goal of this study was to evaluate their effect on human factors of efficient drug delivery in children.

**Methods:** Auto-injectors with 3 different form factors (cylindrical, elliptical, and prismatic) were tested in a laboratory-based repeated measures experiment with 20 participants (aged 8–12 years). Participants applied their maximum possible force onto a force plate positioned over their thigh (maximum force capability task) and practiced an injection using the trainer auto-injector after watching the device’s training video (application task). Participants rated force confidence and preference for all devices.

**Results:** For the maximum force capability task, the elliptical device exhibited significantly higher axial-applied force compared with the prismatic device (43N vs 38N, respectively). The elliptical device also exhibited the lowest difference between force and device angle compared with the other two devices (1° vs 6° and 12°). For the application task, the elliptical device had the fastest time to force. The elliptical and prismatic devices had significantly lower grip effort compared to the cylindrical. The cylindrical form factor was the least preferred. Overall, these general patterns across form factors were similar to patterns observed in a previous study of adults. However, children exhibited lower force, higher grip effort, higher force angle, and more variability in application force, force angle, and grip effort.

**Conclusions:** General trends were similar between adults and children. The results suggest that the elliptical form factor may have better success in drug delivery in children.

**679 Adherence to the Treatment Choices of Anaphylaxis: An Epidemiological View of the Pediatric Patients**

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**Rationale:** Anaphylaxis is a life threatening condition. There is a consensus that the rapid intramuscular injection of adrenaline is life-saving and first line intervention. We aimed to describe adherence to the treatment choices of anaphylaxis besides the epidemiological characteristics.

**Methods:** This retrospective study included the children with available records referred to the outpatient clinics of Pediatric Allergy and Immunology Department of the Karadeniz Technical University from October 2002 to April 2014 for investigation of anaphylaxis.

**Results:** One hundred and forty-five episodes of anaphylaxis were reported in 65 children (45 boys, p < 0.05). The median age was 8 years (range: 1.5 months–17 years). The causative agents were foods in 67 (46.2%), episodes, hymenoptera venom in 33 (22.8%), drugs & medications in 19 (13.1%). In twenty-six episodes (17.9%), the causative agent was unidentified referred as idiopathic. When compared according to age groups, the rate of food anaphylaxis was significantly high in the 0–2 years age group (n = 22, 88% P < 0.01, OR: 0.079, 95% CI: 0.02–0.27), while hymenoptera venom anaphylaxis was high in the 7–11 age group (n = 20, 48.8%, P < 0.01, OR: 0.15, 95% CI: 0.06–0.34). The patients with 123 (84.8%) episodes received antihistamines, the patients with 97 (66.9%) received corticosteroids, the patients with 21 (14.5%) episodes received adrenaline, and the patients with 21 (14.5% for 132 episodes) episodes received beta-2-agonists prior to admission to our clinics.

**Conclusions:** The use of adrenaline as a first line intervention of the treatment of anaphylaxis is not often applied. This seems due to the various barriers related to clinicians, patients, and regulators. It is necessary to overcome those barriers in order to settle and improve emergency management of anaphylaxis.
680 Using Video Technology to Improve Epinephrine Auto-Injector Use
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RATIONALE: Inaccurate use of epinephrine auto-injectors is well documented in the literature. We hypothesized that providing personalized instructional videos that patients can access via email would improve self-administration technique.

METHODS: 102 patients aged 12 and above who were previously prescribed epinephrine auto-injectors were recruited and randomized to receive an instructional video versus no video in this IRB-approved study. All participants received standard of care instruction. Active arm participants were given a video of themselves demonstrating proper self-administration of the auto-injector. They received a bi-weekly email-viewing reminder. At three and six month follow-up, participants demonstrated use of the auto-injector. A blinded investigator evaluated their technique.

RESULTS: Number of missed steps decreased significantly from baseline to three months in both groups (t-test, p<0.0001). The percentage of patients with perfect self-administration increased from 16% to 43% at three months (Fisher’s, p<0.0001) with no difference between active and control arms. At three months, participants who viewed their video two or more times demonstrated correct use more frequently than participants viewing the video less than twice (Fisher’s p=0.03).

CONCLUSIONS: There was significant improvement in epinephrine self-administration technique at three months in both study arms. Receiving the video did not increase likelihood of perfect technique; however, viewing the video multiple times was associated with correct auto-injector technique. We suspect the study made participants accountable for proper medication use, and motivated subjects in both arms to prepare for follow-up assessments. In practice, we suggest that patients should be made accountable by requiring them to demonstrate epinephrine auto-injection technique at each appointment.

681 Parent / Child Perceptions of Children’s Readiness to Self-Inject Epinephrine
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RATIONALE: To decide upon a food-allergic child’s autonomy, the parent and child must be comfortable with the child self-injecting epinephrine; however, little is known about the consistency of parent-child assessment of this responsibility.

METHODS: Children with food allergies ages 8-18 years and their parents attending a food allergy referral center independently completed surveys containing the query: “Can you (your child) use an epinephrine auto-injector on your (his/her) own if needed?” (“Never”, “Sometimes”, “Most of the time,” “Always” or “Don’t know”). We predefined a child that was “very” autonous (ages 8-11 years) and an “adolescent” (ages 12-18 years) age group.

RESULTS: There were 413 parent-child pairs (273 children, 140 adolescents). Overall, 38% of parents and 22% of children reported “don’t know”; those responses were excluded from the correlational analyses. In the child age group, parents and children’s perception about the child’s ability to self-inject generally aligned well with each other (Kappa=.79), but there was substantial disagreement between adolescents and parents (Kappa = .48). 69% of adolescents perceived that they can self-inject “most of the time” or “always”, whereas only 53% of parents thought so. Even in instances in which an auto-injector was previously used (34.9% of the sample), many parents reported that they “don’t know” if their child (33.7%) or adolescent (32.8%) can self-inject, and agreement between parents and adolescents remained low (Kappa=.44).

CONCLUSIONS: Parents and adolescents disagree about whether the adolescent can self-inject epinephrine. This lack of agreement may lead to confusion in decisions related to granting autonomy to the food-allergic adolescent.

682 School Staff Food Allergy (FA) Education Increases Epinephrine Coverage and Recognition of Allergic Reactions
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RATIONALE: Because 5-8% of children have FA, 16-18% experience reactions and 28% of fatalities secondary to anaphylaxis occur in school, improving recognition and treatment of school FA reactions is essential. We hypothesized that expanding FA training to entire school staff would increase school epinephrine device (Epi) coverage (#Epi/#FA students) and prevent food related allergic reactions.

METHODS: A school nurse survey in spring 2013 identified 12 target schools selected for high FA prevalence rates (>6 FA children/school) in the Houston Independent School District. The remaining 68 district schools served as controls. Target school staff received FA training through school nurse didactic sessions. The number of Epi/FA child and food reactions/school for both groups were measured by survey in the spring of 2013 and 2014. Mann Whitney U test was used for analysis.

RESULTS: Mean Epi/FA child in 2013 was 61% (0-100%) and 59% (0-100%) for target and control schools, respectively (p=NS). In 2014, Epi/FA child increased to 76% (0-100%) and 71% (0-100%) for target and controls, respectively (p=0.258, p=0.05). Recorded FA reaction rates decreased for controls from 36% to 11% but increased for target schools from 7.3% to 10% in 2014. Total FA reactions/school decreased from 2013 to 2014 (p=0.003).

CONCLUSIONS: Increasing epinephrine coverage for FA children after training reflects awareness of anaphylaxis preparedness. While the rise in allergic reactions/FA children in target schools would seem to indicate increased exposure, it may be due to heightened awareness and recognition with systematic treatment of food related allergic reactions. Further study will determine the effect of staff education on the incidence of reactions.

683 Barriers to Treatment with Epinephrine for Anaphylaxis By School Nurses
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RATIONALE: Approximately 150-200 fatalities per year occur from anaphylaxis related to food allergies. School nurses have significant barriers associated with administering epinephrine to a student in anaphylaxis including availability of stock epinephrine, expired prescriptions, first episode with previously unknown allergies, state regulations, and legal ramifications. Our survey identifies existing barriers to receiving life-saving epinephrine at school, and to evaluate the need for stock epinephrine.

METHODS: In 2012, an anonymous online survey was distributed via an e-mail newsletter to the National Association of School Nurses. Excel was used to generate descriptive statistics.

RESULTS: 2,439 school nurses completed the survey. For students with known allergies, 25.3% of students have no epinephrine and 24.6% had two unexpired auto-injectors at school. 16.7% of school nurses replied that they would not give an expired epinephrine. 41.3% would not give a different child’s epinephrine if there was nothing else available. 3.6% would not use a non-patient specific stock epinephrine auto-injector if it were available. In each situation, the majority had the concern for legal repercussions. 43.3% already have stock epinephrine available of which 90.8% felt it improved their stock epinephrine at least once and 10.9% used it more than once.

CONCLUSIONS: Significant barriers currently exist which may prevent school nurses from treating episodes of anaphylaxis with life-saving epinephrine. Stock epinephrine available in schools may improve the likelihood that children will receive effective treatment in a timely manner.
EpiPen4Schools Survey: Characteristics of Anaphylaxis and Common Triggers
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RATIONALE: The purpose of this study was to describe the characteristics of anaphylactic events and EAI use in children and adults in US schools.
METHODS: This exploratory, cross-sectional, web-based survey of schools participating in the EpiPen4Schools program captured characteristics of anaphylactic events and EAI use during the 2013-2014 school year.
RESULTS: A total of 5683 schools responded to questions on the occurrence of anaphylactic events. A total of 919 anaphylactic events were reported by 11% of schools (607/5683). Most schools (89%, n = 5076) reported no anaphylactic events, and 10% (n = 543) reported 1 to 2 anaphylactic events. Most anaphylactic events occurred in students, 89% (n = 575), 22% (n = 187) of which occurred in those with no known allergies. In 9% (n = 75), allergy status was unknown. Of the 919 events, triggers were reported for 847 events (92%); most triggers, 62% (n = 529), were listed as food, 10% (n = 81) were listed as insect stings, 7% (n = 56) as environmental/medication/health-related factors, and 1% (n = 9) as latex. Approximately 20% of events (n = 172) had an unknown trigger. Although food allergy triggers were predominant throughout the year, prevalence of certain triggers varied seasonally. Insect stings were relatively less frequent during winter, 4% (n = 5/143), vs fall, 13% (n = 31/243), and spring, 10% (n = 26/268) months, whereas unknown triggers reached a high of nearly 27% (n = 71/268) during spring.
CONCLUSIONS: More than 1 in 10 schools reported an anaphylactic event in a single school year, many of which were associated with unknown triggers. These data indicate the unpredictable nature of anaphylaxis and the importance of anaphylaxis training for staff.

Epinephrine Administration for Cases of Anaphylaxis in a US School Setting: Results from the EpiPen4Schools Survey
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RATIONALE: This study was designed to describe anaphylactic events and epinephrine auto-injector (EAI) use in US schools.
METHODS: This exploratory, cross-sectional, web-based survey of schools participating in the EpiPen4Schools program captured details of all reported occurrences of anaphylactic events and treatment(s) administered at each responding school during the 2013-2014 school year.
RESULTS: A total of 919 anaphylactic events were reported in 607 schools (11%, n = 5683 responding schools). Of the 851 events with data on the use of EAI, 75% (n = 636) were treated with auto-injectors. Of the events treated by EAI, 49% (n = 310) were treated using the EpiPen4Schools® program stock EpiPen® EAI, and 46% (n = 289) were treated using the individual’s EpiPen EAI. Other EAs accounted for approximately 4% of EAI treatments. Fifty-four (9%) received a second epinephrine injection. Of those individuals not treated with an EAI, 77% (n = 157) received antihistamines, 13% (n = 26) received another treatment, and 8% (n = 17) received no treatment. Of the 850 events with data on hospital transport, 80% of individuals (n = 677) were transported to the hospital.
CONCLUSIONS: Over 10% of schools participating in the EpiPen4Schools survey reported an anaphylactic event. Approximately 25% of anaphylactic events were not treated with epinephrine, most receiving antihistamines. Furthermore, 20% of patients were not taken to the hospital after an anaphylactic event. Considering the potential for biphasic reactions, close medical supervision is imperative after an anaphylactic attack. Thus, these data suggest the value of stocking EAs and providing continued education for school personnel and family members.

Occurrence of Anaphylaxis By School Grade Level and Staff Training: Findings from the EpiPen4Schools Survey
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RATIONALE: To conduct an exploratory study of anaphylaxis and epinephrine auto-injector (EAI) use in US schools during the 2013-2014 school year.
METHODS: This exploratory, cross-sectional, web-based survey of schools participating in the EpiPen4Schools program captured characteristics of anaphylactic events and EAI use in children and adults enrolled or working in schools.
RESULTS: Thirty-six percent of responding schools (n = 2146) were grade schools (pre-K to grade 5), 12% (n = 703) were middle schools (grades 6-8), and 18% (n = 1064) were high schools (grades 9-12); the remaining 34% (n = 2088) were other grade combinations. Nearly 50% of students (n = 355) who experienced anaphylaxis were in high school, 32% (n = 234) were in grade school, and 19% (n = 135) were in middle school. Although frequency of food-related triggers was consistent across grade levels, 22% of high school students (n = 79) experienced an event with an unknown trigger, compared with 14% (n = 33) and 15% (n = 20) of grade school and middle school students, respectively. Approximately 36% of schools (n = 2022) trained only the school nurse and select staff to recognize anaphylaxis, whereas 29% (n = 1621) and 31% (n = 1730) trained most staff or all staff, respectively. A majority of schools, 54% (n = 3024), permitted only the school nurse and select staff to administer epinephrine; 16% (n = 879) and 22% (n = 1218) permitted most staff or all staff, respectively, to administer epinephrine.
CONCLUSIONS: Adolescence may be a particularly high-risk developmental stage for anaphylaxis, and some students encounter staff members who are untrained in anaphylaxis recognition or treatment. These findings suggest a need for continued anaphylaxis training for protection of all students, staff, and visitors.

Serum Tryptase Concentrations in Patients with Hymenoptera Venom Allergy in Beekeeping Zone
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RATIONALE: Studies suggest that increased tryptase levels is an individual risk factor for hymenoptera venom allergy (HVA). The purpose of the study was to investigate tryptase levels in HVA patients living in beekeeping zone.
METHODS: Serum tryptase concentrations were measured in 60 adults diagnosed with HVA. Gender, age and having allergic rhinitis and asthma (AR-A), culprit insect, number of systemic reaction, the period after last sting reaction, elapsing time after sting, sting reaction severity, IgE levels were investigated as an effective factor on tryptase level.
RESULTS: The mean age was 41.42±1.84 years. Almost 2/3 of them had AR-A. Culprit insect was bee in 37 (61.7%), wasp in 10 (16.7%) and both in 13 (21.7%) patients. Number of sting reaction was between 1 and 10. The period after last reaction was between 1 and 84 months. The mean elapsing time was 15.83±3.89 minutes (5-180). The severity of sting reaction was moderate in 18 (30%) and severe in 42 (70%) patients. The mean basal tryptase level was 9.21±0.4 ug/l (4-18). There was no significant correlation between basal tryptase levels and features of sting reaction, total IgE, venom specific IgE levels in the whole group. Tryptase level was slightly high in patients with history of both insect. Strong correlation was observed between apis specific IgE and total IgE levels in patients with history of bee sting reaction (r=0.82, p<0.000).
CONCLUSIONS: Basal tryptase level could not always be related with clinical features of HVA, particularly for patients living in beekeeping zone.
688 Physician Recognition and Management of Venom-Induced Anaphylaxis
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RATIONALE: Anaphylaxis is defined as a severe, acute-onset allergic reaction that may lead to death. An important cause of fatal systemic reactions, venom-induced anaphylaxis can be especially severe. Venom immunotherapy significantly decreases the risk of recurrent systemic reactions in hymenoptera allergic patients. Data on physician diagnosis and treatment of anaphylaxis due to hymenoptera venom is underrepresented in the primary literature. The purpose of this study is to examine physician recognition and management of venom-induced anaphylaxis.

METHODS: Physicians in a variety of specialties, including allergy, were invited to complete an electronic case-based survey describing a child experiencing venom-induced anaphylaxis.

RESULTS: There were 105 total participants, including 25 allergists. 29% (30/105 physicians) elected not to treat with epinephrine and 28% (29/105) did not refer the child to an allergist. Of the 75 physicians (71%) who treated with epinephrine, 27% (20/75) responded that the patient was either not experiencing anaphylaxis or they were unsure. While 72% (76/105) of responders referred the patient to allergy, 42% (44/105) did not know the patient should start venom immunotherapy despite positive testing for hymenoptera allergy. When non-allergist physicians are considered separately, 36% (29/80) elected not to treat with epinephrine. 35% (28/80) did not refer the patient to allergy, and 54% (43/80) did not know that the patient should start venom immunotherapy.

CONCLUSIONS: To our knowledge, this is the first survey study on physician diagnosis and management of venom-induced anaphylaxis. The results highlight the need for increased education regarding recognition and treatment of hymenoptera venom allergy, a potentially life-threatening condition.

689 Effect of Statin Drugs on Hymenoptera Venom Anaphylaxis Severity
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RATIONALE: PAF is a mediator of severe or fatal anaphylaxis. The biologic actions of PAF are terminated by an enzyme, PAF acetylhydrolase (PAF-AH), which inactivates PAF. Previous studies have shown that lipid lowering drugs also reduce serum PAF-AH activity which circulates as a complex with low density lipoproteins (LDL). We studied the effect of LDL-lowering drugs on anaphylaxis severity in a cohort of patients with Hymenoptera venom anaphylaxis.

METHODS: A retrospective chart review was undertaken of patients with Hymenoptera venom anaphylaxis seen in an academic allergy clinic between 2008 – 2013. Patients aged 45 years and older were included. Anaphylaxis severity scores were calculated. Use of lipid-lowering statin drugs was recorded.

RESULTS: A total of 90 patients with Hymenoptera venom anaphylaxis were identified. Of these, 17 patients were on statin drugs, either atorvastatin or rosuvastatin. For those patients with grade 1 anaphylaxis (n=30), 5 were on a statin drug (16.7%). Of 43 patients with grade 2 anaphylaxis, 8 patients were on a statin drug (18.6%). For 17 patients with grade 3 anaphylaxis, 3 of 17 patients were on a statin drug (17.6%).

CONCLUSIONS: There is no trend towards increasing anaphylaxis severity with statin drug use. This preliminary analysis did not take into account the LDL and PAF-AH levels at the time of the anaphylactic event. The risk of severe anaphylaxis is expected to increase only if LDL and PAF-AH levels are lowered below normal by statin drug use. This study is now in progress.
Severe Anaphylaxis to Flying Hymenoptera Stings As the Presenting Sign of Indolent Systemic Mastocytosis: News to US?

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RATIONALE: Associations between hymenoptera venom allergy (HVA) and systemic mastocytosis (SM) are widely discussed in European literature, yet garner limited attention in the United States (US). Current practice parameters mention tryptase levels for mastocytosis evaluation in idiopathic anaphylaxis or suspected venom allergy with negative serum skin testing, but no clear recommendation for the role of tryptase in identified hymenoptera anaphylaxis is mentioned. We present a patient with severe anaphylaxis to hymenoptera stings and indolent SM, spotlighting the need for more US focus on this association.

METHODS: Serum specific IgE to flying hymenoptera confirmed venom allergy. Tryptase levels and bone marrow biopsy supported indolent SM diagnosis.

RESULTS: 55 year old male experienced severe anaphylaxis with cardiovascular collapse following an episode of 10-14 stings by flying hymenoptera. Within 10 minutes, he experienced diaphoresis, lightheadedness, vision changes, weakness, and a sense of impending doom before losing consciousness. Due to asystole, EMS performed CPR and administered IV fluids, epinephrine x3 doses, and Vasopressin before return of spontaneous circulation. His tryptase level drawn 8 hours after stings was 44ng/ml. Flying hymenoptera serology revealed evidence of IgE mediated responses to honey bee, wasp, yellow jacket, and hornets. Given the severity of his initial reaction, a subsequent baseline tryptase was obtained (25ng/ml). Bone marrow biopsy confirmed systemic mastocytosis. Patient started venom immunotherapy and has tolerated conventional build up thus far.

CONCLUSIONS: Although not the first such case reported, our patient highlights the need for increased awareness and understanding of the link between identified severe anaphylaxis to hymenoptera and SM.

Cluster Immunotherapy Build-up Has Better Adherence Rates but Greater Risk of Mild to Moderate Systemic Reactions

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RATIONALE: Subcutaneous immunotherapy (SCIT) significantly improves allergic rhinitis symptoms, but adherence rates are poor. Faster build-up may result in improve adherence, allowing more patients to reach maintenance doses.

METHODS: All charts and SCIT records for patients starting on cluster immunotherapy (TI) build-up (1/1-6/30/2014) were reviewed and compared to those starting traditional SCIT build-up from 1/1-6/30/2013 in a large private practice. Demographics, clinical characteristics, numbers of injections and injection visits, and systemic reactions (SRs) during the build-up period were recorded. Patients were categorized as having reached or not reached maintenance. SRs were categorized according to World Allergy Organization severity grades.

RESULTS: The study population (n=167) was 92% Caucasian, 52% female, and had an average age of 34.9±14.3 years. There were no differences in demographics between the groups. A significantly higher proportion of patients reached maintenance on cluster build-up (62 of 76; 81.6%) as compared to traditional SCIT (60 of 91; 65.9%; p=0.02). Cluster build-up took less time (61.0±30.3 vs. 164.0±70.4 days), with fewer visits (12.0±2.0 vs. 37.4±5.3) and injections (25.7±3.9 vs. 37.4±5.3) (p<0.0001 for all). Cluster build-up patients had a significantly higher rate of SRs (9 of 1671 total injections (0.54%) vs. 3 of 3051 total injections (0.10%)); p=0.005), which were generally mild (7 of 9 Grade 1, 2 of 9 Grade 2) but delayed (128.9±61.4 minutes).

CONCLUSIONS: Cluster IT build-up has significantly higher adherence rates, resulting in more patients achieving maintenance doses, but a higher rate of mild/moderate, delayed reactions. Given better adherence, cluster IT build-up may be a preferred approach for appropriate patients.
695 Diurnal Variations in Subcutaneous Allergen Immunotherapy Reactions
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RATIONALE: Circadian rhythms underlie many immune responses and allergic diseases. Diurnal variations exist in skin prick testing wheel sizes and mast cell activity. Subcutaneous Allergen Immunotherapy (SCIT) can result in adverse reactions, but it is unclear whether such reactions have diurnal pattern. We sought to examine whether timing of SCIT administration affected adverse reactions.

METHODS: A retrospective chart review of Northwestern Memorial Hospital patients receiving SCIT was performed. The hypothesis was that all reactions (local/systemic) to subcutaneous immunotherapy would be higher in PM (12:00-17:00) vs AM (07:30-12:30).

RESULTS: A total of 18,184 injections with 578 reactions (442 local+136 systemic) occurred in 295 patients (44% male, μ±SD=12). Immunotherapy reactions occurred more frequently after PM injection (30/8807=3.7%) versus AM (24/8799=2.8%), (χ2=12.11,p<0.01). Systemic reactions did not have diurnal variation, PM(74/8807=0.8%) v. AM (62/8799=0.7%), (χ2=1.08,p=0.30). PM reactions were not larger in size, but is unclear in duration, nor more likely during buildup than AM reactions. Hymenoptera was the most likely allergen to result in reactions in PM v. AM (23/34=68%). Considering extremes of time, injection after 1500 (11/2551=4.4%) was more likely to result in reaction compared to before 1000 (177/1647=2.9%), (χ2=11.35,p<0.01); systemic reactions occurred at similar frequency. Non-systemic cutaneous reactions ≤30 minutes after injection occurred PM (79/8807=1.1%) versus AM (49/8799=0.6%), (χ2=6.54,p=0.01).

CONCLUSIONS: PM injections of SCIT are associated with increased total and cutaneous reaction rates when compared to AM. No diurnal variation in systemic reactions was noted. These findings suggest circadian rhythm of early phase in allergic responses of the skin to SCIT.

696 The Impact of Asthma Control and Higher Maintenance Doses on Immunotherapy Safety: Year 5 of the AAAAI/ACAAI Surveillance Study
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RATIONALE: Clinical practices that influence systemic allergic reactions (SRs) to subcutaneous and sublingual immunotherapy (SCIT and SLIT) are not well defined.

METHODS: From 2008-2013, 27-49% of AAAAI and ACAAI members completed an annual survey of SCIT-related SRs of varying severity (Grade 1 = mild; Grade 2 = moderate; Grade 3 = severe; Grade 4 = very severe anaphylaxis). During 2012-2013 (Year 5), members were queried regarding patient selection based on asthma control and lung function, target doses for SCIT, and use of off-label SLIT.

RESULTS: Between 2008-2013, data were gathered on 29 million SCIT injection visits. Two confirmed fatalities were reported. From 2012-2013, SR rates remained stable at 0.1%, with Grade 4 SRs occurring in 1 in 1 million injection visits. Practices that never started SCIT in patients with uncontrolled asthma (Asthma Control Test <20; 21% of practices) had fewer total and severe/very severe (Grade 3/4) SRs (p<0.05). Similar trends were seen for practices never initiating SCIT in patients with forced expiratory volume in 1 second <70% (p=0.09). Practices giving higher dust-mite doses (>1,000 AU) reported more total and grade 4 SRs (p=0.04 and 0.02). Higher maintenance doses of cat, ragweed, and pasture grass were associated with increased risk for SRs overall (p<0.05). There were 45 SRs reported among 3,343 patients on off-label SLIT, including nine Grade 2 and one Grade 3 SRs.

CONCLUSIONS: Overall, SCIT-related fatalities have remained low. Greater vigilance regarding asthma control among patients initiating SCIT may further improve safety. Further study to determine optimal SCIT dosing is needed. Details of reported SRs to off-label SCIT will be addressed in future studies.

697 Changes in Allergen Specific IgG4 and IgE after Subcutaneous Immunotherapy in Children Under 4 Years of Age
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RATIONALE: Subcutaneous immunotherapy (SCIT) has been shown to increase antigen specific IgG4 levels. The immunologic response to SCIT in children under age 4 has not been prospectively studied.

METHODS: In an ongoing randomized control trial of multiple allergen SCIT in atopic inner city children with asthma between the ages of 18 to 47 months, serum levels of allergen specific IgG4 and IgE were determined before and after 3 years of SCIT. A mixed effect longitudinal model was used to compare immunoglobulin levels between control group (n=11) and immunotherapy group (n=10).

RESULTS: A significant rise in allergen specific IgG4 in response to SCIT was observed for all antigens tested (p<0.006). The most significant responses were seen in mouse IgG4 (coefficient 8.38 ug/l, 95% CI 4.10 to 12.6 ug/l, p <0.001) and dog IgG4 levels (coefficient 3.45 ug/l, 95% CI 2.06 to 4.84 ug/l, p <0.001). In contrast, no significant changes were seen for serum specific IgE levels for most antigens. The only allergen with a statistically significant decrease in serum specific IgE in response to SCIT was cat (coefficient -10.3 kU/l, 95% CI -19.1 to -1.49 kU/l, p =0.016).

CONCLUSIONS: This preliminary study suggests that children who start multiple allergen SCIT between 18 months and 4 years of age can mount a significant increase in allergen specific IgG4 levels after a 3-year course of multiple allergen SCIT, including mouse antigen. A significant decrease in serum specific IgE was only seen for cat antigen.
Subcutaneous Allergen Immunotherapy (SCIT) and Its Effects on Irritability and Sleep in Patients with Allergic Rhinitis (AR) Utilizing a Structured Questionnaire

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RATIONALE: A pilot study to retrospectively assess the effects of SCIT on sleep and irritability in patients with AR.

METHODS: We performed a retrospective analysis of 40 AR patients receiving SCIT at our allergy center from 2004-2014 with the outcomes being rhinitis symptom control, sleep disturbances and irritability assessed by structured questionnaire. Answers were ranked on a scale of 1-4 with (1) signifying “No improvement” thru (4) “Marked improvement” in the build-up phase of SCIT, or after completing 1 year of maintenance. Comparison of sleep and irritability scores were analyzed for total population, and for subpopulations of males, females, age groups, BMI, and those with seasonal or perennial AR using a Wilcoxon t-test pairing.

RESULTS: The male/female ratio was (1:0.1:3.5); age range 4 to 63 years; with a mean age of 39.5 years. Total score demonstrated statistically significant decrease in rhinitis symptoms after the first year of maintenance SCIT (before 1 year mean = 52.29SE = 3.73; after 1 year mean = 66.18SE = 2.94; p < 0.001). Irritability was decreased in total population (before 1 year = 3.10SE = 0.21; after 1 year = 3.60SE = 0.16; p < 0.005) as well as in females (before 1 year = 3.27SE = 0.195; after 1 year = 3.91SE = 0.091; p = 0.01) and there appeared to be a decrease in irritability in all those aged 31-45 (p = 0.07).

CONCLUSIONS: SCIT decreases irritability in patients with AR, especially in women. A larger study is necessary to evaluate the effect on SCIT on sleep disturbances in AR. e is in irritability in all those aged 31-45 (p = 0.07).

Blomia Tropicalis Allergy in Two Different Regions of South Africa

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RATIONALE: Asthma and allergic rhinitis affect 15% and 38% of South African children respectively; house dust mite (HDM) is the most significant aero-allergen involved. The HDM species involved include Dermatophagoides pteronyssinus, Dermatophagoides farinae and Blomia tropicalis. Conventional skin prick tests only for Dermatophagoides and not Blomia tropicalis due to cross-reactivity between the Dermatophagoides species that isn’t shared by Blomia. Blomia tropicalis has only been described in the tropical and subtropical regions of the world. However, it is now coming to light that Blomia tropicalis plays an important role outside the tropical belt and has been proven significant in the Western Cape of South Africa. Testing for Blomia tropicalis isn’t routine practise in South Africa. We hypothesise that Blomia tropicalis is significant in certain regions of South Africa.

METHODS: Skin prick tests (to HDM mix [D. pteronyssinus and D. farinae] and Blomia tropicalis) was conducted in 85 (50 in Kwa-Zulu Natal and 35 in Gauteng) atopic subjects presenting to a private practice in Ballito, northern Kwa-Zulu Natal and Alberton, Gauteng. Kwa-Zulu Natal is a coastal region, experiencing a more tropical climate. Sensitisation was determined if the SPT was 3 mm greater than the negative control.

RESULTS: 52% of subjects in Ballito and 3% of subjects in Alberton were sensitised to Blomia tropicalis (p < 0.05). Of the 52% of patients sensitised to Blomia tropicalis, half were sensitised only to Blomia tropicalis.

CONCLUSIONS: Results of this study indicate that the prevalence of Blomia tropicalis in the Northern Kwa-Zulu Natal region is high and testing should be incorporated in this region.
**Cellular Responses to the Major Allergen of Olea Europaea in Subjects with Local and Systemic Allergic Rhinitis**

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**RATIONALE:** The goal of the study was to thoroughly evaluate the cellular responses to nOle e 1 in allergic rhinitis (AR) and local allergic rhinitis (LAR) patients with sensitization to olive tree pollen (OL) demonstrated by nasal allergen provocation test (NAPT).

**METHODS:** Twelve subjects with AR (+NAPT with OL, + skin testing and specific IgE (sIgE) to OL), 12 subjects with LAR (+ NAPT with OL, - skin testing and sIgE to OL), and 12 subjects as control group (CG) (- NAPT, - skin testing and sIgE to OL) were selected. Basophil activation tests (BAT) with OL and nOle e 1, along with dendritic cell (DC) maturation/proliferation studies in response to nOle e 1 stimulation, were carried out in all subjects.

**RESULTS:** All AR subjects had positive BAT responses to OL and 10/12 to nOle e 1 (83%); 8/12 LAR (66.6%) had a positive BAT with OL and 4/12 (33%) to nOle e 1, with only one subject of the control group with a positive BAT to both OL and nOle e 1 (8%). DC proliferation and maturation were increased in SAR> LAR > CG but with no significant differences (matura- tion: 66.7%/57%/50%; proliferation: 40%/20%/0%). Local ethical committee approved the study.

**CONCLUSIONS:** BAT with OL and nOle e 1 in LAR group showed sensitivity between 66.6 and 33%, demonstrating specific basophil activation with pollens in patients with LAR. DC proliferation and maturation were demonstrated in SAR and LAR subjects although with no significant differences with CG.

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**On-Daily Treatment with Beclomethasone Dipropionate (BDP) Nasal Aerosol Is Effective in Improving Total Nasal Symptom Scores (TNSS) in Children with Seasonal Allergic Rhinitis (SAR) Regardless of Baseline Symptom Severity**

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**RATIONALE:** BDP nasal aerosol (a nonaqueous formulation) is approved for management of seasonal and perennial allergic rhinitis in adolescents and adults. This post hoc analysis evaluated the effectiveness of BDP nasal aerosol by baseline symptom severity in children with SAR.

**METHODS:** This 2-week, double-blind, placebo-controlled study evaluated children with SAR (aged 6-11 years) randomized to BDP nasal aerosol 80μg/day (n=239), 160μg/day (n=242), or placebo (n=234). Efficacy included change from baseline in patient-reported average AM and PM reflective and instantaneous TNSS (rTNSS and iTNSS) by baseline symptom severity: less severe (baseline rTNSS/iTNSS-baseline median) and more severe (baseline rTNSS/iTNSS-baseline median).

**RESULTS:** BDP improved average AM and PM rTNSS over 2 weeks versus placebo in children with more severe baseline symptoms (least squares mean [95% confidence interval] difference: BDP 80μg -0.79 [-1.4, -0.1], P=0.008; BDP 160μg -0.80 [-1.4, -0.2], P=0.006) and less severe baseline symptoms (BDP 80μg -0.60 [-1.1, -0.1], P=0.017; BDP 160μg -0.72 [-1.2, -0.2], P=0.004). BDP improved AM and PM iTNSS versus placebo in children with more severe baseline symptoms (BDP 160μg -0.72 [-1.3, -0.2], P=0.011) and less severe group (BDP 80μg -0.73 [-1.2, -0.3], P<0.001; BDP 160μg -0.74 [-1.2, -0.3], P<0.001). Numerical improvements were apparent with BDP 80μg (-0.49 [-1.1, 0.1]; P=0.084) versus placebo in the more severe group.

**CONCLUSIONS:** In this post hoc analysis, BDP nasal aerosol treatment in children with SAR resulted in significant improvements versus placebo in rTNSS and iTNSS regardless of baseline symptom severity, with the exception of BDP 80μg iTNSS in the more severe group. Study sponsored by Teva.
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**704** Once-Daily Treatment with Beclomethasone Dipropionate Nasal Aerosol Is Effective in Improving Total Nasal Symptom Scores (TNSS) in Children with Perennial Allergic Rhinitis (PAR) Regardless of Baseline Symptom Severity

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**RATIONALE:** Beclomethasone dipropionate (BDP) nasal aerosol (a nonaqueous formulation) is approved for management of seasonal and perennial allergic rhinitis in adolescents and adults. This post hoc analysis evaluated the effectiveness of BDP nasal aerosol based on baseline symptom severity in children with PAR.

**METHODS:** This 12-week, double-blind, placebo-controlled study evaluated children with PAR (aged 4-11 years) randomized to BDP nasal aerosol 80μg/day; n=362) or placebo (n=185). Efficacy assessments included change from baseline in patient-reported average AM and PM reflective and instantaneous TNSS (rTNSS and iTNSS) over 6 weeks for children aged 6-11 years (primary variable) evaluated by baseline symptom severity: less severe (baseline rTNSS or iTNSS-baseline median) and more severe (baseline rTNSS or iTNSS<baseline median).

**RESULTS:** Treatment with BDP nasal aerosol improved average AM and PM rTNSS versus placebo in children with more severe baseline symptoms (least squares mean [95% confidence interval] difference: −0.70 [-1.31, −0.08]; P<0.02) and less severe baseline symptoms (−0.64 [-1.20, 0.08]; P=0.026) over the 6 weeks. Treatment with BDP nasal aerosol improved average AM/PM iTNSS versus placebo in children with more severe baseline symptoms (−0.72 [-1.32, −0.12]; P=0.019); however, in children with less severe baseline symptoms, the improvement did not reach statistical significance (80μg −0.43 [-0.92, 0.07]; P=0.094).

**CONCLUSIONS:** In this post hoc analysis, BDP nasal aerosol treatment in children with PAR resulted in significant improvements versus placebo in rTNSS regardless of baseline symptom severity and in iTNSS in the group with more severe symptoms at baseline. Study sponsored by Teva.

**705** Cetirizine Improves Both Ocular and Nasal Allergy Symptoms in Subjects with Perennial Allergic Rhinitis

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**RATIONALE:** In vitro evaluations using an anatomical model of the human nasal cavity quantified the distribution of azelastine HCl (AZ) and fluticasone propionate (FP) in a single nasal spray (Dymista) compared to sequential sprays of marketed azelastine (Astelin) and either Flonase or generic fluticasone.

**METHODS:** The cast was divided into 4 sections from anterior to posterior of the cast. A single spray of AZ/FP (0.137 mL [137 mcg of azelastine/50 mcg of fluticasone propionate]) or sequential single sprays of azelastine (0.137 mL) followed by generic fluticasone propionate or Flonase nasal spray (0.100 mL) were manually actuated into the model. A vacuum (15 mL/min) was applied during actuation to simulate nasal inhalation. Following extraction from the nasal cast, HPLC was used to quantify drug deposition on the different sections of the cast. Each experiment was repeated three times.

**RESULTS:** A single spray of AZ/FP showed a uniform distribution of close to 100% of applied drug within the nose/nasal valve and turbinates (first 2 sections of the cast); the average % AZ was 61.4% in section 1 and 38.6% in section 2 and the average % FP was 65.4% and 34.6% in sections 1 and 2, respectively. In comparison, single sprays of the individual agents showed uneven distribution of AZ and FP and a substantial amount of dripping from sequential administration.

**CONCLUSIONS:** Application of AZ/FP in a single spray provided more uniform distribution and greater retention in the nasal cavity than sequential sprays of the individual components, potentially allowing for better local absorption of medication.

**706** Quantification of the Distribution of Azelastine HCl/Fluticasone Propionate Nasal Spray in an Anatomical Model of the Human Nasal Cavity

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**RATIONALE:** In vitro evaluations using an anatomical model of the human nasal cavity quantified the distribution of azelastine HCl (AZ) and fluticasone propionate (FP) in a single nasal spray (Dymista) compared to sequential sprays of marketed azelastine (Astelin) and either Flonase or generic fluticasone.

**METHODS:** The cast was divided into 4 sections from anterior to posterior of the cast. A single spray of AZ/FP (0.137 mL [137 mcg of azelastine/50 mcg of fluticasone propionate]) or sequential single sprays of azelastine (0.137 mL) followed by generic fluticasone propionate or Flonase nasal spray (0.100 mL) were manually actuated into the model. A vacuum (15 mL/min) was applied during actuation to simulate nasal inhalation. Following extraction from the nasal cast, HPLC was used to quantify drug deposition on the different sections of the cast. Each experiment was repeated three times.

**RESULTS:** A single spray of AZ/FP showed a uniform distribution of close to 100% of applied drug within the nose/nasal valve and turbinates (first 2 sections of the cast); the average % AZ was 61.4% in section 1 and 38.6% in section 2 and the average % FP was 65.4% and 34.6% in sections 1 and 2, respectively. In comparison, single sprays of the individual agents showed uneven distribution of AZ and FP and a substantial amount of dripping from sequential administration.

**CONCLUSIONS:** Application of AZ/FP in a single spray provided more uniform distribution and greater retention in the nasal cavity than sequential sprays of the individual components, potentially allowing for better local absorption of medication.
Efficacy of Azelastine HCl/Fluticasone Propionate Nasal Spray in the Treatment of Nasal Congestion in Patients with Seasonal Allergic Rhinitis (SAR)

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RATIONALE: The objective of this analysis was to evaluate the efficacy of azelastine/fluticasone propionate (AZ/FP) nasal spray (Dymista) in the treatment of nasal congestion in patients with SAR.

METHODS: This evaluation included four, 2-week, double-blind, placebo- and active-controlled studies (studies 4001, 4002, 4004, and 4006) analyzed individually and as a pooled data set. AZ/FP was compared to monotherapy with AZ and FP and to placebo. All treatments were administered 1 spray per nostril twice daily (AM and PM). Total daily doses of AZ and FP were 548 mcg and 200 mcg, respectively. The primary efficacy variable was change from baseline in the 12-hour reflective total nasal symptom score (rTNSS), which included nasal congestion, sneezing, itchy nose, and runny nose scored twice daily (AM and PM) on a 4-point rating scale such that the maximum daily score was 24.

RESULTS: AZ/FP was statistically superior (P<0.05) to placebo and to all monotherapy for improving rTNSS in all the studies. In the pooled analysis of nasal congestion (n=3999), the mean improvement from baseline with AZ/FP was statistically significant compared to AZ (-0.31; 95% CI, -0.41, -0.21), FP (-0.20; 95% CI, -0.30, -0.10), and placebo (-0.52; 95% CI, -0.62, -0.43). Significant differences were seen vs. AZ in all studies and vs. FP in studies 4001 and 4004. AZ/FP was well tolerated, the most frequently reported adverse events were dysgeusia (4%), epistaxis (2%), and headache (2%).

CONCLUSIONS: Results of these studies demonstrate that AZ/FP provided statistically significant improvement in nasal congestion compared to monotherapy with either AZ or FP.

Patient-Reported Clinical Characteristics in a Randomized Controlled Trial in Seasonal Allergic Rhinitis (SAR)

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RATIONALE: Medical history and positive allergen skin testing are generally considered sufficient to make a diagnosis of allergic rhinitis (AR); however, many individuals with AR are also sensitive to non-allergic triggers. In a clinical trial of azelastine HCL/fluticasone propionate (AZ/FP) nasal spray (Dymista) in patients with SAR, a rhinitis questionnaire was used to identify patient demographic and clinical characteristics (n=831).

METHODS: The rhinitis questionnaire gathered information on age at symptom onset, parental history, types of symptoms, sensitivity to allergic and non-allergic triggers, and response to previous medications.

RESULTS: Mean age of the patients was 37 years with average onset of allergic symptoms at 16 years. Forty-eight percent and 16% reported parental history of allergy and asthma, respectively. Stuffy nose (97%), sneezing (95%), and runny nose (95%) were the most commonly reported symptoms. Sixty-five percent and 45% of patients reported sensitivity to cat and dog, respectively, indicative of PAR. Nearly 90% of patients reported a response to at least one non-allergic trigger, including climate changes (68%), smoke (63%), and fragrances (56%). Less than 30% reported more than a moderate effect from previous medications.

CONCLUSIONS: A large percentage of SAR patients reported symptoms in response to non-allergic triggers suggesting a high percentage of patients in this study had AR with a non-allergic component (i.e., mixed rhinitis). The efficacy of AZ/FP in this study population suggests that it may be effective for treatment of both perennial and non-allergic rhinitis in addition to SAR.

Effect of ONO-4053, a DP1 (prostaglandin D2 receptor) Antagonist, on Antigen-Induced Nasal Congestion

Shinsuke Yamaguchi, Yutaka Okada, Yoko Matsunaga, Fumio Nambu; Ono Pharmaceutical Co., Ltd.

RATIONALE: As prostaglandin D2 (PGD2), a chemical mediator released in large amounts from mast cells, is known to be involved in a number of allergic responses, blockage of PGD2 action might be beneficial for the treatment of nasal allergies. ONO-4053 is an orally active DP1 (PGD2 receptor) antagonist in phase 2 clinical development for the treatment for the allergic rhinitis. The purpose of this study was to evaluate the effect of ONO-4053 on antigen-induced nasal congestion in Ascaris suum sensitive cynomolgus monkeys.

METHODS: Monkeys were anesthetized, intubated and mechanically ventilated. Animals were antigen challenged into the nostril. The nasal volume was evaluated by acoustic rhinometry. The nasal secretion was measured. The changes in the inflammatory parameters in nasal lavage fluid were determined after antigen challenge. ONO-4053 was given orally to animals.

RESULTS: Antigen challenge induced nasal congestion and rhinorrhea with elevated levels of eosinophils, chemical mediators and Th2 cytokines in the nasal cavity. ONO-4053 completely inhibited antigen-induced decrease in nasal volume both in the early and late phases.

CONCLUSIONS: ONO-4053 strongly inhibits antigen-induced nasal congestion. It is therefore believed ONO-4053 would be clinically useful for the treatment of allergic rhinitis.

Effect of ONO-4053, a DP1 (prostaglandin D2 receptor) Antagonist, on Prostaglandin D2–Induced Nasal Congestion

Yutaka Okada, Shinsuke Yamaguchi, Yoko Matsunaga, Fumio Nambu; Ono Pharmaceutical Co., Ltd.

RATIONALE: As prostaglandin D2 (PGD2), a chemical mediator released in large amounts by mast cells, is known to be involved in a number of allergic responses, blockage of PGD2 action might be beneficial for the treatment of nasal allergies. ONO-4053 is an orally active DP1 (PGD2 receptor) antagonist in phase 2 clinical development for the treatment of allergic rhinitis. The purpose of this study was to evaluate the effect of ONO-4053 on PGD2–induced nasal congestion in dogs.

METHODS: Dogs were anesthetized, intubated, and mechanically ventilated. Nasal volume (NV), as indicator of nasal congestion, was determined before and after intranasal application of PGD2 using acoustic rhinometry. ONO-4053 was given orally to PGD2–treated dogs.

RESULTS: Nasal application of PGD2 rapidly decreased NV. This decrease continued for up to 60 min after PGD2 application. ONO-4053, given orally 4 hours prior to PGD2 application, dose-dependently inhibited PGD2–induced decrease in NV. In addition, ONO-4053, given intravenously for 17 minutes after PGD2 application, completely reversed PGD2–induced decrease in NV.

CONCLUSIONS: These findings indicate that PGD2–induced nasal congestion is predominantly mediated via the DP1 receptor and that ONO-4053 could be effective for the treatment of allergic rhinitis.
3-Dimensional and 2-Dimensional CT-Imaging of the Sinus Airways in a Model of Allergic Sinus Congestion

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RATIONALE: Sinus congestion is a prominent symptom of allergic rhinitis, for which congestion measurements and treatment options are relatively limited. A model of allergic sinus airway inflammation was investigated using non-invasive sinus imaging, in the guinea pig (GP) which has relevant histamine-driven allergic similarities to humans, and could potentially be used to test efficacy of putative drugs that might reduce sinus congestion.

METHODS: After systemic ragweed pollen (RWP) sensitization i.p., GP’s were administered either RWP (10 µg/nostril), or vehicle, intranasally, once per day, for 5 days, to induce sinus airway inflammation (AI). Sinus fluid-fill volume (SFFV) was measured as a direct index of allergic sinus congestion, and compared with 2-dimensional (2-D) measures of sinus spatial area (SSA), and 3-dimensional (3-D) measures of sinus image volume (SIV), determined by computed-tomography (CT). RWP-associated AI was verified with BAL fluid eosinophil counts.

RESULTS: As compared to intranasal vehicle, RWP increased BAL eosinophils by over 50% (P<0.05), indicating RWP-associated AI. As measured by decrements in SFFV, RWP produced significant increments in allergic sinus congestion, to levels of ~80% sinus occlusion (P<0.05). CT image measurements likewise indicated significant sinus occlusion with RWP, observed as decreased 2-D SSA (80-90%; P<0.05), and 3-D SIV (averages: 60-70%, P<0.05).

CONCLUSIONS: We conclude that both 2-D and 3-D imaging provide measures of sinus congestion changes comparable with direct fluid-displacement methods of sinus volume assessments, indicating that non-invasive CT imaging may allow reliable assessment of sinus airway congestion, in the pre-clinical evaluation of efficacy of therapeutic drugs for treatment of allergic sinus airway congestion.

Prolonged RSK and RPS6 Phosphorylations By IL-3 Increases Translation in Human Eosinophils

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RATIONALE: Besides IL-5, other cytokines which are part of the β chain family, such as IL-3 or GM-CSF may contribute to eosinophil activation in allergic inflammation. IL-3 is more potent than IL-5 and GM-CSF to enhance the production of the pro-fibrotic GBP protein, semaphorin 7A, on the eosinophil surface. Also, compared to GM-CSF, IL-3 activation is associated with more enrichment of semaphorin7A mRNA within the polyribosomes indicating increased translation. Based on these observations, we hypothesized that among the cytokines of the β chain family, IL-3 triggers a differential intracellular signal that increases translation.

METHODS: We analyzed by western-blot and confocal microscopy the phosphorylation stage of factors implicated in translation in IL-3-, IL-5- or GM-CSF-activated human blood eosinophils. Translation rate was measured using the non-radioactive Click-iT® tool to label nascent protein.

RESULTS: Unlike GM-CSF or IL-5, IL-3 triggered a strong and prolonged signaling via the Erk/RSK/RPS6 pathway. The phosphorylated ribosomal S6 protein (RPS6) was located around the nucleus and was associated with the endoplasmic reticulum compartment. RPS6 phosphorylation was independent of the canonical PI3K/mTOR/p70S6K pathway, while blockade of p90S6K (RSK) activation using a small-molecule inhibitor decreased translation, expression of semaphorin7A, and inhibited the phosphorylation of RPS6 in eosinophils.

CONCLUSIONS: Our findings demonstrate that IL-3 is unique among the β chain family of cytokines, in prolonging RPS6 phosphorylation and consequently increasing translation in eosinophils. IL-3 and its specific downstream intracellular signals, including RPS6 should be considered as potential targets to reduce eosinophil functions and improve negative health consequences associated with eosinophilia.

The Association Between Severity of Total Eosinophilic Count and Disease Categories

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RATIONALE: Eosinophilia is an initial sign of various allergic or non-allergic diseases. The aim of this study was to investigate the relationship between severity of eosinophilia and disease categories.

METHODS: Retrospective, cross-sectional study was conducted in tertiary referral hospital in Japan. Eosinophilia was defined with total eosinophilic count (TEC) > 500/µl. Eosinophilia was categorized into 3 groups; mild (500–1500/µl), moderate (1501–5000/µl), severe (5001–/ µl). The final diagnosis and laboratory data, including maximum TEC (TECmax) were collected.

RESULTS: 18878 patients were diagnosed with hyper eosinophilia between July 2003 and June 2014. 57% was male and the mean age (SD) was 50 years old (17). The mean(SD) of TECmax was 870/µl (92). The number and proportion(95%CI) of each categories of mild, moderate, severe was 93.0% [92.6-93.3], 6.3% [5.9-6.6], 0.7% [0.6-0.9], respectively. In the eosinophilic patients, the prevalence (95%CI) of malignant disease, allergic disease, parasite disease, and other disease was 9.8% [9.4-10.3], 15.4% [14.9-16.0], 0.07% [0.04-0.12], and 74.7% [74.0-75.3] respectively. There was a significant association between four disease categories and severity of eosinophilia (Chi-square test, P < 0.001). The proportions of malignant disease and allergic disease is significantly higher in the patient with moderate and severe eosinophilia than in the patient with mild eosinophilia. This proportion tend to be higher as TECmax become severe (Trend test, P < 0.0001). More than 50% of patients have malignancy or allergic disease in the patients with severe TECmax.

CONCLUSIONS: Malignancy or allergic disease are probable in the adult patients with moderate or severe TECmax.
714 Priming for Degranulation in Eosinophils Stimulated with Interleukin-5 (IL-5) Is Reversible
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RATIONALE: Degranulation of eosinophil, the ultimate activation, is preceded by priming, the state of increased sensitivity to degranulating factors. To test whether eosinophil priming can be reversed pharmacologically, the effect of Lipoxin A4 and inhibitor of PKCβII were compared in cells previously primed with IL-5 in vitro.

METHODS: Peripheral blood eosinophils isolated from healthy donors were stimulated with IL-5 at 1 ng/ml and characterized for changes in cell shape (forward cell scatter), expression of activated αMβ2 integrin (M24 Ab), actin depolymerization (FITC-Phalloidin), PKCβII activation, L-plastin phosphorylation and eosinophil degranulation in response to Eotaxin (EPX and ECP). Lipoxin A4, an eicosanoid with counter-regulatory properties for IL-5 signaling or Ribostaxaurin, an inhibitor of PKCβII were added to eosinophils after priming with IL-5 for 2, 4 and 8 h.

RESULTS: Eosinophils stimulated with IL-5 showed increased degranulation in response to Eotaxin that was detectable 30 min. after stimulation and remained elevated 8 h later. Priming was accompanied by changes in cells shape, actin depolimerization, active conformation of αMβ2 integrin, activation of PKCβII and L-plastin phosphorylation. Both Lipoxin A4 and Ribostaxaurin reversed IL-5-induced changes in cell shape, actin depolimerization, activation of αMβ2 and increased degranulation when added up to 6 h after priming occurred. Neither Lipoxin A4 nor Ribostaxaurin affected eosinophil viability or total αMβ2 expression.

CONCLUSIONS: Our data show that eosinophil priming is not irreversible event. The demonstration of reversal of priming implies therapeutic strategies to control harmful eosinophil functions before they occur, leaving homeostatic functions intact.

715 Cyclophilin D Regulates Eosinophil Survival
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RATIONALE: Eosinophil degranulation and presence of clusters of free extracellular granules have been frequently observed in diverse diseases, including atopic dermatitis, nasal polyps, and eosinophilic esophagitis. However, whether these intact granules are released by accidental necrosis or a biochemically-mediated necrosis is not known. Recently, a prolyl isomerase located within the mitochondrial matrix, cyclophilin D (the Ppif gene product) was shown to regulate necrotic but not apoptotic cell death in vitro. Thus, we hypothesized that cyclophilin D is required for regulated necrosis of eosinophils.

METHODS: Bone marrow (BM)-derived eosinophils from wild type (WT) and PPIF-deficient mice were treated with stimuli for apoptotic and regulated necrotic cell death. After 4-hr stimulation, eosinophils were harvested and necrosis/apoptosis determined by flow cytometry following 7-AAD and Annexin-V-APC staining.

RESULTS: WT and PPIF-deficient eosinophils exhibited comparable development from BM progenitor cells in terms of their proliferation and surface expression levels of Siglec-F and CCR3. Following 4-hr incubation with ionomycin or H2O2, stimuli for Ca2+ overload and oxidative stress-induced regulated necrosis, a significant decrease of necrosis (7-AAD+) but not apoptosis (7-AAD Annexin-V-) was observed in PPIF-deficient eosinophils compared to WT eosinophils. However, when treated with traditional apoptosis inducers, such as anti-Fas mAb, anisomycin, or camptothecin, no significant difference in either apoptosis or necrosis was observed between WT and PPIF-deficient eosinophils.

CONCLUSIONS: Mouse eosinophil necrosis in vitro is regulated by cyclophilin D. Thus, we have identified a pathway which can be manipulated to affect eosinophil necrosis and release of free extracellular granules in eosinophil-associated diseases.

716 A Novel Therapeutic Target for the Treatment of Eosinophilic Disorders
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RATIONALE: Crosslinking of an inhibitory receptor present on eosinophils using a chimeric IgG4 receptor-specific antibody causes eosinophil apoptosis. A chimeric IgG1 antibody to the same receptor also targets receptor-bearing cells for NK-mediated ADCC.

METHODS: To compare the relative efficacy of these two antibodies at inducing eosinophil cell death in peripheral eosinophils from patients with hypereosinophilic syndrome (HES), flow cytometric assays were performed using peripheral blood leukocyte preparations (PBL) from normal and eosinophilic donors: 1) apoptosis assay (purified eosinophils incubated +/-IL-5 +/- anti-receptor antibodies), 2) ADCC assay (purified eosinophils and autologous NK cells (NK:EO=5 +/- antibodies), 3) PBL assay. In the first two assays, cell death was measured by Annexin V-7AAD staining. PBL killing was assessed as eosinophil deplet.

RESULTS: Both anti-receptor antibodies induced eosinophil apoptosis (GM 54 and 53%, respectively, vs 12% for isotype controls) after overnight priming with IL-5 (P<0.0001, n=22). Only the IgG1 antibody-induced IL-5 independent apoptosis and NK-mediated killing (GM 58.6% vs 26.5%, P<0.01, n=14, NK:EO=5:1). In the whole blood assay, eosinophil deplet at 4 hours was seen with IgG1 in PBL from HES donors with an NK:EO>0.25:1 and was improved with IL-5 for both IgG1 (from 0% to 47%) and IgG4 (from 0.1% to 7%) (P<0.01, n=11). Eosinophil deplet occurred after overnight incubation even with low NK:EO ratios.

CONCLUSIONS: Both IgG1 and IgG4 anti-receptor antibodies induce eosinophil apoptosis and this may be enhanced in patients with increased serum IL-5 levels. The IgG1 antibody showed additional killing activity due to ADCC.

717 Eosinophil Peroxidase As an Autoimmune Target in Eosinophilic Airway Disorders
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RATIONALE: Thyroid peroxidase and Myelperoxidases have immunogenic properties. We hypothesized that eosinophil peroxidase (EPX) released by ‘activated’ eosinophils in the airways of patients with eosinophilic respiratory disorders may also be immunogenic.

METHODS: EPX and anti-EPX immunglobulins were analysed in induced sputum (IS) from patients with eosinophilic asthma (EA, n=6, 42.25 ± 10.0% sputum eosinophils), neutrophilic asthma (NA, n=6, 0.6 ± 0.3%), eosinophilic bronchi (EB, n=5, 70 ± 7.8%), and other respiratory disorders (CR, n=6, 0.2 ± 0.1%). Eosinophilic patients were on high doses of inhaled corticosteroids or additional prednison. Endogenous EPX in IS supernatants added to mouse anti-EPX antibody (4 µg/ml, clone MM25-429.1.1) coated wells was detected by biotinylated mouse anti-EPX antibody, clone (0.8 µg/ml, MM25-429.1.1). To detect anti-EPX antibodies, 250 ng/µl EPX (LEE Biosolutions, MO, USA) was added to the wells coated with pooled supernatants for each disease phenotype, blocked overnight with 1% BSA, and detected by the same anti-EPX antibody.

RESULTS: Both EA and EB patients had high amounts of EPX, 1.76 ± 1.3 and 1.4 ± 0.6 µg/ml respectively; otherwise, undetectable in NA and CR phenotypes. After adjusting for the endogenous EPX signals, there were 3.18 ± 0.7 and 2.15 ± 0.2 fold increases in anti-EPX signal detected for EA and EB respectively, w.r.t. CR sputum (p=0.0013, ANOVA). In contrast, 1.57 ± 0.3 times increase in signal for the NA sputum was deemed significant only to the EA phenotype (p<0.001).

CONCLUSIONS: We hereby report a previously unrecognized local autoimmune component underlying severe eosinophilic bronchitis that may relate to disease severity and treatment responses.
Abstracts
J ALLERGY CLIN IMMUNOL
FEBRUARY 2015

AB222

718 Cytokine and Chemokine Analysis in Bronchoalveolar Lavage Fluid of Acute Eosinophilic Pneumonia
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RATIONALE: Acute eosinophilic pneumonia (AEP) is characterized by a massive pulmonary infiltration of eosinophils. Mechanisms regulating the selective accumulation of eosinophils in AEP have not been fully established. The objective of this study was to analyze cytokine and chemokine responses to evaluate the mechanisms of eosinophil accumulation in alveolar spaces.

METHODS: We measured cytokine and chemokine concentrations in bronchoalveolar lavage fluid (BALF) of AEP (n = 10), sarcoidosis (n = 8), or hypersensitivity pneumonitis (n = 10) using ELISA.

RESULTS: The concentrations of IL-5, thymus- and activation-regulated chemokine (TARC), macrophage-derived chemokine (MDC) and monococyte chemotactic protein (MCP)-4 were elevated in BALF of AEP. The concentrations of GM-CSF, eotaxin, regulated upon activation, normal T cell cytoxic (TARC), macrophage-derived chemokine (MDC) and monococyte chemotactic protein (MCP)-4 were elevated in BALF of AEP.

CONCLUSIONS: These findings suggest that IL-5, TARC, MDC, and MCP-4 may play an important role in the accumulation of eosinophils in AEP.

719 Procateler Suppresses Epithelial to Mesenchymal Transition (EMT) of Bronchial Epithelial Cells Induced By Eosinophils
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RATIONALE: A recent study has shown that bronchoconstriction induces airway remodeling and that b2 agonists inhibit it in patients with bronchial asthma. We have previously reported that eosinophils play a critical role in airway remodeling by inducing EMT in asthma (PLoS One 2013; 8: e64281). In this study, we hypothesized that b2 agonists are protective against airway remodeling by suppressing eosinophil-induced EMT.

METHODS: The human bronchial epithelial cell line, BEAS-2B, and primary human peripheral blood eosinophils or the eosinophilic leukemia cell line, Eol-1, were co-cultured in the presence or absence of various concentrations of procateler, a potent b2 agonist. Morphological changes, gene/protein expressions of epithelial and mesenchymal markers were measured by confocal microscopy, PCR and Western blotting. The concentration of TGF-b1 was measured by enzyme immunossay. The expression of integrins on eosinophils/Eol-1 was evaluated by flow cytometry. The effect of the b2 receptor inhibitor, butoxamine, was also examined.

RESULTS: Procateler decreased the EMT changes in BEAS-2B bronchial epithelial cells induced by co-culture with eosinophil including spindle shape change, enhanced expression of vimentin and decreased expression of E-cadherin. TGF-b1 production and eosinophil expressions of CD11b, CD18, CD29 and CD49d were significantly inhibited by procateler. Butoxamine blocked all the effects induced by procateler.

CONCLUSIONS: This study shows that procateler inhibits eosinophil-induced EMT of airway epithelial cells by inhibiting TGF-b1 production, suggesting that b2 agonists may ameliorate airway remodeling in asthma.

720 Increased Expression of Leukotriene C4 Synthase Is a Feature of Circulating CD34+ Hematopoietic Stem Cells but Not Circulating Eosinophils in Aspirin-Exacerbated Respiratory Disease (AERD)
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RATIONALE: Aspirin-exacerbated respiratory disease (AERD) is characterized by over-expression of leukotriene C4 synthase (LTC4S) by tissue eosinophils, driving constitutive over-production of cysteinyl leukotrienes (CysLTs) and the surge of CysLT secretion that occurs following aspirin ingestion. It is unknown whether upregulation of LTC4S is a phenotypic change induced in mature eosinophils or a feature of eosinophils differentiating from CD34+ progenitors. We investigated the expression of enzymes involved in arachidonic acid (AA) metabolism in circulating eosinophils and CD34+ cells and their ability to secrete AA metabolites.

METHODS: Eosinophils and CD34+ cells were enriched from peripheral blood leukocytes obtained from control, aspirin-tolerant asthmatic, and AERD subjects. mRNA was extracted and subjected to reverse transcription followed by quantitative polymerase chain reaction (qPCR) to evaluate expression of genes related to AA metabolism and responsiveness. Separately cells were stimulated and secretion of AA metabolites quantified.

RESULTS: Surprisingly, no differences in LTC4S or other enzymes involved in AA metabolism were observed in circulating eosinophils. In contrast, AERD CD34+ cells were associated with increased LTC4S expression in comparison to controls and aspirin-tolerant asthmatics. Stimulation of AERD-derived CD34+ cells was associated with enhanced AA metabolism.

CONCLUSIONS: The defining phenotype of AERD is the increased expression of LTC4S in tissue-associated eosinophils. This feature appears to develop early, in hematopoietic stem cells, cells that are either newly emerging from the bone marrow or plausibly amongst progenitors recirculating between the upper and lower airway. Fully mature eosinophils remaining in the circulation, although increased in absolute number in AERD, do not express this phenotype.
721 Nasal Allergen Challenge (NAC) Induced Eosinophilia - the Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC)
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RATIONALE: The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) is a Canadian initiative with the goal of performing standardized nasal allergen challenge (NAC) to study the effects of therapeutic agents for allergic rhinitis (AR), while allowing the identification of mechanisms and biomarkers of AR. Various NAC protocols have been described previously. The multiple cumulative allergen concentration (MCAC) NAC protocol was shown to produce more robust symptom scores than a cumulative allergen concentration (CAC) protocol. Here we examined NAC-induced eosinophilia for these two protocols.
METHODS: 17 atopic and 12 non-atopic participants were enrolled for this study. Atopic individuals presented with AR symptoms in ragweed season and a supportive skin test response. During screening incremental concentrations of ragweed allergen were administered until each participant reached the qualifying symptom cut-off. For the subsequent NAC one week later, ten atopics were challenged with one dose of allergen equivalent to the cumulative amount of allergen each received during screening (CAC). Seven atopics received the cumulative of all preceding allergen doses to the qualifying concentration (QC), followed by the QC 15 minutes later (MCAC). Non-atopics were challenged with a 1:2 ragweed concentration. Nasal lavage samples were collected at baseline, 1 hour (1H) and 6 hours (6H) post NAC to determine differential counts.
RESULTS: The eosinophil fraction was significantly increased in atopics following NAC when compared to non-allergics at both 1H and 6H for the CAC-protocol but only at 6H for the MCAC-protocol.
CONCLUSIONS: Even though the MCAC protocol establishes more robust symptom scores, the CAC protocol appears to produce more pronounced eosinophilia.

722 Differential Exposure of Eosinophils to Cytokines Leads to an Activated Phenotype in the Airways Inducing Pulmonary IL-13 Responses That Are Eosinophil Dependent
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RATIONALE: Allergic respiratory inflammation is characterized by immune responses and induced pathologies linked with increased airway IL-13. Mice deficient (CCR3−/−, IL-5−/−) or devoid (PHIL, ΔmGATA-1, IL-5−/−/ectoTNF-1−/−) of eosinophils display reduced pulmonary IL-13 in various mouse models of allergic respiratory inflammation. Moreover, previous studies have demonstrated that eosinophils are capable of both expressing IL-13 and promoting its expression by other resident lung cells.
METHODS: Purified blood-derived wild type or cytokine deficient (IL-4−/− and IL-13−/−) eosinophils were either untreated or cytokine pre-treated prior to adoptive transfer into the lungs of allergen challenged mice deficient (IL-5−/−) or devoid (PHIL) of eosinophils. Allergen-induced pulmonary changes in Th2 cytokine levels, histopathologies, and accumulation of leukocyte populations were assessed.
RESULTS: Eosinophils with activation-state unique activities were achieved by differential cytokine pre-treatment prior to adoptive transfer into the lungs of allergen-treated IL-5−/− or PHIL mice. GM-CSF treated eosinophils activated T cells and DCs in the lung draining lymph nodes, yet did not induce either T cell recruitment to the lung or evidence of pulmonary inflammation. In contrast, eosinophils pre-treated with both IL-33 and GM-CSF were sufficient to restore pulmonary T cell accumulation as well as induced allergen-mediated pathologies. We demonstrated in both IL-5−/− and PHIL mice that the Th2 pulmonary immune responses and induced pathologies mediated by differentially activated eosinophils were specifically dependent on the expression of IL-13 (but not IL-4) by eosinophils.
CONCLUSIONS: Our data demonstrates an underappreciated and significant role for eosinophil-derived IL-13 in inducing Th2 pulmonary pathologies in mouse models of allergic respiratory inflammation.

723 Preferential Production of TNFα, Compared to IL-4 and IFNγ, By Differentiating Eosinophil-Basophil Cord Blood Progenitors
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RATIONALE: Eosinophils are multi-functional leukocytes involved in a number of infectious and inflammatory processes, including allergic diseases. Classical Th1 (IFNγ) and Th2 (IL-4) cytokines negatively impact eosinophil differentiation from cord blood (CB) in vitro. Since we have recently shown that CB progenitor cells produce cytokines that auto-regulate their own differentiation, and we have recently uncovered a novel role for TNFα positively impacting eosinophil-basophil (EoB) colony formation, we investigated the secretion of TNFα relative to these classical Th1 and Th2 cytokines during eosinophil differentiation from cord blood.
METHODS: Fresh CB progenitor cells were stimulated with GM-CSF, IL-5 and IL-3 for 14 days in liquid media to induce eosinophil production. Cells were isolated at days 3, 7 and 10 and cytokine levels of IL-4, TNFα, IFNγ, were evaluated using luminex technologies. Morphological analysis was performed to determine the presence of eosinophils in culture after 14 days.
RESULTS: As compared the Th1 cytokine IFNγ, TNFα was secreted at significantly greater amounts at day 3 (p=0.033), day 7 (p=0.040), and day 10 (p=0.040) of culture. When compared to the Th2 cytokine IL-4, TNFα was secreted at significantly greater amounts at day 3 (p=0.014), day 7 (p=0.013), and day 10 (p=0.019) of culture.
CONCLUSIONS: We show for the first time that CB EoB progenitors preferentially secrete TNFα, compared to classic Th1 and Th2 cytokines, during their own differentiation process. Our work provides novel insights into ‘cytokine signatures’ utilized by CB progenitors that may enhance eosinophil-basophilic inflammation in early life.
724 Pharmacological Characterization of T Cell-Induce Bronchoconstriction in the Mice
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RATIONALE: To investigate a role of Th cells in asthma, T cell-transfer model was analyzed for immediate and late phase asthmatic responses after antigen challenge.

METHODS: OVA specific Th clones were adoptively transferred into unprimed mice. After intranasal or inhalation challenge with OVA, airway resistance was continuously monitored by either unrestrained whole body plethysmography or resistance/compliance analyzer under anesthetized condition. Bronchoalveolar lavage and analysis of airway hyperresponsiveness (AHR) were performed 48 hr after OVA challenge. Supernatants of stimulated Th clones were analyzed for contractile activity using collagen gels embedded with murine primary bronchial smooth muscle cells. Effects of H2R and LTR antagonist were analyzed both in vitro and in vivo.

RESULTS: When unprimed mice were transferred with Th clones, T5-1, T6-1, or T6-10 did not show any change. Airflow limitation was confirmed by a direct measurement of airway resistance under anesthetized, restrained, and intubated conditions. The airflow limitation was also efficiently induced by the challenge with T cell epiopeptide. OVA322-339. Contractile activity was detected in the supernatants of T6-2 stimulated with immobilized anti-CD3. T cell-induced contraction was not affected by H2R or LTR antagonist.

CONCLUSIONS: Activation of Th cells resulted in an airflow limitation besides eosinophilic inflammation, AHR, and mucus hyperplasia. T cell-derived bronchoconstrictor might be a target for treatment-resistant asthma.

725 C5a Mediated Induction of Phosphorylated P38 MAPK Expression By Blood T Lymphocytes in Adults with Allergic Asthma
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RATIONALE: Complement split product C5a levels increase in airways after allergen provocation in asthmatics. We reported plasma C5a levels relate to asthma severity and quality of life scores. Expression of phosphorylated p38 MAP kinase (p38 MAPK) in lymphocytes from allergic adults correlates with total serum IgE. We investigated whether C5a increases p38 MAPK by lymphocytes and its association with IgE production in vitro.

METHODS: PBMCs (1.5 x 10^6/mL) from allergic asthmatics (n = 10, age 35-66 years) were cultured with anti-CD40 antibody and recombinant-human interleukin-4 ± recombinant-human C5a (0.1-10 μg/mL) for 2-10 days. P38 MAPK levels (% expression) by CD4+ and CD8+ T lymphocytes were determined on day 2 and 4 (flow cytometry). IgE levels in supernatants were determined on day 10 (ELISA). Serum IgE levels were determined (fluoroenzymimmunoassay).

RESULTS: Serum IgE levels were 1646.7 ± 1405 U/ml. C5a (1 and 10 μg/ml) significantly increased p38 MAPK levels by CD8+ T lymphocytes at 96 hours compared to without C5a (p = 0.037 and 0.047, respectively). CD4+ T lymphocytes had no significant change in p38 MAPK (p = 0.093, p=0.646). In 7/10 (70%) of subjects, IgE production increased (0.04-3.48 ng/ml, mean 2.08 ± 0.52) from baseline IgE (7.19-45.49 ng/ml, mean 10.48) when cultured with C5a. However, in vitro IgE production had no significant correlation with p38 MAPK or serum IgE levels.

CONCLUSIONS: C5a upregulates p38 MAPK by CD8+ T cells of IgE+ allergic asthmatics. CD8+ T cells with increased P38 MAPK may serve as helpers for IgE response.

726 NMDA Receptor Triggering Leads CD4+ T Cells Cytokine Balance Towards Type 2 Dominant
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RATIONALE: A rate-limiting enzyme, indoleamine 2,3-dioxygenase (IDO), is known to suppress T cell-mediated immune responses, however, its precise mechanisms and its effects on type 1/2 balance remain unclear. We previously showed that human CD4+ T cells express N-methyl-D-aspartate (NMDA) receptor that is known to bind kynurenines, tryptophan catabolites via the activation of IDO, and stimulation of NMDA receptor decreases T cell viability. Thus, we aimed in this study to investigate if NMDA receptor triggering influences on cytokine secretion balance from CD4+ T cells.

METHODS: PBMCs were isolated from consenting adult healthy donors, and CD4+ T cells were isolated using immunomagnetic beads followed by cell activation with anti-CD3/28 antibodies. Cells were then treated with NMDA receptor agonists. Cell-free culture supernatants were collected, and the cytokine/chemokine levels were quantified by multiplex assay after 24 hours of culture.

RESULTS: NMDA receptor triggering was found to decrease proinflammatory cytokines (TNF and IL-6) as well as type 1 cytokines (IFN-gamma). Conversely, type 2 cytokine (IL-5 and IL-13) levels were increased by the same treatment.

CONCLUSIONS: Our results suggest that IDO does not simply suppress T cell-mediated immune responses but skews T cell cytokine secretion pattern towards Th2 through NMDA receptor triggering. In addition, NMDA receptor is found to be a potential therapeutic target of diseases with T cell-mediated type 2 inflammation.
727 Distinct Patterns and Magnitude of T Cell Responses Are Associated with Seasonal Exposure to Timothy Grass Allergens

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RATIONALE: Timothy grass (TG) pollen is a common seasonal airborne allergen associated with symptoms ranging from mild rhinitis to severe asthma. The aim of this study was to characterize TG-specific T-cell responses as a function of seasonality. Moreover, quantitative as well as qualitative differences, in terms of magnitude of the response and involved T-cell subsets were assessed.

METHODS: PBMCs samples, obtained either during the pollen season or out of season, from allergic individuals and non-allergic controls were stimulated either with TG extract or a pool of previously identified immunodominant antigenic regions. The production of IFNγ, IL5, IL10 and IL17 associated with different Th-subsets was evaluated.

RESULTS: PBMCs from in-season allergic individuals exhibit significantly higher IL5 and IL10 responses and a trend towards higher IFNγ production compared to out of season donors. In the case of non-allergic individuals, we observed much lower responses in terms of the Th2 associated cytokine IL-5 and a robust production of Th1-associated IFNγ. Strikingly, the Th1 response in normal individuals was decreased in season compared to out of season. Unexpectedly, a reverse pattern of responses with regard to seasonality was observed in non-allergic compared to allergic donors. Particularly, IFNγ and IL-5 were significantly lower in in-season non-allergics compared to out of season.

CONCLUSIONS: Our data suggest that the magnitude and functionality of T-cell responses differ substantially for in-season versus out of season in allergic and non-allergic individuals. The potential basis of this phenomenon is currently being investigated.

728 The Th2/Th17 Predominant Endotype of Severe Asthma Is Associated with Increased IL1β, C3 and Their Downstream Signaling Molecules in Bronchoalveolar Lavage

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RATIONALE: We recently described a Th2/Th17 endotype of severe asthma based upon the predominance of Th2/Th17 cells in bronchoalveolar lavage (BAL). Th2/Th17 cells demonstrated steroid-resistance. We investigated the mechanism of this endotype.

METHODS: BAL cells were analyzed by flow cytometry. Cytokines and danger-associated molecular patterns (DAMPs) in BAL were assayed by ELISA.

RESULTS: IL1β, IL6, IL21 and IL23 induce Th2/Th17 cells in vitro. The protein level of IL1β, IL6 and IL23 was increased in BAL from asthmatic patients (N=25) as compared to disease controls (N=15). IL21 was undetectable. IL1β (r=0.75, p=0.03) but not IL6 (r=0.23) or IL23 (r=0.37) positively correlated with Th2/Th17 cells. BAL Th2/Th17 cells had increased phosphorylation of IL1R and its downstream effector p38 MAPK. IL1β and Th2/Th17 can be induced by endogenous DAMPs. BAL from asthmatic patients had elevated 3 DAMPs—C3, serum amyloid A and uric acid. Of these, only C3 correlated positively with IL1β (r=0.69, p=0.001) and Th2/Th17 (r=0.46, P=0.03). C3a activates MEK-ERK1/2 signaling. We reported increased expression of MEK in BAL Th2/Th17 cells. The expression of two MEK-inducible and steroid resistance-inducing transcription factors—c-Fos and JunB was elevated in Th2/Th17 cells. The MEK inhibitor trametinib inhibited Th2/Th17. Finally BAL Th2/Th17 cells had increased IRF4 and Batf, two transcription factors that are associated with chromatin reorganization and transdifferentiation of Th2 and Th17 cells.

CONCLUSIONS: Our studies revealed a molecular pathway involving IL1β and C3 that could promote the transdifferentiation of airway Th2 cells into Th2/Th17 cells in a select group of asthmatic patients and contribute to their steroid resistance.

729 Gamma-Secretase Inhibitor Alleviates Acute Airway Inflammation of Allergic Asthma in Mice By Down-Regulating Th17 Cell Differentiation

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RATIONALE: Notch signaling plays a key role in the differentiation of T helper cells, which are involved in the pathogenesis of allergic asthma. γ-secretase inhibitors (GSI) block Notch signaling, making it an appealing target for treatment of allergic asthma. This study was to evaluate the effect of GSI on Th17 Cell differentiation in a mouse model of allergic asthma.

METHODS: BALB/C mice were sensitized by i.p. injection of ovalbumin (OVA) on days 1 and 13. They were then exposed to OVA aerosol challenges for daily for 8 days. GSI was administered intranasally 30 minutes before each OVA challenge. The sham mice were sensitized and challenged with normal saline and treatment with dimethylsulfoxide (DMSO) as a vehicle for GSI. Mice were sacrificed within 24h after the last allergen challenge. Lung tissue was examined with histochemistry for asthma pathology. Notch1 mRNA was assessed by real-time PCR. Notch receptor intracellular domain (NICD) was detected by Western Blotting. Serum IL-17 was measured by ELISA.

RESULTS: The administration of GSI inhibited the development of OVA-induced asthma, including decreasing airway inflammation responses and ameliorating the clinical signs. GSI reduced the expression of Notch1 mRNA and NICD protein. GSI treatment resulted in reduction of serum level of IL-17 with a concomitant decrease of Th17 frequency.

CONCLUSIONS: These findings suggest that GSI directly regulates Th17 responses through a Notch signaling–dependent pathway in allergic asthma mouse model, suggesting GSI as a potential therapeutic agent for the treatment of allergic asthma.
730 Percentages of Activated and Proliferating T-Regulatory Cells Correlate with Peanut-Specific Immunoglobulin-E Level in Peanut Allergic Children

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RATIONALE: IgE-mediated food allergy results from failure of peripheral immune tolerance. Reduced T-regulatory cell (Treg) activity is suggested to result in dysregulated Th1 helper responses with Th2 activation and production of specific-IgE (sIgE). We hypothesise that Treg populations correlate with serum levels of peanut-sIgE in peanut allergic children.

METHODS: Flow cytometry was used to enumerate total Treg (CD4+CD25+Foxp3+), activated Treg (CD4+CD25+Foxp3+CD45RA-) and proliferating Treg (CD4+CD25+Foxp3+CFSElo-), in resting (6-day culture in media) and crude peanut extract (CPE)-stimulated (6-day culture with CPE) peripheral blood mononuclear cells (PBMC) obtained from 26 peanut allergic subjects. Levels of peanut-sIgE, measured by ImmunoCAP, were log2-transformed. Pairwise correlation and age- and sex-adjusted linear regression analyses were performed.

RESULTS: There was strong correlation between peanut-sIgE and total Treg (r = 0.53, p = 0.006), activated Treg (r = 0.56, p = 0.003), proliferating Treg (r = 0.53, p = 0.004) in resting PBMC and similarly with Treg populations in CPE-stimulated PBMC (total Treg (r = 0.45, p = 0.02), activated Treg (r = 0.47, p = 0.01), proliferating Treg (r = 0.50, p < 0.01)). Higher total Treg [beta = 0.20 (95% CI 0.05-0.34); p = 0.01], higher activated Treg [beta = 0.20 (95% CI 0.06-0.34); p = 0.007] and higher proliferating Treg [beta = 0.19 (95% CI 0.05-0.33); p = 0.009] in resting PBMC were associated with higher peanut-sIgE levels. Similar associations between peanut-sIgE and total Treg [beta = 0.19 (95% CI 0.01-0.36); p = 0.04], activated Treg [beta = 0.18 (95% CI 0.03-0.34); p = 0.02] and proliferating Treg [beta = 0.19 (95% CI 0.03-0.34); p = 0.02] were observed in CPE-stimulated PBMC.

CONCLUSIONS: In peanut allergic children, there is a high level of agreement between the percentages of activated and proliferating populations of peripheral blood Treg and serum levels of peanut-sIgE. Longitudinal analyses will be important to confirm whether these associations reflect causal relationships.

731 Phenotypic Analysis of Peanut-Responsive T Cells at Baseline in Subjects Enrolled in CoFAR6, a Randomized Placebo-Controlled Epicutaneous Immunotherapy (EPIT) Trial for the Treatment of Peanut Allergy

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RATIONALE: The role of allergen-specific T-cells in the pathogenesis of food allergy is poorly understood.

METHODS: Baseline blood samples were obtained from challenge-confirmed peanut-allergic subjects (4-25 years) enrolled in CoFAR6 (n = 75), and from healthy controls (n = 8). PBMCs were stimulated with peanut extract and analyzed by flow cytometry. Peanut-responsive CD4+ and CD4+Treg (CD25+CD127-Foxp3+) cells were identified by CD154 expression at 6h and 18h of stimulation, respectively.

RESULTS: PBMCs from peanut-allergic subjects had increased Th2+CD154+ cells after 6h stimulation. Median (lower/upper quartile) values (10th CD4+ cells for peanut- vs. unstimulated were 140 (66/327) vs. 11 (4/20) for IL4+CD154+, 131 (45/295) vs. 4 (1/7) for IL13+CD154+, 7 (3/20) vs. 4 (2/7) for IFNγ+CD154+ and 12 (7/20) vs. 5 (2/8) for IL10+CD154+. Controls had no response to peanut. Peanut-responsive cells at 6h expressed skin, mucosal, or follicle homing molecules at low frequency (CCR4-16% (10/67), CCR6-5.6% (3/52), CXCR5-7.2% (5/71)). At 18h, CD154+ Tregs were increased from peanut-allergic subjects (441/208/686 stimulated vs. 31/20/49 unstimulated) but not from controls. CD154+Tregs expressed CCR4 (52.6% (35/66)) and CCR6 (29.8% (16/42)), but little IL10 (1.8% (0.6/3.7)) or IFNγ (0.4% (0/1.7)). Peanut-specific IgE (median 78.6 kU/L), but not IgG4 correlated with CD154+IL4+ cells (r = 0.68, p < 0.001) and CD154+IL13+ cells (r = 0.69, p < 0.001), but not CD154+ Tregs.

CONCLUSIONS: Peanut-allergic individuals have circulating peanut-responsive Th2 cells that correlate with specific IgE levels. Peanut-responsive Tregs expressing skin and mucosal homing markers are detectable, but do not correlate with IgE or IgG4.

732 Epicutaneous but Not Oral Immunotherapy Induces Antigen-Specific Gastrointestinal Tregs and Protects Against Food-Induced Anaphylaxis

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RATIONALE: Epicutaneous immunotherapy (EPIT) is being investigated for the treatment of food allergies. We studied the mechanism of tolerance induction by EPIT in comparison to oral immunotherapy (OIT) in a mouse model of food-induced anaphylaxis.

METHODS: C3H/HeJ mice were sensitized to ovalbumin (OVA) orally or through the skin and treated with EPIT or OIT using OVA- Viaskin® patches or oral OVA. Mice were orally challenged with OVA to induce anaphylaxis. Antigen-specific Treg induction was assessed by flow cytometry using a transgenic T cell transfer model.

RESULTS: OVA-EPIT induced the appearance of antigen-specific LAP+Foxp3- cells in the mesenteric lymph nodes that were absent in OIT-treated mice. The suppressive activity of the LAP+ cells was confirmed in vitro. LAP+ cells primed in the skin-draining lymph nodes expressed the gut-homing marker CCR9 (85% LAP+ vs 7% LAP-) and the mucosal-homing marker CCR6 (55% LAP+ vs 9% LAP-). Both LAP+ and LAP- cells expressed high levels of the skin-homing marker CCR4 (84% and 88%, respectively). The induction of antigen-specific Tregs in the gut by EPIT was associated with protection from food-induced anaphylaxis. 100% of skin or oral sensitized EPIT-treated mice were protected from anaphylaxis, whereas only skin-sensitized mice were protected by OIT.

CONCLUSIONS: EPIT induces the generation of gastrointestinal-homing antigen-specific Tregs in the skin-draining lymph nodes, which translates into an increased number of Tregs in the gastrointestinal tract. Induction of gastrointestinal Tregs is associated with an enhanced protection of mice from food-induced anaphylaxis by EPIT versus OIT.
733 Effects of Cigarette Smoke Extract and Nicotine on Regulator of G Protein Signaling-2 Expression in Human Airway Smooth Muscle and Bronchial Epithelial Cells

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RATIONALE: Recent studies have shown that reductions of Regulator of G protein signaling 2 (RGS2) are associated with lead to airway hyperresponsiveness in asthma and cigarette-induced lung disease. Human airway smooth muscle cells (HASM) treated with low dose (0.1%) cigarette smoke extract (CSE) for 10 days had a reduction in both mRNA and protein RGS2 expression. We aimed to determine if acute exposure of CSE to HASM and human bronchial epithelial cells (HBEC), which express RGS2, also reduced RGS2 expression. Further, we tested whether acute nicotine exposure alters RGS2 expression in HASM.

METHODS: HASM and HBEC were exposed to either air- or low-tube nicotine CSE (5% or 1%) or nicotine (50 uM and 2 uM) for 24 hrs. RT-PCR and Western blot were used to evaluate RGS2 expression.

RESULTS: Acute challenge of HASM or HBEC with either CSE or nicotine did not significantly affect RGS2 protein or mRNA expression.

CONCLUSIONS: Our data suggest that acute exposure of HASM or HBEC to CSE, at either high or low doses does not significantly alter RGS2 expression, in contrast with chronic low dose exposure in HASM. Since RGS2 regulates airway hyperresponsiveness and controls many important epithelial and smooth muscle cell functions, further study on the effects of cigarette smoke and/or nicotine on airway RGS2 expression and function are warranted.

734 Decreases in GM-CSF Release and Intracellular Levels of GM-CSF By Human Airway Smooth Muscle Cells in Response to Carbon Monoxide and Anoxia

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RATIONALE: Human airway smooth muscle cells (HASMC) may contribute to airway inflammation via production of pro-inflammatory cytokines, including granulocyte macrophage colony stimulating factor (GM-CSF). Based on prior work, we hypothesized that intracellular GM-CSF reductions would track with reductions in GM-CSF release, in response to both carbon monoxide (CO) and anoxia.

METHODS: HASM were exposed to 500 ppm CO, normoxia (control), or anoxia for 8 hours, with serum added (+), or withdrawn (-). ELISA was used to determine GM-CSF that was released from cells (in the conditioned media), or as intracellular levels (within cell lysates).

RESULTS: In serum(+) HASMC exposed to normoxia, the intracellular GM-CSF levels (in pg/mL/10⁶ cells; n=3/treatment) averaged 6296±464 (SE), while GM-CSF release averaged 9697±440. With CO and anoxia exposure, GM-CSF intracellular levels were significantly reduced by 51% and 58%, respectively, while GM-CSF release was also significantly reduced by 34% and 54%, respectively. In serum(-) HASMC exposed to normoxia, the intracellular GM-CSF levels averaged 743±13, while GM-CSF release averaged 2264±115. With CO and anoxia exposure, GM-CSF intracellular levels were significantly reduced by 71% and 78%, respectively, while GM-CSF release was also significantly reduced by 76% and 86%, respectively. There was a significant positive correlation between intracellular and extracellular GM-CSF for both serum(+) (R²=0.734; P<0.05) and serum(-) (R²=0.975; P<0.05) HASMC.

CONCLUSIONS: We conclude that the concomitant decreases in intracellular GM-CSF levels and GM-CSF release by HASMC, with both CO and anoxia, are supportive of a CO mechanism that may be associated with cellular hypoxia.

735 Cataloguing the Effects of Genetic Variants in 5' Upstream Regions of Eicosanoid Related Genes

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RATIONALE: Eicosanoids regulate various biological processes including immune system function, inflammation, cell homeostasis and reproduction. They are produced from the metabolism of arachidonic acid through various pathways and their physiological effects depend on the correct expression of the enzymes involved in their biosynthesis and their receptors. They are implicated in several pathologies, including allergy and hypersensitivity drug reactions. Previous genetic studies of these genes have focused on coding regions. We have investigated the effects of SNPs in the 5' upstream regions of eicosanoid-related genes on gene expression and disease.

METHODS: We obtained SNPs from the 5' upstream regions of enzymes and receptor genes in eicosanoid pathways from dbSNP. We analysed their potential effects on gene expression by analyzing changes to transcription factor binding site (TFBS) motifs, overlap with DNA hypersensitivity regions, overlap with TFBS according to ENCODE/ChIP-seq data. We used 1000 genome project data to investigate genetic linkage with variants associated with diseases according to the NHGRI catalogue of published GWAS data and eQTL using the regulomeDB resource.

RESULTS: We found several SNPs with roles in gene expression regulation, in terms of effects on TFBS and eQTL. We also found SNPs in genetic linkage with disease-associated SNPs according to previous GWAS, including inflammatory conditions and asthma. Associations were also found with obesity and cancer.

CONCLUSIONS: By combining molecular data from different sources we can find SNPs that affect gene expression and that are associated with immune system related pathologies. These variants need further experimental study and validation using well-defined groups of patients.
Maternal Prenatal Intake of Fructose Is Associated with Asthma in Children

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RATIONALE: Intake of fructose may promote inflammation and associated conditions including obesity and asthma. We examined associations of maternal prenatal and child nutrient intake of fructose with childhood asthma, and hypothesized that higher fructose intake was associated with childhood asthma.

METHODS: We studied 1,111 mother-child pairs from Project Viva, a longitudinal pre-birth cohort. Using food frequency questionnaires, we estimated maternal intake of fructose from beverages and foods during the first and second trimesters of pregnancy and child intake at 2 years. In mid-childhood (median age 7.7 years), mothers reported physician diagnosed asthma with current wheeze or use of asthma medication via questionnaire. In a multivariable analysis, we examined associations of total fructose and beverage intake (per internal z-score) with asthma in mid-childhood, adjusted for maternal socio-demographics (age, education, and household income), pre-pregnancy BMI, pregnancy smoking status and child sex and race/ethnicity.

RESULTS: In mid-childhood, asthma was common (19.5%) and mean (SD) BMI was 17.2 (3.1). Fructose intake during the first and second trimesters was associated with greater odds of asthma (OR 1.22; 95%CI, 1.03-1.44). Additionally, child fructose intake at 2 years of age was associated with greater odds of asthma in mid-childhood (OR 1.22; 95% CI:1.02-1.46). Different sources of fructose may underlie these associations. Maternal first and second trimester intake of sugar sweetened beverages (OR 1.20; 95% CI, 1.01-1.42) and child intake of juice, excluding orange juice (OR 1.34; 95% CI, 1.12-1.61) seem to be driving the fructose- asthma associations.

CONCLUSIONS: Early life exposure to fructose may influence asthma development in children.

Higher Serum 5-Methyltetrahydrofolate (5-MTHF) Levels Are Associated with Lower Risk of Wheeze in the National Health and Nutrition Examination Survey (NHANES)

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RATIONALE: While previous cross-sectional studies have demonstrated an inverse relationship between total serum folate levels and wheeze, the relative contribution of various folate forms, such as the biologically active 5-MTHF and unmetabolized folic acid, to this relationship have not yet been examined.

METHODS: Data were obtained from the 2007-2008 and 2011-2012 NHANES, where serum folate forms were measured in a subsample of individuals. Self-reported wheeze over the past year was assessed by questionnaire. Risk factors for wheezing were assessed by logistic regression, using survey weights and strata for all analyses. Models were adjusted for age, gender, race/ethnicity, education, income, smoking, and healthy diet as measured by the Healthy Eating Index.

RESULTS: 10,188 individuals over 1 year old were included. The odds of wheeze decreased across quartiles of both total serum folate and 5-MTHF levels (test for trend p < 0.001 for both). These relationships persisted after adjusting for age, gender, race/ethnicity, education, income, smoking (OR 0.70; 95%CI 0.53–0.92 for total folate and OR 0.53; 95%CI 0.37–0.77 for 5-MTHF, highest vs. lowest quartile). However, after accounting for healthy diet, only the protective effect of 5-MTHF persisted (OR 0.50; 95% CI 0.30–0.85 highest vs. lowest quartile). Unmetabolized folic acid and wheeze were not associated (p=0.62).

CONCLUSIONS: In a nationally representative population, 5-MTHF, the primary bioreavailable form of folate for DNA synthesis and methylation, was found to be protective for self-reported wheeze. Whether this is due to epigenetic modification of DNA or anti-inflammatory properties of this metabolite is unknown and warrants further study.

Relationships Among Eosinophils, Asthma, and Sex in a High-Risk Birth Cohort

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RATIONALE: Peripheral blood eosinophil counts have been reported to track not only with asthma severity, but with response to therapy as well. Since sex differences in lung function and asthma prevalence have also been reported, we evaluated these observations for sex specific developmental differences in peripheral blood eosinophils.

METHODS: 217 children were followed prospectively from birth to 11 years in the COAST (Childhood Origins of ASThma) study, a high-risk birth cohort designed to evaluate genetic and environmental factors associated with the development of atopic diseases. We prospectively sampled peripheral blood eosinophils at annual visits from ages 3 through 11 years. We analyzed relationships among peripheral blood eosinophils (least square mean), asthma at age 11 and sex.

RESULTS: At age 11 years, children with asthma had significantly increased peripheral blood eosinophils [mean eosinophils/μL: asthma 222, no asthma 135, p=0.001]. Sex did not significantly impact eosinophils [girls 160, boys 155, p=0.81]. However, an asthma by sex interaction was noted for eosinophils [boys/no asthma 146, boys/asthma 174, girls/no asthma 124, girls/asthma 315, p=0.01]. Similar patterns of sex by asthma interactions for eosinophils were observed at 6 years.

CONCLUSIONS: These findings indicate that in children with asthma, eosinophilia is more prominent in girls, and suggests that sex should be considered when peripheral blood eosinophil counts are used as a biomarker to assess asthma severity and/or treatment options in school-aged children.
CONCLUSIONS: The prevalence of pediatric asthma has possibly reached a plateau or is showing a slight decrease in the past 10 years.

400 Longitudinal Patterns of Skin Prick Test Sensitization in Early Childhood Predict Risk for Asthma at Age 7
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RATIONAL: Few studies have examined longitudinal patterns of allergic sensitization during early childhood to identify children at risk for developing asthma. We hypothesized that patterns of allergic sensitization based on longitudinal skin prick testing (SPT) can identify children at risk for asthma at age 7.

METHODS: We identified children in the Cincinnati Childhood Allergy and Air Pollution Study birth cohort who completed SPT to four common indoor Aeroallergens: cat, dog, dust mite (DM) and cockroach (CR) at ages 1, 2, 3 and 4 years. Children were defined as having asthma at age 7 by parental report of lower respiratory symptoms with confirmation by pre and post bronchodilator spirometry and/or methacholine testing. Unsupervised clustering techniques were used to identify groups of children having similar longitudinal patterns of Aeroallergen sensitization.

RESULTS: A total of 458 children were included in this analysis; of these 17% (n = 78) were diagnosed with asthma at age 7. Five clusters of children based on SPT results were identified: non-atopic, cockroach-sensitized, persistent cat-sensitized, late persistent DM-sensitized and early transient DM-sensitized. Children in two clusters, persistent cat and early transient DM sensitization, were at significantly increased risk of developing asthma by age 7 (OR [95%CI] 2.6 [1.5-5.2] and 3.8 [1.5-8.8] respectively) compared to non-atopic children.

CONCLUSIONS: Children with persistent cat sensitization and early transient DM sensitization were significantly more likely to develop asthma by age 7; late DM and CR sensitization was not associated with asthma development. Longitudinal SPT may distinguish subgroups of allergic children at highest risk for developing asthma.
Potential Immunoregulatory Roles of Natural Killer Cells in Children with Atopic Dermatitis

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METHODS: The study group consists of children without treatment (n=8, mean age=6.7±3.7 years), under treatment (n=14, mean age=7±4.3 years), and healthy subjects (n=14, mean age=11±4.2 years). Patients were multisensitized and had high serum total IgE levels. NK cells were studied by their cytotoxic capacity, expression of CD16 & CD56, activatory receptors and intracellular IL-4, IL-10 & IFN-g levels by flow cytometry.

RESULTS: The percentages of CD3+CD16+CD56+ NK cells were significantly diminished in patients under treatment compared to healthy subjects. Compared to healthy subjects, CD16+CD56+CD57dimNK cell subset was significantly increased in the group without treatment however decreased in those under treatment. In all patients, IL-10 secreting regulatory and IL-4 secreting NK2 cells were increased, however, expression of activatory receptors, NKG2D and NKp46, cytotoxic capacity of NK cells and NK1 cell subset were significantly decreased compared to healthy subjects.

CONCLUSIONS: Our findings suggested impaired NK cell functions in AD patients and imply a decrease of IFN-g production by NK1 cells, which may partially be responsible for the tendency to develop viral infections.

744 Release of High-Mobility Group Box-1 (HMGB1) in the Airways of Children with Viral Lower Respiratory Tract Infections

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METHODS: Nasopharyngeal secretions (NPS) were collected from 39 children <24 months of age hospitalized for LRTIs from October 2013 to May 2014, and were tested for 17 respiratory viruses using the Lumiplex xTAG RVP multiplex, and for HMGB1 and cytokines by an ELISA and a Bio-Plex 27-targets array, respectively.

RESULTS: One or more viruses were identified in 94.8% of LRTI cases, with respiratory syncytial viruses (RSV, A or B strains) as the most common (66%), followed by enteroviruses/rhinoviruses (38.5%), parainfluenza (10.3%), adenovirus (10.3%), and metapneumovirus (5.1%). Although HMGB1 was detected in patients infected with different viruses, a trend for greater concentrations of HMGB1 was found in patients with RSV bronchiolitis and in bronchiolitis patients requiring oxygen supplementation. Viral co-infections were not associated with greater release of HMGB1 compared to those caused by RSV alone. In RSV bronchiolitis, HMGB1 concentrations significantly correlated with chemokines MIP-1β (P<0.01), MIP-1α (P<0.01), IL-8 (P<0.01), and RANTES (P<0.05).

CONCLUSIONS: Greater levels of HMGB1 in infants with RSV bronchiolitis correlate with inflammatory chemokines that are produced by or activate macrophages suggesting that HMGB1 represents a marker of disease severity and/or directly contributes to the pathogenesis of such infection.
Detection of Airborne Juniperus Pollen By Conventional and Real-Time PCR from Burkard Air Samples

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RATIONALE: Microscopic detection of pollen requires considerable expertise, and identification is limited to genus or family level. Cupressaceae pollen is detected in the Tulsa atmosphere for seven months each year; however, local members of this family only pollinate from February through April. Here, we developed molecular methods to identify multiple Juniperus species from air samples.

METHODS: Air samples from January to March were collected with a Burkard 7-day sampler located in Tulsa, OK. One-day segments of exposed Melenex tape were cut into smaller pieces and DNA extracted. Species-specific marK primers for J.ashei and J.virginiana were developed for conventional PCR and leafy primers/probe pairs were developed for real-time PCR for Juniperus. An air sample collected on 15 January in London, Ontario(CANADA) using a Buck Bioslide sampler was also analyzed.

RESULTS: Serial dilution of J.ashei pollen demonstrated that specific marK primers were able to detect the presence of 2 to 3 pollen grains indicating this method would be useful for Burkard samples. Using marK primers, J.ashei pollen was confirmed from air samples collected in Tulsa in January as well as from Canada. Primers for marK in J.virginiana confirmed the presence of that pollen in Tulsa air sampled in February and March. Primers/probe pairs for leafy were successful in real-time PCR and can be used to quantify Juniperus pollen from Burkard samples.

CONCLUSIONS: This study provides an effective alternative to microscopy for identification of pollen from Burkard air samplers and Bioslide samplers. These data also confirm the long distance transport of J.ashei pollen up to 2,400 km.

Magnifying: The Truth behind Fungal Spore Counts

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RATIONALE: National Allergy Bureau recommends that fungal spore analysis of air samples be performed at 1000x magnification. However, recent publications have calculated concentrations using a magnification of 400x. The aim of this study was to compare the concentration of airborne fungal spores using 400x and 1000x magnifications to determine the accuracy of the lower magnification.

METHODS: Air samples were collected using a Burkard volumetric 7-day spore trap. Sampler drums were changed weekly and slides prepared using standard methods. Slides were analyzed for fungal spores at both 400x and 1000x magnification at chosen traverses. Spore counts were converted to concentrations; resulting data were log transformed and statistically analyzed by t test to compare the concentrations of airborne fungal spores observed at each magnification.

RESULTS: The 1000x magnification produced significantly higher concentrations of total spores as well as 9 individual spore types from July 2013. The mean monthly concentration of total spores counted at 400x was 8,591 spores/m³ and 13,247 spores/m³ at 1000x, r(30)=29.55, p<0.0001. Among individual spore types, the greatest differences in monthly mean concentration were for Cladosporium (r(30)=19.05, p<0.0001), basidiospores (r(30)=12.47, p<0.0001), and ascospores (r(30)=11.56, p<0.0001). The single day with the greatest difference in concentration of total spores was 27 July with levels of 22,827 spores/m³ and 34,852 spores/m³ at 400x and 1000x, respectively.

CONCLUSIONS: Fungal spore counts analyzed at magnifications of 400x and 1000x demonstrate significant differences in calculated atmospheric concentrations, proving the need for an international standard of analysis at 1000x.

A Systematic Analysis of Pollen Transcriptomes from Plant Allergens Reveals Conserved Targets of Immune Responses

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RATIONALE: We have recently identified a set of novel antigens in Timothy grass (TG) pollen using an unbiased, integrated transcriptomic and proteomic analysis, and have shown that these antigens are prominent targets of T-cell responses. Here we determined the degree of conservation of these epitopes across multiple plant species that could be targets of cross-reactive T-cells. This would make them potential candidates for pan-pollen immunotherapy approaches.

METHODS: RNA was extracted and sequenced from nine plant allergens (4-grass, 2-weed, and 3-tree pollens). Peptides from TG were examined for conservation across these pollens using sequence alignments, and conservation was correlated with immunogenicity. T-cell epitope cross-reactivity was determined by generating short term mono-specific T-cell lines and assessing their response across the panel of pollen extracts in ELISPOT assays.

RESULTS: We find that conservation of a peptide across pollens based on transcriptomic analysis correlates with the likelihood that it will elicit Th2 responses in allergic donors. Furthermore, If TG extract elicited high responses after culture with peptide, so did those extracts in which the peptide is conserved. Substitutions of 3 or more residues in the peptide greatly reduced the response. Thus, conserved peptides (with 2 or less substitutions) are capable of inducing cross-reactive T-cell immune responses.

CONCLUSIONS: We have identified peptides that are significantly conserved across multiple plant allergen species and stimulate cross-reactive T-cells. It is possible that inducing tolerance against these peptides may modulate the allergic immune response against a broad range of grass and pollen allergens.

Molecular Characterization & Epitope Mapping of Recombinant Rice Chitinase

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RATIONALE: The prevalence of IgE-mediated rice allergy is about 0.8% in subjects with asthma and rhinitis in India. In the present study, recombinant rice chitinase was expressed, purified and subjected to epitope mapping using in silico tools.

METHODS: Rice chitinase was subcloned in pET28a+ vector, expressed and affinity purified by Ni-NTA resin. It was analyzed for digestibility, heat stability, and IgE binding using atopic patients’ sera. Rice chitinase 3D structure was generated by homology modelling and B cell epitopes were predicted by a combination of sequence and structure based tools.

RESULTS: Purified protein appeared as a single band at 24 kDa on SDS-PAGE and showed significant IgE binding with 7 of 110 patients’ sera positive to different food allergens by ELISA. Rice chitinase remained stable for 60 mins on incubation with pepsin and was resistant to heat treatment at 90°C for 1 h. Homology modeled 3D structure was used for epitope prediction and three B cell epitopes A1 (12–25 aa), A2 (31–45 aa) and A3 (140–166 aa) were identified for chitinase. Property Distance (PD) values were calculated for the predicted epitopes using Structural Database of Allergenic Proteins (SDAP) and showed similarity with allergens from H. brasiliensis, Persea americana (avocado) and Castanea sativa (chest nut).

CONCLUSIONS: Chitinase was identified as a potential allergen and may share cross reactive epitopes with reported food allergens. However, more information needs to be gathered from the epitopic regions in predicting cross reactivity with food allergens.
750 The GIS-Based Ecological Association Between Ambient Ozone and Allergic Diseases at the Sub-District Level in Seoul, Korea

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Rationale: Almost 9 million people suffer from allergic diseases (atopic dermatitis, asthma and allergic rhinitis) in South Korea causing the treatment expenses of USD 600 million per year especially in urban areas. Ambient ozone has been known as one of the major environmental risk factors for allergic diseases. We explored ecological association between the concentration of ozone and the prevalence of allergic diseases at the sub-district level in Seoul.

Methods: The information of patients per 10,000 inhabitants of allergic diseases for 424 sub-districts in Seoul from January 2011 to December 2011 was collected through National Health Insurance Service (NHIS) of South Korea. Ozone was measured once a day in 2011 at the monitoring stations located in 25 districts, which was then interpolated to the entire surface of Seoul by employing universal kriging method to obtain data at the sub-district level. Both multiple linear regression and geographically weighted regression were used for analyses.

Results: The statistical model and resulting map demonstrated spatial patterns of ecological association between the asthma prevalence and each pollutant at the sub-district level in 2011. The level of ambient ozone was significantly associated with the prevalence of atopic dermatitis (P<0.05), asthma (P<0.001) and allergic rhinitis (P<0.05).

Conclusions: Ecological association between allergic diseases and ambient ozone was found. Employment of kriging interpolation could improve the accuracy and applicability of the air quality data. This approach could be widely applicable as it can be expanded to include different kinds of risk indices for other environmental health problems.

751 Adverse Reactions Associated with Oral and Parenteral Cephalosporin Use: A Retrospective Population-Based Analysis

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Rationale: Population-based, route-specific, data on cephalosporin-associated new drug “allergy” reports and severe adverse drug reaction (ADR) incidence is rare. We determined the incidence of new cephalosporin-associated “allergy” reports and serious ADRs.

Methods: All cephalosporins administered to Kaiser Permanente Southern California healthplan members between 1-1-2010 and 12-31-2012, new cephalosporin-associated “allergy” reports, and serious ADRs were identified.

Results: There were 622,456 healthplan members exposed to 901,908 courses of oral cephalosporins and 326,867 healthplan members exposed to 487,630 courses of parenteral cephalosporins over the 3-year study interval. New cephalosporin “allergy” reports were more frequent in females than males, 0.56% [95% CI, 0.54% to 0.57%] versus 0.43% [95% CI, 0.41% to 0.44%], per course (p<0.0001). The most frequent serious cephalosporin-associated ADRs were Clostridium difficile (CDiff) within 90 days (0.91%), nephropathy within 30 days (0.15%), and all cause death within 1 day (0.10%). All were un-correlated to drug “allergy” history. Physician-documented cephalosporin-associated anaphylaxis occurred with 5 oral [95% CI, 1 in 1,428,571 to 1 in 96,154] and 8 parenteral [95% CI, 1 in 200,000 to 1 in 35,971] exposures (p=0.0071). There were 3 cephalosporin-associated serious cutaneous adverse reactions (SCARs) [95% CI, 0 to 1 in 217,291] documented. All were also associated with another antibiotic used at the same time as the implicated cephalosporin.

Conclusions: Cephalosporins are widely and safely used, even in individuals with a history of penicillin “allergy”. Physician-documented cephalosporin-associated anaphylaxis and SCARs are rare compared to CDiff within 90 days, nephropathy within 30 days, and all cause death within one day.

752 Impact of a Clinical Guideline for Prescribing Antibiotics to Inpatients with Reported Penicillin or Cephalosporin Allergies

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Rationale: Self-reported penicillin (PCN) allergy infrequently reflects an inability to tolerate PCNs. Inpatients reporting PCN allergy receive alternative antibiotics that may be broader-spectrum, more toxic or less effective. Inpatient providers are interested in tools to help them prescribe antibiotics to patients with PCN or cephalosporin allergy.

Methods: A guideline was implemented to assist providers with allergy history assessment and prescribing antibiotics for patients with reported PCN or cephalosporin allergy. The guideline utilizes a standard 2-step graded challenge or test dose. We performed a quasi-experimental study assessing safety, feasibility, and impact on antibiotic use, comparing 21 months prior to guideline implementation, when allergy consultation was required for all test doses, to 12 months after guideline implementation.

Results: Significantly more test doses of β-lactam antibiotics were performed monthly in the post-period compared to the pre-period (median 14.5 [IQR 13, 16.25] vs. 2 [IQR 1, 3.25], p<0.001). Seven adverse drug reactions (ADRs) occurred during guideline-driven test doses with no significant difference in rate (p=0.44) or severity (p=0.5) between pre- and post-periods. Guideline-driven test doses decreased alternative antimicrobial therapy after the test dose, including vancomycin (68.3% vs 37.2%, p<0.001), aztreonam (11.5% vs 0.5%, p<0.001), aminoglycosides (6.0% vs 1.1%, p=0.004) and quinolones (15.3% vs 3.3%, p<0.001).

Conclusions: The implementation of an inpatient antibiotic prescribing guideline for patients with PCN or cephalosporin allergy was associated with an almost 7-fold increase in the number of test doses to β-lactams without increased ADRs. Patients assessed with guideline-driven test doses were observed to have significantly decreased alternative antibiotic exposure.
753 The Effect of Misoprostol in Aspirin Exacerbated Respiratory Disease Undergoing Aspirin Challenge

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**RATIONALE:** Prostaglandin E2 (PGE2) is an anti-inflammatory compound that acts as a "brake" on 5-lipoxygenase activity. It is likely that diminished PGE2 regulation in AERD leads to respiratory reactions upon cyclo-oxygenase 1 inhibition. In vitro studies show that exogenous PGE2 stabilizes inflammatory mediator release. We hypothesize that misoprostol, an FDA approved oral prostaglandin E1 analogue, may decrease severity of aspirin induced symptoms occurring after NSAID ingestion and make aspirin desensitization safer for AERD patients.

**METHODS:** This study included a total of 76 patients undergoing aspirin challenge/desensitization at Scripps Clinic who received misoprostol (30), placebo (15) or neither (31) and served as historical controls. After meeting inclusion criteria, 30 patients received misoprostol, which was administered at a dose of 200 mcg orally at time -30 minutes, 90 minutes, and 4 hours after first dose of nasal ketorolac. Measured endpoints included change in FEV1 and peak nasal inspiratory flow rate (PNIF), or number of treatments required for induced symptoms.

**RESULTS:** Following clinical reaction to nasal ketorolac, there was a significantly larger drop in FEV1 measurement in misoprostol treated compared to placebo (p=0.002). No difference was seen with misoprostol compared to historical controls (p=0.189). There was no difference in FEV1 between misoprostol and placebo (p=0.176) and misoprostol and controls (p=0.370) following reaction to oral aspirin. There was no difference in PNIF measurements, treatment requirements, or GI side effects amongst all groups.

**CONCLUSIONS:** The use of misoprostol in patients with AERD undergoing aspirin desensitization has no protective effect in reducing the intensity of symptoms that occur after NSAID ingestion.

754 Clinical Utility of Skin Testing Six Weeks after a Carboplatin Induced Hypersensitivity Reaction

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**RATIONALE:** Skin testing (ST) is used to risk stratify and manage patients after a hypersensitivity reaction (HSR) to carboplatin. Similar to hymenoptera venom allergy, a small subset of patients with carboplatin-induced HSRs have falsely negative ST if performed <6 weeks after HSR. Repeat carboplatin ST outside the 6 week window could identify patients who seroconvert and improve management decisions.

**METHODS:** A retrospective review was conducted of all patients referred between 2006 and 2012 with carboplatin-induced HSR. Carboplatin ST was performed at initial visit and prior to each carboplatin desensitization. Only patients with initial ST <6 weeks after HSR were included. Patients were stratified based on ST results (positive, negative, and seroconverter) and clinical characteristics and outcomes were compared.

**RESULTS:** We identified 52 patients with carboplatin ST < 6 weeks after HSR. All patients were female and 89% had gynecologic malignancies. The mean age was 57.9 years. Twenty two patients (42%) were ST+ initially, 21 (41%) remained ST-, and 9 (17%) seroconverted. Among seroconverters, only 1 patient had a false negative ST outside of the 6 week window. Similar rates of HSRs during desensitization were documented among ST+ patients (45%) and seroconverters (33%) compared to ST- (13%; P=0.016). Ten of 21 patients (47%) who remained ST- safely received carboplatin without desensitization as outpatients.

**CONCLUSIONS:** Carboplatin ST is falsely negative in a subset of patients <6 weeks after HSR. Repeat ST after 6 weeks can identify seroconverters who are at risk for further HSRs and ST negative patients who can safely return to the outpatient setting.

755 Rituximab Hypersensitivity: Evaluation, Implications of Skin Testing, Potential Mechanisms, and Desensitization

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**RATIONALE:** Rituximab sensitivity (RITS) is common with cytokine release syndrome (CRS) and tumor lysis syndrome (TLS) proposed as the major mechanisms. We examined whether IgE mediated mechanisms and mast cell degranulation are also involved.

**METHODS:** Skin testing was performed on 18 out 25 RITS patients admitted for desensitizations and tryptase levels were measured during HSR and a limited number of asymptomatic desensitizations.

**RESULTS:** Seven of the 18 patients (39%) had a positive skin test during at least one point during their treatment. The rates of HSR during desensitizations were similar among skin test positive and skin test negative patients. Tryptase levels were checked in 72% of patients during their desensitizations, with assessment performed during 28/42 (67%) of the HSR desensitizations and 27/130 (21%) of the asymptomatic desensitizations. Excluding one patient with probable mast cell activation syndrome with constant elevated tryptase, tryptase level was elevated in 21% of the HSR desensitizations, confirming mast cell degranulation may participate, along with cytokine release and tumor lysis, in causing HSR in RITS. The testing of tryptase levels in the NSR desensitizations indicated that asymptomatic mast cell degranulation only happened rarely (1/27, 3%).

**CONCLUSIONS:** IgE mediated mechanism and mast cell degranulation, in addition to CRS and TLS, may contribute to a significant portion of HSR among RITS patients. However, positive skin test did not confer a greater likelihood of HSR reactions during desensitizations but mast cell degranulation is higher among HSR than asymptomatic desensitizations. Nearly all the desensitizations were completed successfully.
756 Safety of Food Allergy Clinical Trials: The Consortium for Food Allergy Research’s 10 Years of Experience

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Rationale: The Consortium for Food Allergy Research (CoFAR) has completed 3 treatment protocols and two are actively enrolling. Given that concerns regarding safety have limited clinical trials in food allergy, we describe our experience over the past 10 years.

Methods: CoFAR consists of five clinical sites, a coordinating center and NIADD support. Data from five treatment studies were assessed to quantify enrollment, dosing symptoms, adverse events and oral food challenge (OFC) outcomes. Epinephrine was administered liberally for GI or respiratory symptoms. To minimize risk of adverse reactions due to drug distribution errors, doses were validated before dispersal.

Results: 335 subjects have been screened (253 enrolled) in 5 treatment protocols including peanut sublingual (SLIT) and epicutaneous immunotherapy, egg oral immunotherapy, and a rectal engineered recombinant peanut protein (vaccine). Eleven serious adverse events (SAEs) were reported with 3 dosing related (2 vaccine and 1 SLIT). 536 OFCs were performed with 28.2% receiving epinephrine, 3.9% requiring two doses (baseline OFCs: 39.2% and 6.5% respectively) and 1 requiring 6 doses. Only 2 (0.3%) OFC reactions were considered SAEs (no in-patient hospitalizations, 1 considered life-threatening). In three completed protocols (SLIT, OIT, vaccine), 61,319 active doses were administered (87 subjects) with 80.7% symptom-free, 117 moderate symptoms, 2 severe symptoms and 6 dosing reactions requiring epinephrine. 13 (14.9%) subjects on active treatment discontinued due to symptoms.

Conclusions: CoFAR’s careful protocol development, central data management and monitoring, pharmacovigilance and ultimately expert care at experienced sites provide a safe environment to implement interventional trials for the treatment of food allergy.

757 Increasing Tolerance to Less Extensively Heat-Denatured (baked) Milk Products in Milk-Allergic Children

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Rationale: Increasing tolerance to food allergens during infancy is associated with reduced risk for food allergy in later life. Heat denaturation of food proteins can reduce allergenicity; however, efficacy for improving tolerance to food allergens is not well established. The aim of this study was to evaluate the safety and effectiveness of FAHF-2 as a treatment for food allergy.

Methods: In this double-blind, randomized, placebo-controlled study, 68 subjects, 12-45 years of age, with allergies to peanut, tree nut, sesame, fish, and/or shellfish, confirmed by baseline double-blind, placebo controlled food challenge (DBPCFC), received FAHF-2 (n=46) or placebo (n=22). After 6 months of therapy, subjects underwent DBPCFC. For those who demonstrated improvement, a repeat DBPCFC was performed 3 months after stopping therapy.

Results: Treatment was well-tolerated with no serious adverse events. By intent-to-treat analysis, the placebo group had a higher eliciting dose and cumulative dose (p=0.05) at the end of treatment DBPCFC. There was no difference in the requirement for epinephrine to treat reactions (p=0.55). There were no significant differences in allergen-specific IgE and IgG4, cytokine production by PBMCs or basophil activation between active and placebo groups. In vitro immunological studies showed that FAHF-2 treated-PBMCs produced significantly less IL-5, greater IFN-γ and IL-10 and increased numbers of Tregs than untreated cells. Notably, 44% of subjects had poor adherence for at least one-third of the study period.

Conclusions: FAHF-2 is a safe herbal medication for food allergic individuals and shows favorable in vitro immunomodulatory effects; however, efficacy for improving tolerance to food allergens is not demonstrated at the dose and duration used.
759 Investigation of Peanut Oral Immunotherapy Using CpG/Peanut-Nanoparticles in a Murine Model of Peanut Allergy
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RATIONALE: A cure for peanut allergy remains elusive. Current clinical approaches utilizing peanut oral/sublingual immunotherapy are promising, but concerns about safety and long-term benefit remain a barrier to wider acceptance. Improved methods of delivering peanut-specific immunotherapy are needed.

METHODS: Peanut allergy was established in C3H/HeJ mice by oral sensitization with peanut and cholela toxin. Mice were subsequently given 4 weekly gavages with CpG/PN-nanoparticles or vehicle (PBS). Untreated mice were naïve controls. After completing therapy, mice underwent 5 monthly oral peanut challenges. Anaphylaxis was evaluated by visual assessment of symptom scores and measurement of body temperature. PN-specific serum IgE and IgG2a and plasma histamine and cytokines were measured by ELISA.

RESULTS: PN-allergic mice treated with CpG/PN-nanoparticles but not vehicle were significantly protected from anaphylaxis to all five oral peanut challenges as indicated by lower symptom scores (P<0.05 vs. vehicle), better retention of body temperature (P<0.05-0.001 vs. vehicle) and reduced plasma histamine (P<0.05-0.001 vs. vehicle). Importantly, CpG/PN-nanoparticle treatment did not cause anaphylactic reactions. Treatment was associated with a sustained and significant decrease in PN-specific IgE (P<0.05-0.001 vs. vehicle) and elevation in PN-specific IgG2a (P<0.05-0.01 vs. vehicle). Compared to vehicle controls, peanut recall responses in splenocyte cultures from CpG/PN-nanoparticle treated mice showed significantly decreased Th2 cytokines (IL-4, IL-5 and IL-13, P<0.001 vs. vehicle for all) but increased IFN-γ (P<0.001 vs. vehicle).

CONCLUSIONS: Pre-clinical findings indicate that PN-OIT using CpG/PN-nanoparticles may be a valuable strategy for peanut-specific oral immunotherapy in humans.

760 Pioglitazone Attenuates Peanut Induced Anaphylaxis in a Mouse Model of Peanut Allergy
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RATIONALE: Peroxisome proliferator activated receptor gamma (PPARγ) agonists such as pioglitazone (PIO) have demonstrated multiple roles in inflammation and metabolism; however the role of PPARγ signaling in peanut allergy has not been defined.

METHODS: C3H/HeJ mice were peanut-sensitized by intragastric administration of 1 mg whole peanut extract with 10mg cholela toxin weekly for four weeks. Non-sensitized mice received PBS by oral gavage. Peanut sensitization was assessed by analysis of serum peanut-specific IgE by ELISA. Mice were treated with PIO (10mg/kg/day) or vehicle for 7 days following sensitization. Mice underwent intraperitoneal peanut challenge with 1 mg peanut protein 1 week after PIO treatment; core body temperatures were obtained every 15 minutes and symptoms were quantified using established criteria. Immunologic assessment using cytokine assay and flow cytometry was performed on harvested tissues.

RESULTS: During peanut challenge, peanut sensitized, PIO treated mice had reduced hypothermia with >50% higher core body temperature compared with untreated, peanut sensitized mice (p<0.001). PIO treated mice also had significantly reduced anaphylaxis symptom scores compared with untreated, peanut sensitized mice (P<0.001). Peanut-specific IgE levels were similar in all peanut-sensitized mice and did not differ after 7 days of PIO. (p=NS) Increased CD4+CD25+Foxp3+ Tregs were observed in the spleens of peanut-sensitized, PIO-treated mice by flow cytometry.
Long-Term Adherence to Self-Injectable Epinephrine Prescription
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RATIONALE: Self-injectable Epinephrine (SIE) is essential for immediate treatment of anaphylaxis. Our study aims to determine long-term adherence approximately 10 years after initial SIE prescription.

METHODS: A total of 173 patients who received and filled a SIE prescription between 2002 and 2003 at the VA West Los Angeles Hospital were identified, of which 82 patients were active in the system in 2013. Patient’s gender, race/ethnicity, age, setting of initial SIE prescription, associated diagnosis, and frequency of SIE refills were collected. A prescription for SIE under the active medication list constituted adherence.

RESULTS: Of the 82 patients identified, indications for SIE prescription included 36 for venom allergies, 21 for food allergies, 11 for angioedema, 1 for drug allergy, 3 for immunotherapy, and 10 for unknown etiology. Initial SIE was prescribed at the following clinical sites: 34 from allergy clinic, 40 from primary care clinic, 3 from emergency department, 1 from hospital discharge, and 4 from miscellaneous/unknown clinics. 37 patients (45%) were adherent to SIE prescription with an average of 9 prescription refills over approximately 10 years. 54 patients (55%) were non-adherent to SIE prescription, of which 29 patients (37%) never refilled after their initial prescription. Patients with venom allergies had the highest adherence rate of 62% (23 patients), while those with angioedema had the lowest rate of 27% (3 patients).

CONCLUSIONS: This study shows that most patients do not have an active SIE prescription after approximately 10 years, of which 37% patients do not refill after their initial prescription.

A Cost-Effective Analysis of the U.S. Varicella Zoster Virus (VZV) Vaccination Program with Consideration for Delayed Onset of Asthma Following VZV Infection
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RATIONALE: Cost-analysis models agree that the implementation of universal varicella zoster virus (VZV) vaccination has resulted in widespread cost savings. Recent studies in our laboratory reported that infection by VZV may lead to delayed onset of asthma in children/adolescents. This information could alter the cost-effectiveness of the program. We created a decision analysis model to estimate the costs and health-related effects of the U.S. VZV vaccination program, assuming VZV infection will delay asthma onset.

METHODS: The Markov model (TreeAge Software) considered a birth cohort of 3,957,577 individuals entering the population from a societal perspective over a 20 year time frame. We predicted the number of asthma/VZV cases, asthma/VZV related mortality and costs associated with asthma/VZV. Comparison arms included: 1) VZV vaccination program with no delayed asthma onset 2) VZV vaccination program with delayed asthma onset and 3) no VZV vaccination program with delayed asthma onset. We considered delayed onset ranging from 3-12 years.

RESULTS: The vaccination program proved preferential across outcomes without an assumed delay in asthma onset. When the “Vaccination” and “No Vaccination” arms were compared assuming delayed asthma onset, the vaccination program remained less costly despite increased savings related to asthma without vaccination. Additionally, when the delayed onset was 9 years or greater, a comparison of the “Vaccination” and the “No Vaccination” arms revealed greater overall mortality with vaccination.

CONCLUSIONS: When there was delayed onset of asthma post-VZV infection, the VZV vaccination program proved less costly, although it resulted in increased asthma morbidity and an overall increase in mortality.
Factors Associated with Rates of Influenza Vaccination in Allergy and Primary Care Clinics

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RATIONALE: The influenza vaccine is recommended for patients older than 6 months of age, yet the CDC notes adult vaccination rates to be as low as 45%. Specific reasons for declining vaccination are not well understood.

The aim of this study was to identify factors associated with patients’ likelihood to receive the influenza vaccine to elucidate how to improve vaccination rates.

METHODS: A voluntary, anonymous survey (n=472) was distributed in six internal medicine and allergy outpatient clinics in the metropolitan Detroit area from April to August, 2013. Patients were asked to answer if they had received the influenza vaccine in the previous year, provide demographic information, and answer questions regarding perceptions about the influenza vaccine. Data were analyzed using chi-square tests.

RESULTS: People self-identified as either Asian or White had higher vaccination rates (93.3% and 83.8% respectively) than those who self-identified as Black (62.1%), p = 0.001. Those who believed the influenza vaccine was beneficial had higher rates of vaccination (89.9%), versus those who did not (27.7%), p < 0.001. Those who recalled that a physician recommended the vaccine also had higher rates of vaccination (89.9%) than those who did not (57.7%), p = <0.001. Previous adverse reactions, such as pain, fever or infection, did not significantly affect vaccination rates.

CONCLUSIONS: Interventions to increase flu vaccination rates may need to focus on improving physician recommendations and communicating benefits of the vaccine. Better understanding of vaccine perceptions among Black individuals is warranted.

Complement Activation in Nasal Tissue of Patients with Chronic Rhinosinusitis

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RATIONALE: Chronic rhinosinusitis (CRS) is a chronic inflammatory disease of the upper airway commonly divided into two clinical phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). Recent studies indicate the presence of autointoautobodies in some patients. This study investigates the presence, location and mechanisms of complement activation in nasal tissue from patients with CRS.

METHODS: Uncinate (UT) and/or nasal polyp (NP) tissues were obtained from control patients(n=22) and patients undergoing nasal surgery for CRSsNP(n=78) or CRSwNP(n=37). Tissue homogenates were analyzed by ELISA for C5b-9 (terminal complement complex, common to all three complement pathways), C4d (degradation product of the classical and lectin pathways) and activated C1 (activator of the classical pathway). Frozen tissues were sectioned, fixed and stained for C5b-9 and C4d.

RESULTS: C5b-9 was significantly increased in NP tissue compared to UT of CRSwNP, CRSsNP and control (92.75 vs 6.85, 15.49 and 16.51ng/mg protein respectively, all p<0.05). Similarly, C4d was increased in NP compared to UT of CRSwNP, CRSsNP and control (0.1497 vs 0.02949, 0.04079, and 0.01198ng/mg protein respectively, all p<0.0001). Activated C1 was also increased in NP tissue compared to UT (101.7 vs 67.84, 60.79, 43.57 AU/mg protein respectively, all p<0.01). Immunofluorescence showed C5b-9 and C4d deposition was localized to the basement membrane.

CONCLUSIONS: Deposition of C5b-9, C4d and C1 was significantly increased locally in NP tissue and C5b-9 and C4d were located in the basement membrane of polyp and UT tissue of patients with CRS. This provides evidence that complement activation occurs in CRS via the classical pathway.

Interleukin-25 As a Novel Therapeutic Target in Nasal Polyps of Chronic Rhinosinusitis

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RATIONALE: Interleukin (IL)-25 was reported to induce Th2-type immune responses and contribute to several allergic diseases, such as atopic dermatitis and asthma. However, the role of IL-25 in nasal polyposis is not clearly understood.

METHODS: We investigated IL-25 expression and its cellular origins in NPs of human subjects. Correlations between the expression of IL-25 and the gene expression of other inflammatory markers in NP tissues were also explored. To confirm the function of IL-25 during nasal polyposisgenesis, anti-IL-25 neutralizing antibody was administered in the murine NP model.

RESULTS: IL-25 expression was upregulated in NP mucosa from patients with CRSwNP. Overexpression of IL-25 was confirmed by IHC, and double IHC staining showed that tryptase+ cells were one of the main sources of IL-25 among immune cells. IL-17RB was also increased in immune cells of nasal polyp. In NPs, IL-25 mRNA expression was positively correlated to the expression of several inflammatory markers, including T-bet, RORC, GATA3, ECP, TGF-b1, and TGF-b2. In animal model, IL-25 was more abundant and similar correlations between IL-25 and inflammatory markers to human data were observed. Anti-IL-25 treatment reduced the number of polyps, mucosal thickness, collagen deposition, and infiltration of inflammatory cells, such as eosinophils and neutrophils. This treatment also inhibited the expression of local inflammatory cytokines. Furthermore, the expression of CCL11, CXCL2, ICAM-1, and VCAM-1 in the nasal mucosa was suppressed in the anti-IL-25 treated group.

CONCLUSIONS: Our results suggest IL-25, secreted from the sinonasal epithelia and infiltrating mast cells, plays a crucial role in the pathogenesis of CRSwNP.
768 A Newly Established Murine Model of Nasal Polyps Demonstrates B Cell Activation, Similar to Human Nasal Polyps
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RATIONALE: Animal model systems are valuable for investigating human diseases. Our laboratory recently established a murine model of nasal polyps (NP) and investigated similarities and differences between this murine model and human NP.

METHODS: After induction of an ovalbumin (OVA)-induced allergic rhinosinusitis, 6% OVA and SEB (10 ng) were instilled into the nasal cavity of mice 3 times a week for 8 weeks. The development of NP was confirmed by hematoxylin and eosin staining. The mRNA and protein levels of various inflammatory cell markers and mediators were measured by real-time PCR in nasal tissue, ELISA in nasal lavage fluid (NLF) and immunohistochemical staining (IHC) using paraffin-embedded nasal tissues.

RESULTS: Similar to human NP, there were significant increases in gene expression of inflammatory cell markers such as CD19 (2-fold), CD138 (3-fold), CD11c (9-fold), and MCP-6 (300-fold) in the nasal mucosa of the NP mouse model compared to control (P < 0.05). In further investigations of B cell activation, mRNAs expressions of B-cell Activating Factor (BAFF, 3-fold) and A Proliferation Inducing Ligand (APRIL, 2.5-fold) were found to be significantly increased in murine NP tissue (P < 0.05). BAFF protein concentration in NLF was significantly higher in the NP model than in the control group (1.5-fold, P < 0.05). Representative CD138 IHC finding showed markedly increased positive signals in murine NP.

CONCLUSIONS: The NP mouse model demonstrated enhanced B-cell responses, reminiscent of human NP. This mouse model may enhance our understanding of the pathophysiology of NP and provide a model to test therapeutic targets such as BAFF.

769 Innate Lymphoid Cell and Mast Cell Distributions in Chronic Rhinosinusitis Subtypes
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RATIONALE: Chronic rhinosinusitis (CRS) describes inflammatory diseases of the sinonasal mucosa and is subdivided into Th1-associated CRSwNP and Th2-associated diseases (CRS with NP (CRSwNP)) based on cytokine profiles (IFN-γ vs IL-5, respectively). Allergic fungal rhinosinusitis (AFRS) is a CRSwNP clinical subtype with fungal involvement. Compared to many human inflammatory diseases, CRS provides a unique opportunity to characterize local immune cell populations from tissue surgically removed as part of standard therapy. Innate lymphoid cells (ILCs) secrete cytokines characteristic of Th1 (ILC1), Th2 (ILC2) and Th17 (ILC3) cells. Understanding ILC subtype distribution may represent a novel target in CRS, specifically, and Th1- and Th2-driven diseases overall.

METHODS: Sinonasal tissue was surgically obtained from CRSsNP (n = 3), CRSwNP (n = 7), and AFRS (n = 4) patients. ILC1 (Lineage negative/CRTH2+/CD127+/CXCR3+), ILC2 (Lineage negative/CD127/CRTH2+), and ILC3 (Lineage negative/CRTH2+/CD127+/Nkp44+) populations from dissociated tissue were analyzed by flow cytometry and results are reported as a percentage of lymphocytes.

RESULTS: ILC1 percentage in CRSsNP (.53 +/- .18%) was slightly less than in CRSwNP (1.56 +/- .42%, P = .08) and AFRS (2.44 +/- .89%). The ILC2 percentage in CRSsNP (3.51 +/- 1.63%) was equivalent to CRSwNP (2.6 +/- .7%). ILC3 percentage in CRSwNP (.29 +/- .08%) was higher than in CRSsNP (.16 +/- .12%) and AFRS (.06 +/- .02%).

CONCLUSIONS: The mean percentages of ILC1 and ILC2 were similar among CRS subtypes. Notably, CRSwNP had wide percent distribution for all 3 ILC types not observed in CRSsNP and AFRS, mirroring its clinical heterogeneity. Finally, ILC3 presence in CRSwNP suggests a possible role in its pathophysiology.

770 Allergic Sensitization, High Local IL-5 and IgE Predict Surgical Outcome 12 Years After Endoscopic Sinus Surgery for Chronic Rhinosinusitis with Nasal Polyposis
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RATIONALE: Long-term control of chronic rhinosinusitis with nasal polyposis (CRSwNP) is a challenge despite medical treatment and endoscopic sinus surgery (ESS). We sought to monitor recurrence and revision surgery over 12 years after endoscopic sinus surgery (ESS) in CRSwNP patients and identify predictors of recurrence and revision surgery.

METHODS: In a prospective cohort study we evaluated clinical symptoms and total nasal endoscopic polyp score in 47 patients with CRSwNP, over 12 years after ESS. The inflammatory profile was obtained before surgery based on nasal tissue, nasal secretions and serum.

RESULTS: Twelve years after surgery, the total nasal endoscopic polyp score and the symptoms were significantly better than before surgery. Within the 12-year follow-up period, nasal polyps recurred in 78.9%, whereas 36.8% underwent additional revision surgery, albeit continuous medical treatment. Comorbid allergy, tissue IL-5 and IgE levels were found to be significant predictors for the need of revision surgery.

CONCLUSIONS: This long-term cohort study, investigating the outcome after ESS in CRSwNP, showed that patients with CRSwNP were subject to recurrence of the disease and to revision surgery over a 12-year period. Based on these findings we have identified allergic sensitization, local IL-5 and IgE production as risk factors for the need of revision surgery.
**771 IgE Is Necessary for Pulmonary Vascular Leak during a Respiratory Viral Infection**

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**RATIONALE:** Childhood respiratory viral infections are associated with increased risk of asthma. Severe paramyxoviral (Sendai virus, SeV) respiratory infection in mice translates into post-viral atopic disease. In this model, IgE is critical for development of post-viral atopic disease. This pathway appears operative in humans, as we and others have shown antiviral IgE is part of respiratory antiviral immune responses. Why IgE is part of the antiviral immune response is not known; therefore, we hypothesized it is needed to develop pulmonary vascular leak.

**METHODS:** C57BL6 (WT) or IgE^{−/−} mice were inoculated with SeV. At different days post inoculation (PI) SeV, Evans Blue Dye (EBD) was injected i.v. one hour before euthanasia. After flushing the pulmonary vasculature with PBS, EBD concentration in lung homogenates was determined by spectrophotometry and reported as fold increase in SeV-infected versus uninfected mice.

**RESULTS:** In WT mice, SeV infection induced pulmonary vascular leak (1.74±0.41, p=0.15 versus uninfected; 2.54±0.33, p=0.015; 3.24±0.17, p=0.0016; mean±SEM fold EBD concentration at days 6, 8, and 10 PI SeV, n=2-6). IgE^{−/−} mice failed to demonstrate increased vascular leak at all days PI SeV (1.31±0.40, 0.97±0.20, 1.34±0.27, 1.39±0.28, respectively; n=3). Compared to WT mice vascular leak at days 6, 8, and 10 PI SeV was significantly reduced in IgE^{−/−} mice (p=0.0037, 0.0009, 0.0283, respectively, n=3-5).

**CONCLUSIONS:** IgE is required for development of pulmonary vascular leak during a severe paramyxoviral respiratory infection. Further studies will identify cell(s) responsible for the leak and the specificity of the IgE response.

**772 Rhinovirus Infection Modulates the Activation Status of Circulating Basophils and Dendritic Cells**

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**RATIONALE:** Rhinovirus (RV) infection is a major risk factor for asthma exacerbations in atopic subjects; however, the cellular mechanisms involved remain unclear. Given the pivotal role of basophils and myeloid dendritic cells (DCs) in promoting Th2-driven inflammation, we aimed to explore how RV infection impacts circulating basophils and DCs using an experimental challenge model.

**METHODS:** Atopic asthmatics and non-atopic controls were challenged intranasally with human rhinovirus 16. Circulating basophils and DCs in whole blood were immunophenotyped by flow cytometry in fresh whole blood obtained immediately before inoculation (day 0), and during acute (day 4) and convalescent (day 21) phases post-inoculation.

**RESULTS:** Within both groups of subjects, basophils displayed increased expression of FcεRIα and CD63, as well as increased intracellular Syk (both total and phosphorylated) during acute infection. Moreover, this phenotype was maintained at day 21. Basophils from those subjects who had a high percentage of IL-4 positivity on day 0 (>50%) showed a decrease in IL-4 positivity by day 4. In contrast to basophils, FcεRIα expression decreased on DCs during acute infection in all subjects, whereas Syk levels (total and phosphorylated) increased, but only among asthmatics. Additionally, DCs showed a marked increase in expression of the prostaglandin D_{2} receptor, CRTH2.

**CONCLUSIONS:** The activation status of circulating basophils and DCs is modulated in a dynamic fashion by rhinovirus infection, irrespective of concomitant asthma. Upregulation of the Th2 cell-associated receptor CRTH2, on DCs, points to a heretofore unrecognized role for the CRTH2 pathway in DCs during RV infection.

**773 Alternaria alternata Induces Mast Cell Activation in an IgE-Independent Fashion**

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**RATIONALE:** Alternaria alternata is an environmental fungus implicated in acute exacerbations of asthma and was recently demonstrated to trigger cysteinyl leukotriene (cys-LT) production in the lung of naive mice. As mast cells (MCs) have the capacity to generate significant quantities of cys-LTs, we sought to determine whether A. alternata could induce MC cys-LT production and the pathway that might be involved.

**METHODS:** Human MCs were grown from peripheral blood. Murine bone marrow-derived MCs (BMMCs) were generated from wild type (WT), leukotriene C_{4} synthase (LTC_{4}S)-deficient, and FcεRI{γ}-deficient mice. A. alternata-induced cys-LTs were measured by ELISA. Degranulation was assessed by release of beta-hexosaminidase. WT BMMCs were activated with A. alternata in the presence or absence of inhibitors of spleen tyrosine kinase (Syk), phosphoinositide 3-kinase (PI3K), and protein kinase C (PKC) and pertussis toxin. Cutaneous MC degranulation and ear swelling after intradermal injection of A. alternata was assessed.

**RESULTS:** A. alternata triggered cys-LT generation and degranulation of human peripheral blood MCs and murine BMMCs. Cys-LT production was absent in LTC_{4}S-deficient BMMCs. Cys-LT production was FcεRIγ-independent and was not reduced by inhibitors of Syk, PI3K, PKC{α}, or pertussis toxin. Intradermal ear injection of A. alternata in both BALB/c and C57BL/6 mice led to dose-dependent ear edema associated with MC degranulation.

**CONCLUSIONS:** A. alternata triggers cys-LT generation and degranulation in human and murine MCs through a mechanism that is independent of IgE receptor signaling. This demonstrates an innate pathway by which environmental allergens can trigger pathologic MC activation.
**774** Prostaglandin E2 Deficiency Permits Leukotriene E4-Selective Airway Hyperresponsiveness and Mast Cell Activation

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**RATIONALE:** Aspirin exacerbated respiratory disease (AERD) is characterized by asthma, tissue eosinophilia, dysregulation of cysteinyl leukotrienes (cysLTs) generation, selective airway-hyperresponsiveness to LTE4, and cysLT-driven respiratory reactions to nonselective cyclooxygenase (COX) inhibitors. Previously we have demonstrated that mPGES-1-null (ptges−/−) mice showed bronchoconstriction and high level production of cysLTs when challenged with aspirin following induction of eosinophilic pulmonary inflammation. We hypothesized that the ptges−/− mice would exhibit enhanced end-organ responsiveness to cysLTs, in addition to their overproduction of cysLTs.

**METHODS:** ptges−/− and WT-C57BL/6 mice were treated intranasally with low-dose of house dust mite extract on 6 occasions, then were challenged with aerosolized LTC4, LTD4, or LTE4, and airway resistance (RL) was measured.

**RESULTS:** ptges−/− mice, but not WT controls, exhibited sustained increases in RL in response to cysLTs with a rank order of LTE4 > LTD4 > LTC4. CysLTs elicited the release of both prostaglandin (PG)D2 and thromboxane (TX)A2 with a similar rank order of potency, and also induced the releases of both mMCP-1 and histamine. Administration of a T prostanoid (TP) antagonist blunted the rise in RL induced by LTE4. Quantitative-PCR revealed that the lungs of ptges−/− mice expressed higher levels of mRNA encoding the LTE4 receptor GPR99 than did WT-controls.

**CONCLUSIONS:** Lesions that impair the inducible generation of PGE2 with airway inflammation may dysregulate the expression and function of GPR99, permitting mast cell activation in response to LTE4. Mast cell-derived COX products may then mediate LTE4-induced bronchoconstriction. The findings suggest applications of stabilizer of mast cell or TP receptor antagonists for the treatment of AERD.

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**The FcεRIβ Homologue, MS4A4, Promotes FcεRI-dependent Human Mast Cell Degranulation By Facilitating PLCγ1 Signaling**

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**RATIONALE:** MS4A4 is a member of the MS4A gene family that includes FcεRIβ and CD20. The MS4A gene family are clustered around 11q12, a region previously linked to atopy. FcεRIβ is critical for trafficking FcεRI complexes to the plasma membrane and promotes FcεRI-dependent signaling within lipid rafts via recruitment of Lyn kinase. We predicted analogous roles for the highly homologous MS4A4 protein.

**METHODS:** We used lentiviral-delivered shRNA to silence MS4A4 expression in human LAD2 mast cells (MCs). Cells were monitored by measurement of receptor expression and apoptosis by flow cytometry, degranulation by β-hexosaminidase release, protein trafficking by confocal microscopy and cell signaling using immunoblotting.

**RESULTS:** MS4A4 expression increased as human MCs mature in culture. Silencing MS4A4 did not affect surface FcεRIa expression, but resulted in significant reduction in FcεRI-dependent degranulation without reducing receptor-independent degranulation induced by thapsigargin. We found that FcεRI-induced PLCγ1 and ERK signaling were also significantly reduced, but not signaling through Akt (an activation marker of the PI-3-Kinase pathway). MS4A4 also regulates KIT signaling and trafficking via recruitment to caveolin-1-enriched membrane microdomains and enables SCF-induced PLCγ1 signaling. In addition, MS4A4 silencing promoted proliferation and migration in response to SCF.

**CONCLUSIONS:** MS4A4 promotes MC degranulation by facilitating PLCγ1 signaling pathways most likely within lipid rafts. MS4A4 expression may be involved in MC differentiation as indicated by increased expression during maturation and enhanced proliferation with knockdown. Indeed, silencing MS4A4 may convert MCs from a secretory to a less differentiated and migratory phenotype indicating a significant role for MS4A4 in MC function.